Chapter 3
Laboratory-Induced Apogamy and Apospory in *Ceratopteris richardii*

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3.1 Introduction

A life cycle characterized by an alternation between two generations, each developing a multicellular body, is a feature unique to land plants (Embryophytes). In addition to the sexual life cycle defined by meiosis and fertilization, some species, widely distributed among the embryophytes, complete their life cycle asexually. The best-described variations of the asexual life cycle include apomixis in grasses and obligate apogamy in ferns. Recent studies using model plants to unravel the developmental program of the angiosperm female gametophyte at the molecular level have provided insights into the understanding of apomixis. This chapter begins with a review of the current view of how alternation of generations in the embryophytes evolved, then provides a description of apogamy and apospory in ferns and compares those alternatives to the sexual life cycle with apomixis in angiosperms. Finally, induced apogamy and apospory in the model fern *Ceratopteris richardii* are reviewed.

3.2 Alternation of Generations

All embryophytes progress through a life cycle that alternates between two generations, the haploid gametophyte and the diploid sporophyte. Unlike animals in which meiosis produces single-celled gametes directly, gametes of embryophytes arise from a multicellular entity, the gametophyte generation, which in turn develops from the products of meiosis, the spores. The dominant generation in the basal branch of the embryophytes, the bryophytes, is the gametophyte, which supports a minuscule sporophyte. Conversely, in the most advanced embryophytes, the angiosperms, the gametophyte generation is miniscule and deeply embedded in the

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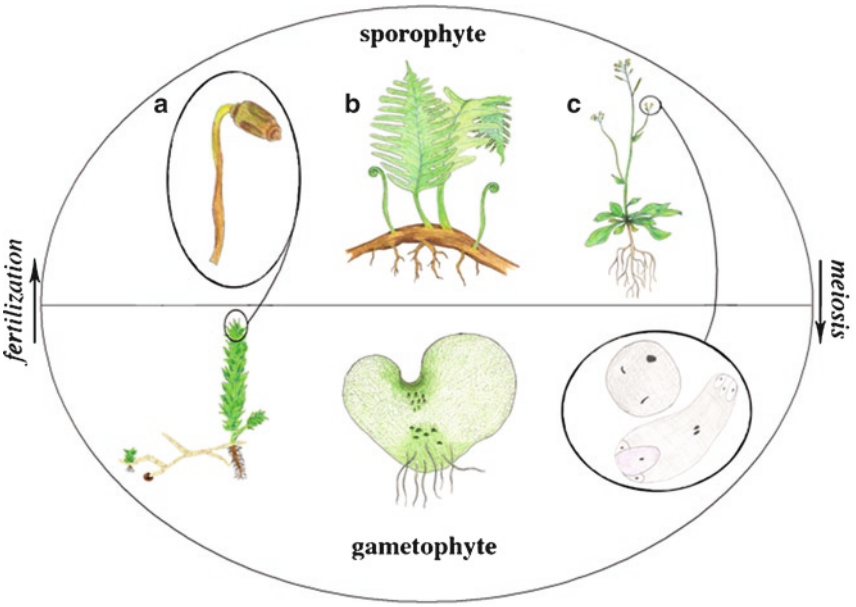

Fig. 3.1 Variation of alternation of generations in embryophytes. The three representative plants are a moss (**a**), a fern (**b**), and *Arabidopsis* (**c**); sporophytes are in the top panel and gametophytes are in the bottom panel

flowers of the sporophyte. Most deserving of the term "alternation of generations" is the life cycle of the seedless vascular plants, the lycophytes and monilophytes, whose two generations grow independently. Both gametophyte and sporophyte are photosynthetic in the monilophytes, the group that includes the ferns. The variation of alternation of generations among embryophytes is depicted in a cartoon (Fig. 3.1).

Although whether multicellularity arose first in the gametophyte or sporophyte in the ancestral green land plant can be argued either way, the current view of evolution of the two generations in land plants can be summarized as follows. Embryophytes belong to a monophyletic clade of photosynthetic eukaryotes that descended from a charophyte-like ancestor (McCourt et al. 2004; Becker and Marin 2009). All charophytes have a life cycle consisting of a single multicellular haploid generation – the only diploid cell is the zygote, which undergoes meiosis immediately (Bateman et al. 1998). Thus, an alternation between two multicellular generations in land plants is a derived condition (Niklas and Kutschera 2010). The ancestral land plants most likely possessed a dominant gametophyte generation that restricted them to dwelling in moist environments. The optimal reproduction and dispersal strategy for early terrestrial plants was via the production of spores, rather than gametes. The multicellular sporophyte, arising from a delay of meiosis, was thought to be the first adaptation to terrestrial life (Becker and Marin 2009). During the Devonian period (approximately 420–350 million years ago), an

