Chapter 1
The Gametophyte of Fern: Born to Reproduce

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1.1 Introduction

I would like to confess with some degree of disappointment, that a not small part of my teaching activity on Plant Physiology at the Faculty, must be dedicated to seduce my students, underlining the enormous and not well recognized yet, importance the plants have for all the organisms living in the Earth. Plants transform the electromagnetic energy into the biosphere, in energy of chemical bounding, providing food to the rest of organisms. There are no doubts about the need to apply the most advanced technologies available so far to their study, in order to face the three major challenges coming up for human being such as food, energy, and environmental changes (Ehrhardt and Frommer 2012). In the order of preferences, our students place plants' interest behind animals and microorganisms and consider the plant research as a poor relative that does not deserve major attention. I have often to remind that plants and animals share many homologous genes, metabolic pathways, and cellular processes. Moreover, plants can go even far away in life spans, environmental resources management, and cellular plasticity. All these aspects make plants good experimental systems to understand the fundamental principles of life and also how we are able to cope with the continuous changes in the planet, inviting us to explore their great adaptive capacity under very stressful situations, some of them derived from the anthropogenic activity such as ultraviolet light, high temperature, etc.

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1.2 Why Is It Important to Lead Studies on Mosses, Lycophytes, and Ferns?

Certainly, a great success in the history of life, especially for green plants, was the acquisition from a single eukaryotic ancestor of a photosynthetic cyanobacterium (the ancestral plastid), according to the endosymbiont theory of Lynn Margulis (Schwartz 2007). Later on, no minus repercussions had the conquest of land from the fresh water, streptophyte green algae, during the Ordovician period, over 470 million years ago, colonizing and dominating terrestrial sceneries (Kernick and Crane 1997; Becker and Marin 2009). Flora expansion between the Silurian and Permian periods leads to the origin of plant groups represented today by bryophytes, lycophytes, and euphyllophytes, which include monilophytes (ferns) and spermatophytes (seed plants).

Bryophytes (which include hornworts, mosses, and liverworts), lycophytes, and monilophytes are remains of the invasion of land by plants, hiding clues capable to shade light on how plant development evolved. The terrestrial environment imposed variations in water availability and temperature, as well as increased exposure to radiation, thus demanding changes in body plan and modifications to cellular, physiological, and regulatory processes (Rensing et al. 2008; Pires and Dolan 2012). Certainly, ferns are among the pioneer vascular plants coping with the new environmental conditions and represent a critical clade for comparative evolutionary studies in land plants. They keep traits of an ancestral life history such as the lack of secondary growth, homospory, motile sperm, and independent free-living gametophyte and sporophyte generations. Moreover, ferns represent an unexplored genetic diversity that could be taken advantage for improving plants by means of genetic transfer technologies (Rathinasabapathi 2006).

Ferns have received minor attention, and to date, only few fern species such as *Adiantum capillus-veneris*, *Anemia phyllitidis*, *Blechnum spicant*, *Dryopteris affinis* ssp. *affinis*, *Ceratopteris richardii*, *Marsilea vestita*, *Matteuccia struthiopteris*, *Onoclea sensibilis*, and *Pteridium aquilinum* have been used to study basic developmental processes such as photomorphogenesis (Wada 2007), germination (Salmi et al. 2005, 2007; Suo et al. 2015), cell polarity (Salmi and Bushart 2010), cell wall composition (Eeckhout et al. 2014), or asexual and sexual reproduction as the gametophyte is an autonomous living organism, for in vitro culture and sample collection (Whittier and Steeves 1960, 1962; von Aderkas 1984; Wen et al. 1999; Fernández and Revilla 2003; Cordle et al. 2007, 2010, 2012; Kezmierczak 2010; López and Renzaglia 2014; Valledor et al. 2014; DeVries et al. 2016; Grossmann et al. 2017).

Apart from the abovementioned processes, we assist recently to the use of ferns to resolve interesting problems in the plant world caused by abiotic and biotic stress. Drought is one of the most severe abiotic stress factors affecting plant growth and productivity and has caused considerable reduction in crop yield worldwide. Several fern and fern-ally species of *Actiniopteridaceae*, *Sinopteridiaceae*, *Pteridaceae*, and *Selaginellaceae* have been associated to desiccation tolerance. Concretely, the fern-ally *Selaginella* is one of the most primitive vascular resurrection plants, which can survive a desiccated state and recover when water becomes available, by morphological adaptations, hormonal regulation, antioxidant protection, and accumulation of osmolytes, which could serve to cope with drought in crops (Wang et al. 2010). Other important adaptations of ferns to extreme environments such as salinity, heavy metal, epiphytism, or invasiveness tolerance are summarized by Rathinasabapathi (2006). More recently, it was published in a fascinating paper based upon the feature that ferns and mosses are rarely infested by phytophagous insect in the field (Hendrix 1980; Markham et al. 2006) and in which an insecticidal protein from the fern species *Tectaria macrodonta* (Fee) C. Chr. was identified and expressed in transgenic cotton lines, conferring protection against whitefly, a sap-sucking pest (Shukla et al. 2016).

