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Reproduction and hybridization in ferns

Ph.D. Thesis

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Annotation

This thesis studies several aspects of fern reproduction and hybridization. Aspects of multiple stages in the fern life cycle are analyzed. Specifically, rates of spore abortion and antheridiogen usage are assessed and compared between various groups. Own cultivation and spore assessment data are compared extensively with existing literature. Finally, the rate of hybridization in natural populations and the symmetry of hybridization is analyzed in buckler ferns (*Dryopteris carthusiana* group).

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. Zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 1.12.2020

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The thesis is based on the following papers (listed chronologically):

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OH and LE conceived the study, collected samples and contributed to the writing of the manuscript, OH performed SAI assessment and statistical analyses.

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OH, WLT, JEW and LE designed the study; OH, WLT, CEC and JP conducted the cultivation experiments; OH compiled the meta-analysis list; and OH, WLT and EBS analyzed the data. All authors contributed to the writing of the manuscript.

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Chapter 1: General introduction

Introducing the life of plants in general, and ferns in particular.

The life cycle of land plants

Compared to the circa three hundred thousand species angiosperms (Christenhusz and Byng, 2016), there are only about twelve thousand species of ferns in the world (PPG I, 2016). But their fascinating life cycle makes them worth studying. Most other land plants have either the haploid (bryophytes) or the diploid (seed plants) life phase as dominant. The sporophyte of bryophytes grows permanently attached to the gametophyte and is nutritionally fully dependent on it. In some instances, the sporophyte is even ephemeral. In contrast, gametophytes of seed plant tend to be highly reduced, generally never leaving the protection of either the microspore (pollen), or the overall sporophyte biomass. Ferns, alongside lycophytes, have both life phases mostly nutritionally and spatially independent, connected only for short durations of time, when spores prepare for release and before the young sporophyte smothers its maternal gametophyte.

The principal difference between the sporophytic and gametophytic life stages is number of chromosome sets. Gametophytes are haploid (n), meaning that they only have one set of chromosomes. Upon merging of a haploid sexual cell, gamete, with another likewise haploid cell, a zygote is formed. The zygote is the first cell of the diploid (2n) life stage, called the sporophyte. Some cells of the diploid organism undergo a reductive type of cell division, meiosis, leading to the initial haploid cell type, spore. The production of gametes and spores is generally localized in organs called gametangia and sporangia, respectively. Throughout the evolution of plants, characteristics of gametes and spores, such as motility and differentiation, have changed (Niklas and Kutschera, 2009; Petersen and Burd, 2017).

Though initially homosporous (one spore type), land plants seem to crave the advantages of heterospory (multiple spore types), which has led to its multiple independent origins (Bateman and DiMichele, 1994; Petersen and Burd, 2017). Modern seed plants are entirely heterosporous, as are two of the three contemporary lineages of lycophytes (Selaginallales and Isöetales). Bryophytes seem to compensate for lack of heterospory via sexual chromosomes (Korpelainen, 1998; Renner et al., 2017), which leads some of the species to have spores equal on the outside, but different inside. Ferns have mostly ignored the selective push towards heterospory.

The specifics of ferns

As most fern lineages are homosporous, this thesis will focus on homospory. The life cycle of a homosporous fern involves a constant shift between independent haploid and diploid life stages. Let us choose the spore as our starting point. This first cell of

the haploid stage tends to be small (<100 μm) and airborne. Spores of most modern ferns are catapulted out of sporangia and may travel up to thousands of kilometers (Kessler, 2010). Upon landing in a suitable habitat, the spore germinates into a tiny green organism, the gametophyte. Gametophytes come in many sizes and shapes but remain small and very fragile. The gametophyte assesses its situation and chooses what type of gametangia are most suitable. This capability is called environmental sexual determination (Korpelainen, 1998), gametophytes may be male, female or bisexual, and their sexuality often changes over time. Females form female gametangia, archegonia, which in turn create female sexual cells, eggs (egg cells). Similarly, males form antheridia loaded with sperm (spermatozoids). The sperm then swim towards archegonia and both gamete types merge to create a zygote. Therein lies one of the main limitations of fern sexuality, sperm need a film of water to swim in. This is why we mostly tend to find ferns in shaded forests and rock crevices, where free water is present long enough. The zygote quickly differentiates into a root and the first leaf of a newborn sporophyte. After several leaves are formed, the gametophyte withers away and the sporophyte continues to grow up to several meters tall. Eventually, leaves form clumps called sori, aggregates of sporangia often protected by an indusium. Upon spore release, the cycle closes.

Fern sporophytes may be beautiful, but almost all interesting events of reproduction happen on gametophytes. For that reason, this thesis will focus on, and start with the gametophytic life stage, specifically its first cell.

The spore, the means of fern travel and the check of genetic integrity

Spores as means of dispersal

Spores of homosporous ferns are tiny propagules usually tens of micrometers in size. In contrast to vegetative growth, they serve as the main method of dispersal, especially over long distances. Due to their minute proportions, they can be carried by wind currents across continents and oceans. For example, the Hawaiian Islands are located about 4000 km from the nearest continent. Its flora of 188 fern species (Palmer, 2003) is the result of at least 140 colonization events (Wagner et al., 1990, Wagner, 1995). The spores have arrived via the subtropical jet stream from the west and by the trade winds from the east and some species have likely arrived multiple times (summarized by Kessler, 2010). Due to the long-distance dispersal of spores, ferns often have low among population genetic differentiation (Sciaretta et al., 2005; Schneller and Liebst, 2007; Ekrt et al., 2020). Nevertheless, most spores fall next to the originating plant (Conant, 1978; Penrod and McCormick, 1996).

Spore size, shape and color

The diversity of spore sizes, shapes and colors reflects the rich evolutionary history of ferns. The largest spores of homosporous ferns (up to 150 μm) are found among the unusual genus *Ceratopteris*, sometimes called water sprites (Lloyd, 1974; Tryon & Lugardon, 1991). Their claim to fame is in the name, they are the only water ferns outside the heterosporous order *Salviniales*. Water environments call for specific adaptations, such as larger nutritional reserves of propagules and shorter generation time (Petersen and Burd, 2017). These requirements were addressed by increasing spore size and a possibly unique antheridiogen system (antheridiogens explained in chapter 3). The increase in spore size was achieved rather simplistically, by reducing the number of spores per sporangium from 64 to 32 or even 16 (Lloyd, 1974). Even terrestrial ferns tend to vary in spore size, mainly because of polyploidy. Polyploid (having more than two chromosome sets) species tend to have larger spores than their diploid (with two chromosome sets) relatives (Barrington et al., 1986; Quintanilla and Escudero, 2006; Barrington et al., 2020).

The shape of spores is mostly dictated by the shape of their scar. As spores are the product of meiosis, four are produced at a time in a tetrad. Based on how the spores are oriented in the tetrad, a scar is created at the point of contact of all four sister spores. The scar is either trilete (Y shaped), leading to a tetrahedral spore, or monolete (I shaped), resulting in an oval spore. The character of scar shape is highly conservative and rarely varies within family (Tryon & Lugardon, 1991). Finally, spores may lack a scar and be of spherical shape. This rare phenomenon is generally agreed to be caused by either

incomplete meiosis or cytokinesis leading to a polyploid spore (Morzenti, 1962; Haufler et al., 1985; Rabe and Haufler, 1992; Ekrt et al., 2020). These aberrant spores can germinate and may play a role in the evolution of polyploidy in ferns.

The variety of colors that ferns produce is not only aesthetically pleasing and a useful identification character, but also plays a crucial role in ecology. The main color split is between green (chlorophyllous) and non-green (achlorophyllous, generally shades of brown or translucent). The taxa with chlorophyllous spores are in a minority but the trait evolved multiple times and is spread throughout the fern phylogeny tree (Sundue et al., 2011). The presence of chlorophyll in the spore indicates its active metabolism and fundamentally changes spore characteristics. Green spores germinate within a couple days (Lloyd and Klekowski, 1970), while achlorophyllous *Plagiogyra* spores may wait up to 210 days to germinate (Stokey and Atkinson, 1956). Green spores tend to result in faster growing gametophytes, although exceptions are known in epiphytic species. As a price for their active metabolism, green spores pay a price in short life spans. Chlorophyllous spores die after average 48 days, compared to the tens of years achlorophyllous spores may remain viable (Lloyd and Klekowski, 1970). Chlorophyllous spores are often employed by ferns of wet environments. The intended strategy is similar to that used by some seed plants such as willows (*Salix spp.*). Sexual propagules are short lived and exploit narrow time windows after massive disturbances (e.g. spring floods). In addition, asexual reproduction, via clonal growth or asexual propagules, carries the full nutritional potential of the adult to succeed even in highly competitive environments.

Some ferns produce spores seem to have an olive color, both green and brown. These spores are called cryptochlorophyllous. Sundue et al. (2011), who first described this phenomenon, have found 43 such species. Olive spores are protected by well-developed perispores (brown color) but carry chlorophyll inside (green color). As such, they likely last longer than regular green spores, some chlorophyll was detected in 110-year-old spores (Sundue et al., 2011). Therefore, the division of spore color should be viewed as a spectrum rather than a dichotomy.

Spore abortion

So far, this chapter has discussed things going according to plan. But aberrations from regular development pathways may serve as an important driver of evolution. Meiosis, through which spores generally form, is an important check of genetic integrity, as chromosomes have to form pairs. In contrast to angiosperms, in which some hybrids may successfully sexually reproduce (Rieseberg and Carney, 1998), fern hybrids are unable to properly pair chromosomes during spore formation and their spores are mostly inviable, aborted. Aborted spores vary greatly in size, and are often very dark, shriveled, with a collapsed exospore. This may be very practical for researchers using aborted spores as

an identification character (Wagner and Chen, 1965; Reichstein, 1981; Ekrt et al., 2010), but hybrid ferns are sterile and potential reproductive dead-ends. Apomictic hybrids (one parent apomictic, one sexual) are a peculiar exception to hybrid sterility. For example, one such pentaploid hybrid, *D.* ×*critica*, formed a significant proportion of viable spores and is capable of producing new and viable entities (Bär and Eschelmüller, 2010; Ekrt and Koutecký, 2016). Apomictic hybrids are generally considered to form 80-95% spores aborted (Fraser-Jenkins, 2007; Ekrt and Koutecký, 2016; Férová, 2018), with one individual of tetraploid *D.* ×*complexa* having SAI of only 60% (Férová, 2018). Such low spore abortion may be hypothetically indicative of a second-generation hybrid, which has already overcome some of its limitations. Apart from hybrids, apomictic taxa may also have a higher proportion of aborted spores, as theoretically predicted based on the way apomicts form spores (Manton, 1950; Gastony and Windham, 1989) and confirmed by several studies (Walker, 1962; Eschelmüller, 1998; Park and Kato, 2003). Nevertheless, low spore abortion rate was found in some apomicts (Khare and Kaur, 1983; Quintanilla and Escudero, 2006; Guo and Liu, 2013). Spore abortion is an incredibly important and variable characteristic. Therefore, it was the focus of the first paper of this thesis, in which spore abortion was compared between several reproductive groups and its methodology was assessed.

Summary

The size, shape, color and viability of spores are important characteristics that are related to not just reproduction, but also ecology, evolution and phylogeny of ferns. But the spore is just the first cell of the haploid life stage. Upon landing in a suitable habitat, a viable spore germinates into a newborn gametophyte, sometimes called prothallium. The gametophyte is a tiny organism that is arguably even more important that the spore and will be the focus of the next chapter.

The gametophyte, the short and turbulent stage in a fern's life

Factors affecting gametophyte growth

The purpose of the gametophyte is the formation of gametes and a suitable spot, where they may merge. However, the ultimate goal is the production of a sporophyte which may be done by alternative means. No matter the strategy, the gametophyte (prothallium) must first emerge from its spore shell and grow. The speed of growth if affected by a multitude of factors, either abiotic or biotic. Abiotic factors affect gametophyte growth in so many ways, it is beyond the scope of this thesis, but illumination and substrate chemistry are the most important (Raghavan, 1989). The most prominent and studied biotic factor is the presence of pheromones called antheridiogens and these will be elaborated on in detail below. The density at which prothalli grow is also important. Predictably, increased density generally reduces gametophyte size (Huang et al., 2004; DeSoto et al., 2008). Antheridiogen research, and its blunders, allows us a glimpse into another understudied but likely important biotic factor, non-specific growth inhibitors. These allelopathic compounds are released by adult gametophytes and stunt the growth of other nearby gametophytes (Petersen and Fairbrothers, 1980; Chiou and Farrar, 1997; Testo et al., 2014). In extreme cases, germinated gametophytes may be stuck at the size of ca 10 cells for months, even if abiotic factors are suitable for indeterminate growth (Hornych, pers. obs.). As fern sex determination is environmental (Korpelainen, 1998), the sexuality of gametophytes is tightly related to their growth. Consequently, all abovementioned factors affect sexuality in some way.

Gametophyte ontogeny

Every gametophyte is theoretically capable of forming two types of gametangia, antheridia (male) and archegonia (female), producing sperm and eggs, respectively. Gametophytes with both types of gametangia are often referred to as bisexual, although the term hermaphroditic may be more appropriate. As described by Klekowski (1969), gametophyte sexuality often changes over time and ferns differ in their ontogeny strategies. Usually, ferns start as male and then develop into females or hermaphrodites, the initial male phase may be skipped. These strategies can differ even between species within one genus (e.g. *Asplenium*, Pangua et al., 1994). However, the main advantage of environmental sex determination is the flexibility of response to outside factors. As mentioned above, various abiotic and biotic factors affect sex expression (Raghavan, 1989). Some generalizations can be made. In habitats with ample light, water and nutrients, archegonia can be made. Less suitable habitats only permit the formation of antheridia. Extremely stressful environments (e.g. crowded environment, non-specific growth inhibitors) may prevent the formation of any gametangia altogether. The correlation between sex and environmental factors is likely associated with the cost of each sex and architectural constraints (Huang et al., 2004; DeSoto et al., 2008).

Archegonia, associated with abundance, may eventually nurture the developing sporophyte and need a solid reserve of resources and a suitable environment for subsequent sporophyte growth. In contrast, antheridia are comparably cheap, research on dark grown gametophytes shows that just the spore itself may store enough resources to form multiple antheridia (Schneller, 1988; Haufler and Welling, 1994).

Antheridiogens

As alluded to above, perhaps the most important biotic factor affecting gametophyte growth and sexuality is the antheridiogen system. First discovered by Walter Döpp in 1950, antheridiogens are pheromones exuded by archegoniate gametophytes, inducing precocious formation of antheridia in surrounding asexuals (Döpp, 1950). Unlike in a general male-first ontogeny, gametophytes affected by antheridiogens are locked into being male as long as the releasing females are present (Näf et al., 1975). This system essentially promotes outcrossing by separating the population into females and males according to their growth capabilities, a proxy of later success in nurturing a sporophyte. Via this system, prothalli are functionally dioecious in populations but retain their potential for monoecy and self-fertilization when isolated. Furthermore, the number of potential sporophytes in a gametophyte population is limited, preventing excessive competition, and the number of sperm in the population increases, facilitating outcrossing. While this system seems advantageous, not all species of ferns utilize it (Yatskievych, 1993; Schneller, 2008). Previously, there was no data-based estimate on what percentage of fern species respond to antheridiogens. This topic is further complicated by the fact that several types of antheridiogens exist, and each type only affects a subset of responsive species (Naf et al., 1975, Schneller et al., 1990; Schneller, 2008). Even the definition of antheridiogen type is not agreed upon.

Additionally, correlations between antheridiogens, polyploidy and apomixis were not properly tested yet. There are theoretical reasons for polyploids and apomicts to abandon antheridiogen usage. For example, polyploids are generally more tolerant of selfing (Masuyama, 1979; Soltis and Soltis, 2000; Pangua et al., 2003; Testo et al., 2015; Sessa et al., 2016), due to their inherent genetic variability. Antheridiogens render gametophytes dioecious, which may slow down fertilization, compared to a single gametophyte forming both gamete types synchronously and in one spot. And, in the world of fern gametophytes, waiting another couple of days may be the difference between life and death. Apomicts do not need any gametes to form sporophytes (apomixis further explained below). So, wasting valuable resources on possibly useless sperm may considerably hinder apomictic species, although sperm production may be potentially advantageous for apomicts. Finally, it is worth noting that we know of three species (one polyploid, two apomicts) that produce antheridiogens, but do not respond to them (Yatskievych, 1993; Testo et al., 2015). Essentially, these species are sending false

signals, possibly suppressing competition, by preventing the formation of archegonia in other species. Therefore, the selective pressures on polyploids and apomicts may be not towards abandoning the antheridiogen system entirely, but towards reducing responsiveness to it. Nevertheless, antheridiogen responsiveness may be a conserved characteristic and any correlation between it and polyploidy/apomixis may be obscured by the fact, that responsiveness is inherited from diploid/sexual ancestors (Haufler and Gastony, 1978; Haufler and Ranker, 1985).

As many facets of antheridiogens were not subject to a proper review, the second paper of this thesis includes a meta-analysis of the team's own cultivation experiments and all available literature. The prevalence, properties and evolution of the antheridiogen system was assessed.

Choosing a mate

Simply choosing what gametes to form is not enough, an appropriate mate must be found. Being homosporous, ferns have three options, brilliantly explained and renamed by Haufler et al. (2016). First, gametophytic selfing involves the merger of gametes from a single gametophyte, i.e. one gametophyte fertilizes itself. An unfortunate consequence of this approach is that the resultant sporophyte is homozygous at all homologous loci (Haufler et al., 2016). Sporophytic selfing is less severe, the two merging gametes are from different gametophytes, but both gametophytes grew out of spores released by a single sporophyte. The most genetically variable progeny is the result of sporophytic outcrossing, the merger of gametes from different gametophytes, which are, in turn, from different sporophytes. In principle, heterosporous plants (e.g. angiosperms) are only capable of the last two strategies. But, the extreme variant of selfing, gametophytic selfing, has inspired, and also discouraged, great interest in ferns. Perhaps the main impulse for fern mating research has been the work of Edward Klekowski (Haufler, 2014), who formulated his theories (Klekowski and Baker, 1966; Klekowski, 1973), that homosporous ferns were mostly polyploid (due to their unusually high chromosome numbers) and reproduced by gametophytic selfing (referred to as intragametophytic selfing; Klekowski, 1969). Consequently, ferns inherently store considerable genetic variation, releasable by the pairing of homeologous chromosomes, which is possible even during gametophytic selfing. A well outlined theory with testable assumptions and a selection of new research methods inspired many papers over the years. For example, now we know, that polyploidy is a very important evolutionary feature of ferns (Wood et al., 2009), but many ferns are functional diploids, even with incredibly high chromosome numbers (Gastony and Gottlieb, 1985; Haufler and Soltis, 1986; Wolf et al., 1987). Ferns also generally employ a mixed mating system, being capable of selfing (including gametophytic), but generally preferring outcrossing and having a high level of genetic variation in natural populations (Soltis and Soltis, 1987; Wubs et al., 2010; de Groot et

al., 2012; Peredo et al., 2013; Sessa et al., 2016). Nevertheless, new polyploids do seem capable of using their extra genomic complement to better tolerate, or even prefer, gametophytic selfing, as mentioned above.

Apomixis

So far, the success of the gametophyte was shown to be predicated on the formation and merging of gametes, a sexual process. However, ca 3% of ferns have chosen an alternative strategy, apomixis (Liu et al., 2012). The term, originally used by Winkler (1908), has evolved in its meaning over time. In fern research, it is generally used to describe a set of processes enabling the production of sporophytes and gametophytes of the same ploidy level, i.e. without cycling between haploid and diploid levels. Such reproduction strategy necessarily involves two processes, the production of gametophytes without the reduction into haploidy and the formation of sporophytes without the doubling of chromosome numbers. Utilizing only one process inevitably leads to unsustainable genome bloating or reduction, but may be beneficial in the short term, especially for researchers studying ferns (Manton and Walker, 1954; Bouharmont. 1972a, b). The process of creating sporophytes directly from gametophytic tissue, without gametes, is called apogamy and is rather straightforward. Gametophytes of apogamous species only rarely form archegonia (Haufler and Welling, 1994) but may form viable antheridia and hybridize (Ekrt et al., 2016). The apogamous sporophyte usually emerges near the lateral meristem, the tissue normally responsible for the formation of archegonia. The formation of unreduced gametophytes is achieved by two known processes, apospory and agamospory. Apospory is rare and simply involved the differentiation of functionally gametophytic (but diploid) tissue directly from the sporophyte. Agamospory, the formation of unreduced spores (diplospores), is more prevalent and has considerable consequences, depending on its type (Grusz, 2016). There are two types of agamospory, explained in more detail in chapter 4.

Summary

Early growth, sex expression, and, eventually, mating of gametophytes are all essential parts of fern life. Observing gametophytes directly has many advantages but cannot fully answer all our questions regarding their reproduction. For example, gametophytes are notoriously hard to observe in natural habitats. But a sporophyte may be viewed as an easily identifiable and observable conclusion of the many incredible features of gametophyte existence. Sporophytes also hold the key to spore formation and play their own important role in the never-ending cycle of fern reproduction. They fully deserve to be the focus of the next chapter.

The sporophyte, the conspicuous and resilient stage in a fern's life

The legacy of sporophytes

Due to their conspicuous nature, fern sporophytes have been studied quite more than gametophytes. Their ultimate purpose is the formation of spores, but many rely extensively on vegetative reproduction (e.g. bracken, Conway, 1949; Sheffield et al., 1989; Ekrt et al., 2020). Before sporogenesis is discussed and the cycle closed, we will look closer at sporophytes as results of gametophyte interactions.

Each sporophyte of sexual species carries in it a legacy of its parents, which profoundly affects its capabilities. Since most ferns seem to prefer outcrossing, the sporophyte is generally a descendant of two other successful sporophytes. However, this continuity of success may end abruptly. If the parents are of different species, hybridization occurs. Fern hybrids, especially between two sexual species, are mostly incapable of creating functional spores (Wagner and Chen, 1965). The phenomenon of hybridization is well-known in ferns, new hybrids are constantly being described (Nitta et al., 2018; Della et al., 2020; Zhang and Zhang, 2020). Many more species are allopolyploids, i.e. hybrids, that restored their fertility by polyploidization. Hybridization has profound positive and negative consequences. Yet is seems that many important questions, appropriately addressed in angiosperms, were left unanswered. For example, numerous hybridization barriers were described in angiosperms (Baack et al., 2015) and even mosses (Natcheva and Cronberg, 2004). In ferns, researchers presume, that hybridization is frequent in ferns, the hybridization barriers are weak (Barrington et al., 1989; Sigel, 2016). But, with a few exceptions (Testo et al., 2015), the nature of these barriers was not properly studied. Furthermore, there was no research quantitatively measuring the frequency of hybridization, hybridization rates, in natural populations. In contrast, asymmetric hybridization was studied in several fern groups.

Asymmetric hybridization is the phenomenon of one parental combination being more likely to occur than the other one, i.e. one species being more likely to be the maternal parent of a hybrid (Rieseberg and Carney, 1998). In general, asymmetric hybridization happens when hybridization barriers act differently based on the parental combination. Therefore, studying this phenomenon may uncover important facts about hybridization barriers. Asymmetric hybridization was found in many fern hybrids (Vogel et al., 1998; Xiang et al., 2000; Hunt et al., 2011; Zhang et al., 2013; Testo et al., 2015) using chloroplast studies. Since chloroplasts are maternally inherited in ferns (Vogel et al., 1998), the chloroplast genome of the hybrid will match the genome of its maternal parent. Several possible hybridization barriers were tested and suggested as the cause of this asymmetry. Many of these are related to ploidy level of parents. For example, diploid

sperm (of tetraploid species) may be unable to enter haploid archegonia (of diploid species), although this was not true for the species tested by Testo et al. (2015). Sperm swimming capability seems correlated with ploidy level, haploid sperm was more motile and, consequently, the diploid species was the predominant paternal parent in taxa studied by Testo et al. (2015). Tetraploids being more likely to reproduce by selfing was also considered as a possible driver or asymmetric hybridization (Xiang et al., 2000; Testo et al., 2015). Other, yet unstudied, factors are also quite possibly at play. Both hybridization rates and asymmetric hybridizations were tested in the third paper of this thesis, focusing specifically on the Central European buckler ferns (*Dryopteris carthusiana* group).

Sporogenesis

As mentioned above, the primary goal of the sporophyte is the formation of spores in sporangia. The evolutionarily primitive sporangia are called eusporangia. They produce hundreds to thousands of spores each and have thicker walls. Eusporangiate ferns form a loose paraphyletic group of four families, whose phylogenetic relationships are still being explored (Shen et al., 2018). The derived sporangial type, leptosporangia, is the defining characteristic of most ferns existing today, the monophyletic leptosporangiate group. Leptosporangia are generally just a single cell thick. They also form a predefined number of spores, usually 64. The number 64 is based on the regulated number of divisions of the original cell of the sporangium. Usually, four mitotic divisions result in 16 spore mother cells. The subsequent meiosis gives rise to 64 spores. There are known exceptions to the rule of 64 spores per sporangium, for example, 32 or even 16 in *Ceratopteris* (Lloyd, 1974), as mentioned above. However, the most consistent exception is in apomicts.

Apomicts generally produce diploid gametophytes using the abovementioned process of agamospory. During this process, 32 diplospores are created per sporangium by one of two known methods (Grusz, 2016). The first discovered, and most prevalent, method is called premeiotic endomitosis (PE), or Döpp-Manton method (Döpp, 1932; Manton, 1950). In PE, the first three mitotic divisions are regular, but the fourth one is incomplete, leading to eight tetraploid spore mother cells. Tetraploid spore mother cells undergo a regular meiosis, leading to 32 diplospores. Interestingly, PE enables recombination between non-identical homologous chromosomes, leading to a potential variability in offspring more akin to selfing, rather than clonality (Ootsuki et al., 2012; Grusz, 2014). The other agamosporous method is called meiotic first division restitution (MFDR), or Braithwaite method (Braithwaite, 1964; Walker, 1985). MFDR includes all four regular mitoses, the first division of meiosis is incomplete, chromosomes do not pair. Thus, meiosis lead to the formation of 32 diplospores in diads. Due to the absence of recombination, MFDR is closer to close to clonal reproduction. Gametophytes of MFDR

species are also unable to form viable sperm (Braithwaite, 1964; Regalado Gabancho et al., 2010).

Apomictic hybrids

Fascinating results were found when observing the reproduction of the apomictic hybrid *Dryopteris ×critica*. This hybrid is the result of mating between the apomictic *D. borreri* and the sexually reproducing *D. filix-mas*. The abortion rate of this pentaploid hybrid is ca 90% (Ekrt and Koutecký, 2016), unusually low for fern hybrids. Non-aborted spores are capable of germination and the resultant gametophytes form apogamous sporophytes. Surprisingly, these sporophytes can be either pentaploid or polyhaploid (2.5x; Ekrt and Koutecký, 2016). Both ploidy levels are viable, and F3 generations were raised (Ekrt, pers. obs.). It seems that the hybrid inherited PE-type agamospory from its apomictic parent. However, apart from forming diplospores, some reduced spores are created via the regular sexual sporogenesis. In such event, the reduced spores are usually aborted in apomictic species (Grusz, 2016). But, in *Dryopteris ×critica*, regular sporogenesis results in some viable reduced spores, in this case the polyhaploids. These discoveries triggered further research on tetraploid apomictic hybrids, which could, in theory, give rise to functional diploids. These diploids would incorporate the genetic variability of both parents and could further hybridize. It was demonstrated that the related tetraploid apomictic hybrid *Dryopteris ×complexa* creates a greater proportion of viable spores, compared to the pentaploid (Férová, 2018), but we were yet unable to find any diploid gametophytes when growing these spores (Hornych, pers. obs.). Nevertheless, hybridization between apomictic and sexual species is likely to have far-reaching consequences on the evolution of ferns and further studies on this topic may reinvent the way we view ferns.

Summary

Now that spores are formed, the cycle is closed. Every step in the life cycle of ferns has its potential obstacles and intriguing processes. This thesis has attempted to show that fern reproduction profoundly affects all aspects of fern existence, their distribution, ecology, evolution, genetics and physiology. Yet much of this topic is still poorly understood. Due to its complexity, any researcher, who wishes to understand fern reproduction, should study multiple aspects of it. This, I have set out to do with my PhD thesis.

Aims of the thesis

This thesis focuses on the several aspects of fern reproduction and hybridization. Both life phases, sporophytic and gametophytic, and their transition, spore, were tested. The thesis frequently targets the genus *Dryopteris*, but its scope spans the entire fern phylogeny.

Paper 1 was aimed at quantitatively assessing spore abortion across multiple genera and reproductive characteristics (polyploidy, apomixis, hybridity). The paper also attempted to test spore assessment methodology.

Paper 2 was aimed at combining cultivation experiments with all available literature in order to create an antheridiogens interaction dataset. The dataset would be utilized to explore various features of antheridiogen usage in ferns.

Paper 3 was aimed at quantitatively estimating hybridization rates in natural populations of buckler ferns (*Dryopteris carthusiana* group). Additionally, asymmetric hybridization was measured via chloroplast sequencing to explore the poorly understood barriers to fern hybridization.

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Chapter 2: Spore abortion index (SAI) as a perspective tool of evaluation of spore fitness in ferns: An insight into sexual and apomictic species.

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Abstract

Ferns reproduce through small and usually haploid spores. The general paradigm states that whereas species produce good shaped spores, hybrids are sterile and form aborted spores. Apomictic fern species represent an unusual case, and it is believed that they produce an unbalanced spore spectrum. Until now, no comprehensive comparison of sexual and apomictic taxa using extensive spore fitness data has been published. Based on a representative data set of 109 plants from 23 fern taxa, we accomplished the first robust analysis of spore fitness using spore abortion index (SAI), the ratio of aborted to all examined spores. One thousand spores were analyzed for each plant. Focusing mainly on two major European fern taxa (*Asplenium*, *Dryopteris*), we compared this trait for different fern reproductive types (sexual/apomicts/hybrids) and ploidy levels (diploid versus polyploid). Our results confirmed the general assumption that shows higher SAI for apomictic taxa (18%) when compared to sexual taxa (3%). Furthermore, hybrids are characterized by having almost all spores aborted (99.8%) with the notable exception of pentaploid *Dryopteris* ×*critica* (93.1%), the hybrid between sexual and apomictic taxa. We found no significant difference in SAI between sexual taxa of various ploidy levels or between sexual taxa of genera *Dryopteris* and *Asplenium*. Additionally, we carried out an optimization of the SAI method, outlying important guidelines for the use of this method in the future.

Introduction

Generative reproduction represents the basic and recurrent evolutionary force of land plants. Evaluation of reproductive success of plants is universally measured by amounts of offspring or figuratively by amounts of formed seeds (Johnson et al. 2010).