explosion of morphological diversification occurred in the elaboration of the sporophyte, such as deep roots and strong vascular supports, allowing the sporophytes with these adaptations to colonize drier lands. Gametophytes, on the other hand, became both smaller and dependent upon the sporophyte generation for growth and development. Eventually the seed habit evolved, permitting the further colonization of new environments (Bateman et al. 1998; Dolan 2009).

Molecular evolution analyses of gene families encoding transcription factors have provided invaluable information for investigating the evolution of homology and novelty in developmental patterns and the complex regulatory circuitry inherited from a common ancestor (Shubin et al. 2009). Some gene families encoding evolutionarily conserved transcription factors important in angiosperm development have been identified. They have shown dynamic changes across the green plant phylogeny. Comparison of their expression patterns revealed that some family members function in development of the haploid reproductive structures in algae, indicating an ancient origin. Over evolutionary time these genes were redeployed for sporophyte development in lower embryophytes and later for the elaboration of more complex sporophytes in angiosperms (Niklas and Kutschera 2009). Take, for example, the *FLORICAULA/LEAFY* (*FLO/LFY*) regulation of the MIKC-type MADS-box transcription factors in angiosperms. In Arabidopsis, MIKC-type genes are positively regulated in the floral meristem once it has been converted from the vegetative state by the action of *FLO/LFY* transcription factors (Weigel et al. 1992). In the fern *Ceratopteris richardii,* which like all seedless vascular plants lacks floral meristems, the expression of *FLO/LFY,* and MIKC-type orthologs is not correlated, suggesting transcription of the MIKC-type genes is not yet under the control of *FLO/LFY* (Himi et al. 2001). In the moss *Physcomitrella patens*, *FLO/LFY* paralogs are required instead for the first division of the zygote and early embryogenesis (Henschel et al. 2002; Tanahashi et al. 2005). In the Charophyte *Chara globularis*, the expression of MIKC-type is highest in gametangia (Tanabe et al. 2005). The dynamic expression pattern of the MIKC-type genes and their relationship with *FLO/LFY* illustrates the gene duplication, loss, and recruitment for expression that had occurred from algae to flowering plants.

Another example is the three amino acid loop extension (TALE) superfamily of homeodomain proteins. Homeodomains are among the most ancient of DNA-binding protein domains (Treisman et al. 1992; Derelle et al. 2007). Classes I and 2 knotted1-like homeobox (*KNOX)* genes and *BELLRINGER* (*BELL*) class genes are all members of this superfamily. In Arabidopsis, class 1 *KNOX* genes and the *BELL* genes interact to specify shoot meristem identity (Hake et al. 2004). In *C. richardii*, the expression of three class 1 *KNOX* genes are expressed in the shoot meristem, leaf primordia, vascular bundles, and leaf margins of the sporophyte. The single class 2 *KNOX* gene is expressed throughout the sporophyte, but no member of either class of the *KNOX* genes is expressed in gametophytes (Sano et al. 2005). Similarly, in *P. patens*, expression of all *KNOX* genes is restricted to sporophytic tissues. Knocking out the two class 1 *KNOX* genes or the single class 2 *KNOX* gene disrupts sporophyte development (Singer and Ashton 2007). Most interestingly, in

the green algae *Chlamydomonas reinhardii*, the *KNOX* ortholog Gsm1 and the BELL-like-protein gene Gsp1 are expressed in *minus* and *plus* vegetative cells, respectively. When these two proteins are brought together upon syngamy, they form heterodimers and translocate to the nucleus to turn on genes for the zygote developmental program. When either protein is ectopically expressed in the other type of vegetative cells, zygote-program genes are turned on (Zhao et al. 2001; Lee et al. 2008). This illustrates how deeply rooted the TALE genes are in "generation" dimorphism and the eventual diversification of the function of family members in flowering plants.