However, ferns are reported to have higher chromosome numbers and larger genomes than mosses and seed plants (Barker and Wolf 2010), making it difficult to establish genetic resources for them such as genomic and transcript sequence data. Recently, the advent of the next-generation sequencing (NGS) technologies, such as Roche'454 GS-FLX Titanium and Illumina HiSeq sequencers, by means of which is possible to characterize the transcriptome in plants, representing a small but information reach-target compared to complete genome (Ward et al. 2012). The variation in gene expression induced by whatever environmental or inner condition can be examined in non-model organisms because these techniques have become more feasible as automation and efficiency has reduced the cost. Until present and recently, some NGS transcriptome data sets have been published for ferns, which include the species *Pteridium aquilinum* (Der et al. 2011), *Ceratopteris richardii* (Bushart et al. 2013), *Lygodium japonicum* (Aya et al. 2015), *Dryopteris affinis* ssp. *affinis* (Grossmann et al. 2017), and some others resulting from the oneKP project (Matasci et al. 2014). Moreover, one of the major genomics centers in the world, BGI (in Beijing) and the China National GeneBank (CNGB), have announced 10KP, their plan to sequence 10,000 genomes or more, crossing every major plant clade and eukaryotic microbes, which will build on oneKP project.

Omics technologies based on comprehensive biochemical and molecular characterizations of an organism, tissue, or cell type and next-generation omics approaches facilitate the analyses of non-model organisms owing to the rapid generation of large amounts of de novo systems biology data, making them attractive options for studying plant development and evolution. In our lab, during the last years, they have published a significant number of reports, dealing with different topics carried out either in model species such as *Arabidopsis* and *Chlamydomonas* or non-model species, the case of *Pinus radiate*, focusing on different aspects of plant development (Valledor et al. 2010; Romero-Rodríguez et al. 2014; Jorrín-Novo et al. 2015; Sánchez-Lucas et al. 2016).

1.3 The Gametophyte: Born to Reproduce Either Sexually or Asexually

During its life cycle, a fern exists in two distinct forms: the small, sample, haploid gametophyte and the large, morphologically complex, diploid sporophyte. In ferns, the gametophyte lives separately from sporophyte (Klekowski and Baker 1966; Haufler et al. 2016) excluding some time after fertilization takes place, supporting the beginning of sporophyte development. In fact, as sporophyte develops, the gametophyte disappears in most cases, reflecting to have a role purely involved on reproduction. In *Thelypteris palustris* (Salisb.) Schott, sporophytes are supported by gametophytes until the first leaves develop; then sporophytes grow on the organic matter they produce themselves (Sakamaki and Ino 2007).

The evolution of sexual reproduction represents a major transition in the evolution of life, and occurred well before plants first ventured onto land some 470 million years ago, when the ancestors already had differentiated male and female gametes (spermatozoids and eggs), inside the sex organs archegonia and antheridia (Pannell 2017). The first land plants had also already evolved an alternation of haploid and diploid generations, gametophyte and sporophyte (Kenrick and Crane 1997), being the gametophyte, the generation when the differentiation of sex organs into egg- or sperm-producing tissues occurs. Actually, in seedless plants, gametophytes may be male or female, with separate individuals producing either antheridia or archegonia, or they may be hermaphroditic, with both antheridia and archegonia present in the same individuals (Okada et al. 2001).

Sex determination is a matter of the differentiation of cells and tissues in different parts of the same individuals. In many bryophytes, sex is determined in gametophytes by (U and V) sex chromosomes (Okada et al. 2001). To my knowledge, no sex chromosomes have been described for any ferns or lycophytes so far. In these plant groups, sex determination differs between "homosporous" species, in which sporophytes produce spores of the same size (as in bryophytes), and "heterosporous" species, in which sporophytes produce both small "microspores" and larger "megaspores". In some homosporous ferns, gametophytes are all functionally hermaphroditic, with both antheridia and archegonia. In these cases, sex determination is a question of cellular and tissue differentiation into different male and female "gametangia" (Klekowski 1969). In other homosporous species, gametophytes may develop a unisexual function (producing either only antheridia or only archegonia) (Klekowski 1969). In "heterosporous" ferns and lycophytes, sex determination acts through the size of the spore from which the gametophytes germinate. The gametophytes do not differ genetically, and the sporophyte controls the sex of its gametophytes by regulating the spore-producing "sporangia" to produce either small "microspores", which develop into male (micro-) gametophytes, or larger "megaspores", which develop into female (mega-) gametophytes. Heterospory has evolved several times in vascular land plants, in ferns and lycophytes, as well as independently in the lineage that gave rise to seed plants (Bateman and Dimichele 1994).