In experimental studies, pollen viability tests, assessing the percentage of viable pollen grains, are widely used to address several topics related to seed plant reproduction (Dafni and Firmage 2000). Similarly, for spore producing vascular plants, spore abortion rate is used as a ratio of aborted spores to all spores in each sample. Unfortunately, until now, this spore abortion index (in this paper abbreviated as SAI) is yet to be standardized and optimized or comparatively and overly evaluated among different ferns types (sexual, apomicts, hybrids).

Ferns are capable of forming spores sexually or via several apomictic ways to form ‘‘normal’’ spores, bad shaped aborted spores or somatic diplospores (Manton 1950; Braithwaite 1964). It was generally believed that sexual species usually form good shaped spores, hybrids are usually sterile (with predominantly aborted spores), and apomictic species are known for an unbalanced spore spectrum (Manton 1950; Lovis 1977). Until now, studies analyzing this topic used only small sample sizes or taxon representation or

were otherwise limited (Park and Kato 2003; Quintanilla and Escudero 2006; Gomes et al. 2006).

No generally encompassing study was done. This made it more difficult to research hybrids of sexual and apomictic species (Bär and Eschelmüller 2010; Dyer et al. 2012; Ekrt and Koutecký 2016). In light of recent studies in ferns, it seems that spore abortion index can represent an important and informative tool in fern population biology and biosystematics (Quintanilla and Escudero 2006; Arosa et al. 2009; Nakato et al. 2012; Hernández et al. 2015; Ekrt and Koutecký 2016).

To this day, no unified method exists for assessing SAI. Various amounts of spores are being used for this goal ranging from 100 (Quintanilla and Escudero 2006; Gomes et al. 2006; Hanušová et al. 2014) through 400 (Arosa et al. 2009) up to 1000 (Nakato et al. 2012; Ekrt and Koutecký 2016). Sometimes abortion rates are estimated without presenting a unified number of spores counted (Hernández et al. 2015).

Apart from SAI, studies have employed an alternative method of assessing viability of spores by estimating spore germination rates. Germination rates and inverted SAI seem to correlate well in *Dryopteris* (Quintanilla and Escudero 2006). *Dicksonia sellowiana* (Pr.) Hook. (Dicksoniaceae) produced <10% of spores that appeared viable but did not germinate (Gomes et al. 2006). For *Cornopteris christenseniana* Tagawa (Woodsiaceae), germination rate of viable spores is roughly proportional to their frequency among all spores (Park and Kato 2003). As such, for the purpose of this study we consider germination rates and inverted SAI comparable. However, germination rates may be affected by storage conditions (Kott and Britton 1982; Aragon and Pangua 2004), type of substrate used for germination tests (Kott and Peterson 1973) and age of the collected specimen (Windham and Ranker 1986). The physical appearance of spores does not change significantly over time or by improper storage conditions. Because of this limitation, misguided data could be obtained. Therefore, SAI may be a better method to estimate the formalized ability to produce viable spores for aged or improperly stored specimens.

Our study focused on the largest European fern genera *Asplenium* and *Dryopteris*. Additionally, the genera *Athyrium*, *Gymnocarpium* and *Phegopteris* were represented by one taxon each. We selected 23 taxa that include 14 sexual species, 5 apomictic species and 4 hybrids. The selection includes 8 diploids, 6 triploids, 8 tetraploids and 1 pentaploid. The main goal of our study was (1) a formalized comparison of taxa with different modes of reproduction (sexual, apomictic, hybrids), ploidy levels and generic affiliation in spore abortion and (2) an optimization of the method of assessing spore abortion index for further research.

Materials and methods

Plants used in the study

A total of 109 specimens from 23 fern taxa with monolete non-chlorophyllous spores were used for the study. The majority of the taxa belong to genera *Asplenium* and *Dryopteris*. Three additional genera *Athyrium*, *Gymnocarpium* and *Phegopteris* were added with one taxon each as they represent sexual diploids, sexual tetraploids and apomictic triploids, respectively. The studied taxa encompass varying modes of reproduction (sexual or apomictic) and ploidy levels. Four hybrid taxa were also included. For the purpose of this study, ‘‘hybrid’’ refers to F1 generation hybrids. For each taxon, 3–5 plants have been analyzed (Table 1).

The majority of plants, including every hybrid, have been determined and used from previous systematic studies (Ekrt and Štech 2008; Ekrt et al. 2009, 2010; Ekrt and Koutecký 2016), and some plants have recently been collected for the purpose of this study in the field. We did not collect plants growing in suboptimal conditions (extreme shade, light exposure, etc.). Fronds were collected in their phenological optimum; fronds with immature spores were avoided. A small minority of rare specimens were obtained from public herbaria. Ploidy levels of studied taxa were determined using flow cytometry or micromorphological characters correlated with ploidy levels (e.g., spore size, stoma measurements). Voucher specimens are deposited in the herbarium of the Faculty of Science, University of South Bohemia in České Budějovice (CBFS), or in other public herbaria (Online Resource 1); herbaria acronyms follow Thiers (2016). The nomenclature of taxa under study follows Danihelka et al. (2012) or Blockeel (2006) for taxa not included in the previous.

Evaluation of spore abortion rates

To prepare the spore sample, dried fronds were used. Parts of the frond, which have shed the majority of their spores, were avoided. Using a thick needle, spore material was gently brushed to move spores onto a microscope slide for examination under dry conditions. Before creating a new set of spores for examination, the microscope slide was thoroughly cleaned to avoid contamination. Light micro- scope (LABO COMFORT 1502, Arsenal) was used to determine the viability of spores under 4009 magnification. The microscope slide was examined while making sure that no spore is calculated twice. Spores were considered aborted when exhibiting abortive traits such as collapsed exospore, overly blackish color or anomalous shape. Spores of uncommon shapes with a stable exospore and natural color were considered developed.

Tab. 1 A list of taxa under study with described characteristics (ploidy level, mode of reproduction / hybrid status).

Species	No. of samples	Ploidy level	Mode of reproduction
Asplenium adiantum-nigrum	5	4x	Sexual
Asplenium cuneifolium	5	2x	Sexual
Asplenium onopteris	5	2x	Sexual
Asplenium ruta-muraria	5	4x	Sexual
Asplenium trichomanes nothosubsp. *lusaticum*	4	3x	Hybrid
Asplenium trichomanes subsp. *quadrivalens*	5	4x	Sexual
Asplenium trichomanes subsp. *trichomanes*	5	2x	Sexual
Asplenium viride	5	2x	Sexual
Athyrium filix-femina	5	2x	Sexual
Dryopteris affinis	3	2x	Apomictic
Dryopteris borreri	5	3x	Apomictic
Dryopteris cambrensis	5	3x	Apomictic
Dryopteris carthusiana	5	4x	Sexual
Dryopteris dilatata	5	4x	Sexual
Dryopteris expansa	5	2x	Sexual
Dryopteris filix-mas	5	4x	Sexual
Dryopteris fragrans	5	2x	Sexual
Dryopteris remota	3	3x	Apomictic
Dryopteris ×ambroseae	5	3x	Hybrid
Dryopteris ×critica	4	5x	Hybrid
Dryopteris ×deweveri	5	4x	Hybrid
Gymnocarpium dryopteris	5	4x	Sexual
Phegopteris connectilis	5	3x	Apomictic

A total of 1000 spores per each sample were checked to calculate spore abortion index (SAI) as a ratio of aborted spores to all spores in each sample expressed as a percentage. For optimization and the most suitable employment of SAI in the future, SAI was calculated for ten sets of 100 spores independently. Additionally, after 500 spores were analyzed a new set of spores was prepared from a different part of the frond to amount for any discrepancies within the frond. Thus, SAI is available for ten sets of 100 spores, two sets of 500 spores and the total SAI from 1000 spores.

Data analyses

Several Nested ANOVA tests were performed in this study. All of these tests had species affiliation as a random factor nested within the main tested factor. The first analysis was employed to show potential differences between SAI of sexual and apomictic taxa. For the purpose of this analysis, only the genus *Dryopteris* could be used as the other well-represented genus *Asplenium* had no sampled apomictic species. Sexually reproducing diploids and tetraploids of all applicable genera were also compared in this manner.

Additionally, an analysis was performed to ascertain discrepancies of total SAI between *D. ×critica* and other *Dryopteris* hybrids. Finally, a set of Nested ANOVAs was also employed to determine the effects of taxon-related factors. The analysis was used to show possible differences between the apomictic *Phegopteris connectilis* and other apomictic species, all from the genus *Dryopteris*. Furthermore, potential differences in SAI between the genera *Asplenium* and *Dryopteris* were analyzed. Only sexual non-hybrid taxa were used. Other genera are represented by a single taxon each and thus could not be put to the same test. For all samples, the values of SAI were arcsine-transformed for every performed Nested ANOVA and no samples were excluded from their respective analyses. All above-mentioned analyses were performed using Statistica 13 (Dell Inc. 2015).

The values of standard error of estimate for SAI calculation were calculated. The following equation was used:

$$w = \sqrt{\frac{pq}{N}}$$

where N stands for the amount of spores used, p and q stand for the ratio of developed and aborted spores, respectively, and w stands for the standard error of estimate. The values of w were calculated for 100, 500 and 1000 analyzed spores as well as for three p values: 5, 50% and the mean SAI of apomictic samples. To exploratively contrast theoretical results with real data, the difference between partial SAI and total SAI was calculated. Calculations of partial SAI were performed for 100, 200, etc., spores up to a 1000, resulting in ten values total. Each sample of the taxon was used.

To show the potential differences between different parts of the frond, a series of permutation tests was performed. Each sample had the difference in SAI between the two sets of 500 spores calculated. Additionally, two random selections from binomial distribution, with frequency being the total SAI of the sample and N being 500 (to represent 1000 spores calculated), were picked and difference between them was calculated. This pair of selections was performed 9999 times for a total of 10,000 numbers. The sample difference between two parts of the frond was then compared with the modeled distribution, and p value was calculated. To compensate for Type 1 error,

p values were adjusted by false discovery rate correction. The permutation analysis and p value adjustments were performed in R 3.1.2 (R Core Team 2014).

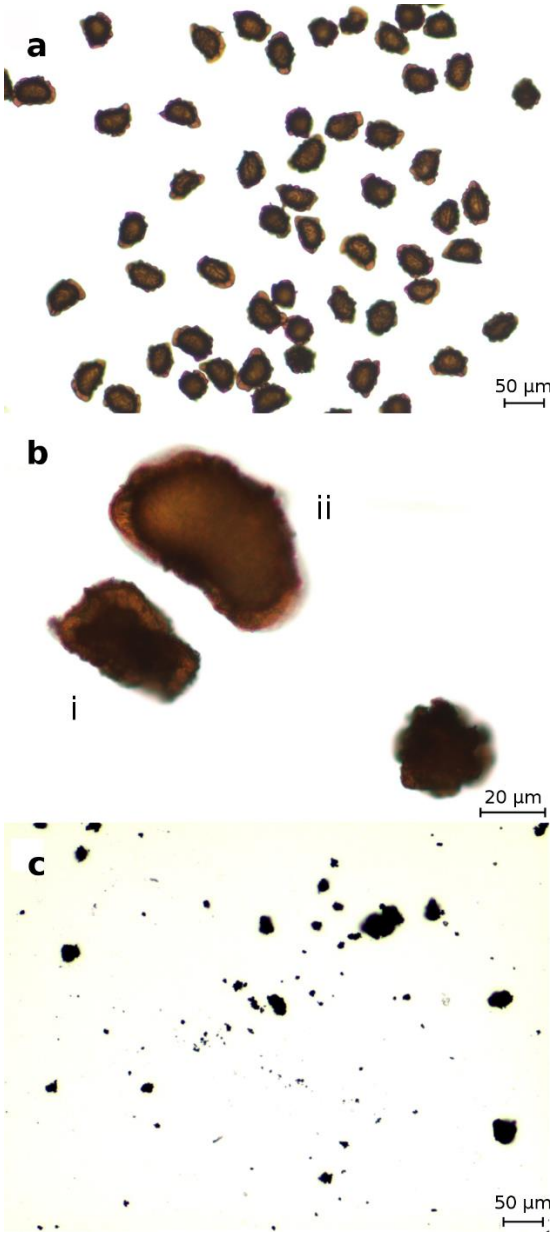

Fig. 1 Different types of spores and its variability in plants under study. **a** *Dryopteris filix-mas*: developed light brown spores observed in most non-hybrid sexual taxa. **b** *Dryopteris borreri*: **i** aborted spore present in darker colors in apomictic taxa **ii** well-developed spore, typical for non-hybrid taxa. **c** *Dryopteris ×ambroseae*: black irregularly shaped and sized aborted spores typical for hybrids with debris scattered around

Results

Determining aborted spores

A total of 109,000 spores were determined as either aborted or developed. A variety of shapes and sizes was found. Most developed spores looked as represented in Fig. 1a, transparent enough to tell the exospore and colored in light brown. We have found two types of aborted spores. Non-hybrid plants (Fig. 1b) have aborted spores with collapsed exospore and darker colors than the surrounding developed spores. This type of spores is often smaller than developed spores and has an irregular shape but sometimes retains a degree of transparency. In hybrid taxa (Fig. 1c), aborted spores are completely black and vary greatly in size sometimes being much larger than developed spores. These spores lack transparency completely. Additionally, a large amount of tiny black debris is scattered around aborted spores of hybrids. Similar findings have been reported by Wagner and Chen (1965) in the genus *Dryopteris*.

Spore abortion index

A variety of SAI values were obtained from the 109 samples tested, ranging from <1 to 100% (Table 2).

Of the total 23 taxa sampled, the diploid sexual *Athyrium filix-femina* has the lowest mean SAI, with all samples having <1% of aborted spores (mean SAI = 0.76%). The sexual tetraploid *Gymnocarpium dryopteris* has mean abortion rate of 1.04%, while having a sample with only three aborted spores out of a thousand (sample 5), the lowest of all sampled plants. Predictably, hybrids occupy the other side of the spectrum with a single sample of both *Dryopteris ×ambroseae* and *D. ×deweveri* having no developed spores. The majority of hybrid plants samples, except those of the distinct *D. ×critica*, have <1% of developed spores.

Overall, sexual taxa have SAI ranging from abovementioned 0.3% (*G. dryopteris*, sample 5) up to 19% (*D. fragrans*, sample 4). Mean SAI for samples of all sexually reproducing taxa is 3.05%. Meanwhile, apomictic taxa occupy a large gradient of SAI ranging from 1.7 (*D. affinis*, sample 3) to 60.9% (*D. borreri*, sample 5). The SAI of the apomictic *P. connectilis* is similar to SAI of studied *Dryopteris* apomictic taxa (species: $p = 0.443854$, genus: $p = 0.634251$; mean SAI 14.36 and 19.25%, respectively). Mean SAI for all apomictic samples is 18.09%. Regarding ploidy, diploid apomicts have mean SAI of 13.4%, while triploid apomicts abort mean 18.87% of spores. However, the number of samples is unbalanced. A comparison between SAI of various modes of reproduction is shown in Fig. 2.

Tab. 2 A summary of total SAI (%) of all samples. Each taxon is represented by three to five samples (see Tab. 1). SAI1–5 denotes individual plants given a number 1-5 for each taxon. The cross indicates that less than five samples have been used for the respective taxon.

Taxon	SAI 1	SAI 2	SAI 3	SAI 4	SAI 5	Mean	s.d.
Asplenium adiantum-nigrum	2.5%	1.6%	4.4%	6.2%	2.3%	3.40%	1.88
Asplenium cuneifolium	2.0%	1.8%	0.8%	1.4%	11.6%	3.52%	4.54
Asplenium onopteris	2.4%	2.4%	2.5%	4.1%	1.8%	2.64%	0.86
Asplenium ruta-muraria	3.3%	0.8%	1.0%	1.2%	5.3%	2.32%	1.95
Asplenium trichomanes nothosubsp. *lusaticum*	99.6%	99.9%	99.9%	99.9%	×	99.83%	0.15
Asplenium trichomanes subsp. *quadrivalens*	3.3%	0.6%	1.5%	0.7%	1.3%	1.48%	1.09
Asplenium trichomanes subsp. *trichomanes*	0.9%	1.0%	0.8%	0.7%	1.8%	1.04%	0.44
Asplenium viride	4.2%	3.0%	1.1%	2.3%	1.4%	2.40%	1.25
Athyrium filix-femina	0.9%	0.6%	0.5%	0.9%	0.9%	0.76%	0.19
Dryopteris affinis	5.0%	33.5%	1.7%	×	×	13.40%	17.49
Dryopteris borreri	4.3%	10.2%	33.4%	21.1%	60.9%	25.98%	22.46
Dryopteris cambrensis	28.2%	10.1%	7.2%	13.5%	8.2%	13.44%	8.59
Dryopteris carthusiana	0.6%	1.2%	1.7%	2.5%	0.2%	1.24%	0.91
Dryopteris dilatata	3.3%	3.3%	6.9%	7.9%	3.5%	4.98%	2.24
Dryopteris expansa	2.6%	7.6%	1.9%	5.3%	7.5%	4.98%	2.67
Dryopteris filix-mas	11.5%	4.4%	1.8%	4.1%	15.2%	7.40%	5.68
Dryopteris fragrans	1.2%	3.0%	1.1%	19.0%	3.4%	5.54%	7.60
Dryopteris remota	30.2%	20.9%	19.6%	×	×	23.57%	5.78
Dryopteris ×ambroseae	99.0%	99.8%	100%	99.5%	99.8%	99.62%	0.39
Dryopteris ×critica	92.6%	97.3%	89.5%	93.5%	×	93.23%	3.21
Dryopteris ×deweveri	95.6%	100.0%	99.8%	99.8%	96.6%	98.36%	2.09
Gymnocarpium dryopteris	1.8%	1.5%	1.3%	0.4%	0.3%	1.06%	0.67
Phegopteris connectilis	14.5%	7.4%	32.2%	5.4%	12.4%	14.38%	10.62

Comparing SAI of different groups

Highly significant differences in SAI exist between sexual and apomictic taxa of the genus *Dryopteris* (species: p = 0.3418, reproduction mode: p = 0.0022). For this genus, median SAI values for apomictic and sexual taxa are 19.6 and 3.3%, respectively (Fig. 3). Apomictic taxa form aborted spores with higher frequency.

Potential effects of other taxon-related factors on SAI were tested. Our analyses showed no effect of ploidy level on SAI when comparing sexual taxa (species: p = 0.0008, ploidy level: p = 0.8976), genera *Athyrium* and *Gymnocarpium* included. There seems to be no difference in SAI regarding ploidy levels for sexual species. However, there are significant differences between species. SAI values not standard for hybrid taxa were found in the *Dryopteris ×critica* (hybrid of apomictic and sexual taxa). This hybrid differs significantly in SAI from others studied *Dryopteris* hybrids (species: p = 0.3357, hybrid origin: p = 0.0012). Median SAI value for *Dryopteris ×critica* is 93.05%, while other hybrids (with sexually reproducing parents) have median SAI 99.8%. Most of these other hybrids have SAI close to 100% with a notable exception of samples 1 and 5 of *D. ×deweveri* having SAI of 95.6 and 96.6%, respectively (Fig. 4).

Marginally, significant differences in SAI were found between sexual species of the genera *Asplenium* and *Dryopteris* (species: p = 0.0925, genus p = 0.05353). The genus *Dryopteris* has a higher median of 3.3% compared to 1.8% of the genus *Asplenium*. Nevertheless, this difference is comparable to the difference between the species within their respective genus.

Optimization of SAI assessment method

Standard error of estimate was calculated (Table 3). This error increases with the proximity of real SAI value to 50% and decreases with the amount of spores used to estimate SAI. At the least optimal scenario (100 spores calculated, real SAI 50%), the standard error of mean is equal to 5%, suggesting that the calculated value will on average be 5 aborted spores off the real value in either direction.

The change in the difference between cumulatively calculated partial SAI and total SAI demonstrates the variance in data (Fig. 5). The following taxa represent the different levels of mean deviation from total SAI in increasing order. The hybrid *D. ×ambroseae* (Fig. 5a) is very uniform, and SAI never differs more than 1% from total SAI. The example of *A. ruta-muraria* (Fig. 5b) shows little change in estimate after ca 400 spores are calculated. The number of spores needed to provide a close estimate increases to approximately 600 and 900 for D. dilatata (Fig. 5c) and *D. cambrensis* (Fig. 5d), respectively.

After p value adjustment, 12 of 109 (11%) samples significantly differed ($p < 0.05$) between the two sets of 500 spores, and each estimated from a distinct part of the frond; hence, the SAI value of these plants varies within the frond. Furthermore, ten plants are marginally significantly different ($0.05 < p > 0.1$). Of the significantly differing plants, eight were apomicts, three were sexually reproducing, and one sample was of hybrid origin. One sample (*D. remota* 2) has a surprising 18.2% difference between two parts of the frond, while total SAI for the sample is 20.9%. See Online Resource 2 for the results of individual tests alongside other measures of variation within sample.

Tab. 3 Calculated values for standard error of estimate of SAI at varying numbers of calculated spores and real SAI values. The value of 18% reflects the mean SAI of sampled apomicts.

	100 spores	**500 spores**	**1000 spores**
5% SAI	2.18%	0.97%	0.69%
18% SAI	3.84%	1.72%	1.21%
50% SAI	5.00%	2.24%	1.58%

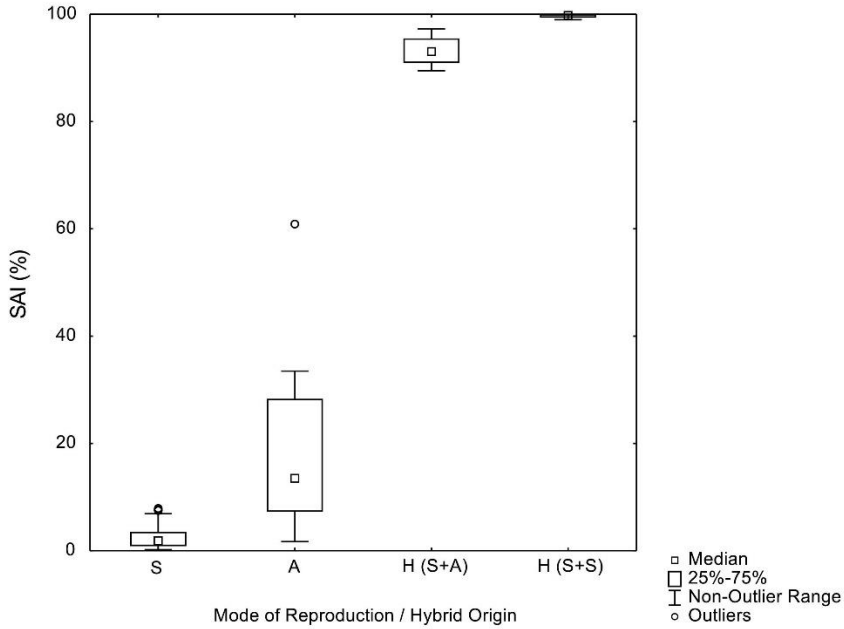

Fig. 2 Spore abortion index (SAI) for all reproduction modes using all samples. S sexual taxa, A apomictic taxa, H (S + A) hybrids of both apomictic and sexually reproducing parents (represented by *Dryopteris* ×*critica* only), H (S + S) hybrids of two sexually reproducing parents

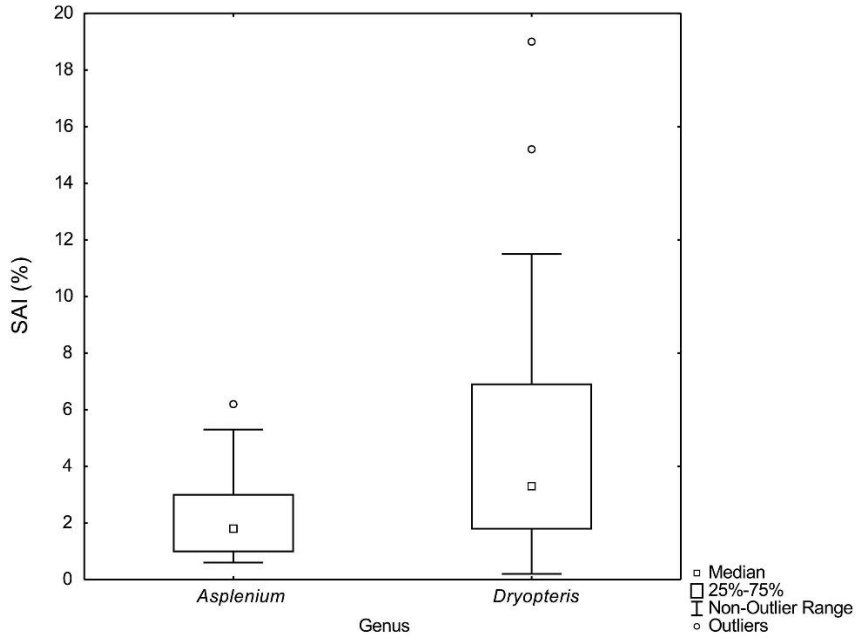

Fig. 3 Spore abortion index (SAI) between sexual and apomictic taxa of the genus *Dryopteris*

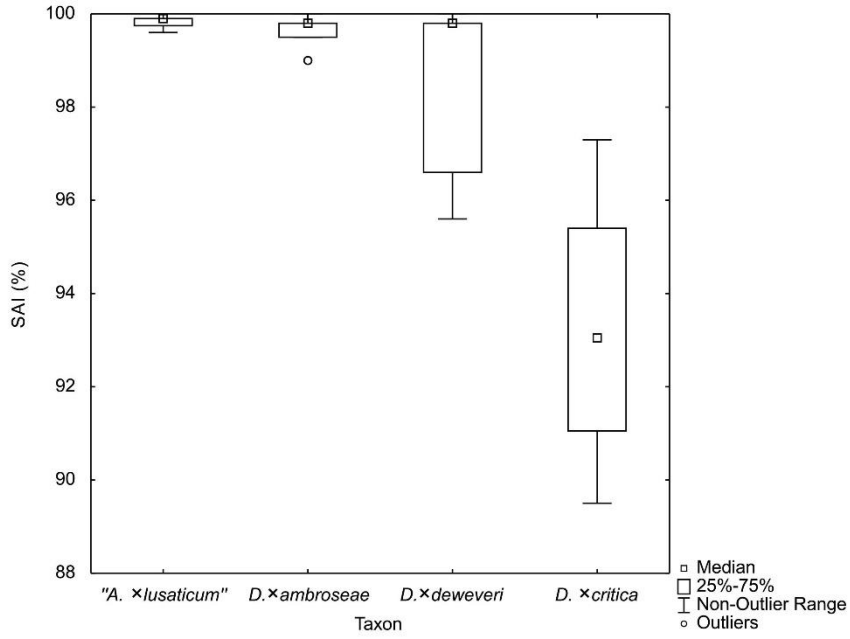

Fig. 4 Spore abortion index (SAI) between hybrid taxa under study, the name "A. ×lusaticum" refers to the hybrid *Asplenium trichomanes* nothosubsp. *lusaticum*

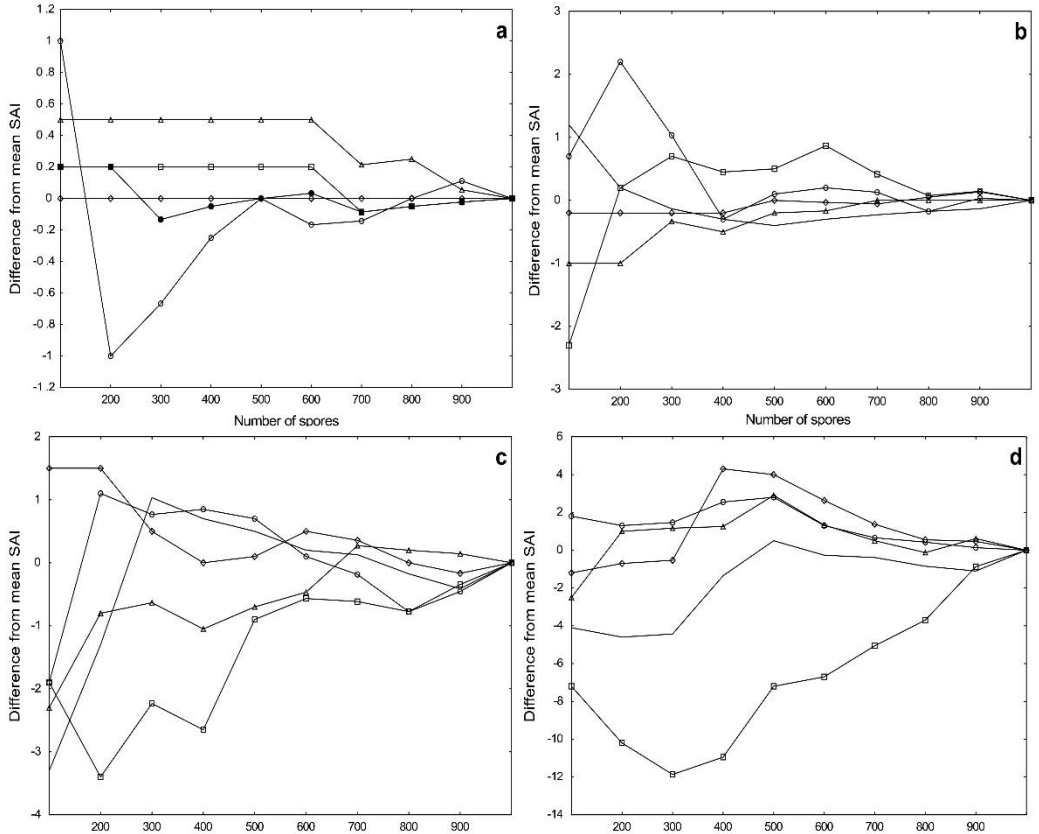

Fig. 5 Change in the difference between partial and total SAI with the increasing amount of spores used to calculate partial SAI. The change is presented for each sample in *Dryopteris ×ambroseae* (a), *Asplenium ruta-muraria* (b), *D. dilatata* (c) and *D. cambrensis* (d)

Discussion

Effects of reproduction mode on spore abortion

Our results suggest a high degree of variability in SAI for apomictic taxa. Studied apomictic species had SAI ranging from 1.7 to 60.9% with the mean abortion rate being around 18%. Apomictic taxa of both *Dryopteris* and *Phegopteris* showed similar SAI and wide pattern. Therefore, the rate of aborted spores in apomictic species might permeate throughout family boundaries and is probably not just limited to either genus.