3.3 Apogamy and Apospory in Ferns

In addition to the normal land plant life cycle marked by meiosis and fertilization, some species of moss and fern undergo generational transitions asexually through apogamy or apospory. In these processes, the morphological development of one generation to another is uncoupled from the changing of ploidy during meiosis and gamete fusion. A long debated but still unresolved question concerning the evolution of the gametophyte and the sporophyte generations is whether the ancestral land plant possessed an isomorphic or dimorphic life cycle. The capacity for naturally occurring apogamy and apospory in some present-day mosses and ferns species suggests that both haploid and diploid genomes contain the information required for constructing the body plan of gametophyte and sporophyte. This argues for a land plant ancestry with an isomorphic life cycle (Niklas and Kutschera 2009).

In nature, many fern species are obligatorily agamosporous, so that they undergo alternation of generations asexually (Bell 1992). These ferns typically are sterile due to nonfunctional archegonia or antheridia, and without fertilization, sporophytes are regenerated from a vegetative cell of the gametophyte by apogamy (Smith 1979; White 1979). Such apogamous sporophytes possess the same chromosome numbers as the gametophytes. During sporogenesis, a compensatory mechanism acts, giving rise to diplospores that retain the same number of chromosomes as the apogamous sporophyte. In most sexually reproducing ferns, the initial archesporial cell divides four times mitotically, resulting in 16 spore mother cells (SMC). Each SMC then undergoes meiosis, generating a total of 64 spores in 16 tetrads. The two types of compensatory mechanisms that are recurrent, allowing the life cycle to be completed, are the Dopp-Manton and the Braithwaite schemes (Klekowski 1979; Walker 1979). In the Dopp-Manton scheme, most commonly, the archespore first undergoes three rounds of mitosis, generating eight cells. During the fourth mitosis, the chromosomes double without cytoplasmic division, thus resulting in restitution nuclei that contain twice the number of the chromosomes. Subsequent meiosis proceeds with the formation of bivalents and produces 32 diplospores as tetrads. In the Braithwaite scheme, the archespore undergoes four normal mitoses to give rise to 16 spore mother cells; subsequently, during meiosis

I, the chromosomes do not pair and the cytoplasm fails to divide, resulting in restitution nuclei with doubled chromosomes. Meiosis II proceeds normally to give rise to 32 diplospores in dyads. Gametophytes develop from these diplospores but are defective in reproductive functions, producing sporophytes apogamously to complete the asexual life cycle. Such a life cycle does not require a water film for sperm to swim for fertilization and is thought to have evolved as an adaptation to a drier environment (White 1979). Indeed many Cheilanthoid ferns use this strategy in order to persist in xeric environments (Mickel 1979).

In apospory, gametophytes are produced by somatic cells of the sporophyte without going through sporogenesis (Sheffield and Bell 1981; Raghavan 1989; Bell 1992). Apospory only occurs in nature sporadically (Walker 1979) but is easily induced in the laboratory. In contrast to induced apogamy, which typically produces abnormal sporophytes, aposporous gametophytes appear normal, heart-shaped, and with functional antheridia and archegonia (Walker 1979; Ambrozic-Dolinsek et al. 2002). However, induced apospory in a normally sexual species cannot be repeated indefinitely from generation to generation because the chromosome numbers would be doubled with each fertilization event (Walker 1979).

3.4 Comparison of Apogamy and Apospory in Ferns with Apomixis in Angiosperms

While the majority of flowering plants reproduce sexually to form seeds, some 400 species from 40 families of angiosperms have evolved pathways bypassing the gametophyte to produce seeds, collectively called apomixis (Nogler 1982). Ferns, while being seedless, are nonetheless embryophytes with an experimentally accessible gametophyte generation, making them relevant for comparing their alternate life cycles to apomixis in angiosperms.

A successfully developed apomictic seed requires the maturation of endosperm, derived with or without fertilization. Because ferns completely lack an endosperm, its importance will not be discussed here. The origins of the embryo in plants with one of the three types of apomixis have been reviewed recently (Ozias-Akins 2006; Tucker and Koltunow 2009) and are summarized below (and in Fig. 3.2). In adventitious embryony, asexual embryos may arise from diploid nucellar or integument cells adjacent to the sexual embryo. In diplospory, the megaspore mother cell (MMC) either fails to initiate or complete meiosis before the onset of mitosis, resulting in an embryo sac containing an egg-like cell that proceeds onto embryogenesis without fertilization. In apospory, the MMC may or may not complete meiosis; in either case, one or more somatic cells in close proximity to the MMC differentiate into aposporous initial cells that develop into asexual embryos. In all the three types of apomixis, neither fertilization nor meiotic recombination occurs during the production of the asexual embryo, thus giving rise to a diploid embryo genetically identical to the parent.