In angiosperms (flowering plants) and gymnosperms, the gametophytic phase has become even more highly modified and reduced, and differentiation between the male and female structures takes place in sporophytic structures in which the gametophytes form. In contrast to non-seed plants, seed-plant gametophytes are always either male or female, and it is the sporophyte that determines their sex (Pannell 2017).

1.4 Blechnum spicant: A Sexual Species

Blechnum spicant L. belongs to one of the most ancient groups of ferns. Sexual development in the gametophyte of this species is carried out by the action of the unknown antheridium-inducing substances (Fig. 1.1). When cultured in vitro, initially the gametophyte develops female sexual organs or archegonia and produces and excretes antheridiogens into the medium that induces the formation of male sexual organs or antheridia in the youngest filamentous or spatulate-shaped gametophytes (Klekowski 1969; Cousens 1979; Fernández et al. 1997, 1999; Menéndez et al. 2006a).

In Pteridophyta, sporophyte formation occurs by the means of the fusion of sexual cells, either of the same or different prothalli, and we can distinguish between intro- and intergametophytic crosses, respectively (Klekowski 1969). Natural systems have been reported to favor intergametophytic crosses, promoting genetic interchange at different levels, such as morphological, population, or genetic. In many ferns, a chemical messenger, antheridiogen, controls the onset of antheridium formation in young gametophytes, and it has been also found in *B. spicant* L. (Cousens 1979; Fernández et al. 1997, 1999).

It is clear that this induction should be reflected in a complete reprogramming of the cell fate and function, since there are not only functional but structural changes during this process. Proteomic analyses have been conducted in flowering plants in relation to reproduction, and, concretely, several reports have been published on male gametophyte development in higher plants (Kerim et al. 2003; Dai et al. 2007; Sheoran et al. 2007; Mayank et al. 2012; Ischebeck et al. 2014; Chaturvedi et al. 2016a, b). Valledor et al. (2014) compared the proteomic profiles between female and male gametophytes of *B. spicant*. Female gametophytes were obtained in MS medium (Murashige and Skoog 1962) directly from spores, and the males ones were obtained growing the gametophytes in the same medium supplemented with an extract of mature gametophytes showing antheridiogenic effects. The induction of male gametophytes by antheridiogens correlated to a global increase in stress- and defense-related pathways, which affects also flavonoid signaling and cell division while reducing protein biosynthesis pathways (Fig. 1.2). On the other hand, the photosynthesis and other energy-related pathways are affected also during this induction, being an indicator of how stressed are the gametophytes during induction process.