Fern apomicts are formed via apogamy (formation of sporophytes from somatic cells of the prothallium) followed by agamospory (production of unreduced spores). There are several ways of how spores are formed in fern apomicts. Aborted spores are formed via unbalanced meiosis, and (diplo)spores are formed via regular meiosis. Both processes are present simultaneously, so it is generally expected that apomicts usually have a higher incidence of aborted spores when compared to sexual species (Manton 1950; Gastony and Windham 1989). There are several studies that confirm higher spore abortion and greater SAI variability for apomicts. Study of apomictic *Cornopteris christenseniana* revealed 8–99% of aborted spores (Park and Kato 2003). A more detailed examination of apomicts was carried out by Walker (1962). He compared natural apomicts and synthetic apomictic hybrids of *Pteris* resulting in 15–43% and 45–81% SAI, respectively. An extensive series of studies were carried out by Eschelmüller analyzing germination rates of apomicts. These studies suggest very erratic and highly variable germination rates for *Dryopteris affinis* complex (Eschelmüller 1998) and *Dryopteris remota* (Eschelmüller 1993).Our results with a robust and highly comparable dataset confirmed the generally expected notion that apomictic species are mostly capable of forming a high proportion of viable spores but are prone to high levels of abortion. However, published literature is equivocal. There are apomicts with evidence of little or no spore abortion, e.g., triploid species of *Cyrtogonellum* Ching (Dryopteridaceae) (Guo and Liu 2013), tetraploid *Pteris vittata* L. (Pteridaceae) with stated 100% germination rate, therefore, supposed 0% SAI (Khare and Kaur 1983). High germination rates and <10% SAI were revealed in apomictic diploid *Dryopteris affinis* (Quintanilla and Escudero 2006). Similarly 8–10% of aborted spores are produced by apomictic triploid *Argyrochosma nivea* var. *tenera* (Gillies ex Hook.) Ponce (Adiantaceae) (Hernández et al. 2015).

In our study, sexual taxa produced a lesser amount of aborted spores (mean SAI 4.83%) when compared to apomicts (mean SAI 19.25%), in the genus *Dryopteris*. This trend applies more broadly to all studied taxa (mean SAI 3.05 and 18.09%, respectively). The apomictic diploid *Dryopteris affinis* was found to have comparable SAI (mostly around or below 5%) and germination rates to sexual *Dryopteris* species (Quintanilla and Escudero 2006). To our knowledge, no other comparisons of sexual and apomictic taxa

in either SAI or germination rates have been published. While results of various germination tests may vary wildly, as demonstrated below, published data suggest low SAI for sexual taxa. Arosa et al. (2009) reported mean SAI lower than 8% for *Culcita macrocarpa* C. Presl (Dicksoniaceae) and *Woodwardia radicans* (L.) Sm. (Blechnaceae). A set of 55 samples of *Dicksonia sellowiana* produced mean 3.8% of aborted spores (Gomes et al. 2006). It is apparent that sexual taxa commonly produce a vast majority of well-developed spores. Nevertheless, our results show a potential of abortion rates as high as 19% (*Dryopteris fragrans*, sample 4). Although not yet backed by proper experiments, environmental stress is sometimes evoked to explain these abnormalities (Arosa et al. 2009) as various environmental or seasonal factors are known to affect spore production (Odland 1998; Greer and McCarthy 2000; Mesipuu et al. 2009). Braithwaite (1964) studied the apomictic *Aspleniun aethiopicum* Bech. (Aspleniaceae), which produced a high amount of aborted spores after producing an overabundance of viable spores the previous season. It is certainly possible that similar mechanisms can affect SAI in sexual taxa as well. Further studies on the effect of various external and internal conditions on SAI are needed to properly explain abnormal spore abortion of some plants.

In ferns, aborted spores are usually used as an important character for the detection of hybrids (Wagner and Chen 1965; Ekrt et al. 2010). In concordance with general expectations, our study confirmed very high spore abortion rates in both triploids and tetraploid hybrids of sexual species (SAI more than 98%). However, *Dryopteris ×critica* represents a special case as a pentaploid hybrid of sexual *D. filix-mas* and apomictic *D. borreri*. This taxon is capable of forming a proportion of developed spores thus produce new entities (Bär and Eschelmüller 2010; Ekrt and Koutecký 2016). Spore abortion rate of *Dryopteris ×critica* reached mean 93.2% in this study, and published data indicate 80–95% SAI (Eschelmüller 1998; Fraser-Jenkins 2007; Ekrt and Koutecký 2016). Furthermore, the existence of a minor portion of developed spores in fern hybrids was revealed in several other studies in *Polystichum* Roth. (Dryopteridaceae) (Pinter 1995), *Osmunda* L. (Osmundaceae) (Yatabe et al. 2011) and *Cystopteris* Bernh. (Cystopteridaceae) (Kawakami et al. 2010; Hanušová and Ekrt unpublished data). Further detailed reproductive studies are needed to fully understand this problem, and a standardization of the SAI estimate method may help in future endeavors.

Effects of ploidy levels on spore abortion

No difference in SAI was observed in our study between sexual diploids and tetraploids. Significant differences were observed for the random nested factor of species. Regarding ploidy levels, similar results were reached in several other studies. This factor had no effect on germination rates of herbaria specimens of *Pellaea* Link. (Adiantaceae) (Windham and Ranker 1986). In *Psilotum nudum* (L.) P.Beauv. (Psilotaceae), plants producing either haploid or diploid spore did not differ in both SAI and germination rates

(Whittier and Braggins 1994). Notably Quintanilla and Escudero (2006) observed no difference between diploid and tetraploid *Dryopteris* in both germination rates and SAI. However, in the same study, the authors found a higher SAI in two samples of *D. corleyi* Fraser-Jenk. presuming that the increase in SAI is a result of a relatively recent origin of the not yet stabilized allotetraploid. Some of our samples of sexual species also had higher SAI, including the diploid *Dryopteris fragrans* (sample 4) at 19% abortion. Therefore, it is possible that other factors (environmental, seasonal) may be at play, as mentioned in the chapter above.

Several studies dealing with spore germination rates show a difference between diploids and polyploids. Comparably lower germination rates were found in diploids for the *Polystichum aculeatum* group (Pangua et al. 2003), *Polypodium virginianum* L. (Polypodiaceae) (Kott and Peterson 1973) and Iso*ëtes* L. (Isoetaceae), where germination rates increased with ploidy level among diploids, tetraploids and decaploids (Kott and Britton 1982). Polyploids tend to have alternate or wider distribution, ecological niches and are more efficient colonizers, when compared to diploids (Vogel et al. 1999; Haufler et al. 2016). As Kott and Peterson (1973) suggest, the difference in germination rates between diploids and polyploids may be a result of various factors, including substrate preferences of viable spores.

Different rates of spore abortion among genera

Our results show a marginally significant difference in SAI between sexual taxa of the species richest genera *Asplenium* and *Dryopteris*. However, this difference is comparable to the difference between the species within their respective genus. Our sampling covers a phylogenetical cross section of species in Asplenium (Schneider et al. 2004) as well as species from several groups within *Dryopteris* including the most basal *D. fragrans* (Sessa et al. 2012). The marginally significant differences could reflect different habitat preferences or different position of phylogeny tree. Spore retention during the season may also be reflected in SAI estimates.

Spore abortion index (SAI) as an informative and standardized tool

We employed SAI in a wide and representative dataset of 109 specimens from 23 fern taxa. The result denoted a robust comparison among particular taxa or particular groups to verify hypotheses of differing amounts of aborted spores in species with different reproduction mode. According to our results, we consider SAI a very promising tool in the study of reproduction in spore producing plants.

Theoretically calculated values of standard error of estimate demonstrate the considerable potential error made by using an insufficient amount of spores. While the error may seem low when counting 100 spores with real SAI being 5%, it is important to consider the proportion of the mistake to the actual SAI. Additionally, exploring cumulatively

calculated partial SAI for sampled taxa, it is clear that some sample's partial SAI started approaching total SAI only after more than 500 spores had been calculated. Calculating SAI using only 100 spores is highly insufficient, and for appropriate accuracy of results 1000 spores should be analyzed.

Furthermore a significant level of variation of SAI within a single frond was found for about 10% of plants with almost as much being marginally significant. One sample had the difference between the two parts almost as high as its total SAI, 18.2 and 20.9%, respectively. This factor may considerably affect SAI estimate accuracy when only one part of the frond is used, which is, to our knowledge, common practice. Therefore, using at least two distinct parts of the frond is suitable, at least for apomictic taxa. We also recommend avoiding fronds or parts of fronds that have already shed a majority of spores as well as damaged plants or plants growing in extremely suboptimal conditions. Following these guidelines will hopefully provide an accurate estimate of total SAI taking into account several factors analyzed in this study.

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Information on Electronic Supplementary Material

Online Resource 1. We list all of the plants used alongside herbaria specimen data and SAI (spore abortion index).

Online Resource 2. We list all of the samples used presented with several measures of variation. Among them are the range of SAI within individual sets of 100 spores as well as the difference between the two parts of the frond.

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Online Resource 1 The list of all plants used in the study alongside herbaria specimen data. Location column presents the original author description prefixed by country code and suffixed by herbarium code. SAI (spore abortion index) and the ID of the plants within taxon are also present.

Taxon	ID	SAI	Date	Collector	Location
Asplenium adiantum-nigrum	1	0.025	2014-09-11	Ekrt, L.	GBR: Wales, Abergwyngregyn - Coedydd Aber National Nature Reserve, ca 1,2 km SE of the village centrum man-made wall along touristic path; 75 m s.m.; 53°13'41"N, 004°00'15"E; CBFS
Asplenium adiantum-nigrum	2	0.016	2014-09-07	Ekrt, L.	GBR: North Scotland, Durness - limestone cliffs near above see near Smoo Cave ca 1,6 km ESE of the Durness village centrum; 2 m s.m.; 58°33'51"N, 004°43'13"E; CBFS
Asplenium adiantum-nigrum	3	0.044	2002-05-07	Ekrt, L.	CZE: Žďár u Mnichova Hradiště, Příhrazy, na hřbetu pískovcových skal asi 1,5 km SZ centra obce Příhrazy, asi 1 km SV vrcholu kopce Mužský, osluněná spára pískovcových skal s vápnitým tmelem; 385 m s.m. 5456cb; 50°32'37"N, 015°04'48"E; CBFS
Asplenium adiantum-nigrum	4	0.062	2010-07-16	Ekrt, L.	MAK: Mavrovo Rostuše (NP Mavrovo) - along path to the Duf waterfall ca 700 m south of the village; 800 m s.m.; 41°36'15"N, 020°35'56"E; CBFS
Asplenium adiantum-nigrum	5	0.023	2014-09-02	Ekrt, L.	DEU: Germany, Pfalzen Wald, Erlenbach bei Dahn - walls of Berwartstein castle ca 0.5 km SSE of the town centrum.; 49°06'23"N, 007°51'46"E; CBFS
Asplenium cuneifolium	1	0.02	1927-10-09	Suza, J.	CZE: Moravia occid. Tišnov: in valle rivi Libochovka prope p. Rojetín in fissuris rupium serpentinacearum.; OP
Asplenium cuneifolium	2	0.018	1932-08-21	Suza, J.	CZE: Moravia occid.: Velké Meziříčí, in rupibus serpentinicis apud molam Těšikův mlýn prope pagum Horní Bory, in Pineto lucido.; OP
Asplenium cuneifolium	3	0.008	1937-08	Laus, H.	CZE: M. Schönberg aüf Serpentin b. Nikles.; OP
Asplenium cuneifolium	4	0.014	1906-06	Servit, M.	CZE: Moravia austro-occidentalis: montes Českomoravská vysočina, in rupibus et lapidibus serpentinicis prope Rožná.; OP
Asplenium cuneifolium	5	0.116	1929-09	Wihan, R.	CZE: Bohemia septentrionali-occidentalis: montes Císařský Les (Kaiserwald), in monte Wolfstein prope thermas Mariánské Lázně (Marienbad), solo serpentinico, 900 m n.m.; OP
Asplenium trichomanes nothosubsp. *lusaticum*	1	0.996	1949-09-05	Medlinová, M.	BGR: Vápencové skály - záp. Rhodopy; PR

Asplenium trichomanes nothosubsp. *lusaticum*	2	0.999	2013-09-08	Ekrt, L.	CZE: Praha-západ Slapy nad Vltavou, Nové Třebenice - svah nad Vltavou ca 3,7 km VSV od zámku v obci Slapy svah se silikátovými skalkami nad řekou, 225 m n.m.; 49°49'20"N, 014°26'26"E; CBFS
Asplenium trichomanes nothosubsp. *lusaticum*	3	0.999	2005-05-31	Ekrt, L.	SVK: Cerová vrchovina, Rimavská Sobota Gortva - Steblová skála, rocky steppes near the peak of Stéblová skala hill ca 1 km ESE of the village, ca 3,2 km NNE of the Hajnáčka village, 380 m s.m.; 7588b; 48°29'34"N, 020°29'13"E; CBFS
Asplenium trichomanes nothosubsp. *lusaticum*	4	0.999	2002-09-04	Ekrt, L.	CZE: Plzeň - sever Brdo u Manětína - siliceous slate rocks over the Manětínský potok stream ca 1,1 km S of the Brdo village, 375 m s.m.; 5545ba; 50°29'28"N, 013°15'37"E; CBFS
Asplenium onopteris	1	0.024	2015-05-03	Ekrt, L.	ESP: South Spain, Andalusia, Benamahoma - shady rocks in forest along small road ca 1.7 km ENE of the village centrum, 660 m s.m.; 36°46'15"N, 005°26'58"E; CBFS
Asplenium onopteris	2	0.024	2015-05-27	Ekrt, L.	ESP: Tenerife Spain, Tenerife Island, Icod de los Vinos - forest above town ca 1.5 km SSW of Cueva del Viento, 890 m s.m.; 28°20'16"N, 016°42'19"E; CBFS
Asplenium onopteris	3	0.025	2015-05-03	Ekrt, L.	ESP: South Spain, Andalusia, Benamahoma - shady rocks in forest along small road ca 1.7 km ENE of the village centrum, 660 m s.m.; 36°46'15"N, 005°26'58"E; CBFS
Asplenium onopteris	4	0.041	s. d.	Fernandes, A.; Fernandes, R.; Mates, J.	PRT: Estrada de Saboia, Barrance de Pisees (Algarve).; PR
Asplenium onopteris	5	0.018	1949-09-05	Medlinová, M.	BGR: Vápencové skály - záp. Rhodopy; PR
Asplenium ruta-muraria	1	0.033	2015-11-06	Hornych, O.	CZE: Kutná Hora: kamenná zídka v ulici Kotkova patřící k budově Vojtěšská 11 (klinika), cca 450 m ZSZ Katedrály sv. Barbory, 300 m n.m..; 49°56'46"N, 015°15'28"E; CBFS
Asplenium ruta-muraria	2	0.008	2012-08-12	Ekrt, L.	KGZ: Jalal-Abad province Bajkaška Terek, Tava Say - valley ca 17 km SSW of the central part of Sary Chelek lake 1350 m s.m.; 41°45'03"N, 071°52'39"E; CBFS
Asplenium ruta-muraria	3	0.01	2014-09-07	Ekrt, L.	GBR: North Scotland, Durness - limestone cliffs near above see near Smoo Cave ca 1,6 km ESE of the Durness village centrum, 2 m s.m.; 58°33'51"N, 004°43'13"E; CBFS

Asplenium ruta-muraria	4	0.012	2011-07-28	Ekrt, L.	GEO: Georgia, Vanis Kvabi rock caves complex ca 1,9 km E of the Vardzia cave complex, ca 1,5 km NE of the village of Gogasheni, 1430 m s.m.; 41°22'54"N, 043°18'27"E; CBFS
Asplenium ruta-muraria	5	0.053	2011-07-15	Ekrt, L.	SRB: Serbia, Smederovo - walls of old fortress in N part of the town near river Dunaj, 80 m s.m.; 44°40'09"N, 020°55'30"E; CBFS
Asplenium trichomanes subsp. *quadrivalens*	1	0.033	2002-04-27	Ekrt, L.	CZE: Broumovsko, Náchod Maršov nad Metují - plaener rocks called Poradní skála rock in the Maršovské údolí valley, ca 1,5 km SE of the Maršov village, 430 m s.m.; 5363cc; 50°30'N, 016°12'E; CBFS
Asplenium trichomanes subsp. *quadrivalens*	2	0.006	2004-08-24	Ekrt, L.	CZE: Frýdek Místek Sklenov, Hukvaldy - walls in the deer-park of Hukvaldy ruins area, ca 30 m of the entrance, ca 100 m SE of the church of the Hukvaldy village, 355 m s.m.; 6375cd; 49°37'22"N, 018°13'22"E; CBFS
Asplenium trichomanes subsp. *quadrivalens*	3	0.015	2002-10-07	Ekrt, L.	CZE: Křivoklátsko, Rakovník Křivoklát - siliceous rocks in the W part of the Nezabudické skály reserve, ca 2,5 km SW od the Křivoklát village, 250 m s.m.; 5949cc; 50°01'21"N, 013°50'09"E; CBFS
Asplenium trichomanes subsp. *quadrivalens*	4	0.007	2010-09-28	Ekrt, L.	CZE: Brno-venkov Veverská Bitýška - na zdech hradu Veveří ca 3 km JV od centra obce, 345 m s.m.; 49°15'24"N, 016°27'38"E; CBFS
Asplenium trichomanes subsp. *quadrivalens*	5	0.013	2014-09-01	Šumberová, K.; Ducháček, M.	CZE: okres České Budějovice: České Budějovice, Mlýnská stoka u mostu mezi Husovou ulicí a křižovatkou ulic Panská a Mlýnská, zeď nad mlýnskou stokou. 386 m n.m.; 48°58'39"N, 014°28'17"E; CBFS
Asplenium trichomanes subsp. *trichomanes*	1	0.009	2004-09-03	Ekrt, L.	SVK: Malá Fatra Žilina Krasňany, dolina Kúr, spodní část doliny asi 3,5 km JV od kláštera v obci silikát, zářez lesní cesty; 605 m s.m.; 6879ba; 49°11'34"N, 018°56'02"E; CBFS
Asplenium trichomanes subsp. *trichomanes*	2	0.01	2007-09-24	Ekrt, L.	CZE: Šumava, Klatovy Rejštejn - dry slopes in NE part of the village, ca 990 m NE of the centrum siliceous rocks 660 m s.m.; 6847ca; 49°08'29"N, 013°31'43"E; CBFS
Asplenium trichomanes subsp. *trichomanes*	3	0.008	2010-11-06	Ekrt, L.	CZE: České Budějovice Nuzice - na skalce v Židově strouze ca 650 m SV od kostela v obci, 370 m s.m.; 6752bb; 49°16'33"N, 014°27'45"E; CBFS
Asplenium trichomanes subsp. *trichomanes*	4	0.007	s. d.	Deyl, M.	CZE:Bohemia occidentalis: In rupibus serpentinicis Schwarzholz et Steinhügel dictis inter vicos Drahotín et Poběžovice.; CBFS
Asplenium trichomanes subsp. *trichomanes*	5	0.018	1966-06-29	Contardo, A.	ITA: 176. exsicata Friuli - Tarcento (Udine): ad rupes et muros prope Vedronza, 320 m s.m.; Pl. Italicae Exs.; PR

Asplenium viride	1	0.042	2015-09-01	Ekrt, L.	NOR: Norway, Rago national parkm Lakshol: bottom of river valley ca 1,5 km of the Laskhol settlement (ESE) 60 m s.m.; 67°26'49"N, 015°48'15"E; CBFS
Asplenium viride	2	0.03	2015-08-24	Ekrt, L.	NOR:Norway, Steinkjer, Asphaugen: limestone rocks on the margin of Rungstadvatnet lake ca 1,2 km SSW of the Asphaugen village, 85 m s.m.; 64°03'20"N, 011°29'45"E; CBFS
Asplenium viride	3	0.011	2014-09-08	Ekrt, L.	GBR: Scotland, Ardarroch, Tornapress - limestone wet gorge ca 3.1 km NE of the village of Ardarroch centrum.; 57°25'19"N, 005°35'11"E; CBFS
Asplenium viride	4	0.023	2004-04-03	Ekrt, L.	CZE: Český Krumlov Holubov, rez. Holubovské hadce, asi 750 m S od železniční zastávky v obci, hadcové skály nad Křemžským potokem, 450 m s.m.; 7152a; CBFS
Asplenium viride	5	0.014	2014-09-03	Ekrt, L.	GBR: England, Yorkshire Dales national park, Malham: entrance to the Gordale Scar ca 2 km NE of the Malham village centrum, 345 m s.m.; 54°04'22"N, 002°07'50"E; CBFS
Athyrium filix-femina	1	0.009	2014-08-21	Hornych, O.	CZE: Rejštejn: Smrčina u mostu přes Otavu 1,8 km S od soutoku Křemelné a Vydry, 600 m n.m.; 49°07'32"N, 013°29'42"E; CBFS
Athyrium filix-femina	2	0.006	2015-07-24	Hornych, O.	CZE: Kutná Hora: Vlhký příkop podél lesní silničky mezi mlýnem Denemark a pilou u Vrbova mlýna, na levém břehu Vrchlice, 750 m SSZ od žel. zastávky Poličany, 250 m n.m.; 49°55'47"N, 015°15'15"E; CBFS
Athyrium filix-femina	3	0.005	2015-07-22	Hornych, O.	CZE: Borová Lada: U silnice mezi pramenem Volyňky a Světlohorskou nádrží 650 m V od vrcholu Světlá hora (1123), 1,8 km JJZ od žel. stanice Lipka, 975 m n.m.; 49°00'16"N, 013°43'41"E; CBFS
Athyrium filix-femina	4	0.009	2015-07-29	Hornych, O.	CZE: Horní Stropnice: Lesní cesta 450 m SV od vrchu Kraví hora (953), 500 m SZ od zastávky v obci Hojná Voda, 825 m n.m.; 48°43'59"N, 014°43'26"E; CBFS
Athyrium filix-femina	5	0.009	2015-10-01	Hornych, O.	CZE: Branišov: V příkopě lesní cesty Mokré - Branišov, 500 m JZ od samoty "U Lesa", 425 m n.m.; 48°58'21"N, 014°24'26"E; CBFS
Dryopteris affinis	1	0.05	2011-07-24	Ekrt, L.	GEO: Georgia, Adjaria region, Keda - forest along forst path ca 3,4 km NE of the town centrum 350 m s.m.; 41°37'18"N, 041°37'06"E; CBFS
Dryopteris affinis	2	0.335	1869-09-01	Meandon, G.	PRT: Pl. Maderenses In umborsis S. Antoio das Serrera.; PR
Dryopteris affinis	3	0.017	1899-08	Lösch, A.	DEU: Flora von Baden. In silvis umborsis et humosis montis nigri meridionalis prope Zastler.; PR

Dryopteris borreri	1	0.043	2015-07-22	Hornych, O.	CZE: Borová Lada: Smrčina na S svahu Světlé hory (1123), nedaleko křižovatky lesní cesty od pramene Volyňky a silnice z Borových Lad do Lipky, 1,7 km JZ od žel. stanice Lipka, 1000 m n.m.; 49°00'42"N, 013°42'58"E; CBFS
Dryopteris borreri	2	0.102	2015-08-04	Hornych, O.	CZE: Hostašovice: Olšina podél potoka 350 J od vlakové stanice Hostašovice, 400 m n.m.; 49°31'04"N, 018°00'58"E; CBFS
Dryopteris borreri	3	0.334	2015-08-03	Hornych, O.	CZE: Čeladná: U křižovatky lesních silnic na začátku žluté a červené tur. cesty 1,2 km S od vrcholu Kněhyně (1257), 950 m n.m.; 49°30'24"N, 018°18'41"E; CBFS
Dryopteris borreri	4	0.211	2010-08-01	Ekrt, L.	CZE: Vysočina, Třebíč Radonín - při lesní cestě ve smrkovém lesním komplexu ca 1,6 km JV od kostela/kaple v obci, 570 m n.m.; 49°16'05"N, 015°44'08"E; CBFS
Dryopteris borreri	5	0.609	2004-09-13	Ekrt, L.	CZE: Šumava, Prachatice Stožec, v rezervaci Stožec asi 750 m V od vrcholu kopce Stožec, květnatá bučina místy s žulovými rozpady, 995 m n.m.; 48°52'56"N, 013°49'53"E; CBFS
Dryopteris cambrensis	2	0.101	2007-09-05	Ekrt, L.; Lepší, M.	CZE: Pohorská Ves, Žofín - ca 1,3 km W of the ancient Žofín village, 835 m s.m.; 7354aa; 48°40'34"N, 014°40'19"E; CBFS
Dryopteris cambrensis	1	0.282	2007-09-17	Ekrt, L.	CZE: Moravskoslezský kraj, Bruntál Krnov, Brantice - fir forest in small debris ca 1,4 km SSE of the church in the village of Brantice. 460 m s.m. ; 50°03'09"N, 017°38'16"E; CBFS
Dryopteris cambrensis	3	0.072	2008-10-15	Ekrt, L.	CZE: 33. Branžovský hvozd: Liščí, foothill of Jezvinec hill, c. 1.8 km NE of the village centre, c. 590 m. alt.; 49°19'19"N, 013°03'42"E; CBFS
Dryopteris cambrensis	4	0.135	2007-09-17	Ekrt, L.	CZE: Krnov, Brantice - ca 1,5 km SE of the church in the village of Bratnice; 50°03'09"N, 017°38'16"E; CBFS
Dryopteris cambrensis	5	0.082	2000-08-26	Boublík, K.	CZE: Kaproun - edge of the path below the Kaproun railway station near the village, 660 m s.m.; 49°04'N, 015°10'E; CBFS
Dryopteris carthusiana	1	0.006	2010-07-10	Ekrt, L.	CZE: Řídelov - olšina v přírodní památce Lukšovská ca 1,3 km SSZ od centra obce; 630 m s.m.; 49°14'43"N, 015°23'50"E; CBFS
Dryopteris carthusiana	2	0.012	2006	Čejková, A.	RUS: Altaj - Larix-Abies tajga ca 56 km south of peak of Belucha 1360 m s.m.; 49°18'N, 086°32'E; CBFS
Dryopteris carthusiana	3	0.017	2005-07-16	Ekrt, L.	CZE: Vysočina, Jihlava Třešť - NPR Velký Špičák reserve, ca 3,5 km NNE of the railway station in the village of Třešť beech forest with the gneis rock; 695 m s.m.; 49°18'45"N, 015°30'37"E; CBFS
Dryopteris carthusiana	4	0.025	1939-07-07	Deyl, M.	CZE: Bohemia australis; In silvis turfosis Blata dictis prope vicum Mažice procul oppidum Soběslav.; PR

Dryopteris carthusiana	5	0.002	1954-07-31	Klášterský, I.	CZE: Bohemia meridionalis: circulus Sušice: in declivibus saxosis in valle fluvii Otava (Vydra) ad Turnerova chata, 790 m s.m.; PR
Dryopteris dilatata	1	0.033	2014-07-24	Hornych, O.	CZE: České Budějovice: Smrčina u PP Kaliště mezi Kalištěmi a Zalinami, 1 km ZJZ od kostela v obci Zaliny, 500 m n.m.; 48°57'23"N, 014°35'27"E; CBFS
Dryopteris dilatata	2	0.033	2010-07-10	Ekrt, L.	CZE: Řídelov - olšina v přírodní památce Lukšovská ca 1,3 SSZ od centra města, 630 m s.m.; 49°14'43"N, 015°23'50"E; CBFS
Dryopteris dilatata	3	0.069	2005-08-08	Ekrt, L.	CZE: Kostelní Myslová - souh edge of the Velký Hulišťský rybník pond ca 1,6 km WNW of the centrum of the village, 520 m s.m.; 49°09'09"N, 015°24'25"E; CBFS
Dryopteris dilatata	4	0.079	2015-07-22	Hornych, O.	CZE: Lenora: Podél lesní cesty na vrchol Zátoňská hora (1034), ve smrčině na SZ svahu hory, 500 m VJV žel. zastávky Zátoň, 925 m n.m.; 48°56'59"N, 013°49'43"E; CBFS
Dryopteris dilatata	5	0.035	1975-07-07	Hájková, A.	CZE: Podbeskydská pahorkatina, Vyšní Lhoty Prašivá, údolí potoka Hlisník; 6376d; CBFS
Dryopteris expansa	1	0.026	2014-07-15	Hornych, O.	CZE: Řídelov: Podmáčená olšina v PP Lukšovská 250 m SSV od křižovatky "Malý pařezitý rybník" nedaleko tohoto rybníku, 625 m n.m.; 49°14'45"N, 015°23'48"E; CBFS
Dryopteris expansa	2	0.076	2015-07-22	Hornych, O.	CZE: Borová Lada: U silnice mezi pramenem Volyňky a Světlohorskou nádrží 650 m V od vrcholu Světlá hora (1123), 1,8 km JJZ od žel. stanice Lipka, 975 m n.m.; 49°00'16"N, 013°43'41"E; CBFS
Dryopteris expansa	3	0.019	2015-07-25	Hornych, O.	CZE: Paseky nad Jizerou: V suťové bučině na JV svahu u Klokotivého potoka mezi vrchy Kapradník (910) a Hromovka (916), nad modrou t. stezkou podél Jizery, 650 m JV od vrcholu Kapradník, 675 m n.m.; 50°44'52"N, 015°23'55"E; CBFS
Dryopteris expansa	4	0.053	2015-07-25	Hornych, O.	CZE: Paseky nad Jizerou: V suťové bučině na JV svahu u Klokotivého potoka mezi vrchy Kapradník (910) a Hromovka (916), nad modrou t. stezkou podél Jizery, 650 m JV od vrcholu Kapradník, 675 m n.m.; 50°44'52"N, 015°23'55"E; CBFS
Dryopteris expansa	5	0.075	2015-08-03	Hornych, O.	CZE: Čeladná: Podél červené tur. trasy 500 m Z od vrchu Kněhyně (1257), 2,2 km V-VSV od vrchu Tanečnice (1084), 1125 m n.m.; 49°29'46"N, 018°18'19"E; CBFS
Dryopteris filix-mas	1	0.115	2014-07-24	Hornych, O.	CZE: České Budějovice: Smrčina u PP Kaliště mezi Kalištěmi a Zalinami, 1 km ZJZ od kostela v obci Zaliny, 500 m n.m.; 48°57'25"N, 014°35'24"E; CBFS
Dryopteris filix-mas	2	0.044	2014-08-21	Hornych, O.	CZE: Rejštejn: Smrčina u mostu přes Otavu 1,8 km S od soutoku Křemelné a Vydry, 600 m n.m.; 49°07'32"N, 013°29'42"E; CBFS