Obligate Apogamy in Ferns	Apomixis in Angiosperms
Direct generation of sporophytes from gametophyte prothallus cells  No comparable variation in ferns	*Aposporous embryo*: developed from a nucellus or integument cell autonomously  *Adventitious embryo*: developed from a nucellus or integument cell adjacent to the fertilized embryo 
Sporophytes produce restitution nuclei by abnormal sporogenesis to produce unreduced spores 	*Diplosporous embryo*: developed from a unreduced-egg-like cell from abnormal gametogenesis 

Fig. 3.2 Comparison of apogamy in ferns with apomixis in angiosperms. Bright green indicates diploid, pale green, haploid. Embryo sacs are outlined in red and red circles represent cells that will develop into embryos

In contrast to angiosperms, the production of spores and sexual gametes in ferns are partitioned in two independent plants, the sporophyte and the gametophyte, respectively. The interval between the two events is also much greater than in angiosperms, depending largely on a favorable environment for the spores to break their dormancy and germinate. During sporogenesis in apogamosporous ferns, mishaps in mitosis or meiosis give rise to restitution nuclei, which allow the plant to complete the asexual life cycle. This aberration may be compared to diplospory apomixis in angiosperms; the difference is, of course, the direct formation of the asexual embryonic sporophyte in angiosperms and the formation of a free-living gametophyte in ferns prior to formation of the new sporophyte by apogamy (Fig. 3.2). Although apomixis has not been observed in any model plant species, insight has been gained toward understanding the mechanism of this sexual bypass by studies of female gametophyte development in model plants. Which genes and how they may function in model angiosperms during MMC differentiation and the subsequent meiosis have been reviewed recently (Curtis and Grossniklauss 2008; Tucker and Koltunow 2009) and are summarized below. The *MULTIPLE SPOROCYTE (MSP1)* and *TAPETUM DETERMINANT (TPD1)* genes may function together as part of an intercellular signaling mechanism regulating sporogenic cell fate; *SPOROCYTELESS (SPL)*, *WUSCHEL (WUS),* and downstream genes act in coordination with an auxin gradient (Sundaresan and Alandete-Saez 2010) on MMC cell differentiation. The *MEIOSIS ARRESTED AT LEPTOTENEI (MEL1)* mutation, affecting one of the

ARGONAUTE (AGO) proteins, suggests a role in siRNA regulation of target mRNAs in young sporogenous tissues. The *DYAD/SWITCH1 (SWI1)* genes are required in the subsequent meiosis of the MMC for sister chromatid cohesion and centromere organization (Mercier et al. 2001). Interestingly, in the *dyad* mutant, diploid "dyad" cells form, and those that are located closest to the chalazal end of the ovule are the most likely to generate an unreduced embryo sac with an egg that upon fertilization gives rise to a triploid embryo (Koltunow and Tucker 2008; Ravi et al. 2008). Although fertilization is involved in producing the embryo, the successful development of a gamete with unreduced chromosome numbers after a failed meiosis is similar to that in displospory apomixis. In addition to *DYAD*, a triple mutant defective in *OMISSION OF SECOND DIVISION (OSD1)*, *ATSP11*, and *ATREC*, which are known to be required for meiosis, turn meiosis into mitosis and produce diploid gametes apomeiotically (d'Erfurth et al. 2009).

Obligatory apogamy occurs in nature in those fern species whose gametophytes lack functional archegonia or antheridia, making sexual reproduction impossible. Apogamy can be forced to occur by preventing fertilization in ferns that normally reproduce sexually (Lang 1898). During induction of apogamy in *C. richardii*, Cordle et al. observed that only hermaphrodites, and not male gametophytes, could be made to produce apogamous sporophytes. In light of these observations, it is tempting to compare the direct generation of a sporophyte from a gametophyte cell in ferns to that of an egg-like cell undergoing displospory in angiosperms (Fig. 3.2). It is worth noting that in obligate apogamy, as in displospory, spores with unreduced chromosomes are produced, whereas in the case of induced apogamy, spores with reduced chromosomes are produced through normal meiosis. Arabidopsis loss-of-function mutants in the genes *FERTILIZATION-INDEPENDENT SEED 2* (*FIS2*), *MEDEA* (*MEA*), and *MULTICOPY SUPPRESSOR OF IRA 1* (*MSI1*), all encoding members of the polycomb repressive complex 2 (PRC2) (Hsieh et al. 2003; Guitton and Berger 2005a), exhibit embryogenesis from unfertilized egg cells (Chaudhury et al. 1997; Guitton and Berger 2005b). The most telling results connecting apogamy and displospory come from *P. patens*: when the moss ortholog of the Arabidopsis *CURLY LEAF* (*CLF*)/*MEA*/*SWINGER* (*SWN*) gene, also encoding a component of the PRC2, is deleted, the moss becomes apogamic (Okano et al. 2009).