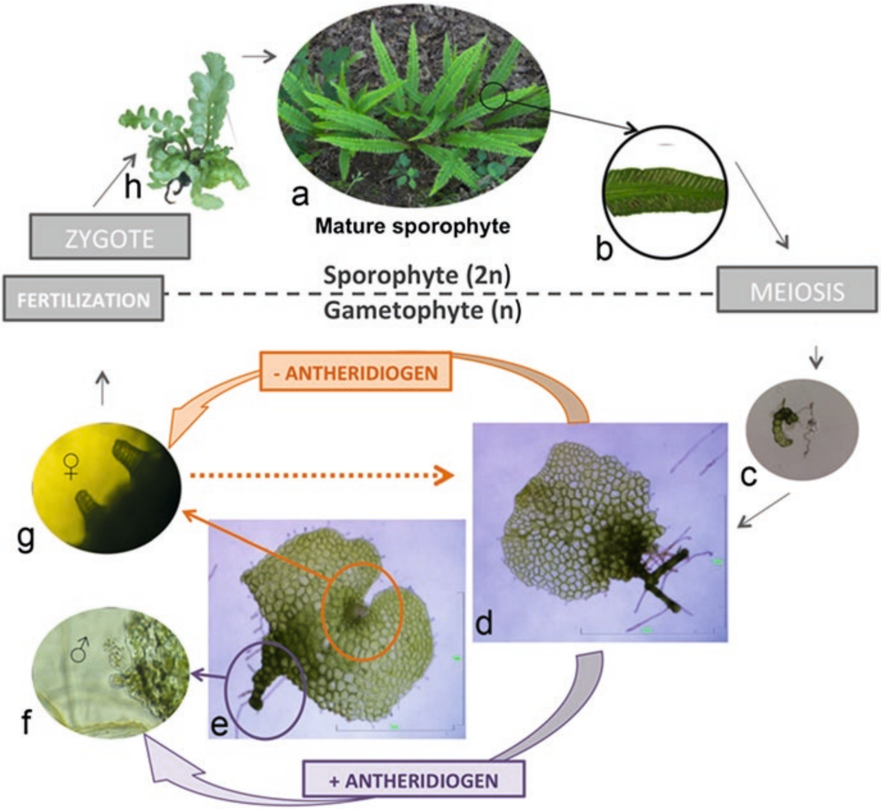

Fig. 1.1 *Blechnum spicant* sexual life cycle. Mature sporophytes (**a**) have reproductive leaves with sori (**b**). Each sorus is a cluster of sporangia in which spores are produced via meiosis. The fern spores develop into a small gametophyte (**c**) that sustains itself by photosynthesis. Young gametophytes are spatulate-shaped (**d**) and quickly maturate into a heart shape (**d**). The gametophytes with fastest growth differentiate as a female (archegonia, **e**), and, along with this maturation, they produce and excrete the antheridiogen to the media. The presence of this pheromone will induce the male differentiation of the surrounding immature gametophytes (antheridia, **e**). Anteridium produces fern sperm (**f**) that uses flagella to swim through moisture from antheridia to fertilize the eggs in the archegonia (**g**). After fertilization a zygote develops into a new young sporophyte (**h**)

1.5 *Dryopteris affinis* ssp. *affinis*: An Apomictic Species

Sexual reproduction generates new genetic individuals by combining the genetic material of two parental individuals, while asexual reproduction is limited to one genetic entity. Most angiosperms reproduce sexually through seeds, where a single generative cell (archesporial cell or megaspore mother cell) undergoes meiosis to produce four chromosomally reduced cells (megaspores). After significant cellular enlargement, the nucleus of the functional megaspore usually undergoes three

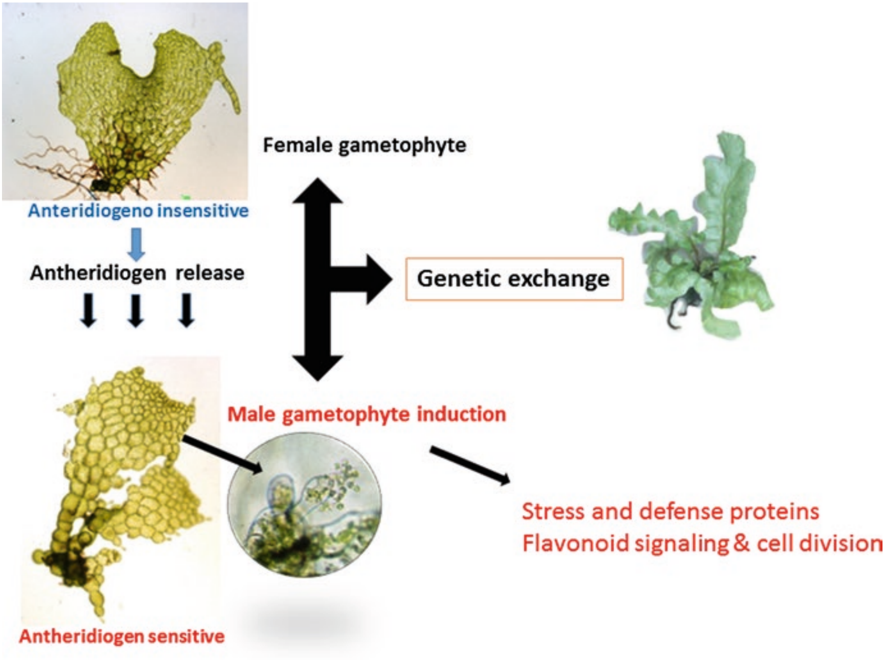

Fig. 1.2 Scheme illustrating the antheridiogen-mediated maleness operating in *Blechnum spicant* L. and the proteins induced

rounds of mitosis before giving rise to a gametophyte composed of seven cells: two companion synergids, the egg cell, a binucleated central cell, and three antipodals. Double fertilization of both the egg and central cell is necessary to trigger embryogenesis and endosperm development, respectively (Taiz and Zeiger 2015; Rodriguez-Leal and Vielle-Calzada 2012).

In plants, as in animals, transition to asexuality may occur; however, despite of its importance, little is known about the mechanisms that cause these transitions and why some taxa experience much higher transition rates than others, especially in plants, where more intensively it has been studied, due to the potential use of asexuality in crop plants to improving agriculture (Neiman et al. 2014). Asexual reproduction in plants can occur either through budding and vegetative growth (e.g., shoots and runners) or by seeds, referred as apomixis, which was a great obstacle for Mendel, when he decided to corroborate his results of genetic inheritance in peas (*Pisum*) in hawkweed (*Hieracium*) and was astonished that *Hieracium* offspring were morphologically identical to their mother plants (Neiman et al. 2014).