Dryopteris filix-mas	3	0.018	2015-08-08	Hornych, O.	CZE: Nové Hrady: NPP Terčino údolí, podél naučné stezky, 800 m JZ parkoviště a rozcestníku "Terčino údolí - vstup", 525 m n.m.; 48°46'47"N, 014°45'34"E; CBFS
Dryopteris filix-mas	4	0.041	2015-07-29	Hornych, O.	CZE: Horní Stropnice: Lesní cesta 450 m SV od vrchu Kraví hora (953), 500 m SZ od zastávky v obci Hojná Voda, 825 m n.m.; 48°43'59"N, 014°43'26"E; CBFS
Dryopteris filix-mas	5	0.152	2014-06-22	Ekrt, L.	SRB: East Serbia, Djerdap National Park, Tekija - forested hillside of Mali Strbac Mt. ca 8.5 km WSW of the town centrum, 300 m s.m.; 44°38'46"N, 022°18'57"E; CBFS
Dryopteris fragrans	1	0.012	1969-09-07	Vašák, V.	RUS: Sibiria centralis, distr. Irkutsk: in vicinitate pagi Bolshie Koty apud la. Baical, 455-600 m s.m.; PR
Dryopteris fragrans	2	0.03	1968-10-05	Vašák, V.	RUS: Buriatia: in locis humidis apud rivulum 15 km versus septentr. Ab oppido Ulan Ude.; PR
Dryopteris fragrans	3	0.011	1913-06-08	Gorbovetz, I.	RUS: Prov. Krasnojarsk. prope pag. Ossipovo. In rupium umbrosorum fissuris ad ripam meridionalen fl. Ossipovka.; PR
Dryopteris fragrans	4	0.19	1867	Fowler	CAN: Canada: Mount Prospect Resteonche.; PR
Dryopteris fragrans	5	0.034	s.d.	coll.?	RUS: Sachalin skály v lesu na sopke blíž Gomona.; PR
Dryopteris remota	1	0.302	2015	Ekrt, L.	AUT: Höllengebirge Unterach am Attersee - old beach forest ca 700 m NNE of the town 590 m s.m.; Plant 1/2015, in cultivation; 47°48'38"N, 013°29'26"E; CBFS
Dryopteris remota	2	0.209	2011-09-28	Ekrt, L.	AUT: Höllengebirge Unterach am Attersee - old beach forest ca 700 m NNE of the town 590 m s.m.; Plant 2/2011, in cultivation; 47°48'38"N, 013°29'26"E; CBFS
Dryopteris remota	3	0.196	2011-09-28	Ekrt, L.	AUT: Höllengebirge Unterach am Attersee - old beach forest ca 700 m NNE of the town 590 m s.m.; Plant 1/2011, in cultivation; 47°48'38"N, 013°29'26"E; CBFS
Dryopteris ×ambroseae	1	0.99	2014-07-17	Hornych, O.	CZE: Studená: Smrčina u lesní silnice ze Skrýchova do Brandlína, 1,1 km JV od kostela ve Skrýchově, 650 m n.m.; 49°09'44"N, 015°18'50"E; CBFS
Dryopteris ×ambroseae	2	0.998	2015-07-17	Hornych, O.	CZE: Strážné: Lesní potok 1,1 km VSV od rozcestí "Hříběcí boudy", 900 m n.m.; 50°40'55"N, 015°38'27"E; CBFS
Dryopteris ×ambroseae	3	1	2010-07-05	Ekrt, L.	CZE: Jindřichův Hradec - Slavonice - starší jedlový les u hranic ca 2,1 km JV od kostela v obci, 530 m n.m. 7058a; 48°58'54"N, 015°22'08"E; CBFS
Dryopteris ×ambroseae	4	0.995	2005-09-06	Ekrt, L.	CZE: Teplice nad Metují town - NPR Teplické skály rocks, Teplické skalní město, Sibiř gorge ca 3,25 km E of the centrum of the town, moist and cold sandstone gorge 5462b; 50°35'N, 016°07'E; CBFS

Dryopteris ×ambroseae	5	0.998	2005-07-14	Ekrt, L.	CZE: Klatovy Špičák - ca 3,7 km NW of the centrum of Špičák village, ca 0,9 km SSW of the dike of Černé jezero lake; middle part of forested corrie of Jezerní stěna rocky wall; 1125 m s.m.; 6845ac; 49°10'27"N, 013°10'50"E; CBFS
Dryopteris ×critica	1	0.926	2007	Vinter, V.	CZE: Rajnochovice - PR Čerňava, údolí Rosošského potoka 600-650 m s.m.; 49°22'50"N, 017°47'10"E; CBFS
Dryopteris ×critica	2	0.973	2004-09-13	Ekrt, L.	CZE: Šumava, Prachatice Stožec, v rezervaci Stožec asi 750 m V od vrcholu kopce Stožec, 950 m s.m.; 48°52'56"N, 013°49'53"E; CBFS
Dryopteris ×critica	3	0.895	2004-09-30	Ekrt, L.	SVK: Malá Fatra, Žilina Krasňany, dolina Kúr, spodní část doliny asi 3,5 km JV od kláštera v obci, 605 m n m.; 49°11'34"N, 018°56'01"E; CBFS
Dryopteris ×critica	4	0.935	2007	Vinter, V.	CZE: Hostýnské vrchy PR Čerňava: údolí Rosošného potoka, 600-650 m. n.m.; 49°22'50"N, 017°47'10"E; CBFS
Dryopteris ×deweveri	1	0.956	2014-07-15	Hornych, O.	CZE: Řídelov: Podmáčená olšina v PP Lukšovská 250 m SSV od křižovatky "Malý pařezitý rybník" nedaleko tohoto rybníku, 625 m n.m.; 49°14'45"N, 015°23'48"E; CBFS
Dryopteris ×deweveri	2	1	2014-07-15	Hornych, O.	CZE: Řídelov: Podmáčená olšina v PP Lukšovská 250 m SSV od křižovatky "Malý pařezitý rybník" nedaleko tohoto rybníku, 625 m n.m.; 49°14'45"N, 015°23'48"E; CBFS
Dryopteris ×deweveri	3	0.998	2010-07-10	Ekrt, L.	CZE: Řídelov - olšina v přírodní památce Lukšovská ca 1,3 SSZ od centra města, 630 m s.m.; 49°14'43"N, 015°23'50"E; CBFS
Dryopteris ×deweveri	4	0.998	2006-07-11	Ekrt, L.	CZE: Vysočina, Pelhřimov Polesí u Počátek - alder forest ca 980 m SE of the village; 630 m s.m.; 6757ba; 49°17'16"N, 015°15'24"E; CBFS
Dryopteris ×deweveri	5	0.966	2006-07-13	Ekrt, L.	CZE: Vysočina, Jihlava Stonařov - alder forest ca 2 km W of the village, 630 m s.m.; 6459a; 49°16'N, 015°33'E; CBFS
Gymnocarpium dryopteris	1	0.018	2014-07-17	Hornych, O.	CZE: Studená: Smrčina u rozcestníku "Březka" na SZ svahu Malého vrchu (717), 1,4 km JJV od kostela ve Skrýchově, 700 m n.m.; 49°09'44"N, 015°19'39"E; CBFS
Gymnocarpium dryopteris	2	0.015	2015-07-17	Hornych, O.	CZE: Strážné: Lesní potok 1,1 km SV od rozcestí "Hříběcí boudy", 925 m n.m.; 50°40'57"N, 015°38'26"E; CBFS
Gymnocarpium dryopteris	3	0.013	2015-07-16	Hornych, O.	CZE: Horní Maršov: Smrčina nad Suchým potokem 900 m Z od vrchu Mravenečník (1005), 900 m n.m.; 50°39'55"N, 015°50'50"E; CBFS
Gymnocarpium dryopteris	4	0.004	2015-08-03	Hornych, O.	CZE: Čeladná: Podél červené tur. trasy 1 km S-SSZ od vrchu Kněhyně (1257). Asi 150 m V od vrchu Folvark (1060), 1025 m n.m.; 49°30'17"N, 018°18'33"E; CBFS

Gymnocarpium dryopteris	5	0.003	1998-08-14	Ekrt, L.	NOR: Rondane Rondane National Park, area near Selsverket town; CBFS
Phegopteris connectilis	1	0.145	2014-07-17	Hornych, O.	CZE: Studená: Smrčina u rozcestníku "Březka" na SZ svahu Malého vrchu (717), 1,4 km JJV od kostela ve Skrýchově, 700 m n.m.; 49°09'42"N, 015°19'30"E; CBFS
Phegopteris connectilis	2	0.074	2014-07-24	Hornych, O.	CZE: České Budějovice: Podmáčená smrčina u PP Kaliště mezi Kališětmi a Zalinami, 1 km ZJZ od kostela v obci Zaliny, 500 m n.m.; 48°57'26"N, 014°35'28"E; CBFS
Phegopteris connectilis	3	0.322	2014-08-21	Hornych, O.	CZE: Rejštejn: Smrčina ve svahu na pravém břehu Vydry, 1,3 km JJV od soutoku Křemelné a Vydry, 700 m n.m.; 49°05'54"N, 013°29'48"E; CBFS
Phegopteris connectilis	4	0.054	2013-09-05	Ekrt, L.	CZE: Krkonoše, Trutnov Špindlerův Mlýn, Svatý Petr - podél turistické cesty u Bílého Labe ca 2 km Z od Luční boudy 1225 m s.m. balvaniště u turistické cesty 5260ca; 50°44'14"N, 015°40'10"E; CBFS
Phegopteris connectilis	5	0.124	2015-08-03	Hornych, O.	CZE: Čeladná: Podél červené tur. trasy 1 km S-SSZ od vrchu Kněhyně (1257). Asi 150 m V od vrchu Folvark (1060), 1025 m n.m.; 49°30'17"N, 018°18'33"E; CBFS

Online Resource 2 Results of SAI counting (%) and data analysis for all taxa under study. The range of SAI for the ten constituent sets of 100 spores as well as the two sets of 500 spores, obtained from two distinct parts of the frond, is present. Min-max value represents the difference between the highest and the lowest SAI values for individual sets of 100 spores within a sample.

Taxon	ID within taxon	Range of mininal to maximal	Difference between 500s	Total SAI	Standard deviation	Min-max
Asplenium adiantum-nigrum	1	0-5	2.2-2.8	2.5	1.51	5
Asplenium adiantum-nigrum	2	0-4	1.4-1.8	1.6	1.43	4
Asplenium adiantum-nigrum	3	2-7	4.0-4.8	4.4	2.01	5
Asplenium adiantum-nigrum	4	4-9	6.0-6.4	6.2	1.87	5
Asplenium adiantum-nigrum	5	0-5	2.2-2.4	2.3	1.49	5
Asplenium cuneifolium	1	1-4	2.0-2.0	2	0.82	3
Asplenium cuneifolium	2	0-5	1.4-2.2	1.8	1.69	5
Asplenium cuneifolium	3	0-2	0.8-0.8	0.8	0.79	2
Asplenium cuneifolium	4	0-3	0.4-2.4	1.4	1.35	3
Asplenium cuneifolium	5	5-16	9.8-13.4	11.6	2.91	11
Asplenium onopteris	1	0-4	2.2-2.6	2.4	1.43	4
Asplenium onopteris	2	1-5	1.4-3.4	2.4	1.35	4
Asplenium onopteris	3	0-6	1.6-3.4	2.5	1.84	6
Asplenium onopteris	4	0-9	3.0-5.2	4.1	2.73	9
Asplenium onopteris	5	0-4	1.0-2.6	1.8	1.32	4
Asplenium ruta-muraria	1	1-6	2.8-3.8	3.3	2.00	5
Asplenium ruta-muraria	2	0-2	0.4-1.2	0.8	0.79	2
Asplenium ruta-muraria	3	0-2	0.8-1.2	1	0.82	2
Asplenium ruta-muraria	4	0-2	1.2-1.2	1.2	0.63	2
Asplenium ruta-muraria	5	1-9	5.2-5.4	5.3	2.26	8
Asplenium trichomanes nothosubsp. *lusaticum*	1	98-100	99.2-100.0	99.6	0.70	2
Asplenium trichomanes nothosubsp. *lusaticum*	2	99-100	99.8-100.0	99.9	0.32	1
Asplenium trichomanes nothosubsp. *lusaticum*	3	99-100	99.8-100.0	99.9	0.32	1
Asplenium trichomanes nothosubsp. *lusaticum*	4	99-100	99.8-100.0	99.9	0.32	1
Asplenium trichomanes subsp. *quadrivalens*	1	1-5	3.2-3.4	3.3	1.70	4

Asplenium trichomanes subsp. *quadrivalens*	2	0-2	0.2-1.0	0.6	0.70	2
Asplenium trichomanes subsp. *quadrivalens*	3	0-5	0.8-2.2	1.5	1.43	5
Asplenium trichomanes subsp. *quadrivalens*	4	0-2	0.4-1.0	0.7	0.67	2
Asplenium trichomanes subsp. *quadrivalens*	5	0-4	0.6-2.0	1.3	1.16	4
Asplenium trichomanes subsp. *trichomanes*	1	0-2	0.8-1.0	0.9	0.88	2
Asplenium trichomanes subsp. *trichomanes*	2	0-4	0.8-1.2	1	1.33	4
Asplenium trichomanes subsp. *trichomanes*	3	0-3	0.6-1.0	0.8	1.03	3
Asplenium trichomanes subsp. *trichomanes*	4	0-2	0.2-1.2	0.7	0.67	2
Asplenium trichomanes subsp. *trichomanes*	5	0-4	1.0-2.6	1.8	1.32	4
Asplenium viride	1	0-10	4.0-4.4	4.2	3.05	10
Asplenium viride	2	1-6	2.2-3.8	3	1.56	5
Asplenium viride	3	0-3	1.0-1.2	1.1	1.10	3
Asplenium viride	4	0-5	1.2-3.4	2.3	1.77	5
Asplenium viride	5	0-5	1.2-3.4	2.3	1.77	5
Athyrium filix-femina	1	0-2	0.8-1.0	0.9	0.74	2
Athyrium filix-femina	2	0-2	0.6-0.6	0.6	0.70	2
Athyrium filix-femina	3	0-3	0.0-1.0	0.5	1.08	3
Athyrium filix-femina	4	0-3	0.4-1.4	0.9	0.99	3
Athyrium filix-femina	5	0-3	0.2-1.2	0.7	0.95	3
Dryopteris ×ambroseae	1	96-100	99.0-99.0	99	1.33	4
Dryopteris ×ambroseae	2	98-100	99.6-100.0	99.8	0.63	2
Dryopteris ×ambroseae	3	100-100	100.0-100.0	100	0.00	0
Dryopteris ×ambroseae	4	98-100	99.0-100.0	99.5	0.85	2
Dryopteris ×ambroseae	5	99-100	99.8-99.8	99.8	0.42	1
Dryopteris ×critica	1	85-96	91.6-93.6	92.6	3.13	11
Dryopteris ×critica	2	92-100	96-98.6	97.3	2.63	8
Dryopteris ×critica	3	83-93	88.4-90.6	89.5	2.72	10
Dryopteris ×critica	4	90-98	92.8-94.2	93.5	2.59	8
Dryopteris ×deweveri	1	90-98	93.6-97.6	95.6	3.47	8
Dryopteris ×deweveri	2	100-100	100.0-100.0	100	0.00	0

Dryopteris ×deweveri	3	98-100	99.6-100.0	99.8	0.63	2
Dryopteris ×deweveri	4	99-100	99.6-100.0	99.8	0.42	1
Dryopteris ×deweveri	5	93-99	95.8-97.4	96.6	1.58	6
Dryopteris affinis	1	3-8	4.4-5.6	5	1.63	5
Dryopteris affinis	2	21-44	32.8-34.2	33.5	6.70	23
Dryopteris affinis	3	0-4	1.0-2.4	1.7	1.42	4
Dryopteris borreri	1	2-7	3.0-5.6	4.3	1.83	5
Dryopteris borreri	2	2-16	9.2-11.2	10.2	4.26	14
Dryopteris borreri	3	20-45	29.6-37.2	33.4	7.18	25
Dryopteris borreri	4	14-34	17.6-24.6	21.1	6.23	20
Dryopteris borreri	5	37-75	60.4-61.4	60.9	10.58	38
Dryopteris cambrensis	1	13-50	21.0-35.4	28.2	11.53	37
Dryopteris cambrensis	2	5-20	9.6-10.6	10.1	6.03	15
Dryopteris cambrensis	3	1-13	4.4-10.0	7.2	3.52	12
Dryopteris cambrensis	4	7-23	10.6-16.4	13.5	5.54	16
Dryopteris cambrensis	5	2-27	4.2-12.2	8.2	7.18	25
Dryopteris carthusiana	1	0-2	0.4-0.8	0.6	0.84	2
Dryopteris carthusiana	2	0-3	0.8-1.6	1.2	1.23	3
Dryopteris carthusiana	3	0-4	1.4-2.0	1.7	1.42	4
Dryopteris carthusiana	4	1-4	2.2-2.8	2.5	1.27	3
Dryopteris carthusiana	5	0-1	0.2-0.2	0.2	0.42	1
Dryopteris dilatata	1	0-9	2.8-3.8	3.3	2.79	9
Dryopteris dilatata	2	1-8	2.6-4.0	3.3	2.00	7
Dryopteris dilatata	3	2-13	6.0-7.8	6.9	3.41	11
Dryopteris dilatata	4	3-12	7.2-8.6	7.9	2.96	9
Dryopteris dilatata	5	1-6	3.4-3.6	3.5	1.72	5
Dryopteris expansa	1	1-4	2.2-3.0	2.6	1.07	3
Dryopteris expansa	2	2-13	6.2-9.0	7.6	3.41	11
Dryopteris expansa	3	0-6	1.4-2.4	1.9	1.66	6
Dryopteris expansa	4	1-14	3.6-7.0	5.3	4.14	13
Dryopteris expansa	5	2-14	4.0-11.0	7.5	4.20	12
Dryopteris filix-mas	1	8-17	11.2-11.8	11.5	3.03	9
Dryopteris filix-mas	2	1-7	4.2-4.6	4.4	1.65	6
Dryopteris filix-mas	3	0-5	0.6-3.0	1.8	1.62	5
Dryopteris filix-mas	4	2-7	2.6-5.6	4.1	1.91	5

Dryopteris filix-mas	5	5-23	12.6-17.8	15.2	5.37	18
Dryopteris fragrans	1	0-5	0.8-1.6	1.2	1.48	5
Dryopteris fragrans	2	1-4	2.4-3.6	3	1.15	3
Dryopteris fragrans	3	0-3	0.8-1.4	1.1	0.74	3
Dryopteris fragrans	4	7-41	11.0-27.0	19	10.50	34
Dryopteris fragrans	5	0-8	1.4-5.4	3.4	2.55	8
Dryopteris remota	1	13-52	24.2-36.2	30.2	12.32	39
Dryopteris remota	2	7-46	11.8-30.0	20.9	12.24	39
Dryopteris remota	3	12-28	19.4-19.8	19.6	4.30	16
Gymnocarpium dryopteris	1	1-4	1.6-2	1.8	1.23	3
Gymnocarpium dryopteris	2	0-3	1.2-1.8	1.5	0.85	3
Gymnocarpium dryopteris	3	0-3	1.2-1.4	1.3	1.16	3
Gymnocarpium dryopteris	4	0-2	0.2-0.6	0.4	0.70	2
Gymnocarpium dryopteris	5	0-1	0.2-0.4	0.3	0.48	1
Phegopteris connectilis	1	7-22	13.6-15.4	14.5	4.95	15
Phegopteris connectilis	2	2-11	6.8-8.0	7.4	3.17	9
Phegopteris connectilis	3	24-40	28.0-36.4	32.2	5.18	16
Phegopteris connectilis	4	0-13	2.0-8.8	5.4	4.67	13
Phegopteris connectilis	5	5-27	6.6-18.2	12.4	7.41	22

Chapter 3: Insights into the evolutionary history and widespread occurrence of antheridiogen systems in ferns.

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Summary

- Sex expression of homosporous ferns is controlled by multiple factors, one being the antheridiogen system. Antheridiogens are pheromones released by sexually mature female fern gametophytes, turning nearby asexual gametophytes precociously male. Nevertheless, not all species respond. It is still unknown how many fern species use antheridiogens, how the antheridiogen system evolved, and whether it is affected by polyploidy and/or apomixis.
- We tested the response of 68 fern species to antheridiogens in cultivation. These results were combined with a comprehensive review of literature to form the largest dataset of antheridiogen interactions to date. Analyzed species also were coded as apomictic or sexual and diploid or polyploid.
- Our final dataset contains a total of 498 interactions involving 208 species (c. 2% of all ferns). About 65% of studied species respond to antheridiogen. Multiple antheridiogen types were delimited and their evolution is discussed. Antheridiogen responsiveness was not significantly affected by apomixis or polyploidy.
- Antheridiogens are widely used by ferns to direct sex expression. The antheridiogen system likely evolved multiple times and provides homosporous ferns with the benefits often associated with heterospory, such as increased rates of outcrossing. Despite expectations, antheridiogens may be beneficial to polyploids and apomicts.

Introduction

Homospory (the production of a single spore type at meiosis) is presumed to be the ancestral state in land plants, yet the majority of extant species are heterosporous (producing two types of spores, typically male and female, at meiosis) and heterospory evolved a minimum of 11 times (Bateman & DiMichele, 1994). Heterospory promotes genetic diversity by limiting inbreeding (Qiu *et al.*, 2012). In contrast, gametophytes of homosporous plants can be bisexual and are theoretically capable of gametophytic selfing, that is the fusion of two gametes originating from a single gametophyte via mitosis (Haufler *et al.*, 2016). As the gametophyte grows from a spore originating via a single meiotic event, the sporophyte arising from gametophytic selfing is completely homozygous (Klekowski & Lloyd, 1968). Nevertheless, contemporary homosporous lineages maintain their genetic diversity by mechanisms that reduce the rate of gametophytic selfing. Some bryophyte gametophytes have their sex determined via sex chromosomes (Renner *et al.*, 2017), whereas fern gametophytes often use a dynamic system controlling sex expression via pheromones called antheridiogens (Schneller, 2008).

Walter Döpp first discovered antheridiogen (hereafter abbreviated AG) in 1950, originally named ‘A–substanz’. During his experiments with gametophyte cultivation, Döpp noted

that reusing agar media previously used to cultivate *Pteridium aquilinum* gametophytes caused precocious formation of antheridia in young gametophytes of *Dryopteris filix-mas* (Döpp, 1950). This effect was confirmed by Näf in 1956 and attributed to a pheromone exuded by older gametophytes that was later named antheridiogen (Näf et al., 1975). Since the discovery of AG, evidence of the utilization of the pheromone has been documented in some (but not all) fern species that have been tested across phylogenetically disparate lineages (Schneller, 2008).

Available evidence suggests that AG production and response varies considerably among fern taxa and that the system involves complex inter- and intraspecific interactions. This has been evident since Döpp's initial discovery of AG as his report involved taxa belonging to two different families. Later studies revealed that AGs often have a gibberellin-like structure (Yamane, 1998) and indicated that various types of AGs occur across the fern clade (Schneller *et al.*, 1990). Generally, AGs have been classified either by the species producing them (e.g. AAn for AG released from *Anemia phyllitidis*) or in broad groups/types according to the taxa that they affect. Three main types of AGs typically are recognized under the second classification scheme (Schneller et al., 1990). First, A or A_{Pt} type is used widely by many species throughout the order Polypodiales, notably by *P. aquilinum* and *Onoclea sensibilis*. Second, B or A_{An} type is used only within the order Schizaeales, notably by *Anemia* and *Lygodium*. Interestingly, gibberellins known from seed plants can evoke the same response as the A_{An} type (Voeller, 1964; Weinberg & Voeller, 1969). Finally, C or A_{Ce} type is used exclusively by the genus *Ceratopteris*. Several other types have been described by a limited number of studies, for example in *Asplenium ruta-muraria* by Schneller & Hess (1995).

Although several distinct types of AGs were described, the primary function of all AGs is the stimulation of precocious formation of antheridia. When a gametophyte of an AG-responsive species grows in the absence of this pheromone, it first develops archegonia (i.e. becomes female; Döpp, 1950). However, right before the gametophyte reaches the archegoniate phase, it begins exuding AGs into its environment (Näf *et al.*, 1975). At the same time, the gametophyte loses the ability to respond to AGs (Näf, 1958). Younger or slow-growing asexual gametophytes in the immediate surroundings of the first gametophyte respond to the AGs by halting growth and forming antheridia (i.e. becoming male). The population ends up composed of a few larger female gametophytes and many smaller male gametophytes (Tryon & Vitale, 1977). As fern sperm are flagellated and need to swim through water to reach archegonia, a greater abundance of sperm due to the AG system may help overcome the limitations of dry environments (Schneller & Hess, 1995). Likewise, AG leads to a greater number of unisexual gametophytes, therefore limiting self-fertilization and facilitating outcrossing, the exchange of gametes between

gametophytes, and therefore maintaining heterozygosity in fern populations (Schedlbauer & Klekowski, 1972). Through the AG system, homosporous ferns gain these advantages, which are usually afforded to heterosporous plants because of their pre-determined sexes and consequent inability to undergo the extreme form of selfing found in homosporous plants (Bateman & DiMichele, 1994). Additionally, larger gametophytes may be able to pheromonally suppress the ability of smaller gametophytes to bear sporophytes, thus reducing competition. However, smaller gametophytes may use the system to contribute to the next generation despite being unable to form archegonia or support young sporophytes owing to unfavorable conditions (Willson, 1981).

Generally, fern spores require light to germinate, but AG was found to replace the need for light in spores cultivated in complete darkness (Raghavan, 1989). In nature, spores buried under a thin layer of soil or detritus affected by AG may form tiny gametophytes and reach the surface or use their limited resources to form a small number of antheridia, skipping the archegonial phase (Schneller, 1988). The sperm from those gametophytes then can reach female gametophytes aboveground (Haufler & Welling, 1994). Therefore, AG enables the mobilization of the genetic and sperm-producing potential of spores buried underground. The concentrations of AG needed to stimulate the precocious formation of antheridia and germination in darkness may differ (Schraudolf, 1962; Weinberg & Voeller, 1969; Endo *et al.*, 1972). If the two effects, germination in darkness and precocious antheridium formation, are tightly correlated, dark germination could be used to test the response to AGs in multiple species, as was done by Weinberg & Voeller (1969). However, most authors comparing the two effects of AGs within one study have only focused on a few species (Yamane *et al.*, 1987; Chiou & Farrar, 1997; Chiou *et al.*, 1998) and a thorough review is necessary.

In theory, some fern species may gain very little but lose a lot by responding to AGs. For example, neopolyploid species (sensu Vida, 1976; herein after referred to as polyploid), having more than two sets of chromosomes and therefore the potential to 'buffer' against the deleterious effects of gametophytic selfing, may reproduce by self-fertilization and still retain genetic variation (Klekowski & Baker, 1966; Hickok, 1978). So, polyploids should tend to self-fertilize more than diploids (Masuyama, 1979; Soltis & Soltis, 2000; Sessa *et al.*, 2016). As the AG system limits self-fertilization, polyploid species may be more likely to stop using the pheromone, potentially allowing all polyploid gametophytes to bear sporophytes and thus avoid any negative adverse effects. However, no comparison of AG response between diploids and polyploids has been conducted until now. A more extreme case of AGs as a potential liability exists in apomictic ferns. Apomictic gametophytes form sporophytes apogamously from a somatic cell, without the need for fertilization. This renders any extra sperm present in a population as a response to AGs

presumably useless. Nevertheless, the ability to suppress surrounding gametophytes may be potentially advantageous from the standpoint of reducing competition. The limited number of tested apomicts were found either to respond to AGs (*Bommeria pedata*, Haufler & Gastony, 1978; *Dryopteris affinis*, Schneller, 1981) or ignore AGs (*Cyrtomium* spp., Yatskievych, 1993). However, a thorough study of AG response in apomictic ferns has not yet been conducted.

Despite the apparently widespread occurrence of AG systems in ferns and their potentially large evolutionary significance via their effects on population structure and mating behavior, our understanding of their evolution and distribution across the fern phylogeny remains limited. Several authors have put together lists of all ferns tested for AG response (Näf et al., 1975; Raghavan, 1989; Schneller, 2008) but we are unaware of any attempt to evaluate AG systems in a broader evolutionary context (with the exception of Greer *et al.*, 2009 which incorporated only the handful of species responding to gibberellins). To determine how widespread the involvement of AGs is among ferns, we combined results of our cultivation experiments with a meta-analysis of all published results of similar assays available to us, including 208 species in total. Using this large dataset, we address the following questions: How many fern species have been tested for AG response and how many of those respond? How many different types of AGs appear to exist and what is their evolutionary history? How tightly are the two effects of antheridium induction and germination in darkness correlated? How are AG production and response correlated with ploidy level and reproductive mode?

Materials and Methods

Cultivation

Frond material with mature sporangia of 69 fern species from 19 families was collected from wild or cultivated plants (Supporting Information Table S1) from tropical and temperate regions. Fronds were allowed to air-dry in paper envelopes to facilitate spore release.

Spores were sown on 1% agar supplemented with standard Bold's medium (Bold, 1957) modified with Nitsch's micronutrients (Nitsch, 1951) in 100 9 25 mm Petri plates. Surface sterilization of spores was not performed, and no fungal contamination was observed within the test period. For all cultivation experiments (with or without mature gametophytes), spores were sown at an approximate density of 25 spores per 100 9 25 mm Petri plate (ThermoFisher Scientific, Waltham, MA, USA) by dispersing them through pinholes from glassine envelopes. Cultures were kept at 25°C and exposed to a 12 h : 12 h, light : dark photoperiod achieved with fluorescent grow bulbs (65 lmol m^{-2} s^{-1}) in growth

chambers (BioChambers, Winnipeg, MB, Canada and Percival Scientific, Perry, IA, USA).

In order to test the potential influence of antheridiogen (AG), spores of each species were sown in the presence of either a single conspecific or a single *Pteridium aquilinum* archegoniate (mature) gametophyte. Since the discovery of AGs in this species (Döpp, 1950), *P. aquilinum* commonly has been utilized as a source of AGs and a 'positive control' to test whether a given species is capable of responding to them (e.g. Yatskievych, 1993). Spores from up to four sporophytes per species were tested and each combination (paired with conspecific or *P. aquilinum*) was replicated three times. As a control, spores of each species also were sown without any mature gametophytes present. All plates were checked under a stereoscope for the presence of gametangia (Fig. 1) once a week for 12 wk. A species was considered as responding to either *P. aquilinum* or conspecific AGs if (1) archegonia were formed only in the absence of influencing gametophytes and (2) antheridia were formed in the presence of influencing gametophytes.

Antheridiogen response meta-analysis

In order to provide a broader sampling of taxa, we combined the data from our experiments with the results of previous studies (88 papers; Table S2) to create a dataset of all known AG production and responsiveness across ferns (Table S3). For each tested (target) species, we scored the following: the types of response tested ('antheridium formation' or 'dark germination'), the outcome of the response ('positive' or 'negative'; implicit evidence sometimes was considered), and the source species used to induce the response (e.g. the same species, a congener, or a control AG source such as P. aquilinum). Some species were tested with multiple source species. In cases where different responses were recorded from the same species combination, we recorded each as a separate data point. For some data points obtained from the literature, assays for responses were carried out not with exogenous AGs produced from gametophytes, but with gibberellins (which are known to produce an AG-like effect in some responsive species; Näf et al., 1975). In these cases, 'gibberellin' was provided in place of the inducing species. In addition, we also scored ploidy level (diploid or polyploid) and reproductive mode (sexual or apomictic) for each species (Table S4). Ploidy data were obtained from floristic treatments, monographs and published chromosome counts; in some cases, data were not available. The reproductive mode of each taxon was obtained from Liu *et al.* (2012). Only species that are obligately apomictic were scored as apomictic.