The process of apospory in ferns and apospory in angiosperms are quite different. The major difference is the type of somatic cells that undergo apospory. In ferns, cells that most readily give rise to gametophytes are cells from new leaves of a young sporophyte whereas in angiosperms, it is the somatic cells in proximity to the MMC, i.e., the nucellus or integument cells, that give rise to the apomictic embryo. How these somatic cells gain their ability to become embryogenic is not clear. What has been demonstrated in flowering plants is the triggering of embryogenesis in young *Arabidopsis* seedlings and roots by over-expression of transcription factors that play important roles in major developmental processes, such as *BABYBOOM (BBM), LEAFY COTYLEDON1 (LEC1), LEC2*, *WUS,* and *SOMATIC EMBRYOGENESIS RECEPTOR KINASE1 (SERK1)* (Curtis and Grossniklauss 2008). This phenomenology may be more comparable to apospory in ferns where

a somatic leaf cell gives rise to a gametophyte than in apospory of flowering plants because these genes, except *SERK1* (Hecht et al. 2001), do not appear to be expressed in egg cells or the zygote of wild-type Arabidopsis.

3.5 Induction of Apogamy and Apospory in *C. richardii*

The homosporous fern *C. richardii* has emerged as a productive model system for studying developmental processes (Hickok et al. 1995; Banks 1999; Chatterjee and Roux 2000). Two obligately apogamous laboratory strains of *Ceratopteris* have been described, both of which produce nonfunctional spermatozoids. One strain resulted from the aneuploid gametophyte of a triploid hybrid and another from a diploid hybrid between *C. richardii* and *C. pteridoides* (Hickok 1977; Hickok 1979). In order to take advantage of the model fern *C. richardii*, Cordle et al. (2007)) developed an experimental system with which apogamy can be induced. This process involves growing gametophytes on a high-sugar medium, while preventing fertilization and thus the production of zygote-derived sporophytes. Fertilization is prevented by physically removing male gametophytes from the medium and by inverting Petri dishes to minimize water condensation on the hermaphrodites. Mutants may also be used to facilitate this process. The *hermaphrodite 1* (*her1*) mutant does not produce male gametophytes (Banks 1994), eliminating the need for their physical removal. If *feminization 1 (fem1)* mutant is used, fertilization is not a concern because the *fem1* spores only produce females in the absence of antheridiogen (Banks 1997); any sporophytes produced from these gametophytes will be apogamous.

In classic studies, apogamy was readily induced in several species of homosporous ferns by culturing gametophytes on exogenous sugars (Whittier and Steeves 1962). In the case of *C. richardii*, apogamy can be induced using various concentrations of sucrose, glucose, or trehalose, but it was found that basal medium (1% agarose supplemented with 0.5 × Murashige and Skoog salts at pH 6) supplemented with 2.5% glucose was optimal for the induction of apogamy. Under this condition, the highest percentage of gametophytes produce apogamous sporophytes in the shortest period of time (Cordle et al. 2007). Grown on this medium, gametophytes become thickened and proliferate, growing an extensive net of rhizoids and clusters of antheridia on the margins of the prothalli. After approximately 25–30 days of growth, apogamous outgrowths begin to appear as isolated sporophyte-like organs, taking the shape of leaves, stems, or sometimes roots. These outgrowths have three dimensional structure, vascular tissue, and stomata, all features that are hallmarks of sporophytes and never present in gametophytes. DNA content and chromosome counts remain at haploid levels, indicating that they are apogamous. Similar to apogamous sporophytes in other sexual fern species (Lang 1898; Whittier and Steeves 1960), the apogamous outgrowths of *C. richardii* may persist, but do not mature or produce spores.