There are examples of asexual seed formation (apomixis), where seeds form without meiosis and fertilization (Nogler 1984; Koltunow et al. 1995; Koltunow and Grossniklaus 2003; Bicknell and Koltunow 2004; Ozias-Akins 2006; Rodriguez-Leal and Vielle-Calzada 2012; Barcaccia and Albertini 2013). In its simplest form (adventitious embryony, also called sporophytic apomixis), apomictic plants form embryos directly from a somatic cell from nucellus or inner integuments, and in species with endosperm development, the endosperm is formed by polar nuclei. Apomictic plants can also form embryos directly from a chromosomally unreduced female gametophyte (apomeiosis) in which the egg cell develops autonomously into an embryo by parthenogenesis (gametophytic apomixis), by means of two types, apospory and diplospory. In apospory, the embryo and endosperm develop in unreduced embryo sac in the ovule. In this case, the megaspore mother cell in the sexual ovule starts to develop but stops at some stage, and one or more somatic cells in the ovule and their nuclei start to develop, resembling megaspore mother cells. Before the mature embryo sac formation, the megaspore or young embryo is aborted and replaced by developing aposporous sacs. Apospory is by far the most common mechanism in higher plants and has been reported in *Beta*, *Brachiaria*, *Cenchrus*, *Chloris*, Compositae, *Eriochloa*, *Heteropogon*, *Hieracium*, *Hyparrhenia*, *Hypericum*, *Panicum*, *Paspalum*, *Pennisetum*, Poaceae, *Ranunculus*, *Sorghum*, *Themeda*, and *Urochloa* (Barcaccia and Albertini 2013). In diplospory, the embryo and endosperm develop in an unreduced embryo sac derived from the megaspore mother cell, which differentiates as in sexual ovules but does not undergo meiosis. Diplospory is found in *Tripsacum*, *Eragrostis*, and *Taraxacum* (Kandemír and Saygili 2015). Apomictic female gametes (2n) undergo embryogenesis autonomously, without fertilization. Apomictic plants carry functional pollen, which they sometimes need for endosperm formation as the formation of endosperm is still dependent on fertilization of the central cell (pseudogamy) (Rodriguez-Leal and Vielle-Calzada 2012; Kandemír and Saygili 2015). Observation of apomixis is difficult since it is generally accompanied by sexual reproduction or facultative apomixis (Kandemír and Saygili 2015).

In ferns, apomixis is an important mode of asexual reproduction (Döpp 1939; Manton 1950), which has evolved several times independently and being its frequency at least 3%, a value much higher than in other major plant groups (Ekrt and Koutecký 2016). However, most apomictic fern species are concentrated in just four families (Liu et al. 2012). Apomixis in ferns (Fig. 1.1) includes apogamy, the formation of sporophytes from somatic cells of the prothallium, and agamospermy (or diplospory), the production of unreduced (diplo) spores (Manton 1950; Ekrt and Koutecký 2016).The archesporial cell of sexual fern species usually undergoes 4 mitoses to produce 16 spore mother cells that undergo regular meiosis, resulting in 64 reduced spores in 16 tetrads. Under the prevailing type of agamospermy (Döpp-Manton scheme), the last (premeiotic) mitosis fails, resulting in 8 spore mother cells that undergo regular meiosis, producing 32 diplospores in 8 tetrads (Döpp 1939; Manton 1950). Rarely, the first meiotic division fails, which results in 32 diplospores in 16 diads (Braithwaite 1964). Genetic variation among apomictic offspring has been documented (Peredo et al. 2013).

Apogamy in ferns may be obligate, when gametophytes produce nonfunctional gametes, facultative, or induced by exogenous factors Menéndez et al. 2006b; Cordle et al. 2007). Contrary to ferns, apogamy does not occur naturally in angiosperms (Yang and Zhou 1992), but apogamous sporophytes can be induced by the culture of pollen or embryo sacs, indicating that the developmental plasticity necessary to overcome meiosis and fertilization barriers is not restricted to ferns (Seguí-Simarro 2010; Germanà 2011). In apogamy, somatic cells of the gametophyte are reprogrammed to start the sporophytic developmental program (Okano et al. 2009).

Because apomixis allows the fixation of complex genotypes, including that of highly productive F1 hybrids, many researchers have extolled the tremendous potential that apomixis holds for plant improvement, whose benefits could surpass those of the green revolution (Grossniklaus et al. 1998; Spillane et al. 2004; Marimuthu et al. 2011). Apomixis combines the advantages of propagation by seed (higher multiplication rate, easier storage and planting, suitability for machine planting, less seed material use, and less bearing of diseases) with those of propagation by clone (maintaining genetic structure and hence fixing superior genotypes after crossing) (Kandemír and Saygili 2015).