The dataset enabled us to score each taxon as AG responsive or unresponsive (Table S4). Each species was considered as responsive to AG if at least one of these combinations yielded positive results. Five species were excluded from all additional analyses because they were either tested only against a type of AG inappropriate for the species (*Cibotium*

barometz – gibberellin + *Pteridium aquilinum*, *Cyathea australis* – gibberellin + *P. aquilinum*, *Pentarrhizidium orientale* – gibberellin, *Radiovittaria stipitata* – *P. aquilinum*) or because conflicting data made it impossible to clearly label the species as responsive or not (*Phlebodium aureum*; Näf, 1956; Voeller, 1964; Weinberg & Voeller, 1969; Gemmrich, 1986; Chiou & Farrar, 1997). The first four species mentioned were removed as they are almost certainly false negatives and further testing is needed for a proper assessment of all five excluded taxa. Additionally, our dataset allowed for re-evaluating previously described AG groups/types. There are two conditions for each type to be unique. First, each taxon produces and/or reacts to only one AG type. If, for instance, one species reacted to two potential types, the types were merged into one. Second, taxa do not have to react to every single AG source within a type. As they represent a wide array of different chemicals, we do not consider the 'gibberellin' group to be AGs for the purposes of type delimitation.

In order to examine whether precocious antheridium formation and dark germination are tightly linked, we used our dataset to find any potential correlation. Species tested for both effects were evaluated as consistent (either affected under light and dark conditions or never) or inconsistent (affected only under one condition). Species tested for only one effect or against an inappropriate AG type (usually gibberellins failing to affect members of Polypodiales) were excluded from this comparison. The correlation of AG production and response with species attributes (e.g. apomict/sexual, diploid/polyploid) was evaluated using chi-square tests performed in R v.3.4.3 (R Core Team, 2017). Species whose ploidy level could not be determined were excluded from the diploid/polyploid comparison.

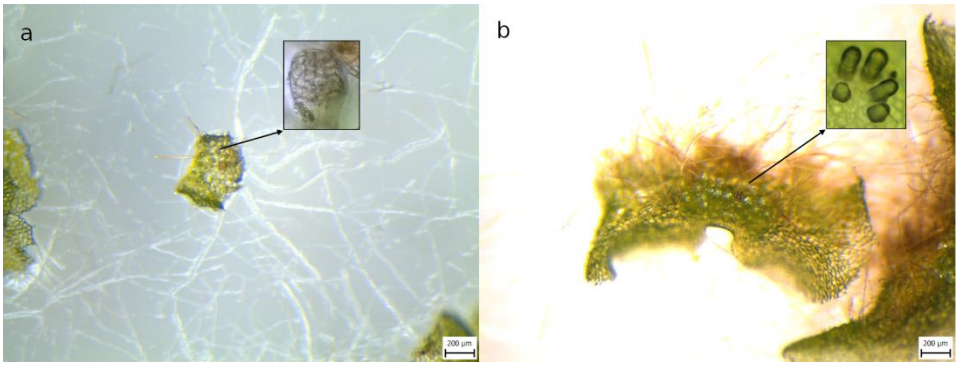

Fig. 6 Gametophytes of *Asplenium ruta-muraria* with gametangia at magnification used for sex determination in this study with details at higher magnification presented in rectangles: (a) male gametophyte bearing antheridia and (b) female gametophyte bearing archegonia.

Results

Cultivation

A total of 68 species were cultivated and tested for AG response (Table 1). Of those, 56 species were tested with a conspecific AG source, and 25 reacted. Additionally, 44 species were tested using P. aquilinum as the source, and 22 reacted. Six species from the genera *Asplenium*, *Ctenitis*, *Cyathea* and *Pityrogramma* responded to conspecific but not to *P. aquilinum* AG. The opposite case, reacting only to *P. aquilinum* but not to conspecific AG, occurred for *Odontosoria* and *Phlebodium*.

AG meta-analysis

Datasets The final dataset (Table S3) included a total of 208 species from 26 families, involved in a total of 498 pairings, either with a conspecific or another taxon (Figs 2, 3). After the exclusion of five species (see the Materials and Methods section), 64.5% of the 203 taxa responded to AGs (Fig. 4). Interestingly, three species (*Cyrtomium fortunei*, *C. macrophyllum* and *Polystichum lonchitis*) seemed to produce AG but did not react to any tested AG source. Tested representatives of five families (Culcitaceae, Equisetaceae, Hymenophyllaceae, Lomariopsidaceae and Osmundaceae) did not appear to produce or respond to AG at all (Fig. 2).

Antheridiogen types From our dataset, we identified two main AG types, corresponding to the *Pteridium* and *Anemia* types affecting Polypodiales and Schizaeales, respectively (Fig. 3a). In total, 64.6% of the tested representatives of Polypodiales responded to AG (usually from *P. aquilinum*; Fig. 3b) and response to gibberellin was extremely rare (Fig. 3c). All representatives of Schizaeales responded to some form of AG (Fig. 4) and to supplemented gibberellins (Fig. 3c), if tested. Using our definition (in Methods), we also identified several different potential minor AG types, affecting only a single species or genus. Based on our definition, many species were considered as having their own type only because they have not been tested against any other species (*Davallia fejeensis*, *Elaphoglossum latifolium*, *Gymnocarpium disjunctum*, *Oleandra articulata*, *Parapolystichum excultum*, *Polypodium cambricum*, *Sadleria* spp., *Woodwardia radicans*) or were cross-tested within a small group of species (*Cheilanthes* spp.). These taxa all belong to the order Polypodiales. *Thelypteris ovata* and *Hemionitis palmata* only failed to respond to gibberellins and *Pteris vittata*, respectively, but their congener responded to *P. aquilinum*. Three species of *Asplenium* failed to react to *P. aquilinum* (*Asplenium auritum*, *Asplenium serratum*) and gibberellins (*Asplenium ruta-muraria*), but they are not phylogenetically closely related and other *Asplenium* species (*A. cuneifolium*, *A. septentrionale*) respond to Pteridium-type AG. *Pityrogramma calomelanos* successfully influenced itself and two species of *Onychium* but failed to react to *P. aquilinum*. Related *Pityrogramma* species also did not respond to *Pteris vittata* AG.

These results indicate that a distinct AG system may be operating within *Pityrogramma*. Likewise, *Vittaria* spp. gemmae responded to exudates of long-lived congeneric gametophytes and supplemented gibberellins by forming antheridia. *Pteridium aquilinum* AG failed to induce the same response. This would indicate an AG system similar to the Anemia type. *Ceratopteris* spp. were affected only by conspecific AG but failed to respond to *Anemia* spp., *P. aquilinum* and *Pteris vittata*. Ceratopteris AG also failed to influence *Bommeria* species responsive to *P. aquilinum*. It is uncertain whether *Ceratopteris* AG would be needed in higher concentrations for any effect to occur, or perhaps it is distinct or different enough to be unable to affect the few species tested but still within the Pteridium type. Outside of Polypodiales, three tree fern (Cyatheales) species (*Cibotium menziesii*, *Cyathea microdonta*, *Cyathea multiflora*) responded to conspecific AG but not to P. aquilinum. Related species (*Cibotium barometz*, *Cyathea australis*) also failed to respond to gibberellins, indicating that tree ferns may utilize chemically and phylogenetically different AGs belonging to one or multiple types.

Dark germination Data on dark germination were obtained for 53 taxa. Data were sufficient (see Methods) to evaluate 32 taxa (20.4% of all taxa with determined AG response). In this subset, 26 taxa (81.3%) germinated in darkness. In three cases, the dark germination response was different than the observed antheridium induction response: *Ceratopteris thalictroides* and *Thelypteris ovata* did not germinate in darkness despite being influenced under light, and *Polypodium cambricum* germinated in darkness despite not responding to AG under light.

Table 1. Overview of the response of 68 tested species to the cultivation experiment.

Tested species	Family	Response to self[1]	Response to *Pteridium aquilinum*[1]
Adiantum radicans	Pteridaceae	Yes	Yes
Asplenium adiantum-nigrum	Aspleniaceae	Not Tested	Yes
Asplenium auritum	Aspleniaceae	Yes	No
Asplenium cuneifolium	Aspleniaceae	Not Tested	Yes
Asplenium ruta-muraria	Aspleniaceae	Not Tested	No
Asplenium scolopendrium	Aspleniaceae	Not Tested	No
Asplenium septentrionale	Aspleniaceae	Not Tested	Yes
Asplenium serratum	Aspleniaceae	Yes	No
Blechnum occidentale	Blechnaceae	Yes	Yes
Blechnum polypodioides	Blechnaceae	Yes	Yes
Bolbitis portoricensis	Dryopteridaceae	No	No

Tested species	Family	Response to self[1]	Response to *Pteridium aquilinum*[1]
Campyloneurum aphanophlebium	Polypodiaceae	No	No
Campyloneurum brevifolium	Polypodiaceae	Yes	Not Tested
Christella dentata	Thelypteridaceae	Yes	Yes
Cibotium menziesii	Cibotiaceae	Yes	No
Ctenitis sloanei	Dryopteridaceae	No	Not Tested
Cyathea microdonta	Cyatheaceae	Yes	No
Cyathea multiflora	Cyatheaceae	Yes	No
Davallia fejeensis	Davalliaceae	Yes	Not Tested
Diplazium striatastrum	Athyriaceae	No	No
Draconopteris draconoptera	Tectariaceae	No	Not Tested
Dryopteris carthusiana	Dryopteridaceae	Not Tested	Yes
Dryopteris caucasica	Dryopteridaceae	Not Tested	Yes
Dryopteris dilatata	Dryopteridaceae	Not Tested	No
Dryopteris expansa	Dryopteridaceae	Not Tested	Yes
Dryopteris filix-mas	Dryopteridaceae	Not Tested	Yes
Dryopteris oreades	Dryopteridaceae	Not Tested	Yes
Elaphoglossum latifolium	Dryopteridaceae	Yes	Not Tested
Elaphoglossum peltatum	Dryopteridaceae	No	No
Equisetum arvense	Equisetaceae	No	No
Equisetum fluviatile	Equisetaceae	No	No
Equisetum palustre	Equisetaceae	No	Not Tested
Equisetum sylvaticum	Equisetaceae	No	Not Tested
Goniopteris curta	Thelypteridaceae	No	Not Tested
Goniopteris nicaraguensis	Thelypteridaceae	Yes	Yes
Hypoderris brauniana	Tectariaceae	Yes	Yes
Lomariopsis japurensis	Lomariopsidaceae	No	Not Tested
Lomariopsis vestita	Lomariopsidaceae	No	No
Lygodium japonicum	Lygodiaceae	Yes	Not Tested
Lygodium microphyllum	Lygodiacea	Yes	Not Tested
Macrothelypteris torresiana	Thelypteridaceae	No	No
Meniscium lingulatum	Thelypteridaceae	Yes	Yes
Mickelia nicotianifolia	Dryopteridaceae	No	Not Tested
Microgramma lycopodioides	Polypodiaceae	No	Not Tested

Tested species	Family	Response to self[1]	Response to *Pteridium aquilinum*[1]
Zealandia pustulata	Polypodiaceae	No	No
Nephrolepis biserrata	Nephrolepidaceae	No	No
Odontosoria c.f. gymnogrammoides	Lindsaeaceae	No	Yes
Oleandra articulata	Oleandraceae	Yes	Not Tested
Olfersia cervina	Dryopteridaceae	No	No
Osmunda claytoniana	Osmundaceae	No	Not Tested
Osmunda regalis	Osmundaceae	No	Not Tested
Osmundastrum cinnamomeum	Osmundaceae	No	Not Tested
Parapolystichum excultum	Dryopteridaceae	Yes	Not Tested
Pecluma pectinata	Polypodiaceae	No	Not Tested
Phlebodium pseudoaureum	Polypodiaceae	No	Yes
Pityrogramma calomelanos	Pteridaceae	Yes	No
Pleopeltis furfuracea	Polypodiaceae	No	Not Tested
Polybotrya osmundacea	Dryopteridaceae	No	Not Tested
Polystichum munitum	Dryopteridaceae	No	No
Polystichum setiferum	Dryopteridaceae	Not Tested	Yes
Pteris propinqua	Pteridaceae	Yes	Yes
Saccoloma elegans	Saccolomataceae	Yes	Yes
Saccoloma inaequale	Saccolomataceae	Yes	Not Tested
Salpichlaena volubilis	Blechnaceae	No	No
Serpocaulon triseriale	Polypodiaceae	Yes	Yes
Tectaria heracleifolia	Tectariaceae	No	Not Tested
Thelypteris kunthii	Thelypteridaceae	Yes	Yes
Trichomanes diversifrons	Hymenophyllaceae	No	Not Tested

[1] Taxa that responded formed antheridia first when exposed to an archegoniate conspecific or *Pteridium aquilinum* gametophyte, despite forming archegonia first under control conditions.

Reproductive types and polyploidy Overall, 12 (6%) of the taxa sampled were obligately apomictic. Apomictic and sexual taxa had similar (i.e. not significantly different) response rates to AGs of 66.7% and 64.4%, respectively (Chi-square < 10^{-6}; df = 1; P = 1; Fig. 4). Of the 208 taxa included in the dataset, ploidy level and AG response were determined for 172 taxa. The 100 diploid and 72 polyploid taxa had nearly equal response rates of 67.0% and 68.1%, respectively (Chi-square = 0; df = 1; P = 1; Fig. 4).

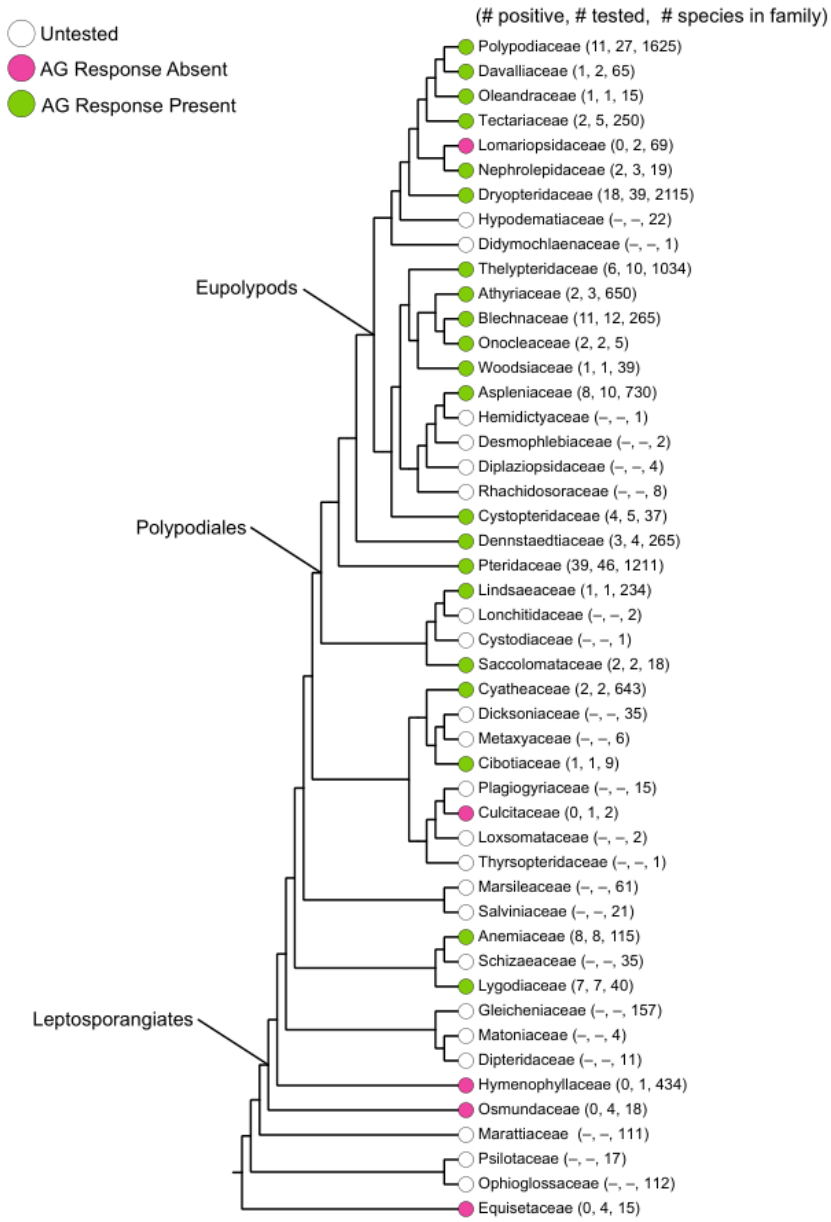

Fig. 7 Fern phylogeny (with relationships based on PPG 1, 2016) indicating the families tested for antheridiogen (AG) response. The number of responsive, tested and total species in a family also is given.

Fig. 8 Overview of antheridiogen interactions (response or no response) between tested fern taxa on a family level (phylogeny tree based on PPG 1, 2016): (a) interactions between all families, excluding *Pteridium aquilinum* (Dennstaedtiaceae) as antheridiogen producer (families tested labelled blue); (b) response of taxa *to P. aquilinum* (Dennstaedtiaceae, labelled blue); and (c) response to gibberellins.

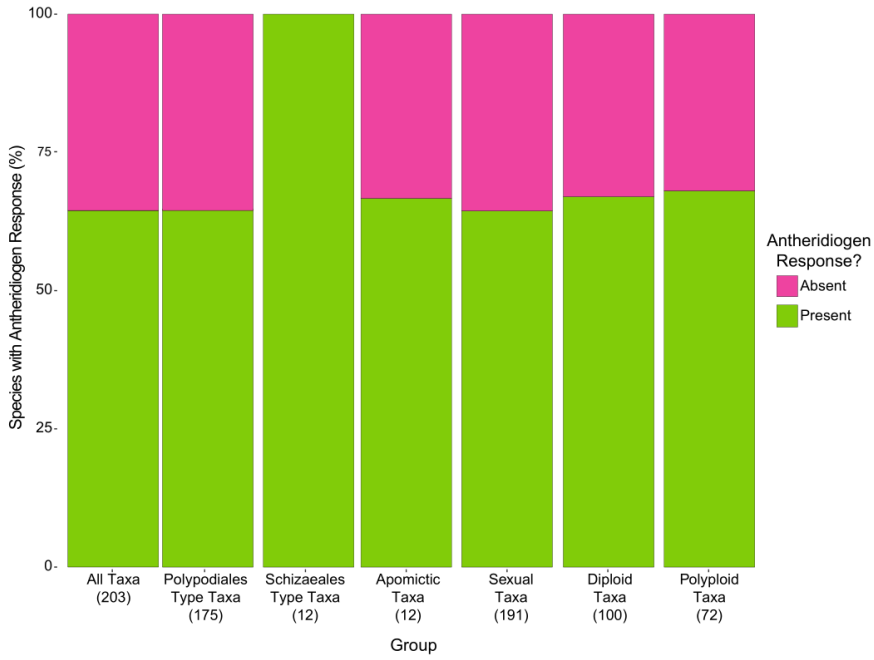

Fig. 9 Overview of the percentage of fern taxa reacting to antheridiogens. The percentage of all studied taxa is listed alongside those for various subgroups based on antheridiogen type (Polypodiales-type AG (AGPo)/ Schizaeales-type AG (AGSc); others not listed), ploidy level (diploid/polyploid) and reproductive types (apomictic/sexually reproducing).

Discussion

Antheridiogen data synthesis and meta-analysis

Combining our results with published data from the literature, we present an updated list of 208 fern species from 26 families that have been tested for antheridiogen (AG) response or production (Table S4). Unlike previous major reviews on the topic (Näf *et al.*, 1975; Raghavan, 1989; Schneller, 2008), we have recorded the response of each species to all tested AG sources (Table S3). A recent estimate puts the number of fern species at 10 578 (PPG 1, 2016), meaning that 2% of all known fern species have now been tested for AG activity. This is a substantial increase from the < 1% tested that was estimated by Kirkpatrick & Soltis (1992). However, the vast majority of fern diversity remains unstudied. Athyriaceae and Cyatheaceae deserve special attention as these are species-rich families with only three species tested each.

About two-thirds (64.5%) of all tested species responded positively to some type of AG. Additionally, three species produced AG but did not react to the pheromone. Clearly, AGs play a major role in the lives of fern gametophytes. Nevertheless, the real percentage of fern species responding may be different. Responsive species may be over-represented in our dataset, as negative results are less likely to be published. However, some species may be incorrectly labelled as nonresponsive if they failed to respond to some of the model AG sources. For example, *Pentarrhizidium orientale* failed to react to gibberellins (Weinberg & Voeller, 1969). Labelling the species as nonresponsive based only on this result could be misleading (the species was excluded as a false negative) as the closely related *Onoclea sensibilis* reacts to AG of ≥ 27 other species, but not to gibberellins. Not all cases can be as clear as that of *Pentarrhizidium orientale* and AG systems are too complex to accurately assign species as nonresponsive based on a limited number of pairings.

Antheridiogen types

Our dataset clearly demonstrates two major AG types, one affecting the order Polypodiales and the other affecting Schizaeales. Among the many minor types described, most would likely fall within the main Polypodiales type, if more pairings are conducted. Some of these minor types (e.g. *Asplenium*, *Pityrogramma*, *Vittaria*) are supported with inconclusive evidence and require further study. *Ceratopteris* is generally considered to have its own AG type (Schneller et al., 1990) and this is supported by the most convincing evidence, such as the lack of response to multiple other species and no germination in darkness. However, we would like to caution against an unambiguous distinction of the *Ceratopteris* type until more pairings are done with other Polypodiales species, especially from Pteridaceae. Some sort of AG system also operates in tree ferns (Cyatheales) that seems distinct from the two major AG types. However, we have insufficient data on the chemical nature and the extent of influence of this system. Tree ferns clearly deserve more study.

In accordance with our merger of many minor types to the larger ones, we would like to suggest the following naming convention: The types should be named after the broadest group under their influence; for example, Polypodiales-type AG, Schizaeales-type AG, Cyatheales-type AG (for a possible unified tree fern AG type) and Ceratopteris-type AG (for the potential *Ceratopteris* type). To allow for easier reading and understanding we advise abbreviating the types as AGPo, AGSc, AGCy and AGCe, respectively, avoiding the usually applied subscript.

Evolution of antheridiogens

Pheromonal control of sex expression via AGs is widespread among leptosporangiate ferns and has likely evolved multiple times. To understand the evolution of AGs,

phylogeny must be considered. For the purpose of this analysis, we presume that only three main types (one being a unified Cyatheales type) described above are distinct. All pairings involving *Equisetum* indicate that it has no AG system, suggesting that AGs evolved within the ferns after the divergence of *Equisetum*. No other eusporangiate ferns have yet been tested, but studies of sexual expression in Osmundaceae, which are sister to all other leptosporangiate ferns, indicate a potential pheromonal control different from AG (Hollingsworth *et al.*, 2012). Phylogenetically, the three types of AG system we recognize could represent three separate origins. The first true AG system is that found in the order Schizaeales, chemically based on gibberellins (Fig. 3c). The origin of this AG type is uncertain until denser sampling of non-polypod leptosporangiates is achieved. Nevertheless, it could be either that the order represents an independent acquisition of AGs, or that AGs were present in the common ancestor of the entire group (Schizaeales through Polypodiales), and the system was later lost in water ferns and its chemical nature was changed considerably in other groups. Therefore, it seems more likely that the Schizaeales type system evolved independently within that order. The second origin, or perhaps multiple origins, appeared in Cyatheales. However, our knowledge in this group is scarce and further research is required. The third origin of AG, the Polypodiales type, could have evolved right at the origin of Polypodiales, potentially as a key innovation of this highly diverse lineage. Our results indicate the presence of AG activity in *Saccoloma* and Lindsaeaceae (Fig. 3a,b). Antheridiogens are certainly well-established within Pteridaceae, as four of its five subfamilies, including the Cryptogrammoideae, which are sister to the other four (Schuettpelz *et al.*, 2007), have species responsive to AG, and members of other families in Polypodiales react to Pteridaceae AG (Fig. 3a). Further studies of *Saccoloma*, *Cystodium*, *Lonchitis* and Lindsaeaceae will be critical for establishing the origins of AGs within Polypodiales, and studies of non-leptosporangiates are necessary to understand the evolution of antheridiogens across all ferns.

Although we advocate for a broader AG type concept, it is important to note that AGs likely diversify considerably within each type. A considerable number of various distinct chemical entities were described within Schizaeales (Nakanishi *et al.*, 1971; Endo *et al.*, 1972; Nester *et al.*, 1987; Yamane *et al.*, 1988; Yamauchi *et al.*, 1996; Wynne *et al.*, 1998). In this order, all species reacted positively to congeners, if tested. However, the compatibility between the two families Anemiaceae and Lygodiaceae was limited. No chemical compound was fully described within Polypodiales, but the lack of compatibility across some families is reminiscent of what is seen in Schizaeales. For example, members of Pteridaceae are capable of inducing a response in members of Onocleaceae and Blechnaceae, but not Aspleniaceae (Fig. 3a). This phenomenon could be caused by the lack of selective pressure to conserve the chemical structure of AG.

The chemical compounds may diversify in each lineage, being less capable of affecting evolutionarily distant species. A possible result would be the evolution of new types, for example in *Ceratopteris*.

Ceratopteris is of particular interest when considering the evolution of ferns in association with AG systems. This genus is the only representative of homosporous aquatic ferns. Its species also form the largest spores of all homosporous ferns (Tryon & Lugardon, 1991) and it may have its own unique AG type. In theory, plants in aquatic environments benefit from propagules with higher energy reserves to speed up the life cycle and help survive in a carbon-dioxide-poor environment (Petersen & Burd, 2017). A greater abundance of male gametes also is beneficial to increase the chance of mating in water. Heterospory, in which a few large megaspores and many small microspores are produced, fits perfectly into this environment. Unsurprisingly, the known cases of heterospory in ferns involve the true water ferns (Salviniales) and *Pteris platyzomoides*, a unique species growing in seasonally waterlogged habitats (Tryon, 1964). *Ceratopteris* represents an alternative solution to this problem. Large spores provide the energy reserves, and the AG system guarantees an overabundance of male gametes in populations whereas single spores can still grow into bisexuals and colonize new habitats. In *Ceratopteris*, the AG system does not just substitute the genetic variation aspect of heterospory, but also confers the benefits of heterospory in aquatic environments. It is possible that the ancestors of Salviniales developed heterospory, in part, due to a lack of AG system in their lineage. In turn, *Ceratopteris* might have become fully heterosporous were it not for AGs.

Germination of spores in darkness

In addition to stimulating precocious formation of antheridia, AGs also enable germination of spores in darkness. Of the 32 species evaluated, 80% reacted to AG, compared to the 64% overall reaction. This higher response rate is likely caused by the over-representation of Schizaeales (which all react) in the dark germination subset. Furthermore, 29 of 32 species (90.6%) were consistent in their response. The first exception was *Polypodium cambricum* germinating in darkness, but without induced antheridia in illuminated cultures (Welling & Haufler, 1993). However, some members of Polypodiaceae are known to respond only to high concentrations of AG (Chiou & Farrar, 1997) and it is possible that the concentration of AG used by Welling & Haufler (1993) was insufficient to promote antheridium formation in illuminated cultures. The second exception, *Thelypteris ovata*, failed to germinate in darkness despite responding to AG in light (Nester-Hudson et al., 1997). However, germination percentages were checked only 7 d after sowing, a duration equal to the time needed for germination in light for the species. As dark germination may take longer than germination under normally illuminated conditions (Weinberg & Voeller, 1969), it is possible that AG-induced dark

germination would have been observed at a later point. Finally, *Ceratopteris thalictroides* does not induce germination in darkness (Schedlbauer, 1976). Spores of *Ceratopteris* generally do not germinate in darkness, although some exceptions have been reported (Scott & Hickok, 1987). From our data, this is the only clear example of AG being capable of inducing antheridiogenesis but not germination in darkness. With the notable exception in *Ceratopteris*, germination in darkness seems to be a reliable indicator of antheridiogen response in fern species. If done properly, assays of dark germination could be used as this method is less demanding than its light counterpart and lends itself to mass analysis of the many species yet to be studied.

Antheridiogens in apomictic ferns

Apomictic ferns, which produce sporophytes spontaneously from gametophytes without fertilization (Grusz, 2016), can produce and respond to AG. Usage of AG in apomicts presents an interesting evolutionary dilemma. On the one hand, apomictic gametophytes that respond to AGs and produce antheridia may be wasting valuable resources to do so, and any subsequent slowed growth might limit their ability to form sporophytes apomictically. On the other, an appealing possibility is that production of AGs may confer a competitive advantage to apomictic fern gametophytes over co-occurring sexual taxa, as some apomictic gametophytes grow faster than their sexual competitors (Whittier, 1968; Haufler & Gastony, 1978). Theoretically, a disturbance revealing a new niche for ferns could be colonized by fast-growing apomictic gametophytes that would suppress sexual gametophytes of similar age and any latecomers. Response to AG in apomicts would then be irrelevant, as older gametophytes producing AG themselves are insensitive to it (Näf, 1958). Provoking dark germination and subsequent antheridium formation in subterranean spores would have the added effect of depleting the spore bank, thus limiting potential future competition.

Like sexual species, about two thirds of apomictic species respond to AG. To date, assessment of AG responsiveness in apomict-containing lineages is too limited to draw broad conclusions, and evaluation of their responses needs to be tested in a phylogenetic context. However, we present several possible explanations for the similar response between apomictic and sexually reproducing taxa. First, apomicts arise from sexual ancestors (Grusz, 2016) and AG response is therefore inherited from them, and thus may be evolutionarily conserved within some lineages. In *Cheilanthes a*nd *Cystopteris*, the AG-responsiveness of an allotetraploid species was reported as the average of its diploid progenitors, suggesting a legacy of AG activity in descendants (Haufler & Ranker, 1985; Pajarón *et al.*, 2015). Many apomicts start as hybrids (Grusz *et al.*, 2009; Liu *et al.*, 2012; Ekrt & Koutecký, 2016) and likely follow a similar pattern. Yatskievych (1993) found that two apomictic *Cyrtomium* species retained the ability to produce AG but did

not react to it, thus keeping the advantage, but losing the liability. The simple inheritance of AG systems from ancestors may be the most parsimonious explanation of the equal usage of AGs among sexual and apomictic taxa. Nevertheless, the presence of apomictic species that have lost sensitivity to AG despite being able to synthesize it indicates that adaptive pressures affect the use of the AG system in apomicts.