This induced-apogamy system was used to determine the minimum time required on glucose for gametophytes to commit to apogamous development. Spores were

germinated and grown on glucose-containing medium for various lengths of time and then moved to basal medium for the remaining time to observe and score for apogamous sporophytes. The first visible sign of sporophyte outgrowth occurred around day 25 and the numbers continued to increase until around 40 days after plating. It was found that from 10 to 12 days on glucose, there was a fourfold increase in the percentage of gametophytes that produced apogamous sporophytes. Interestingly, for gametophytes grown continuously on basal medium, a basal level of apogamy of up to 2% was observed. Whatever factor other than glucose in the experimental condition might be conducive for apogamy is unknown. What this "control" revealed is the plasticity of *C. richardii* development. This experimental system can be useful for isolating genes that are being turned on or off during the commitment period (day 12 versus day 10). It is also useful for examining cell-specific expression by in situ hybridization during apogamy commitment for genes identified this way and for other candidate genes, such as some of the genes described in this review.

It is interesting to note that day 12, when the gametophytes grown on inductive medium are becoming committed to apogamous development, is also the time hermaphroditic gametophytes are producing their first mature archegonia. Under this induction system, apogamy has never been observed from male or *tra1fem1* intersex gametophytes. The latter produce antheridia and non-functional archegonia due to interactions between the signaling pathways involving the *TRA* and *FEM* genes (Eberle and Banks 1996). Combining these observations, we suspect that the trigger of sporophyte development is conferred from mature and functional archegonia.

Apospory, the formation of gametophytes from vegetative tissue of sporophyte leaves, can be induced readily in many ferns by culturing sporophyte leaves on medium without or with low concentrations of added sugars (Hirsch 1975; Raghavan 1989; DeYoung et al. 1997). Because aposporous gametophytes are fertile and they have the same chromosome number as the parental sporophytic tissue, they provide a means of inducing polyploidy series (Walker 1979). DeYoung et al. (1997) used induced apospory in *C. richardii* to generate autotetraploid sporophytes and used this system in genetic analyses of mutations affecting gametophyte development. With the goal of identifying genes that play a role in apospory, we set out to optimize the conditions for apospory induction in *C. richardii*. Apospory could be induced using detached leaves on various concentrations of sucrose, glucose, or trehalose. It was found that basal medium (0.8% agarose supplemented with 0.5 × Murashige and Skoog salts at pH 6) supplemented with 0.01% sucrose was optimal for the induction of apospory. Assays using leaves of various ages from young plants revealed that leaf 1 from 7-day-old sporophytes was most likely to undergo apospory. In addition, removing ethylene released by the plants increased aposporous gametophytes by 15%. Under this condition, 35% of excised sporophyte leaves formed at least one aposporous gametophyte after 49 days. Such gametophytes are fertile and produced tetraploid progeny that survived to produce spores.

Endowed with two independent generations and now with the establishment of induced apogamy and apospory, *C. richardii* offers a versatile system to join other model plants for understanding the development and evolution of alternation of generations in embryophytes.

References

Ambrozic-Dolinsek, J., Camloh, M., Bohanec, B., and Zel, J. 2002. Apospory in leaf culture of staghorn fern (*Platycerium bifurcatum*). Plant Cell Rep. 20:791–796.

Banks, J. A. 1994. Sex-determining genes in the homosporous fern *Ceratopteris*. Development 120:1949–1958.

Banks, J. A. 1997. Sex determination in the fern *Ceratopteris*. Trends Plant Sci. 2:175–180.

Banks, J. A. 1999. Gametophyte development in ferns. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:163–186.

Bateman, R. M., Crane, P. R., DiMichele, W. A., Kenrick, P. R., Rowe, N. P., Speck, T., and Stein, W. E. 1998. Early evolution of land plants: phylogeny, physiology, and ecology of the primary terrestrial radiation. Annu. Rev. Ecol. Syst. 29:263–292.

Becker, B. and Marin, B. 2009. Streptophyte algae and the origin of embryophytes. Ann. Bot. 103:999–1004.

Bell, P. R. 1992. Apospory and apogamy: implications for understanding the plant life cycle. Int. J. Plant Sci. 153:S123–S136.

Chatterjee, A. and Roux, S. J. 2000. *Ceratopteris richardii*: A productive model for revealing secrets of signaling and development. J. Plant Growth Regul. 19:284–289.

Chaudhury, A. M., Ming, L., Miller, C., Craig, S., Dennis, E. S., and Peacock, W. J. 1997. Fertilization-independent seed development in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 94:4223–4228.

Cordle, A. R., Irish, E. E., and Cheng, C. L. 2007. Apogamy induction in *Ceratopteris richardii*. Int. J. Plant Sci. 168:361–369.