Over the last decades, several studies focusing on apomixis in model species of angiosperms concluded that sexual and apomictic pathways share gene expression profiles and, thus, common molecular regulatory features, indicating that they are not distinct pathways (Grossniklaus et al. 2001; Tucker et al. 2003). The initiation of apomixis invariably occurs during early ovule ontogeny; sexual and apomictic development can coexist within the same ovule, or within different ovules of a same individual, suggesting that apomixis could have originated as a modified form of sexual reproduction that has undergone deregulation of key developmental steps during gametogenesis (Koltunow and Grossniklaus 2003). So far it is a truth that very few crop species are apomictic, and attempts to introduce this trait by crossing have failed. The alternative would be to de novo engineer apomixis, but for this strategy to be applied, the genes that confer elements of apomixis must be identified.

Apomixis research can be faced by different approaches in each of its three major stages: apomeiosis, parthenogenesis, and seed formation. In most species under study, the basic components of apomixis can be explained by a few genes that control unreduced gamete formation and parthenogenesis, respectively; however, polyploidy, segregation distortion, suppressed recombination, epistatic interactions, naturally active modifiers, and environmental effects complicate their genetic analyses (Rodriguez-Leal and Vielle-Calzada 2012).

There are various ways of converting crop plants into apomictic ones: (a) wide crosses with apomictic wild relatives, (b) mutation, and (c) genetic transformation. Transfer of apomixis from wild relatives via sexual hybridization depends on the presence of relatives with which interspecific hybridizations can be made but this is not possible for most cultivated species. Although significant developments have been done recently, it is generally accepted that apomixis transfer via wide crosses has been unsuccessful so far (Spillane et al. 2004). Cloning of LOA and LOP genes of *Hieracium* is underway, and, after their cloning and transfer, apomixis could be introgressed into other crop species (Kotani et al. 2014). On the other hand, studies in the model plant Arabidopsis revealed that apomixis can also be achieved through artificial mutations. One of them involves the gen Osd1, which controls the entering into the second meiotic division (d’Erfurth et al. 2009). By combining a mutation in this gene with two other mutations, one that eliminates recombination and pairing (Atspo11-1) and another that modifies chromatid segregation (Atrec8), a new genotype was created in which meiosis is totally replaced by mitosis without affecting subsequent sexual processes and called MiMe for "mitosis instead of meiosis" (d'Erfurth et al. 2009; Marimuthu et al. 2011). The induction of apomeiosis by the creation of the MiMe genotype is an important step toward understanding and engineering apomixis, giving place to a genotype called MiMe in which meiosis was replaced with mitosis. In another mechanism, a mutation in the Arabidopsis SWI1 gene leads to apomeiosis and diploid egg formation (Ravi et al. 2008). Chaudhury et al. (1997) reported seed development in mutations of Arabidopsis FIS1, FIS2, and FIS3 genes in the absence of fertilization.

Dryopteris affinis (Lowe) Fraser-Jenkins ssp. *affinis* is a diploid fern with an apomictic life cycle, and it probably originates from the crossing of *D. wallichiana* and *D. oreades*, being broadly distributed in the Mediterranean, Macaronesia, and western Eurosiberian regions (Salvo 1990). When cultured in vitro, apogamy in this species is evident as gametophyte develops a brown meristematic area near the apical indentation that evolves into a new sporophyte. Sexual reproduction is not possible due to the lack of archegonia (Fernández et al. 1996) (Fig. 1.3). Apogamy in ferns can be seen as an opportunity to investigate on embryogenesis, one of the more powerful tools in plant biotechnology. On the other hand, in both apomixis and apogamy, unreduced cells form an embryo without fertilization, and it might be expected that they share some common features. Moreover, the mechanism of asexual reproduction in lower and higher plants appears to be controlled by overlapping sets of genes (Cordle et al. 2012). How somatic cells, either of sporophytic or gametophytic origin, become and develop as embryogenic is still poorly understood (Radoeva and Weijers 2014). Somatic embryogenesis research in ferns is scarce so far and limited to a few reports (Mikuła et al. 2015; Domżalska et al. 2017) and even minor is the number of reports involving embryogenesis linked to apogamy (Bui et al. 2017; Grossmann et al. 2017). In general terms, we can assume that the molecular basis of embryogenesis by sexual or asexual means, in plant kingdom, is far to be understood (De Smet et al. 2010). In seed plants, several groups of genes, mostly encoding transcription factors, such as BABY BOOM, AINTEGUMENTA-like 5, FUSCA3, LEAFY COTYLEDON, etc., have been associated to embryogenesis even though they are required either for embryogenesis itself or plan cell viability and would need still further explanation (Radoeva and Weijers 2014).