Second, apomicts may adapt not by losing the ability to respond to AGs, but instead by increasing the needed concentration of the pheromone. Once the species is insensitive enough that common competitors and their typical AG output cannot influence it, there would be no selective pressures to reduce sensitivity further. Schneller (1981) reported AG effects to be weaker in apomictic members of the *Dryopteris affinis* group compared to sexually reproducing *D. filix-mas*. In the same genus, sexually reproducing *D. carthusiana* reacts to AG of *Pteridium aquilinum*, but not to congeneric species that it competes with (Testo et al., 2015). Thus, in laboratory experiments, unusually high concentrations or slightly different sources of AG may result in a positive response in otherwise insensitive species.

Finally, response to AG may be adaptive for apomicts. As mentioned above, apomicts may use AGs to suppress sexual competitors. Furthermore, related apomictic and sexually reproducing ferns may hybridize to form semi-fertile hybrids, which then reproduce via apomixis (Ekrt & Koutecký, 2016). This peculiar merger likely happens via fertilization of an egg from a sexual species by an apomict's sperm, as most apomicts do not form archegonia (Döpp, 1959; Whittier, 1968; Yatskievych, 1993, but see Hori & Murakami, 2019). This way, sporophyte-bearing apomictic gametophytes not only reduce future competition by suppressing conspecific gametophytes, but the suppressed apomictic gametophytes also flood sexual gametophytes that made it to the archegoniate phase with interspecific sperm. Likewise, a sexual archegoniate gametophyte growing on top of an apomictic spore bank may be forced to hybridize this way. In both cases, sexually reproducing gametophytes are either denied fully functioning sporophytic offspring or end up propagating the genes of the apomict. However, owing to the lack of testing in field conditions, we cannot be sure how important this competition between apomicts and sexually reproducing species really is.

Antheridiogens and polyploidy

Polyploidy plays an important role in fern evolution (Vida, 1976; Wood *et al.*, 2009; Liu *et al.*, 2019). Most ferns use a mixed-mating system (Haufler *et al.*, 2016), forming sporophytes by self-fertilization (gametophytic selfing) or by exchanging gametes with other gametophytes (sporophytic selfing or outcrossing). The use of AG promotes the latter option as unisexual gametophytes are more likely to occur. However, polyploid ferns should in theory be more tolerant of forming progeny by self-fertilization on bisexual

gametophytes (Masuyama, 1979; Soltis & Soltis, 2000; Pangua *et al*., 2003; Flinn, 2006; Testo *et al*., 2015; Sessa *et al.*, 2016). As sensitivity towards AGs limits the ability to form bisexual gametophytes, polyploids might be more likely to abandon the use of AGs. That way, each polyploid gametophyte can self-fertilize and take advantage of their inherent genetic diversity (Hickok, 1978). However, the ratio of responsive diploids and polyploids is nearly identical (Fig. 4). As in apomicts, the optimal strategy for a predominantly selfing species may be to exude AGs but not react to them. This strategy has been described in the tetraploid *D. carthusiana* (Testo *et al.*, 2015). As mentioned above, removing the inherited sensitivity towards AG may be a long and difficult process, but polyploids are often evolutionarily young. Alternatively, Schneller & Hess (1995) suggest that AGs in tetraploid *Asplenium ruta-muraria* may be used to increase the quantity of available sperm in their environments, where water is a factor limiting fertilization (such as in the rock walls that *A. ruta-muraria* typically inhabits). The gametophytic community in such a habitat might be founded by a single sporophyte, so the end goal is not increased genetic diversity but an increased chance of fertilization. Finally, polyploids may still benefit from the outcrossing supported by AGs and the positives of retaining their AG sensitivity outweigh the potential negatives of selfing with little genetic risk.

Conclusion

This comprehensive meta-analysis of 88 published papers together with new data from cultivation experiments has focused on the occurrence of antheridiogens in ferns, especially from the perspective of phylogeny, dark germination, mating modes and ploidies. The meta-analysis shows that the AG system is widespread among ferns. About two-thirds (64.5%) of all tested species responded positively to AGs. This finding demonstrates their far-reaching importance, likely related to consequences that affect many aspects of fern reproduction. This unique system of sex determination and ensuing population demographic control deserves more interest. Seventy years after the discovery of AGs by Walter Döpp (Döpp, 1950) the vast majority of fern species (98%) remains unexplored. We suggest that large, species-rich families such as Athyriaceae and Cyatheaceae, with barely any species tested, should be the subject of future inquiries, as should the non-leptosporangiate fern lineages. Several AG types are well-established by now, but others still require thorough testing to determine their scope, distinctness, and features.

Despite expectations, the majority (66.7%) of apomictic species surveyed to date respond to AG. The consequences of this may play an important role in survival and competition among fern gametophytes in nature as well as interactions between apomictic and sexually reproducing taxa. Our study also suggests that there is no difference between diploids

(67.0%) and polyploids (68.1%) in response to AGs, so the pheromonal system may be advantageous even to species capable of being predominantly selfing. Finally, there is a strong correlation between germination in darkness and precocious antheridium formation in light. Testing for dark germination can be done through more expedient methods with binary results. These methods may be key to mass testing AG response in many of the yet unstudied species. We are now beginning to understand how AGs operate on the molecular level (Valledor *et al.*, 2014; Ganger *et al.*, 2015; Attalah *et al.*, 2018; Chen *et al.*, 2019) but many questions about their distribution and evolution remain unanswered. Hopefully, our comprehensive dataset can provide a starting point for fern researchers to learn whether their species of interest use this intriguing system of pheromonal control over sexual determination.

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Author contributions

OH, WLT, JEW and LE designed the study; OH, WLT, CEC and JP conducted the cultivation experiments; OH compiled the meta-analysis list; and OH, WLT and EBS analyzed the data. All authors contributed to the writing of the manuscript.

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Supporting Information Table S1. List of taxa cultivated in this study.

Taxon	Collectors	Location	Herbarium (according to Index Herbariorum)
Asplenium adiantum-nigrum	Ekrt, Libor	Turkey: Altindere.	CBFS
Asplenium adiantum-nigrum	Ekrt, Libor	Italy: Cardia.	CBFS
Asplenium adiantum-nigrum	Ekrt, Libor	Scotland: Durness.	CBFS
Asplenium adiantum-nigrum	Ekrt, Libor	Serbia: Tekija.	CBFS
Asplenium cuneifolium	Hornych, Ondřej	Czech Republic: Holubov.	CBFS
Asplenium cuneifolium	Hornych, Ondřej	Czech Republic: Holubov.	CBFS
Asplenium cuneifolium	Hornych, Ondřej	Czech Republic: Holubov.	CBFS
Asplenium cuneifolium	Hornych, Ondřej	Czech Republic: Holubov.	CBFS
Asplenium ruta-muraria	Ekrt, Libor	Austria: Thörl.	CBFS
Asplenium ruta-muraria	Hornych, Ondřej	Czech Republic: Kutná Hora.	CBFS
Asplenium septentrionale	Ekrt, Libor	Czech Republic: Rájov.	CBFS
Asplenium septentrionale	Ekrt, Libor	Italy: Cardia.	CBFS
Asplenium septentrionale	Hornych, Ondřej	Czech Republic: Kutná Hora.	CBFS
Dryopteris carthusiana	Ekrt, Libor	Czech Republic: Řídelov.	CBFS
Dryopteris carthusiana	Hornych, Ondřej	Czech Republic: Nové Hrady.	CBFS
Dryopteris carthusiana	Hornych, Ondřej; Ekrt, Libor	Germany: Dittersdorf.	CBFS
Dryopteris carthusiana	Hornych, Ondřej; Ekrt, Libor	Germany: Dittersdorf.	CBFS
Dryopteris caucassica	Ekrt, Libor	Armenia. In cult., L. Ekrt, private garden	CBFS
Dryopteris dilatata	Ekrt, Libor	Czech Republic: Řídelov.	CBFS
Dryopteris dilatata	Hornych, Ondřej	Czech Republic: Cikháj.	CBFS

Taxon	Collectors	Location	Herbarium (according to Index Herbariorum)
Dryopteris dilatata	Hornych, Ondřej	Czech Republic: Nové Hrady.	CBFS
Dryopteris dilatata	Hornych, Ondřej; Ekrt, Libor	Germany: Suhl.	CBFS
Dryopteris expansa	Hornych, Ondřej; Ekrt, Libor	Germany: Oberschönau.	CBFS
Dryopteris expansa	Ekrt, Libor	Czech Republic: In cult., L. Ekrt, private garden	CBFS
Dryopteris filix-mas	Hornych, Ondřej; Ekrt, Libor	Germany: Dittersdorf.	CBFS
Dryopteris filix-mas	Ekrt, Libor	Czech Republic: In cult., L. Ekrt, private garden	CBFS
Dryopteris filix-mas	Hornych, Ondřej	Czech Republic: Nové Hrady.	CBFS
Dryopteris filix-mas	Hornych, Ondřej	Czech Republic: Hovězí.	CBFS
Dryopteris oreades	Ekrt, Libor	Scotland. In cult., L. Ekrt, private garden	CBFS
Polystichum setiferum	Ekrt, Libor	Serbia. In cult., L. Ekrt, private garden	CBFS
Adiantum radicans	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Asplenium auritum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Asplenium scolopendrium var. *scolopendrium*	Testo, Weston	Switzerland: Zurich.	FLAS
Asplenium serratum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Blechnum occidentale	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Blechnum polypodioides	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Bolbitis portoricensis	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Campyloneurum aphanophlebium	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Campyloneurum brevifolium	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Christella dentata	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Cibotium menziesii	Watkins, James	Commercial Source. In cult., Colgate University	Living collection, Colgate University
Ctenitis sloanei	Watkins, James	Commercial Source. In cult., Colgate University	Living collection, Colgate University

Taxon	Collectors	Location	Herbarium (according to Index Herbariorum)
Cyathea microdonta	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Cyathea multiflora	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Davallia fejeensis	Watkins, James	Commercial Source. In cult., Colgate University	Living collection, Colgate University
Diplazium striatastrum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Draconopteris draconoptera	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Elaphoglossum latifolium	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Elaphoglossum peltatum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Equisetum arvense	Watkins, James	In cult., Colgate University and Fairchild Botanical Garden	Living collection, Colgate University
Equisetum fluviatile	Watkins, James	In cult., Colgate University and Fairchild Botanical Garden	Living collection, Colgate University
Equisetum palustre	Watkins, James	In cult., Colgate University and Fairchild Botanical Garden	Living collection, Colgate University
Equisetum sylvaticum	Watkins, James	In cult., Colgate University and Fairchild Botanical Garden	Living collection, Colgate University
Goniopteris curta	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Goniopteris nicaraguensis	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Hypoderris brauniana	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Lomariopsis japurensis	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Lomariopsis vestita	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Lygodium japonicum	Watkins, James	USA: Florida.	FLAS
Lygodium microphyllum	Watkins, James	USA: Florida.	FLAS
Macrothelypteris torresiana	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Meniscium lingulatum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Mickelia nicotianifolia	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University

Taxon	Collectors	Location	Herbarium (according to Index Herbariorum)
Microgramma lycopodioides	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Zealandia pustulata	Watkins, James	Commercial Source. In cult., Colgate University	Living collection, Colgate University
Nephrolepis biserrata	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Odontosoria c.f. *gymnogrammoides*	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Oleandra articulata	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Olfersia cervina	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Osmunda claytoniana	Watkins, James	USA: New York.	Living collection, Bewkes Center, Colgate University
Osmunda regalis	Watkins, James	USA: New York.	Living collection, Bewkes Center, Colgate University
Osmunda cinnamomeum	Watkins, James	USA: New York.	Living collection, Bewkes Center, Colgate University
Parapolystichum excultum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Pecluma pectinata	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Phlebodium pseudoaureum	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Pityrogramma calomelanos	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Pleopeltis furfuracea	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Polybotrya osmundacea	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Polystichum munitum	Watkins, James	USA: California.	Living collection, Colgate University
Polystichum setiferum	Watkins, James	Commercial Source. In cult., Colgate University	Living collection, Colgate University
Pteris propinqua	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Saccoloma elegans	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Saccoloma inaequale	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Salpichlaena volubilis	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University

Taxon	Collectors	Location	Herbarium (according to Index Herbariorum)
Serpocaulon triseriale	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Tectaria heracleifolia	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Thelypteris kunthii	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Trichomanes diversifrons	Watkins, James	Costa Rica: Prov. Heredia. In cult., Colgate University	Living collection, Colgate University
Pteridium aqulinum	Testo, Weston	Switzerland, Zurich.	FLAS

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Supporting Information Table S2. List of literature used to compile interactions dataset (Tab S3).

Abbreviation	Reference
Atallah et al., 2018	**Attalah NM, Viter O, Gaiti F, Tanurdzic M, Banks JA. 2018.** Sex determination in *Ceratopteris richardii* is accompanied by transcriptome changes that drive epigenetic reprogramming of the young gametophyte. *G3: Genes, Genomes, Genetics* **8**: 2205–2214.
Ayrapetov & Ganger, 2009	**Ayrapetov A, Ganger MT. 2009.** Nutrient levels do not affect male gametophyte induction by antheridiogen in *Ceratopteris richardii*. *American Fern Journal* **99**: 273–278.
Banks et al., 1993	**Banks JA, Hickok L, Webb MA. 1993.** The programming of sexual phenotype in the homosporous fern *Ceratopteris richardii*. *International Journal of Plant Sciences* **154**: 522–534.
Barker & Willmot, 1985	**Barker J, Willmot A. 1985.** Preliminary studies on the breeding systems of *Dryopteris filix-mas* (L.) Schott and *D. dilatata* (Hoffm) A. Gray. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences* **86**: 455–456.
Chiou, 1996	**Chiou W-L. 1996.** *The biosystematics of pteridophytes: aspects of morphology and reproductive biology of some epiphytic ferns.* PhD thesis, Iowa State University, Ames, IA, USA.
Chiou & Farrar, 1997	**Chiou W-L, Farrar DR. 1997.** Antheridiogen production and response in Polypodiaceae species. *American Journal of Botany* **84**: 633–640.
Chiou et al., 1998	**Chiou W-L, Farrar DR, Ranker TA. 1998.** Gametophyte morphology and reproductive biology in *Elaphoglossum*. *Canadian Journal of Botany* **76**: 1967–1977.
Chiou et al., 2002	**Chiou W-L, Farrar DR, Ranker TA. 2002. The** mating systems of some epiphytic Polypodiaceae. *American Fern Journal* **92**: 65–79.
Corey et al., 1986	**Corey EJ, Myers AG, Takahashi N, Yamane H, Schraudolf H. 1986.** Constitution of antheridium-inducing factor of *Anemia phyllitidis*. *Tetrahedron Letters* **27**: 5083–5084.
Cousens, 1979	**Cousens MI. 1979.** Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*. *American Journal of Botany* **66**: 116–132.
Döpp, 1950	**Döpp W. 1950.** Eine die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.) Kuhn. *Berichte der Deutschen Botanischen Gesellschaft* **63**: 139–147.
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Haufler & Gastony, 1978	**Haufler CH, Gastony GJ. 1978.** Antheridiogen and the breeding system in the fern genus *Bommeria*. *Canadian Journal of Botany* **56**: 1594–1601.
Haufler & Ranker, 1985	**Haufler CH, Ranker TA. 1985.** Differential antheridiogen response and evolutionary mechanisms in *Cystopteris*. *American Journal of Botany* **72**: 659–665.
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Hickok et al., 1995	**Hickok LG, Warne TR, Fribourg RS. 1995.** The biology of the fern *Ceratopteris* and its uses as a model system. *International Journal of Plant Sciences* **156**: 332–345.
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Hornych et al.	This abbretiation refers to the results of cultivation presented in this paper.
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Klekowski & Lloyd, 1968	**Klekowski EJ, Lloyd RM. 1968.** Reproductive biology of the Pteridophyta 1. General considerations and a study of *Onoclea sensibilis* L. *Botanical Journal of the Linnean Society* **60**: 315–324.
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Abbreviation	Reference
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Nester & Schedlbauer, 1982	**Nester JE, Schedlbauer MD. 1982.** Antheridiogen activity of *Anemia mexicana*. *Canadian Journal of Botany* **60**: 1606–1610.
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Voeller, 1964	**Voeller BR. 1964.** Antheridiogens in ferns. . In: Regulateurs naturels de la croissance vegetale. Gif s/Yvette, Paris, France, 665–684.
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Welling & Haufler, 1993	**Welling CB, Haufler CH. 1993.** Antheridiogen and its puzzling effects on *Polypodium australe*. *American Journal of Botany* **8**: 113.
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Supporting Information Table S3. List of antheridiogen interactions between fern taxa (meta-analysis + cultivation results). References found in Tab. S2. Hornych et al. refers to the results of this study. Light response refers to precocious formation of antheridia in light-grown gametophytes exposed to antheridiogens. Dark response refers to germination in darkness caused by antheridiogens.

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Acrostichum danaeifolium	*Acrostichum danaeifolium*	No	Lloyd, 1975	Yes	No	
Adiantum hispidulum	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Adiantum pedatum	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Adiantum pedatum	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Adiantum polyphyllum	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Adiantum radicans	*Adiantum radicans*	Yes	Hornych et al.	Yes	No	
Adiantum radicans	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Adiantum tenerum "Scutum Roseum"	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Adiantum trapeziforme	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Aglaomorpha meyeniana	*Pteridium aquilinum*	Yes	Näf, 1966	Yes	No	Requires very high concentrations
Anemia collina	*Anemia phyllitidis*	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia collina	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia flexuosa	*Anemia hirsuta*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia flexuosa	*Anemia phyllitidis*	Yes	Yamane et al., 1987	Yes	Yes	
Anemia flexuosa	*Anemia rotundifolia*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia flexuosa	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Anemia hirsuta	*Anemia phyllitidis*	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia hirsuta	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia mexicana	*Anemia mexicana*	Yes	Nester & Schedlbauer, 1982; Nester & Coolbaugh, 1986	Yes	Yes	
Anemia mexicana	*Anemia phyllitidis*	Yes	Nester & Coolbaugh, 1986	No	Yes	
Anemia mexicana	Gibberelin	Yes	Nester & Coolbaugh, 1986	Yes	Yes	
Anemia phyllitidis	*Anemia flexuosa*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia phyllitidis	*Anemia mexicana*	Yes	Nester & Schedlbauer, 1982; Nester & Coolbaugh, 1986	No	Yes	Nester & Schedlbauer, 1982 - Not tested in light due to methods (did not germinate at all under white light)
Anemia phyllitidis	*Anemia phyllitidis*	Yes	Näf, 1959; Schraudolf, 1962; Näf, 1966; Näf, 1968; Weinberg & Voeller, 1969; Nakanishi et al., 1971; Endo et al., 1972; Schedlbauer, 1974; Schedlbauer, 1976; Haufler & Gastony, 1978; Corey et al., 1986; Nester & Coolbaugh, 1986; Yamane et al., 1987	Yes	Yes	Tested in light (Näf, 1959; Schraudolf, 1962; Näf, 1966; Näf, 1968; Endo et al., 1972; Haufler & Gastony, 1978; Corey et al., 1986; Yamane et al., 1987); tested in darkness (Schraudolf, 1962; Näf, 1966; Weinberg & Voeller, 1969; Endo et al., 1972; Corey et al., 1986; Nester & Coolbaugh, 1986; Yamane et al., 1987); Corey et al., 1986 - "details will be given in a separate paper"
Anemia phyllitidis	*Anemia rotundifolia*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia phyllitidis	*Ceratopteris thalictroides*	No	Schedlbauer, 1974; Schedlbauer, 1976	Yes	Yes	Tested in light (Schedlbauer, 1974; Schedlbauer, 1976); tested in darkness (Schedlbauer, 1976)

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Anemia phyllitidis	Gibberelin	Yes	Voeller, 1964; Schraudolf, 1964; Näf, 1966; Schraudolf, 1966a; 1966b; Weinberg & Voeller, 1969; Schedlbauer, 1974; Corey et al., 1986; Nester & Coolbaugh, 1986; Sugai et al., 1987; Kaźmierczak, 2019	Yes	Yes	Tested in light (Voeller, 1964; Schraudolf, 1964; Schraudolf, 1966a; 1966b; Schedlbauer, 1974; Corey et al., 1986; Kaźmierczak, 2019); tested in darkness (Näf, 1966; Weinberg & Voeller, 1969; Corey et al., 1986; Nester & Coolbaugh, 1986; Sugai et al., 1987); Corey et al., 1986 - "details will be given in a separate paper"
Anemia phyllitidis	*Lygodium japonicum*	No	Naf & Trager, 1960; Näf, 1966	Yes	Yes	Tested in light (Naf & Trager, 1960; Näf, 1966); tested in darkness (Näf, 1966)
Anemia phyllitidis	*Onoclea sensibilis*	No	Näf et al., 1969	Yes	No	
Anemia phyllitidis	*Pteridium aquilinum*	No	Schedlbauer, 1976	No	Yes	
Anemia phyllitidis	*Pteridium aquilinum*	No	Näf, 1959; Voeller, 1964	Yes	No	
Anemia phyllitidis	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Anemia rotundifolia	*Anemia flexuosa*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia rotundifolia	*Anemia phyllitidis*	Yes	Weinberg & Voeller, 1969; Yamane et al., 1987	Yes	Yes	Tested in light (Yamane et al., 1987); tested in darkness (Weinberg & Voeller, 1969; Yamane et al., 1987)
Anemia rotundifolia	*Anemia rotundifolia*	Yes	Yamane et al., 1987	Yes	Yes	Noted as result of preliminary study
Anemia rotundifolia	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia tomentosa	*Anemia phyllitidis*	Yes	Weinberg & Voeller, 1969	No	Yes	
Anemia tomentosa	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Asplenium adiantum-nigrum	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Asplenium auriculatum	*Asplenium auriculatum*	Yes	Tryon & Vitale, 1977	Yes	No	Implicit from natural populations
Asplenium auritum	*Asplenium auritum*	Yes	Hornych et al.	Yes	No	
Asplenium auritum	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Asplenium ceterach	*Pteridium aquilinum*	No	Döpp, 1959	Yes	No	
Asplenium cuneifolium	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Asplenium ruta-muraria	*Asplenium ruta-muraria*	Yes	Schneller & Hess, 1995	Yes	No	
Asplenium ruta-muraria	Gibberelin	No	Schneller & Hess, 1995	Yes	No	
Asplenium ruta-muraria	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	Referenced as Schneller, unpublished
Asplenium scolopendrium	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Asplenium septentrionale	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Asplenium serratum	*Asplenium serratum*	Yes	Hornych et al.	Yes	No	
Asplenium serratum	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Asplenium trichomanes	*Pteridium aquilinum*	No	Döpp, 1959	Yes	No	
Asplenium trichomanes	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Astrolepis sinuata	*Pteridium aquilinum*	Yes	Döpp, 1959	Yes	No	
Athyrium filix-femina	*Athyrium filix-femina*	Yes	Schneller, 1979; Schneller, 1988	Yes	Yes	Tested in light (Schneller, 1979); tested in darkness (Schneller, 1979; Schneller, 1988)
Athyrium filix-femina	*Dryopteris filix-mas*	Yes	Schneller, 1979; Schneller, 1988	Yes	Yes	Tested in light (Schneller, 1979); tested in darkness (Schneller, 1979; Schneller, 1988)
Athyrium filix-femina	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Athyrium filix-femina	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Athyrium thelypteridoides	*Pteridium aquilinum*	Yes	Näf, 1956	Yes	No	
Blechnum brasiliense	Gibberelin	No	Voeller, 1964	Yes	No	
Blechnum brasiliense	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Blechnum medium	Gibberelin	No	Voeller, 1964	Yes	No	
Blechnum medium	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Blechnum occidentale	*Blechnum occidentale*	Yes	Hornych et al.	Yes	No	
Blechnum occidentale	Gibberelin	No	Voeller, 1964	Yes	No	
Blechnum occidentale	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Blechnum occidentale	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Blechnum polypodioides	*Blechnum polypodioides*	Yes	Hornych et al.	Yes	No	
Blechnum polypodioides	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Blechnum spicant	*Blechnum spicant*	Yes	Cousens, 1979; Fernández et al., 1999; Menéndez et al., 2006; Menéndez et al., 2009; Valledor et al., 2014	Yes	No	
Blechnum spicant	Gibberelin	Yes	Menéndez et al., 2006	Yes	No	
Bolbitis portoricensis	*Bolbitis portoricensis*	No	Hornych et al.	Yes	No	
Bolbitis portoricensis	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Bommeria ehrenbergiana	*Anemia phyllitidis*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria ehrenbergiana	*Bommeria ehrenbergiana*	Yes	Haufler & Gastony, 1978; Haufler & Welling, 1994	Yes	Yes	Tested in light (Haufler & Gastony, 1978; Haufler & Welling, 1994); tested in darkness (Haufler & Gastony, 1978)
Bommeria ehrenbergiana	*Bommeria hispida*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria ehrenbergiana	*Bommeria pedata*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria ehrenbergiana	*Ceratopteris thalictroides*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria ehrenbergiana	*Pteridium aquilinum*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria hispida	*Anemia phyllitidis*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria hispida	*Bommeria ehrenbergiana*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria hispida	*Bommeria hispida*	Yes	Haufler & Gastony, 1978; Haufler & Welling, 1994	Yes	Yes	Tested in light (Haufler & Gastony, 1978; Haufler & Welling, 1994); tested in darkness (Haufler & Gastony, 1978)
Bommeria hispida	*Bommeria pedata*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria hispida	*Ceratopteris thalictroides*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria hispida	*Pteridium aquilinum*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Anemia phyllitidis*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Bommeria ehrenbergiana*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Bommeria hispida*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Bommeria pedata*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Ceratopteris thalictroides*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria pedata	*Pteridium aquilinum*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Anemia phyllitidis*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Bommeria ehrenbergiana*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Bommeria hispida*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Bommeria pedata*	Yes	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Ceratopteris thalictroides*	No	Haufler & Gastony, 1978	Yes	No	
Bommeria subpaleacea	*Pteridium aquilinum*	Yes	Haufler & Gastony, 1978	Yes	No	
Campyloneurum angustifolium	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Campyloneurum angustifolium	*Campyloneurum angustifolium*	Yes	Chiou & Farrar, 1997	Yes	Yes	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Campyloneurum angustifolium	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Campyloneurum angustifolium	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Campyloneurum aphanophlebium	*Campyloneurum aphanophlebium*	No	Hornych et al.	Yes	No	
Campyloneurum aphanophlebium	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Campyloneurum brevifolium	*Campyloneurum brevifolium*	Yes	Hornych et al.	Yes	No	
Campyloneurum phyllitidis	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Campyloneurum phyllitidis	*Campyloneurum phyllitidis*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Campyloneurum phyllitidis	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Campyloneurum phyllitidis	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Ceratopteris richardii	*Ceratopteris richardii*	Yes	Hickok, 1983; Scott & Hickok, 1987; Warne et al., 1988; Banks et al., 1993; Ganger & Sturey, 2012; Atallah et al., 2018	Yes	No	
Ceratopteris thalictroides	*Anemia mexicana*	No	Nester & Schedlbauer, 1982	Yes	Yes	
Ceratopteris thalictroides	*Anemia phyllitidis*	No	Schedlbauer, 1976	No	Yes	
Ceratopteris thalictroides	*Anemia phyllitidis*	No	Schedlbauer, 1974	Yes	No	
Ceratopteris thalictroides	*Ceratopteris thalictroides*	No	Schedlbauer, 1976	No	Yes	
Ceratopteris thalictroides	*Ceratopteris thalictroides*	Yes	Schedlbauer & Klekowski, 1972; Schedlbauer, 1974; 1976; Haufler & Gastony, 1978	Yes	No	
Ceratopteris thalictroides	Gibberelin	No	Schedlbauer, 1974	Yes	No	
Ceratopteris thalictroides	*Pteridium aquilinum*	No	Schedlbauer, 1976	No	Yes	
Ceratopteris thalictroides	*Pteridium aquilinum*	No	Schedlbauer, 1974	Yes	No	
Ceratopteris thalictroides	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Cheilanthes distans	*Pteridium aquilinum*	Yes	Döpp, 1959	Yes	No	
Cheilanthes hispanica	*Cheilanthes hispanica*	Yes	Pajarón et al., 2015	Yes	No	Strong response
Cheilanthes hispanica	*Cheilanthes maderensis*	Yes	Pajarón et al., 2015	Yes	No	Strong response
Cheilanthes hispanica	*Cheilanthes tinaei*	Yes	Pajarón et al., 2015	Yes	No	Intermediate response
Cheilanthes maderensis	*Cheilanthes hispanica*	Yes	Pajarón et al., 2015	Yes	No	Very weak response
Cheilanthes maderensis	*Cheilanthes maderensis*	Yes	Pajarón et al., 2015	Yes	No	Very weak response
Cheilanthes maderensis	*Cheilanthes tinaei*	Yes	Pajarón et al., 2015	Yes	No	Very weak response
Cheilanthes tinaei	*Cheilanthes hispanica*	Yes	Pajarón et al., 2015	Yes	No	Intermediate response
Cheilanthes tinaei	*Cheilanthes maderensis*	Yes	Pajarón et al., 2015	Yes	No	Intermediate response
Cheilanthes tinaei	*Cheilanthes tinaei*	Yes	Pajarón et al., 2015	Yes	No	Intermediate response
Christella dentata	*Christella dentata*	Yes	Hornych et al.	Yes	No	
Christella dentata	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Cibotium barometz	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Cibotium barometz	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Cibotium menziesii	*Cibotium menziesii*	Yes	Hornych et al.	Yes	No	
Cibotium menziesii	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Cosentinia vellea	*Pteridium aquilinum*	Yes	Döpp, 1959	Yes	No	
Cryptogramma crispa	*Cryptogramma crispa*	Yes	Pajarón et al., 1999	Yes	No	Two different populations, same results.
Cryptogramma crispa	*Pteridium aquilinum*	Yes	Döpp, 1959	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Ctenitis sloanei	*Ctenitis sloanei*	No	Hornych et al.	Yes	No	
Culcita macrocarpa	*Culcita macrocarpa*	No	Quintanilla et al., 2005	Yes	No	Implicit evidence
Cyathea australis	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Cyathea australis	*Pteridium aquilinum*	No	Naf & Trager, 1960; Voeller, 1964	Yes	No	
Cyathea microdonta	*Cyathea microdonta*	Yes	Hornych et al.	Yes	No	
Cyathea microdonta	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Cyathea multiflora	*Cyathea multiflora*	Yes	Hornych et al.	Yes	No	
Cyathea multiflora	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Cyclosorus dentatus	Gibberelin	No	Voeller, 1964	Yes	No	
Cyclosorus dentatus	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Cyrtomium falcatum	*Cyrtomium falcatum*	No	Yatskievych, 1993	Yes	No	
Cyrtomium falcatum	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Cyrtomium falcatum	*Pteridium aquilinum*	No	Voeller, 1964; Yatskievych, 1993	Yes	No	
Cyrtomium falcatum	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Cyrtomium fortunei	*Cyrtomium fortunei*	No	Yatskievych, 1993	Yes	No	
Cyrtomium fortunei	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Cyrtomium macrophyllum	*Cyrtomium macrophyllum*	No	Yatskievych, 1993	Yes	No	
Cyrtomium macrophyllum	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Cystopteris bulbifera	*Cystopteris protrusa*	No	Haufler & Ranker, 1985	Yes	No	
Cystopteris bulbifera	*Pteridium aquilinum*	No	Haufler & Ranker, 1985	Yes	No	
Cystopteris protrusa	*Cystopteris protrusa*	Yes	Haufler & Ranker, 1985	Yes	No	
Cystopteris protrusa	*Pteridium aquilinum*	Yes	Haufler & Ranker, 1985	Yes	No	
Cystopteris tennesseensis	*Pteridium aquilinum*	Yes	Haufler & Ranker, 1985	Yes	No	Effect lesser than in C. protrusa
Davallia fejeensis	*Davallia fejeensis*	Yes	Hornych et al.	Yes	No	
Dennstaedtia bipinnata	Gibberelin	No	Voeller, 1964	Yes	No	
Dennstaedtia bipinnata	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Dennstaedtia punctilobula	*Pteridium aquilinum*	Yes	Näf, 1959	Yes	No	
Diplazium striatastrum	*Diplazium striatastrum*	No	Hornych et al.	Yes	No	
Diplazium striatastrum	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Draconopteris draconoptera	*Draconopteris draconoptera*	No	Hornych et al.	Yes	No	
Drynaria quercifolia	Gibberelin	No	Voeller, 1964	Yes	No	
Drynaria quercifolia	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Drynaria rigidula	Gibberelin	No	Voeller, 1964	Yes	No	
Drynaria rigidula	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Dryopteris aemula	*Dryopteris aemula*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris aemula	*Dryopteris corleyi*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris aemula	*Dryopteris oreades*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris affinis	*Dryopteris affinis*	Yes	Schneller, 1981	Yes	Yes	Little dark germination
Dryopteris affinis	*Dryopteris filix-mas*	Yes	Schneller, 1988	No	Yes	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Dryopteris carthusiana	*Dryopteris carthusiana*	No	Testo et al., 2015	Yes	No	
Dryopteris carthusiana	*Dryopteris cristata*	No	Testo et al., 2015	Yes	No	
Dryopteris carthusiana	*Dryopteris intermedia*	No	Testo et al., 2015	Yes	No	
Dryopteris carthusiana	*Pteridium aquilinum*	Yes	Testo et al., 2015; Hornych et al.	Yes	No	
Dryopteris caucasica	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Dryopteris corleyi	*Dryopteris aemula*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris corleyi	*Dryopteris corleyi*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris corleyi	*Dryopteris oreades*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris cristata	*Dryopteris carthusiana*	No	Testo et al., 2015	Yes	No	
Dryopteris cristata	*Dryopteris cristata*	No	Testo et al., 2015	Yes	No	
Dryopteris cristata	*Dryopteris intermedia*	No	Testo et al., 2015	Yes	No	
Dryopteris cristata	*Pteridium aquilinum*	No	Testo et al., 2015	Yes	No	
Dryopteris dilatata	*Dryopteris dilatata*	No	Barker & Willmot, 1985	Yes	No	
Dryopteris dilatata	Gibberelin	No	Voeller, 1964	Yes	No	
Dryopteris dilatata	*Pteridium aquilinum*	No	Voeller, 1964; Hornych et al.	Yes	No	
Dryopteris expansa	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Dryopteris filix-mas	*Dryopteris filix-mas*	Yes	Schneller, 1981; Barker & Willmot, 1985; Schneller, 1988	Yes	Yes	Schneller Dark; Barker light Tested in light (Barker & Willmot, 1985); tested in darkness (Schneller, 1981; Schneller, 1988)
Dryopteris filix-mas	Gibberelin	Yes	Schraudolf, 1966b	Yes	No	Strange results, different effect of various gibberelins compared to Anemia
Dryopteris filix-mas	*Pteridium aquilinum*	Yes	Döpp, 1950; Voeller, 1964; Hornych et al.	Yes	No	
Dryopteris filix-mas	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Dryopteris intermedia	*Dryopteris carthusiana*	Yes	Testo et al., 2015	Yes	No	
Dryopteris intermedia	*Dryopteris cristata*	No	Testo et al., 2015	Yes	No	
Dryopteris intermedia	*Dryopteris intermedia*	Yes	Testo et al., 2015	Yes	No	
Dryopteris intermedia	*Pteridium aquilinum*	Yes	Testo et al., 2015	Yes	No	
Dryopteris oreades	*Dryopteris aemula*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris oreades	*Dryopteris corleyi*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris oreades	*Dryopteris oreades*	Yes	Jiménez et al., 2008	Yes	No	
Dryopteris oreades	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Elaphoglossum callifolium	*Elaphoglossum callifolium*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum callifolium	*Elaphoglossum crassifolium*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum callifolium	Gibberelin	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum callifolium	*Pteridium aquilinum*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum crassifolium	*Elaphoglossum callifolium*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum crassifolium	*Elaphoglossum crassifolium*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum crassifolium	Gibberelin	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum crassifolium	*Pteridium aquilinum*	No	Chiou et al., 1998	Yes	Yes	
Elaphoglossum latifolium	*Elaphoglossum latifolium*	Yes	Hornych et al.	Yes	No	
Elaphoglossum peltatum	*Elaphoglossum peltatum*	No	Hornych et al.	Yes	No	
Elaphoglossum peltatum	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Equisetum arvense	*Equisetum arvense*	No	Hornych et al.	Yes	No	
Equisetum arvense	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Equisetum fluviatile	*Equisetum fluviatile*	No	Hornych et al.	Yes	No	
Equisetum fluviatile	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Equisetum palustre	*Equisetum palustre*	No	Hornych et al.	Yes	No	
Equisetum sylvaticum	*Equisetum sylvaticum*	No	Hornych et al.	Yes	No	
Goniophlebium subauriculatum	Gibberelin	No	Voeller, 1964	Yes	No	
Goniophlebium subauriculatum	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Goniopteris curta	*Goniopteris curta*	No	Hornych et al.	Yes	No	
Goniopteris nicaraguensis	*Goniopteris nicaraguensis*	Yes	Hornych et al.	Yes	No	
Goniopteris nicaraguensis	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Gymnocarpium disjunctum	*Gymnocarpium disjunctum*	Yes	Kirkpatrick & Soltis, 1992	Yes	No	
Gymnocarpium robertianum	*Pteridium aquilinum*	Yes	Döpp, 1959	Yes	No	
Hemionitis arifolia	Gibberelin	No	Voeller, 1964	Yes	No	
Hemionitis arifolia	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Hemionitis arifolia	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Hemionitis palmata	*Hemionitis palmata*	Yes	Ranker, 1987	Yes	No	Variation in reaction within one population (one plant reacts, two do not)
Hemionitis palmata	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Hypoderris brauniana	*Hypoderris brauniana*	Yes	Hornych et al.	Yes	No	
Hypoderris brauniana	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Lepisorus thunbergianus	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Lepisorus thunbergianus	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Lepisorus thunbergianus	*Lepisorus thunbergianus*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Lepisorus thunbergianus	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Lomariopsis japurensis	*Lomariopsis japurensis*	No	Hornych et al.	Yes	No	
Lomariopsis vestita	*Lomariopsis vestita*	No	Hornych et al.	Yes	No	
Lomariopsis vestita	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Lygodium circinatum	Gibberelin	Yes	Yamauchi et al., 1996; Wynne et al., 1998	No	Yes	
Lygodium flexuosum	*Anemia phyllitidis*	No	Weinberg & Voeller, 1969	No	Yes	
Lygodium flexuosum	Gibberelin	Yes	Yamauchi et al., 1996	No	Yes	
Lygodium heterodoxum	*Lygodium heterodoxum*	Yes	Tryon & Vitale, 1977	Yes	No	Implicit from natural populations
Lygodium japonicum	*Anemia mexicana*	Yes	Nester & Schedlbauer, 1982	Yes	Yes	Weaker effect
Lygodium japonicum	*Anemia phyllitidis*	No	Näf, 1966; Weinberg & Voeller, 1969	No	Yes	Näf, 1966 - very weak positive response at high concentrations
Lygodium japonicum	*Anemia phyllitidis*	Yes	Naf & Trager, 1960; Näf, 1966	Yes	No	
Lygodium japonicum	Gibberelin	Yes	Voeller, 1964; Näf, 1966; Weinberg & Voeller, 1969; Takeno & Furuya 1975; Takeno & Furuya 1977; Yamauchi et al., 1996	Yes	Yes	Tested in light (Voeller, 1964; Näf, 1966; Takeno & Furuya 1977); tested in darkness (Näf, 1966; Weinberg & Voeller, 1969; Takeno & Furuya 1975; Yamauchi et al., 1996)