Curtis, M. D. and Grossniklauss, U. 2008. Molecular control of autonomous embryo and endosperm development. Sex Plant Reprod. 21:79–88.

DeYoung, B., Weber, T., Hass, B., and Banks, J. A. 1997. Generating autotetraploid sporophytes and their use in analysing mutations affecting gametophyte development in the fern *Ceratopteris*. Genetics 147:809–814.

D'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., and Mercier, R. 2009. Turning meiosis into mitosis. PLoS Biol. 7:e1000124.

Derelle, R., Lopez, P., Le Guyader, H., and Manuel, M. 2007. Homeodomain proteins belong to the ancestral molecular toolkit of eukaryotes. Evol. Dev. 9:212–219.

Dolan, L. 2009. Body building on land-morphological evolution of land plants. Curr. Opin. Plant Biol. 12:4–8.

Eberle, J. R. and Banks, J. A. 1996. Genetic interactions among sex-determining genes in the fern *Ceratopteris richardii*. Genetics 142:973–985.

Guitton, A. E. and Berger, F. 2005a. Control of reproduction by Polycomb Group complexes in animals and plants. Int. J. Dev. Biol. 49:707–716.

Guitton, A. E. and Berger, F. 2005b. Loss of function of MULTICOPY SUPPRESSOR OF IRA 1 produces nonviable parthenogenetic embryos in *Arabidopsis*. Curr. Biol. 15:750–754.

Hake, S., Smith, H. M., Holtan, H., Magnani, E., Mele, G., and Ramirez, J. 2004. The role of knox genes in plant development. Annu. Rev. Cell Dev. Biol. 20:125–151.

Hecht, V., Vielle-Calzada, J. P., Hartog, M. V., Schmidt, E. D. L., Boutilier, K., Grossniklauss, U., and De Vries, S. C. 2001. The *Arabidopsis* somatic embryogenesis receptor kinase 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol. 127:803–816.

Henschel, K., Kofuji, R., Hasebe, M., Saedler, H., Münster, T., and Theißen, G. 2002. Two ancient classes of MIKC-type MADS-box genes are present in the moss *Physcomitrella patens*. Mol. Biol. Evol. 19:801–814.

Hickok, L. G. 1977. An apomictic mutant for sticky chromosomes in the fern *Ceratopteris*. Can. J. Bot. 55:2186–2195.

Hickok, L. G. 1979. Apogamy and somatic restitution in the fern *Ceratopteris*. Am. J. Bot. 66:1074–1078.

Hickok, L. G., Warne T. R., and Fribourg, R. S. 1995. The biology of the fern *Ceratopteris* and its use as a model system. Int. J. Plant Sci. 156:332–345.

Himi, S., Sano, S., Nishiyama, T., Tanahashi, T., Kato, M., Ueda, K., and Hasebe, M. 2001. Evolution of MADS-Box gene induction by *FLO/FLY* genes. J. Mol. Evol. 53:387–393.

Hirsch, A. M. 1975. The effect of sucrose on the differentiation of excised fern leaf tissue into either gametophyte or sporophytes. Plant Physiol. 56:390–393.

Hsieh, T. F., Hakim, O., Ohad, N., and Fischer, R. L. 2003. From flour to flower: How Polycomb group proteins influence multiple aspects of plant development. Trends Plant Sci. 8:439–445.

Klekowski, E. J. 1979. The genetics and reproductive biology of ferns. In The Experimental Biology of Ferns, ed. A. F. Dyer, pp. 133–165. Academic Press, New York.

Koltunow, A. M. G. and Tucker, M. R. 2008. Functional embryo sac formation in *Arabidopsis* without meiosis – one step towards asexual seed formation (apomixis) in crops? J. Biosci. 33: 309–311.

Lang, W. H. 1898. On apogamy and development of sporangia upon fern prothallia. Philos. Trans. R. Soc. Lond. 190:187–238.

Lee, J. H., Lin, H. W., Joo. S., and Goodenough, U. 2008. Early sexual origins of homeodomain heterodimerization and evolution of the plant KNOX/BELL family. Cell 133:829–840.

McCourt, R. M., Delwiche, C. F., and Karol, K. G. 2004. Charophyte algae and land plant origins. Trends Ecol. Evol. 19:661–666.

Mercier, R., Vezon, D., Bullier, E., Motamayor, J. C., Sellier, A., Lefevre, F., Pelletier, G., and Horlow, C. 2001. SWITCH1 (SWI1): a novel protein required for the establishment of sister chromatid cohesion and for bivalent formation at meiosis. Genes Dev. 15:1859–1871.