Performing proteomic studies on non-model organisms with little or no genomic information is still difficult. In our lab, recently, both transcriptomic and proteomic analyses were performed to increase our knowledge on the molecular basis of apogamy in *D. affinis* ssp. *affinis*, by using next-generation sequencing (NGS) and shotgun proteomics by tandem mass spectrometry (Grossmann et al. 2017). This way 1397 protein clusters with 5865 unique peptide sequences were identified, identifying homologs of proteins involved in several activities (Fig. 1.4a) from several taxa (Fig. 1.4b) and in particular on reproduction of higher plants, including proteins with a potential role in apogamy. Furthermore, in this study, we detected some fern

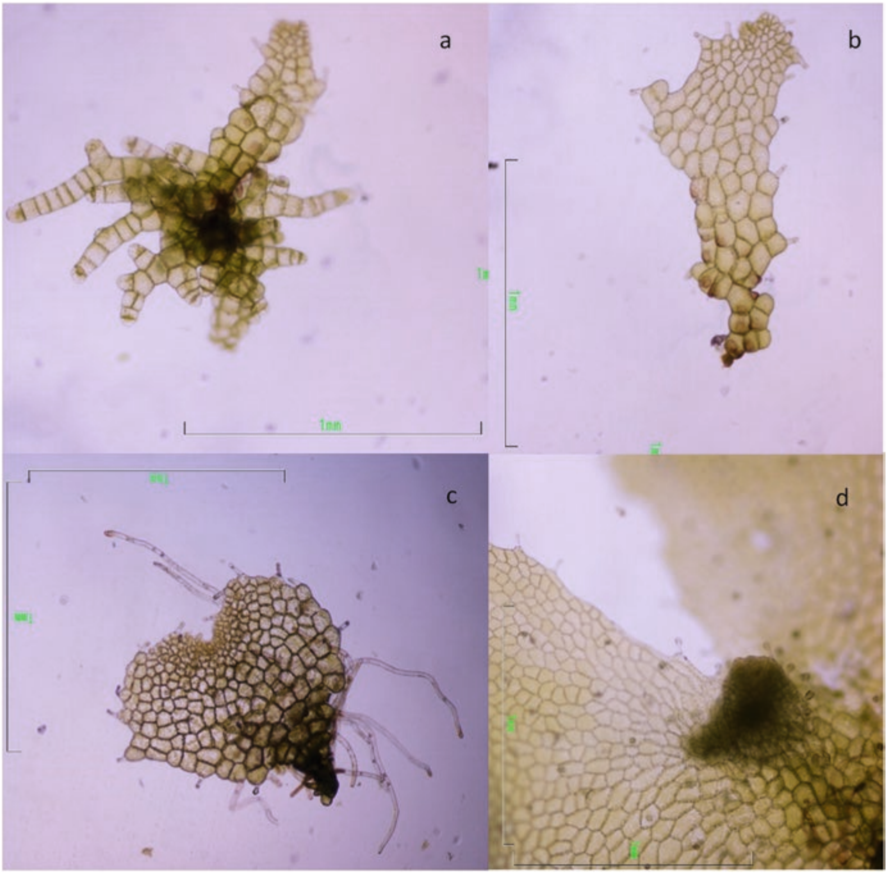

Fig. 1.3 Vegetative developmental and apogamy in the gametophyte of the fern *Dryopteris affinis* ssp. *affinis*. Successive morphological appearance of gametophyte: (**a**) filamentous, (**b**) spatulate- and (**c**) heart-shaped, and (**d**) apogamous embryo

protein homologous to ARGONAUTE10/PINHEAD/ZWILLE and also to the *A. thaliana* SERRATE (SE) RNA effector protein which could participate in the meristematic activity of the incipient apogamic embryo or unknown roles in the switch between sexual and asexual reproduction and perhaps in the regulation of apogamy in ferns (Grossmann et al. 2017). Additionally, proteins involved in gene silencing or enzymes involved in cell wall modifications such as pectinecterases were also identified, which could hide some role on apogamy (Li et al. 2011). Recently, we have done a RNA-Seq approach to compare gene expression profiles of one- and two-dimensional gametophytes of this species, finding several thousands of genes differentially expressed and related to different aspects of either vegetative or reproductive behavior of the gametophyte (Fernández, personal communication).