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Lygodium japonicum	*Lygodium japonicum*	Yes	Näf, 1959; Naf & Trager, 1960; Näf, 1966; 1968; Takeno et al., 1979; Takeno & Furuya 1980; Sugai et al., 1987; Takeno & Furuya 1987; Yamane et al., 1988; Hornych et al.	Yes	Yes	Tested in light (Näf, 1959; Naf & Trager, 1960; Näf, 1966; Näf, 1968; Takeno et al., 1979; Takeno & Furuya 1980; 1987; Sugai et al., 1987; Yamane et al., 1988; Hornych et al.); tested in darkness (Näf, 1959; Näf, 1966; Sugai et al., 1987; Yamane et al., 1988); Näf, 1966 - very weak response in dark germination
Lygodium japonicum	*Onoclea sensibilis*	No	Näf et al., 1969	Yes	No	
Lygodium japonicum	*Pteridium aquilinum*	No	Näf, 1959; Voeller, 1964	Yes	No	
Lygodium japonicum	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Lygodium microphyllum	*Lygodium microphyllum*	Yes	Hornych et al.	Yes	No	
Lygodium palmatum	*Anemia phyllitidis*	No	Weinberg & Voeller, 1969	No	Yes	
Lygodium palmatum	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Lygodium scandens	*Anemia phyllitidis*	No	Weinberg & Voeller, 1969	No	Yes	
Lygodium scandens	Gibberelin	Yes	Weinberg & Voeller, 1969	No	Yes	
Macrothelypteris torresiana	*Macrothelypteris torresiana*	No	Hornych et al.	Yes	No	
Macrothelypteris torresiana	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Matteuccia struthiopteris	*Pteridium aquilinum*	Yes	Döpp, 1959; Näf, 1956	Yes	No	Näf, 1956 tested Matteuccia struthiopteris var. pensylvanica
Matteuccia struthiopteris	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Meniscium lingulatum	*Meniscium lingulatum*	Yes	Hornych et al.	Yes	No	
Meniscium lingulatum	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Mickelia nicotianifolia	*Mickelia nicotianifolia*	No	Hornych et al.	Yes	No	
Microgramma heterophylla	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Microgramma heterophylla	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Microgramma heterophylla	*Microgramma heterophylla*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Microgramma heterophylla	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Microgramma lycopodioides	*Microgramma lycopodioides*	No	Hornych et al.	Yes	No	
Microlepia speluncae	Gibberelin	No	Fellenberg-Kressel, 1969	Yes	No	
Microlepia speluncae	*Microlepia speluncae*	Yes	Fellenberg-Kressel, 1969	Yes	No	
Microlepia speluncae	*Pteridium aquilinum*	Yes	Fellenberg-Kressel, 1969	Yes	No	
Microsorum polycarpon	Gibberelin	No	Voeller, 1964	Yes	No	
Microsorum polycarpon	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Mohria caffrorum	*Anemia phyllitidis*	Yes	Weinberg & Voeller, 1969	No	Yes	
Mohria caffrorum	Gibberelin	Yes	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Mohria caffrorum	*Pteridium aquilinum*	No	Naf & Trager, 1960	Yes	No	
Nephrolepis biserrata	*Nephrolepis biserrata*	No	Hornych et al.	Yes	No	
Nephrolepis biserrata	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Nephrolepis cordifolia	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Nephrolepis cordifolia	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Nephrolepis hirsutula	*Pteridium aquilinum*	Yes	Naf & Trager, 1960	Yes	No	
Niphidium crassifolium	Gibberelin	No	Voeller, 1964; Schraudolf, 1966b	Yes	No	
Niphidium crassifolium	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Niphidium crassifolium	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Odontosoria c.f. gymnogrammoides	*Odontosoria c.f. gymnogrammoides*	No	Hornych et al.	Yes	No	
Odontosoria c.f. gymnogrammoides	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Oleandra articulata	*Oleandra articulata*	Yes	Hornych et al.	Yes	No	
Olfersia cervina	*Olfersia cervina*	No	Hornych et al.	Yes	No	
Olfersia cervina	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Onoclea sensibilis	*Blechnum gibbum*	Yes	Näf, 1956	Yes	No	
Onoclea sensibilis	*Bommeria ehrenbergiana*	Yes	Haufler & Gastony, 1978	Yes	No	
Onoclea sensibilis	*Bommeria hispida*	Yes	Haufler & Gastony, 1978	Yes	No	
Onoclea sensibilis	*Bommeria pedata*	Yes	Haufler & Gastony, 1978	Yes	No	
Onoclea sensibilis	*Campyloneurum angustifolium*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Campyloneurum phyllitidis*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Cyrtomium falcatum*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Cyrtomium fortunei*	Yes	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Cyrtomium macrophyllum*	Yes	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Cystopteris protrusa*	Yes	Haufler & Ranker, 1985	Yes	No	
Onoclea sensibilis	*Dryopteris carthusiana*	Yes	Testo et al., 2015	Yes	No	
Onoclea sensibilis	*Dryopteris cristata*	No	Testo et al., 2015	Yes	No	
Onoclea sensibilis	*Dryopteris intermedia*	Yes	Testo et al., 2015	Yes	No	
Onoclea sensibilis	*Elaphoglossum callifolium*	No	Chiou et al., 1998	Yes	No	
Onoclea sensibilis	*Elaphoglossum crassifolium*	No	Chiou et al., 1998	Yes	No	
Onoclea sensibilis	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Onoclea sensibilis	*Gymnocarpium disjunctum*	Yes	Kirkpatrick & Soltis, 1992	Yes	No	
Onoclea sensibilis	*Lepisorus thunbergianus*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Lygodium japonicum*	No	Naf & Trager, 1960	Yes	No	
Onoclea sensibilis	*Microgramma heterophylla*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Onoclea sensibilis*	Yes	Näf, 1956; Klekowski & Lloyd, 1968; Näf et al., 1969	Yes	No	
Onoclea sensibilis	*Phanerophlebia auriculata*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia juglandifolia*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia jungaldifolia*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia macrosora*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia nobilis*	Yes	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia pumila*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia remotispora*	Yes	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phanerophlebia umbonata*	Yes	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Phlebodium aureum*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Phymatosorus scolopendria*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Polypodium pellucidum*	Yes	Chiou & Farrar, 1997	Yes	No	
Onoclea sensibilis	*Polystichum acrostichoides*	Yes	Yatskievych, 1993	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Onoclea sensibilis	*Polystichum imbricans* subsp. *curtum*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Polystichum lonchitis*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Polystichum munitum*	No	Yatskievych, 1993	Yes	No	
Onoclea sensibilis	*Pteridium aquilinum*	Yes	Näf, 1956; 1958; 1968; Voeller, 1964; Haufler & Gastony, 1978; Haufler & Ranker, 1985; Stevens & Werth, 1999; Testo et al., 2015	Yes	No	Stevens & Werth, 1999 - six populations
Onoclea sensibilis	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	Yes	Very weak response in darkness
Onoclea sensibilis	*Sadleria cyatheoides*	Yes	Ranker et al., 1996	Yes	No	
Onoclea sensibilis	*Sadleria pallida*	Yes	Ranker et al., 1996	Yes	No	
Onoclea sensibilis	*Thelypteris ovata*	No	Nester-Hudson et al., 1997	No	Yes	Checked only after 7 days
Onoclea sensibilis	*Thelypteris ovata*	Yes	Nester-Hudson et al., 1997	Yes	No	
Onychium japonicum	*Pityrogramma calomelanos*	Yes	Dubey & Roy, 1985	Yes	Yes	
Onychium siliculosum	*Pityrogramma calomelanos*	Yes	Dubey & Roy, 1985	Yes	Yes	
Oreopteris limbosperma	Gibberelin	No	Voeller, 1964	Yes	No	
Oreopteris limbosperma	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Osmunda claytoniana	*Anemia phyllitidis*	No	Näf, 1959	Yes	No	
Osmunda claytoniana	Gibberelin	No	Voeller, 1964	Yes	No	
Osmunda claytoniana	*Lygodium japonicum*	No	Naf & Trager, 1960	Yes	No	
Osmunda claytoniana	*Osmunda claytoniana*	No	Hornych et al.	Yes	No	
Osmunda claytoniana	*Pteridium aquilinum*	No	Voeller, 1964; Näf, 1959	Yes	No	
Osmunda regalis	*Osmunda regalis*	No	Hornych et al.	Yes	No	
Osmunda regalis	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Osmundastrum cinnamomeum	Gibberelin	No	Voeller, 1964; Hollingsworth et al., 2012	Yes	No	
Osmundastrum cinnamomeum	*Osmundastrum cinnamomeum*	No	Hollingsworth et al., 2012; Hornych et al.	Yes	No	
Osmundastrum cinnamomeum	*Pteridium aquilinum*	No	Näf, 1959; Voeller, 1964	Yes	No	
Parapolystichum excultum	*Parapolystichum excultum*	Yes	Hornych et al.	Yes	No	
Pecluma pectinata	*Pecluma pectinata*	No	Hornych et al.	Yes	No	
Pellaea calomelanos	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Pellaea calomelanos	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Pellaea glabella	*Pteridium aquilinum*	Yes	Whittier, 1968	Yes	No	Influencer referenced only as "antheridial hormone", Whittier listed Näf, 1958 (On the physiology of antheridium formation in the bracken fern [...].) in literature cited but did not cite the paper in text. Therefore we presume, that the source of AG was bracken, but it could also be native.
Pellaea glabella apo	*Pteridium aquilinum*	Yes	Whittier, 1968	Yes	No	Influencer referenced only as "antheridial hormone", Whittier listed Näf, 1958 (On the physiology of antheridium formation in the bracken fern [...].) in literature cited but did not cite the paper in text. Therefore we presume, that the source of AG was bracken, but it could also be native.
Pellaea rotundifolium	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pellaea viridis	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Pellaea viridis	*Pteridium aquilinum*	Yes	Döpp, 1959; Voeller, 1964	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Pellaea viridis	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	Yes	
Pentarhizidium orientale	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Phanerophlebia auriculata	*Phanerophlebia auriculata*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia auriculata	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia juglandifolia	*Phanerophlebia juglandifolia*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia juglandifolia	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia jungaldifolia	*Phanerophlebia jungaldifolia*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia jungaldifolia	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia macrosora	*Phanerophlebia macrosora*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia macrosora	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia nobilis	*Phanerophlebia nobilis*	Yes	Yatskievych, 1993	Yes	No	
Phanerophlebia nobilis	*Pteridium aquilinum*	Yes	Yatskievych, 1993	Yes	No	
Phanerophlebia pumila	*Phanerophlebia pumila*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia pumila	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Phanerophlebia remotispora	*Phanerophlebia remotispora*	Yes	Yatskievych, 1993	Yes	No	
Phanerophlebia remotispora	*Pteridium aquilinum*	Yes	Yatskievych, 1993	Yes	No	
Phanerophlebia umbonata	*Phanerophlebia umbonata*	Yes	Yatskievych, 1993	Yes	No	
Phanerophlebia umbonata	*Pteridium aquilinum*	Yes	Yatskievych, 1993	Yes	No	
Phegopteris hexagonoptera	*Pteridium aquilinum*	Yes	Näf, 1956	Yes	No	
Phlebodium aureum	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Phlebodium aureum	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Phlebodium aureum	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Phlebodium aureum	*Phlebodium aureum*	No	Chiou & Farrar, 1997	Yes	Yes	
Phlebodium aureum	*Pteridium aquilinum*	No	Chiou & Farrar, 1997	No	Yes	
Phlebodium aureum	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	No	
Phlebodium aureum	*Pteridium aquilinum*	No	Näf, 1956; Voeller, 1964	Yes	No	
Phlebodium aureum	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Phlebodium pseudoaureum	*Phlebodium pseudoaureum*	No	Hornych et al.	Yes	No	
Phymatosorus scolopendria	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Phymatosorus scolopendria	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Phymatosorus scolopendria	*Phymatosorus scolopendria*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Phymatosorus scolopendria	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Pityrogramma calomelanos	*Pityrogramma calomelanos*	Yes	Dubey & Roy, 1985; Hornych et al.	Yes	Yes	Tested in light (Dubey & Roy, 1985; Hornych et al.); tested in darkness (Dubey & Roy, 1985)
Pityrogramma calomelanos	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Pityrogramma hybrida	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Pityrogramma sulphurea	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Platycerium alcicorne	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Platycerium bifurcatum	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pleopeltis furfuracea	*Pleopeltis furfuracea*	No	Hornych et al.	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Polybotrya osmundacea	*Polybotrya osmundacea*	No	Hornych et al.	Yes	No	
Polypodium appalachianum	*Polypodium appalachianum*	No	Testo et al., 2014	Yes	No	No positive control.
Polypodium appalachianum	*Polypodium virginianum*	No	Testo et al., 2014	Yes	No	No positive control.
Polypodium cambricum	*Polypodium cambricum*	Yes	Welling & Haufler, 1993	No	Yes	
Polypodium cambricum	*Polypodium cambricum*	No	Welling & Haufler, 1993	Yes	No	
Polypodium pellucidum	*Anemia phyllitidis*	No	Chiou & Farrar, 1997	Yes	No	
Polypodium pellucidum	Gibberelin	No	Chiou & Farrar, 1997	Yes	Yes	
Polypodium pellucidum	*Polypodium pellucidum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Polypodium pellucidum	*Pteridium aquilinum*	Yes	Chiou & Farrar, 1997	Yes	Yes	
Polypodium virginianum	*Polypodium appalachianum*	No	Testo et al., 2014	Yes	No	No positive control.
Polypodium virginianum	*Polypodium virginianum*	No	Testo et al., 2014	Yes	No	No positive control.
Polypodium vulgare	Gibberelin	No	Voeller, 1964	Yes	No	
Polypodium vulgare	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Polypodium vulgare	*Pteris vittata*	No	Gemmrich, 1986	Yes	No	
Polystichum acrostichoides	Gibberelin	No	Voeller, 1964	Yes	No	
Polystichum acrostichoides	*Polystichum acrostichoides*	Yes	Yatskievych, 1993; Greer & McCarthy, 1997	Yes	No	
Polystichum acrostichoides	*Pteridium aquilinum*	Yes	Näf, 1956; Voeller, 1964; Yatskievych, 1993	Yes	No	
Polystichum aculeatum	*Polystichum aculeatum*	Yes	Pangua et al., 2003	Yes	No	
Polystichum aculeatum	*Polystichum lonchitis*	No	Pangua et al., 2003	Yes	No	
Polystichum aculeatum	*Polystichum setiferum*	Yes	Pangua et al., 2003	Yes	No	
Polystichum imbricans subsp. *curtum*	*Polystichum imbricans* subsp. *curtum*	No	Yatskievych, 1993	Yes	No	
Polystichum imbricans subsp. *curtum*	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Polystichum lonchitis	*Polystichum lonchitis*	No	Yatskievych, 1993	Yes	No	
Polystichum lonchitis	*Pteridium aquilinum*	No	Yatskievych, 1993	Yes	No	
Polystichum munitum	*Polystichum munitum*	No	Yatskievych, 1993; Hornych et al.	Yes	No	
Polystichum munitum	*Pteridium aquilinum*	No	Yatskievych, 1993; Hornych et al.	Yes	No	
Polystichum setiferum	*Polystichum aculeatum*	Yes	Pangua et al., 2003	Yes	No	
Polystichum setiferum	*Polystichum lonchitis*	Yes	Pangua et al., 2003	Yes	No	Weak response
Polystichum setiferum	*Polystichum setiferum*	Yes	Pangua et al., 2003	Yes	No	
Polystichum setiferum	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Polystichum tsus-simense	Gibberelin	No	Voeller, 1964	Yes	No	
Polystichum tsus-simense	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Polystichum tsus-simense	*Pteridium aquilinum*	Yes	Näf, 1966	Yes	No	Reacts to very high concentrations
Pteridium aquilinum	*Anemia mexicana*	No	Nester & Schedlbauer, 1982	Yes	Yes	
Pteridium aquilinum	*Ceratopteris thalictroides*	No	Schedlbauer, 1974; Schedlbauer, 1976	Yes	Yes	Tested in light (Schedlbauer, 1974; Schedlbauer, 1976); tested in darkness (Schedlbauer, 1976)
Pteridium aquilinum	Gibberelin	No	Voeller, 1964; Schraudolf, 1966b	Yes	No	
Pteridium aquilinum	*Microlepia speluncae*	Yes	Fellenberg-Kressel, 1969	Yes	No	
Pteridium aquilinum	*Pteridium aquilinum*	Yes	Döpp, 1950; Näf, 1958; Schedlbauer, 1974; Schedlbauer, 1976; Hornych et al.	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Pteridium aquilinum	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	Yes	
Pteris cretica	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Pteris cretica	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Pteris cretica	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris ensiformis	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Pteris incompleta	*Pteris incompleta*	Yes	Prada et al., 2008	Yes	Yes	
Pteris incompleta	*Pteris vittata*	Yes	Prada et al., 2008	Yes	Yes	
Pteris longifolia	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Pteris longifolia	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Pteris multifida	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris podophylla	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris propinqua	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Pteris propinqua	*Pteris propinqua*	Yes	Hornych et al.	Yes	No	
Pteris quadriaurita	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris tremula	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Pteris tremula	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Pteris tremula	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris tripartita	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Pteris vittata	*Pteris incompleta*	Yes	Prada et al., 2008	Yes	Yes	
Pteris vittata	*Pteris vittata*	Yes	Gemmrich, 1986; Prada et al., 2008	Yes	Yes	Tested in light (Gemmrich, 1986; Prada et al., 2008); tested in darkness (Gemmrich, 1986)
Radiovittaria stipitata	*Pteridium aquilinum*	No	Emigh & Farrar, 1977	Yes	No	
Saccoloma elegans	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Saccoloma elegans	*Saccoloma elegans*	Yes	Hornych et al.	Yes	No	
Saccoloma inaequale	*Saccoloma inaequale*	Yes	Hornych et al.	Yes	No	
Sadleria cyatheoides	*Sadleria cyatheoides*	Yes	Ranker et al., 1996	Yes	No	Two populations
Sadleria pallida	*Sadleria pallida*	Yes	Ranker et al., 1996	Yes	No	Two populations, one has weaker response
Salpichlaena volubilis	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Salpichlaena volubilis	*Salpichlaena volubilis*	No	Hornych et al.	Yes	No	
Scyphularia pentaphylla	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Scyphularia pentaphylla	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Selliguea feei	Gibberelin	No	Voeller, 1964; Weinberg & Voeller, 1969	Yes	Yes	Tested in light (Voeller, 1964); tested in darkness (Weinberg & Voeller, 1969)
Selliguea feei	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	Voeller lists as +?, discussing that no Polypodiaceae react to Apt…
Serpocaulon triseriale	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Serpocaulon triseriale	*Serpocaulon triseriale*	Yes	Hornych et al.	Yes	No	
Tectaria heracleifolia	Gibberelin	No	Voeller, 1964	Yes	No	
Tectaria heracleifolia	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Tectaria heracleifolia	*Tectaria heracleifolia*	No	Hornych et al.	Yes	No	

Taxon influenced	Taxon influencing	Response	Reference	Light	Dark	Notes
Tectaria incisa	*Pteridium aquilinum*	Yes	Näf et al., 1975	Yes	No	Mentioned as Näf, unpubl., presumably only in light
Tectaria macrodonta	Gibberelin	No	Voeller, 1964	Yes	No	
Tectaria macrodonta	*Pteridium aquilinum*	No	Voeller, 1964	Yes	No	
Thelypteris kunthii	*Pteridium aquilinum*	Yes	Hornych et al.	Yes	No	
Thelypteris kunthii	*Thelypteris kunthii*	Yes	Hornych et al.	Yes	No	
Thelypteris ovata	Gibberelin	No	Nester-Hudson et al., 1997	Yes	Yes	
Thelypteris ovata	*Thelypteris ovata*	No	Nester-Hudson et al., 1997	No	Yes	Checked only after 7 days
Thelypteris ovata	*Thelypteris ovata*	Yes	Nester-Hudson et al., 1997	Yes	No	
Todea barbara	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Trichomanes diversifrons	*Trichomanes diversifrons*	No	Hornych et al.	Yes	No	
Vittaria dimorpha	Gibberelin	Yes	Emigh & Farrar, 1977	Yes	No	
Vittaria dimorpha	*Pteridium aquilinum*	No	Emigh & Farrar, 1977	Yes	No	
Vittaria graminifolia	Gibberelin	Yes	Emigh & Farrar, 1977	Yes	No	
Vittaria graminifolia	*Pteridium aquilinum*	No	Emigh & Farrar, 1977	Yes	No	
Vittaria graminifolia	*Vittaria lineata*	Yes	Emigh & Farrar, 1977	Yes	No	
Vittaria lineata	Gibberelin	Yes	Emigh & Farrar, 1977	Yes	No	
Vittaria lineata	*Pteridium aquilinum*	No	Emigh & Farrar, 1977	Yes	No	
Vittaria lineata	*Vittaria lineata*	Yes	Emigh & Farrar, 1977	Yes	No	
Woodsia obtusa	Gibberelin	No	Weinberg & Voeller, 1969	No	Yes	
Woodsia obtusa	*Pteridium aquilinum*	Yes	Näf, 1959; Voeller, 1964	Yes	No	
Woodwardia areolata	*Pteridium aquilinum*	Yes	Näf, 1956	Yes	No	
Woodwardia radicans	*Woodwardia radicans*	Yes	Quintanilla et al., 2005; 2007	Yes	No	
Woodwardia virginica	Gibberelin	No	Voeller, 1964	Yes	No	
Woodwardia virginica	*Pteridium aquilinum*	Yes	Voeller, 1964	Yes	No	
Woodwardia virginica	*Pteris vittata*	Yes	Gemmrich, 1986	Yes	No	
Zealandia pustulata	*Pteridium aquilinum*	No	Hornych et al.	Yes	No	
Zealandia pustulata	*Zealandia pustulata*	No	Hornych et al.	Yes	No	