Mickel, J. T. 1979. How to know the ferns and fern allies. Iowa: Wm. C. Brown Co. Publ.

Niklas, K. J., and Kutschera, U. 2009. The evolutionary development of plant body plans. Funct. Plant Biol. 36:682–695.

Niklas, K. J., and Kutschera, U. 2010. The evolution of the land plant life cycle. New Biol. 185:27–41.

Nogler, G. A. 1982. How to obtain diploid apomictic *Ranunculus auricomus* plants not found in the wild state. Bot. Helv. 92:13–22.

Okano, Y., Aonoa, N., Hiwatashia, Y., Murataa, T., Nishiyamac, T., Ishikawaa, T., Kuboc, M., and Hasebe, M. 2009. A polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution. Proc. Natl. Acad. Sci. USA 106: 16321–16326.

Ozias-Akins, P. 2006. Developmental characteristics and genetics. Crit. Rev. Plant Sci. 25:199–214.

Raghavan, V. 1989. Developmental biology of fern gametophytes. pp. 280–294. Cambridge: Cambridge University Press.

Ravi, M., Marimuthu, M. P. A., and Siddiqi, I. 2008. Gamete formation without meiosis; Nature 451:1121–1124.

Sano, R., Jua´rez, C. M., Hass, B., Sakakibara, K., Ito, M., Banks, J. A., and Hasebea, M. 2005. KNOX homeobox genes potentially have similar function in both diploid unicellular and multicellular meristems, but not in haploid meristems. Evol. Dev. 7:69–78.

Sheffield, E. and Bell, P. R. 1981. Experimental studies of apospory in ferns. Ann. Bot. 47:187–195.

Singer, S. D. and Ashton, N. W. 2007. Revelation of ancestral roles of KNOX genes by a functional analysis of Physcomitrella homologues. Plant Cell Rep. 26:2039–2054.

Shubin, N., Tabin, C., and Carroll, C. 2009. Deep homology and the origins of evolutionary novelty. Nature 457:818–823.

Smith, D. L. 1979. Biochemical and physiological aspects of gametophyte differentiation and development. In The Experimental Biology of Ferns, ed. A. F. Dyer, pp. 355–385. Academic Press, New York.

Sundaresan, V. and Alandete-Saez, M. 2010. Pattern formation in miniature: the female gametophyte of flowering plants. Development 137:179–189.

Tanahashi, T., Sumikawa, N., Kato, M., and Hasebe, M. 2005. Diversification of gene function: homologs of the floral regulator FLO/LFY control the first zygotic division in the moss *Physcomitrella patens*. Development 132:1727–1736.

Tanabe, Y., Hasebe, M., Sekimoto, H., Nishiyama, T., Kitani, M., Henschel, K., Munster, T., Theißen, G., Nozaki, H., and Ito, M. 2005. Characterization of MADS-box genes in charophycean green algae and its implication for the evolution of MADS-box genes. Proc. Natl. Acad. Sci. USA 102:2436–2441.

Treisman, J., Harris, E., Wilson, D., and Desplan, C. 1992. The homeodomain- a new face for the helix-turn-helix? Bioessays 13:145–15-.

Tucker, M. R. and Koltunow, A. H. G. 2009. Sexual and asexual (apomictic) seed development in flowering plants: molecular, morphological and evolutionary relationships. Funct. Plant Biol. 36:490–504.

Walker, T. G. 1979. The cytogenetics of ferns. In The Experimental Biology of Ferns, ed. A. F. Dyer, pp. 87–123. Academic Press.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F., and Meyerowitz, E. M. 1992. *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell 69:843–859.

White, R. A. 1979. Experimental investigations of fern sporophyte development. In The Experimental Biology of Ferns, ed. A. F. Dyer, pp. 505–541. Academic Press, New York.

Whittier, D. P. and Steeves, T. A. 1960. The induction of apogamy in the Bracken fern. Can. J. Bot. 38:925–930.

Whittier, D. P. and Steeves, T. A. 1962. Further studies on induced apogamy in ferns. Can. J. Bot. 40:1525–1531.

Zhao, H., Lu, M., Singh, R., and Snell, W. J. 2001. Ectopic expression of a *Chlamydomonas* mt+-specific homeodomain protein in mtS gametes initiates zygote development without gamete fusion. Genes Dev. 15:2767–2777.