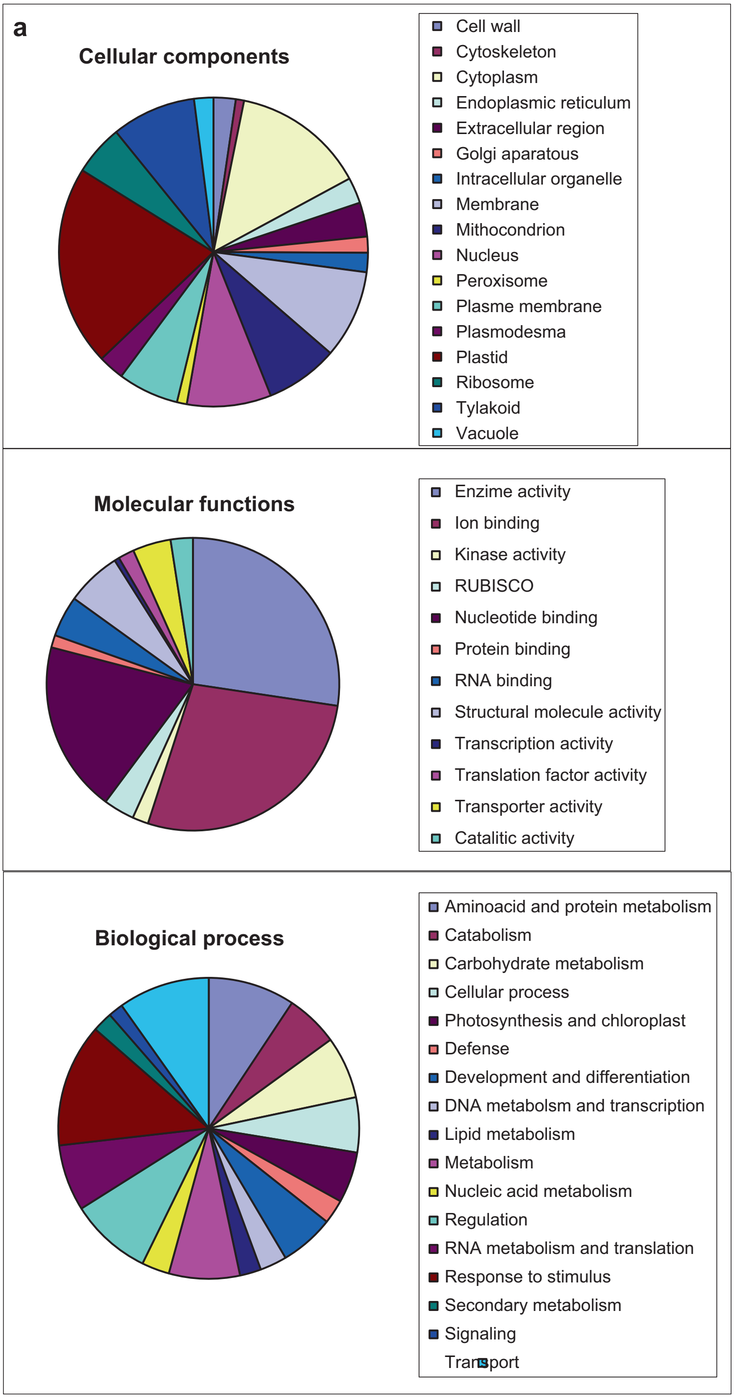

Fig. 1.4 Proteomic profile from the gametophyte of *Dryopteris affinis* ssp. *affinis*. (**a**) Distribution of plant GO-slim functional categories; (**b**) matching species

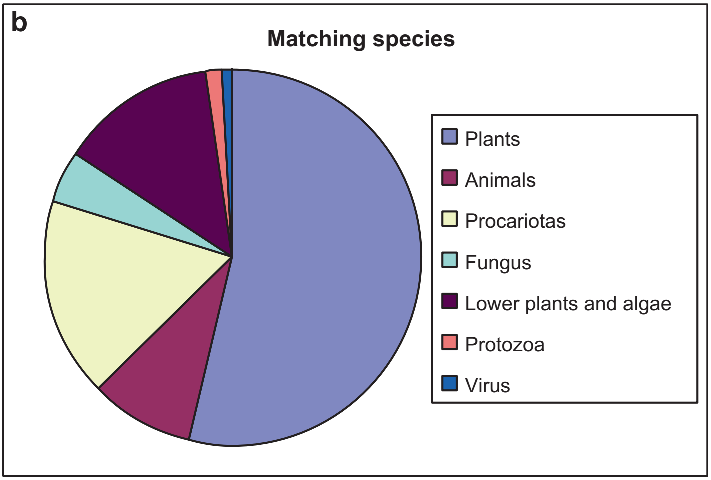

Fig. 1.4 (continued)

In summary, with the increasing availability of genomic data from non-model species, similar proteogenomics approaches will improve the sensitivity in protein identification for species only distantly related to models.

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