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Supporting Information Table S4. List of all taxa (meta-analysis + cultivation results) determined as responsive or not to antheridiogens (based on the interaction dataset - Tab. S3) with additional information for each taxon. AGPo - Polypodiales type antheridiogen, APSc - Schizaeales type antheridiogen, AGCy - Cyatheales type antheridiogen

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Acrostichum danaeifolium	Langsd. & Fisch.	Pteridaceae	diploid	sexual	No	AGPo
Adiantum hispidulum	Sw.	Pteridaceae	polyploid	sexual	No	AGPo
Adiantum pedatum	Z.	Pteridaceae	polyploid	sexual	Yes	AGPo
Adiantum polyphyllum	Willd.	Pteridaceae	polyploid	sexual	No	AGPo
Adiantum radicans	Fée	Pteridaceae	unknown	sexual	Yes	AGPo
Adiantum tenerum "Scutum Roseum"	Sw.	Pteridaceae	diploid	sexual	Yes	AGPo
Adiantum trapeziforme	L.	Pteridaceae	diploid	sexual	Yes	AGPo
Aglaomorpha meyeniana	Schott	Polypodiaceae	unknown	sexual	No	AGPo
Anemia collina	Raddi	Anemiaceae	unknown	sexual	Yes	AGSc
Anemia flexuosa	(Savigny) Sw.	Anemiaceae	unknown	sexual	Yes	AGSc
Anemia hirsuta	(L.) Sw.	Anemiaceae	polyploid	sexual	Yes	AGSc
Anemia mexicana	Klotzsch	Anemiaceae	diploid	sexual	Yes	AGSc
Anemia phyllitidis	(L.) Sw.	Anemiaceae	polyploid	sexual	Yes	AGSc
Anemia rotundifolia	Schrad.	Anemiaceae	diploid	sexual	Yes	AGSc
Anemia tomentosa	(Savigny) Sw.	Anemiaceae	polyploid	apomict	Yes	AGSc
Asplenium adiantum-nigrum	L.	Aspleniaceae	diploid	sexual	Yes	AGPo
Asplenium auriculatum	Sw.	Aspleniaceae	polyploid	sexual	Yes	AGPo
Asplenium auritum	Sw.	Aspleniaceae	polyploid	sexual	Yes	AGPo
Asplenium ceterach	L.	Aspleniaceae	polyploid	sexual	No	AGPo
Asplenium cuneifolium	Viv.	Aspleniaceae	diploid	sexual	Yes	AGPo
Asplenium ruta-muraria	L.	Aspleniaceae	polyploid	sexual	Yes	AGPo
Asplenium scolopendrium	L.	Aspleniaceae	diploid	sexual	Yes	AGPo
Asplenium septentrionale	(L.) Hoffm.	Aspleniaceae	polyploid	sexual	Yes	AGPo
Asplenium serratum	L.	Aspleniaceae	polyploid	sexual	Yes	AGPo
Asplenium trichomanes	L.	Aspleniaceae	unknown	sexual	No	AGPo
Astrolepis sinuata	(Lag. ex Sw.) D.M. Benham & Windham	Pteridaceae	polyploid	sexual	Yes	AGPo
Athyrium filix-femina	(L.) Roth.	Athyriaceae	diploid	sexual	Yes	AGPo
Athyrium thelypteridoides	(Michaux) Desvaux	Athyriaceae	diploid	sexual	Yes	AGPo
Blechnum brasiliense	Desv.	Blechnaceae	diploid	sexual	Yes	AGPo
Blechnum gibbum	(Labill.) Mett.	Blechnaceae	diploid	sexual	Yes	AGPo

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Blechnum medium	(R.Br.) Christenh.	Blechnaceae	polyploid	sexual	Yes	AGPo
Blechnum occidentale	L.	Blechnaceae	polyploid	sexual	Yes	AGPo
Blechnum polypodioides	Raddi	Blechnaceae	polyploid	sexual	Yes	AGPo
Blechnum spicant	(L.) Sm.	Blechnaceae	diploid	sexual	Yes	AGPo
Bolbitis portoricensis	(Spreng.) Hennipman	Dryopteridaceae	diploid	sexual	No	AGPo
Bommeria ehrenbergiana	(Klotzsch) Underw.	Pteridaceae	diploid	sexual	Yes	AGPo
Bommeria hispida	(Mett. ex Kuhn) Underw.	Pteridaceae	diploid	sexual	Yes	AGPo
Bommeria pedata	(Sw.) Fourn.	Pteridaceae	polyploid	apomict	Yes	AGPo
Bommeria subpaleacea	Maxon	Pteridaceae	diploid	sexual	Yes	AGPo
Campyloneurum angustifolium	(Swartz) Fee	Polypodiaceae	polyploid	sexual	Yes	AGPo
Campyloneurum aphanophlebium	(Kunze) T. Moore	Polypodiaceae	unknown	sexual	No	AGPo
Campyloneurum brevifolium	(Lodd. ex Link) Link	Polypodiaceae	diploid	sexual	Yes	AGPo
Campyloneurum phyllitidis	(L.) Presl	Polypodiaceae	polyploid	sexual	Yes	AGPo
Ceratopteris richardii	Brongn.	Pteridaceae	diploid	sexual	Yes	AGPo
Ceratopteris thalictroides	(L.) Brongn.	Pteridaceae	polyploid	sexual	Yes	AGPo
Cheilanthes distans	(R. Br.) Mett.	Pteridaceae	polyploid	apomict	Yes	AGPo
Cheilanthes hispanica	Mett.	Pteridaceae	diploid	sexual	Yes	AGPo
Cheilanthes maderensis	Lowe	Pteridaceae	diploid	sexual	Yes	AGPo
Cheilanthes tinaei	Tod.	Pteridaceae	polyploid	sexual	Yes	AGPo
Christella dentata	(Forssk.) Brownsey & Jermy	Thelypteridaceae	polyploid	sexual	Yes	AGPo
Cibotium barometz	(L.) J. Sm.	Cibotiaceae	diploid	sexual	Unknown	
Cibotium menziesii	Hook.	Cibotiaceae	unknown	sexual	Yes	AGCy
Cosentinia vellea	(Aiton) Tod.	Pteridaceae	polyploid	sexual	Yes	AGPo
Cryptogramma crispa	(L.) R.Br.	Pteridaceae	polyploid	sexual	Yes	AGPo
Ctenitis sloanei	(Poepp. ex Spreng.) C.V. Morton	Dryopteridaceae	polyploid	sexual	No	AGPo
Culcita macrocarpa	C. Presl	Culcitaceae	diploid	sexual	No	AGCy
Cyathea australis	R. Br.	Cyatheaceae	diploid	sexual	Unknown	
Cyathea microdonta	(Desv.) Domin	Cyatheaceae	diploid	sexual	Yes	AGCy
Cyathea multiflora	Sm.	Cyatheaceae	diploid	sexual	Yes	AGCy
Cyclosorus dentatus	(Forssk.) Ching	Thelypteridaceae	polyploid	sexual	No	AGPo
Cyrtomium falcatum	(Thunb. ex L. f.) C. Presl	Dryopteridaceae	polyploid	apomict	No	AGPo
Cyrtomium fortunei	J. Smith	Dryopteridaceae	polyploid	apomict	No	AGPo
Cyrtomium macrophyllum	(Makino) Tagawa	Dryopteridaceae	polyploid	apomict	No	AGPo
Cystopteris bulbifera	(L.) Bernh.	Cystopteridaceae	diploid	sexual	No	AGPo
Cystopteris protrusa	(Weatherby) Blasdell	Cystopteridaceae	diploid	sexual	Yes	AGPo
Cystopteris tennesseensis	Shaver	Cystopteridaceae	polyploid	sexual	Yes	AGPo

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Davallia fejeensis	Hook.	Davalliaceae	unknown	sexual	Yes	AGPo
Dennstaedtia bipinnata	(Cav.) Maxon	Dennstaedtiaceae	polyploid	sexual	No	AGPo
Dennstaedtia punctilobula	(Michx.) Morre	Dennstaedtiaceae	diploid	sexual	Yes	AGPo
Diplazium striatastrum	Lellinger	Athyriaceae	unknown	sexual	No	AGPo
Draconopteris draconoptera	(D.C. Eaton) Li Bing Zhang & Liang Zhang	Tectariaceae	unknown	sexual	No	AGPo
Drynaria quercifolia	(L.) J. Sm.	Polypodiaceae	diploid	sexual	No	AGPo
Drynaria rigidula	(Sw.) Bedd.	Polypodiaceae	diploid	sexual	No	AGPo
Dryopteris aemula	(Ait.) Kuntze	Dryopteridaceae	diploid	sexual	Yes	AGPo
Dryopteris affinis	(Lowe) Fraser-Jenkins	Dryopteridaceae	diploid	apomict	Yes	AGPo
Dryopteris carthusiana	(Vill.) H.P.Fuchs	Dryopteridaceae	polyploid	sexual	Yes	AGPo
Dryopteris caucasica	(A. Braun) Fraser-Jenk. & M.F.V. Corley	Dryopteridaceae	diploid	sexual	Yes	AGPo
Dryopteris corleyi	Fraser-Jenk.	Dryopteridaceae	polyploid	sexual	Yes	AGPo
Dryopteris cristata	(L.) A.Gray	Dryopteridaceae	polyploid	sexual	No	AGPo
Dryopteris dilatata	(Hoffm.) A. Gray	Dryopteridaceae	polyploid	sexual	No	AGPo
Dryopteris expansa	(C. Presl) Fraser-Jenk. et Jermy	Dryopteridaceae	diploid	sexual	Yes	AGPo
Dryopteris filix-mas	(L.) Schott	Dryopteridaceae	polyploid	sexual	Yes	AGPo
Dryopteris intermedia	(Willd.) A.Gray	Dryopteridaceae	diploid	sexual	Yes	AGPo
Dryopteris oreades	Fomin	Dryopteridaceae	diploid	sexual	Yes	AGPo
Elaphoglossum callifolium	(Bl.) Moore	Dryopteridaceae	polyploid	sexual	No	AGPo
Elaphoglossum crassifolium	(Gaud.) Anderson & Crosby	Dryopteridaceae	diploid	sexual	No	AGPo
Elaphoglossum latifolium	(Sw.) J. Sm.	Dryopteridaceae	diploid	sexual	Yes	AGPo
Elaphoglossum peltatum	(Sw.) Urb.	Dryopteridaceae	unknown	sexual	No	AGPo
Equisetum arvense	L.	Equisetaceae	diploid	sexual	No	
Equisetum fluviatile	L.	Equisetaceae	diploid	sexual	No	
Equisetum palustre	L.	Equisetaceae	diploid	sexual	No	
Equisetum sylvaticum	L.	Equisetaceae	diploid	sexual	No	
Goniophlebium subauriculatum	(Blume) C. Presl	Polypodiaceae	diploid	sexual	No	AGPo
Goniopteris curta	(Christ) A.R. Sm.	Thelypteridaceae	unknown	sexual	No	AGPo
Goniopteris nicaraguensis	(E. Fourn.) Salino & T.E. Almeida	Thelypteridaceae	unknown	sexual	Yes	AGPo
Gymnocarpium disjunctum	(Rupr.) Ching	Cystopteridaceae	diploid	sexual	Yes	AGPo
Gymnocarpium robertianum	Newman	Cystopteridaceae	polyploid	sexual	Yes	AGPo
Hemionitis arifolia	(Burm.) Moore	Pteridaceae	polyploid	sexual	Yes	AGPo
Hemionitis palmata	L.	Pteridaceae	diploid	sexual	Yes	AGPo
Hypoderris brauniana	(H. Karst.) F.G. Wang & Christenh.	Tectariaceae	unknown	sexual	Yes	AGPo
Lepisorus thunbergianus	(Kaulf.) Ching	Polypodiaceae	unknown	sexual	Yes	AGPo
Lomariopsis japurensis	(Mart.) J. Sm.	Lomariopsidaceae	diploid	sexual	No	AGPo

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Lomariopsis vestita	E. Fourn.	Lomariopsidaceae	unknown	sexual	No	AGPo
Lygodium circinatum	(Burm. f.) Sw.	Lygodiaceae	diploid	sexual	Yes	AGSc
Lygodium flexuosum	(L.) Sw.	Lygodiaceae	polyploid	sexual	Yes	AGSc
Lygodium heterodoxum	Kunze	Lygodiaceae	unknown	sexual	Yes	AGSc
Lygodium japonicum	(Thunb.) Sw.	Lygodiaceae	diploid	sexual	Yes	AGSc
Lygodium microphyllum	(Cav.) R. Br.	Lygodiaceae	diploid	sexual	Yes	AGSc
Lygodium palmatum	(Bernh.) Sw.	Lygodiaceae	diploid	sexual	Yes	AGSc
Lygodium scandens	(L.) Sw.	Lygodiaceae	diploid	sexual	Yes	AGSc
Macrothelypteris torresiana	(Gaudich.) Ching	Thelypteridaceae	polyploid	sexual	No	AGPo
Matteuccia struthiopteris	(L.) Tod.	Onocleaceae	diploid	sexual	Yes	AGPo
Meniscium lingulatum	(C. Chr.) Pic. Serm.	Thelypteridaceae	unknown	sexual	Yes	AGPo
Mickelia nicotianifolia	(Sw.) R.C. Moran, Labiak & Sundue	Dryopteridaceae	unknown	sexual	No	AGPo
Microgramma heterophylla	(L.) Wherry	Polypodiaceae	diploid	sexual	Yes	AGPo
Microgramma lycopodioides	(L.) Copel.	Polypodiaceae	diploid	sexual	No	AGPo
Microlepia speluncae	(L.) T. Moore	Dennstaedtiaceae	diploid	sexual	Yes	AGPo
Microsorum polycarpon	(Cav.) Tardieu	Polypodiaceae	unknown	sexual	No	AGPo
Mohria caffrorum	(L.) Desv.	Anemiaceae	diploid	sexual	Yes	AGSc
Nephrolepis biserrata	(Sw.) Schott	Nephrolepidaceae	diploid	sexual	No	AGPo
Nephrolepis cordifolia	(L.) K. Presl	Nephrolepidaceae	diploid	sexual	Yes	AGPo
Nephrolepis hirsutula	(Forst.) Presl	Nephrolepidaceae	diploid	sexual	Yes	AGPo
Niphidium crassifolium	(L.) Lellinger	Polypodiaceae	polyploid	sexual	No	AGPo
Odontosoria c.f. gymnogrammoides	Christ.	Lindsaeaceae	unknown	sexual	Yes	AGPo
Oleandra articulata	(Sw.) C. Presl	Oleandraceae	diploid	sexual	Yes	AGPo
Olfersia cervina	(L.) Kunze	Dryopteridaceae	diploid	sexual	No	AGPo
Onoclea sensibilis	L.	Onocleaceae	diploid	sexual	Yes	AGPo
Onychium japonicum	(Thunb.) Kunze	Pteridaceae	polyploid	sexual	Yes	AGPo
Onychium siliculosum	(Desv.) C. Chr.	Pteridaceae	diploid	sexual	Yes	AGPo
Oreopteris limbosperma	(All.) Holub	Thelypteridaceae	diploid	sexual	No	AGPo
Osmunda claytoniana	L.	Osmundaceae	diploid	sexual	No	
Osmunda regalis	L.	Osmundaceae	diploid	sexual	No	
Osmundastrum cinnamomeum	(L.) C. Presl	Osmundaceae	diploid	sexual	No	
Parapolystichum excultum	(Mett.) Labiak, Sundue & R.C. Moran	Dryopteridaceae	unknown	sexual	Yes	AGPo
Pecluma pectinata	(L.) M.G. Price	Polypodiaceae	diploid	sexual	No	AGPo
Pellaea calomelanos	(Sw.) Link	Pteridaceae	polyploid	sexual	No	AGPo
Pellaea glabella	Mett. ex Kuhn	Pteridaceae	diploid	sexual	Yes	AGPo
Pellaea glabella [apomict]	Mett. ex Kuhn	Pteridaceae	polyploid	apomict	Yes	AGPo

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Pellaea rotundifolia	(G. Frost)	Pteridaceae	diploid	sexual	Yes	AGPo
Pellaea viridis	(Forssk.) Prantl	Pteridaceae	polyploid	apomict	Yes	AGPo
Pentarhizidium orientale	(Hook.) Hayata	Onocleaceae	diploid	sexual	Unknown	
Phanerophlebia auriculata	Underw.	Dryopteridaceae	polyploid	sexual	No	AGPo
Phanerophlebia juglandifolia	(Humb. & Bonpl. ex Willd.) J. Smith	Dryopteridaceae	diploid	sexual	No	AGPo
Phanerophlebia jungaldifolia	(Humb. & Bonpl. ex Willd.) J. Smith	Dryopteridaceae	polyploid	sexual	No	AGPo
Phanerophlebia macrosora	Underw.	Dryopteridaceae	diploid	sexual	No	AGPo
Phanerophlebia nobilis	(Schlecht. & Cham.) C. Presl	Dryopteridaceae	diploid	sexual	Yes	AGPo
Phanerophlebia pumila	(Mart & Gal.) Fee	Dryopteridaceae	polyploid	sexual	No	AGPo
Phanerophlebia remotispora	(Fourn.) Underw	Dryopteridaceae	diploid	sexual	Yes	AGPo
Phanerophlebia umbonata	Underw.	Dryopteridaceae	diploid	sexual	Yes	AGPo
Phegopteris hexagonoptera	(Michx.) Fée	Thelypteridaceae	diploid	sexual	Yes	AGPo
Phlebodium aureum	(L.) J. Smith	Polypodiaceae	polyploid	sexual	Unknown	
Phlebodium pseudoaureum	(Cav.) Lellinger	Polypodiaceae	unknown	sexual	Yes	AGPo
Phymatosorus scolopendria	(Burm.) Pichi Serm.	Polypodiaceae	polyploid	sexual	Yes	AGPo
Pityrogramma calomelanos	L. (Link)	Pteridaceae	polyploid	apomict	Yes	AGPo
Pityrogramma hybrida	Domin.	Pteridaceae	polyploid	sexual	No	AGPo
Pityrogramma sulphurea	(Sw.)	Pteridaceae	diploid	sexual	No	AGPo
Platycerium alcicorne	(Sw.)	Polypodiaceae	diploid	sexual	Yes	AGPo
Platycerium bifurcatum	(Cav.) C. Chr.	Polypodiaceae	diploid	sexual	Yes	AGPo
Pleopeltis furfuraca	(Schltdl. & Cham.) A.R. Sm.	Polypodiaceae	unknown	sexual	No	AGPo
Polybotrya osmundacea	Humb. & Bonpl. ex Willd.	Dryopteridaceae	diploid	sexual	No	AGPo
Polypodium appalachianum	Haufler & Windham	Polypodiaceae	diploid	sexual	No	AGPo
Polypodium cambricum	L.	Polypodiaceae	diploid	sexual	Yes	AGPo
Polypodium pellucidum	Kaulf.	Polypodiaceae	diploid	sexual	Yes	AGPo
Polypodium virginianum	L.	Polypodiaceae	polyploid	sexual	No	AGPo
Polypodium vulgare	L.	Polypodiaceae	polyploid	sexual	No	AGPo
Polystichum acrostichoides	(Michx.) Schott	Dryopteridaceae	diploid	sexual	Yes	AGPo
Polystichum aculeatum	(L.) Roth	Dryopteridaceae	polyploid	sexual	Yes	AGPo
Polystichum imbricans subsp. *curtum*	(Ewan) D.H. Wagner	Dryopteridaceae	diploid	sexual	No	AGPo
Polystichum lonchitis	(L.) Roth	Dryopteridaceae	diploid	sexual	No	AGPo
Polystichum munitum	(Kaulf.) C. Presl	Dryopteridaceae	diploid	sexual	No	AGPo
Polystichum setiferum	(Forssk.) Woynar	Dryopteridaceae	polyploid	sexual	Yes	AGPo
Polystichum tsus-simense	(Hook.) J. Smith	Dryopteridaceae	polyploid	apomict	Yes	AGPo
Pteridium aquilinum	(L.) Kuhn	Dennstaedtiaceae	diploid	sexual	Yes	AGPo
Pteris cretica	L.	Pteridaceae	polyploid	sexual	Yes	AGPo

Taxon	Author	Family	Ploidy level	Reproduction mode	Response to AG	Type
Pteris ensiformis	Burm. f.	Pteridaceae	polyploid	apomict	No	AGPo
Pteris incompleta	Cav.	Pteridaceae	diploid	sexual	Yes	AGPo
Pteris longifolia	L.	Pteridaceae	polyploid	sexual	Yes	AGPo
Pteris multifida	Poir	Pteridaceae	polyploid	sexual	Yes	AGPo
Pteris podophylla	Swartz	Pteridaceae	diploid	sexual	Yes	AGPo
Pteris propinqua	J. Agardh	Pteridaceae	unknown	sexual	Yes	AGPo
Pteris quadriaurita	Retz	Pteridaceae	polyploid	sexual	Yes	AGPo
Pteris tremula	R. Br.	Pteridaceae	polyploid	sexual	Yes	AGPo
Pteris tripartita	Sw.	Pteridaceae	polyploid	sexual	Yes	AGPo
Pteris vittata	L.	Pteridaceae	polyploid	sexual	Yes	AGPo
Radiovittaria stipitata	(Kunze) E. H. Crane	Pteridaceae	unknown	sexual	Unknown	
Saccoloma elegans	Kaulf.	Saccolomataceae	unknown	sexual	Yes	AGPo
Saccoloma inaequale	(Kunze) Mett.	Saccolomataceae	unknown	sexual	Yes	AGPo
Sadleria cyatheoides	Kaulf.	Blechnaceae	diploid	sexual	Yes	AGPo
Sadleria pallida	Hook. & Arn.	Blechnaceae	unknown	sexual	Yes	AGPo
Salpichlaena volubilis	(Kaulf.) J. Sm.	Blechnaceae	diploid	sexual	No	AGPo
Scyphularia pentaphylla	(Blume) Fée	Davalliaceae	unknown	sexual	No	AGPo
Selliguea feei	Bory	Polypodiaceae	diploid	sexual	No	AGPo
Serpocaulon triseriale	(Sw.) A.R. Sm.	Polypodiaceae	polyploid	sexual	Yes	AGPo
Tectaria heracleifolia	(Willd) Underw.	Tectariaceae	polyploid	sexual	No	AGPo
Tectaria incisa	Cav.	Tectariaceae	polyploid	sexual	Yes	AGPo
Tectaria macrodonta	(Fée) C. Chr.	Tectariaceae	diploid	sexual	No	AGPo
Thelypteris kunthii	(Desv.) C.V. Morton	Thelypteridaceae	polyploid	sexual	Yes	AGPo
Thelypteris ovata	R.P. St. John	Thelypteridaceae	diploid	sexual	Yes	AGPo
Todea barbara	T. Moore	Osmundaceae	diploid	sexual	No	
Trichomanes diversifrons	(Bory) Mett. ex Sadeb.	Hymenophyllaceae	unknown	sexual	No	
Vittaria dimorpha	Müll. Berol.	Pteridaceae	diploid	sexual	Yes	AGPo
Vittaria graminifolia	Kaulf.	Pteridaceae	diploid	sexual	Yes	AGPo
Vittaria lineata	(L.) Sm.	Pteridaceae	polyploid	sexual	Yes	AGPo
Woodsia obtusa	(Spr.) Torrey	Woodsiaceae	polyploid	sexual	Yes	AGPo
Woodwardia areolata	(L.) T. Moore	Blechnaceae	diploid	sexual	Yes	AGPo
Woodwardia radicans	(L.) Sm.	Blechnaceae	diploid	sexual	Yes	AGPo
Woodwardia virginica	(L.) Sm.	Blechnaceae	diploid	sexual	Yes	AGPo
Zealandia pustulata	(G.Forst.) Testo & A.R.Field	Polypodiaceae	unknown	sexual	No	AGPo

Chapter 4: Asymmetric hybridization in Central European populations of the *Dryopteris carthusiana* group.

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PREMISE: Hybridization is a key process in plant speciation. Despite its importance, there is no detailed study of hybridization rates in fern populations. A proper estimate of hybridization rates is needed to understand factors regulating hybridization.
METHODS: We studied hybridization in the European *Dryopteris carthusiana* group, represented by one diploid and two tetraploid species and their hybrids. We sampled ~100 individuals per population in 40 mixed populations of the *D. carthusiana* group across Europe. All plants were identified by measuring genome size (DAPI staining) using flow cytometry. To determine the maternal parentage of hybrids, we sequenced the chloroplast region trnL–trnF of all taxa involved.
RESULTS: We found hybrids in 85% of populations. Triploid *D.* ×*ambroseae* occurred in every population that included both parent species and is most abundant when the parent species are equally abundant. By contrast, tetraploid *D.* ×*deweveri* was rare (15 individuals total) and triploid *D.* ×*sarvelae* was absent. The parentage of hybrid taxa is asymmetric. Despite expectations from previous studies, tetraploid *D. dilatata* is the predominant male parent of its triploid hybrid.
CONCLUSIONS: This is a thorough investigation of hybridization rates in natural populations of ferns. Hybridization rates differ greatly even among closely related fern taxa. In contrast to angiosperms, our data suggest that hybridization rates are highest in balanced parent populations and support the notion that some ferns possess very weak barriers to hybridization. Our results from sequencing cpDNA challenge established notions about the correlation of ploidy level and mating tendencies.

Polyploidization (whole-genome duplication) plays a major role in plant speciation (Arnold, 1997; Otto and Whitton, 2000; Landis et al., 2018). Many polyploid species originally appear as infertile interspecific hybrids that undergo whole-genome duplication to regain fertility (Barrington et al., 1989; Arnold, 1992; Soltis et al., 2000). The resultant allopolyploids are reproductively isolated from their progenitors and combine their characteristics. Therefore, the study of processes that influence hybridization is essential to understanding plant speciation via allopolyploidization (Twyford and Ennos, 2012), especially in ferns, as more speciation events are correlated with polyploidy in ferns than in angiosperms (Wood et al., 2009).

Hybridization is restricted by two types of barriers: prezygotic (limiting mating and fertilization) and postzygotic (limiting the viability of hybrids from zygote onward; Rieseberg and Carney, 1998). The combined strength of these barriers affects hybridization rates (i.e., the frequency of hybrids). A wealth of literature exists describing hybridization rates in seed plants from several different perspectives (e.g., Bacilieri et al., 1996; Lepais et al., 2009; Koutecký et al., 2011; Ma et al., 2014), and the frequency of

hybrids and recombinants has been studied in mosses (Shaw, 1994; van der Velde and Bijlsma, 2004). Hybridization is considered to be common in ferns (Barrington et al., 1989; Sigel, 2016); however, surprisingly, no accurate estimation of hybridization rates in natural populations has been published for ferns. Many studies have described fern hybrid taxa, including intergeneric ones (e.g., Brownsey, 1977; Reichstein, 1981; Knobloch et al., 1984; Rothfels et al., 2015), or studied the presence of hybrids in natural fern populations (Zhang et al., 2013; Testo et al., 2015; Ekrt and Koutecký, 2016; Hori et al.,

2018; Hanušová et al., 2019). However, hybridization rates have only been described qualitatively in these studies, without any precise evaluation. Various hybridization barriers were described for mosses (Natcheva and Cronberg, 2004) and angiosperms (Baack et al., 2015). However, apart from general genetic incompatibilities (Maheshwari and Barbash, 2011), very few hybridization barriers have been suggested to exist in ferns (Xiang et al., 2000; Testo et al., 2015), and there are almost no data gauging their strength. This missing information is preventing us from better understanding hybridization and allopolyploid speciation in ferns.

A key factor affecting hybridization is the relative abundance of the parent species. Presuming a complete lack of hybridization barriers, a given hybrid should be most abundant in balanced populations in which both parent species have an equally high prevalence of interspecific interactions (Rieseberg et al., 1998). In angiosperms we see the opposite trend: even weak barriers decrease the chance of hybridization in balanced populations (Arnold et al., 1993; Carney et al., 1994; Rieseberg et al., 1995; Emms et al., 1996), and hybridization is frequent only when one species is in the minority and the overabundance of foreign pollen overwhelms the barriers (Prentis et al., 2007; Lepais et al., 2009; Koutecký et al., 2011). Nevertheless, the situation could be different in ferns, in which very few barriers have been described. Therefore, understanding hybridization rates will require considering the parent ratio.

In general, both prezygotic and postzygotic barriers can act differently, based on the direction of the cross. This is commonly termed "asymmetric hybridization," meaning that viable hybrid individuals are more likely to have received one type of gamete from one parent taxon rather than the other (Rieseberg et al., 1998). Among the prezygotic barriers that can lead to asymmetric hybridization are, for example, differences in mating systems and gamete performance (Lewis and Crowe, 1958; Buggs and Pannell, 2006; Testo et al., 2015; Nieto-Lugilde et al., 2018). Various genetic incompatibilities can function as an asymmetric postzygotic barrier and affect the viability and fertility of plant hybrids (Arnold and Bennett, 1993; Peng and Chiang, 2000; Hamzeh et al., 2007). The presence of asymmetric hybridization provides an additional perspective and can help explain results of hybridization studies.

Our study group, the *Dryopteris carthusiana* complex (Fig. 1), is a sexually reproducing fern complex represented in continental Europe by *D. carthusiana* (Vill.) H. P. Fuchs (tetraploid), *D. dilatata* (Hoffm.) A. Gray (tetraploid), and *D. expansa* (C. Presl) Fraser-Jenk. & Jermy (diploid). Because these species are among the most abundant ferns in European forests, considerable effort has been put into studying their ecology (Rünk et al., 2010, 2012; Bennert et al., 2012), phylogeny and evolution (Stein et al., 2010; Juslén et al., 2011; Sessa et al., 2012a), and cytology (Ekrt et al., 2010; Bennert et al., 2012). Within the group, all three possible hybrid combinations exist: *D. ×ambroseae* Fraser-Jenk. & Jermy (triploid) = *D. dilatata* × *D. expansa*; *D. ×deweveri* (Jansen) Jansen & Wacht. (tetraploid) = *D. carthusiana* × *D. dilatata*; *D. ×sarvelae* Fraser-Jenk. & Jermy (triploid) = *D. carthusiana* × *D. expansa*. *Dryopteris ×ambroseae* and *D. ×deweveri* are widespread in Europe, whereas *D. ×sarvelae* is very rare and has only been reported from northern parts of Europe (reviewed in Ekrt et al., 2010). Hybrid individuals mostly form aborted spores (Wagner and Chen, 1965; Ekrt et al., 2010; Hornych and Ekrt, 2017) and are therefore generally incapable of forming subsequent generations. In Central European forests, this group often forms a mixed population in which hybrids have frequently been found (Ekrt et al., 2010). The availability of mixed populations and the formation of three different hybrid combinations make the *D. carthusiana* group useful for analyzing hybridization patterns in ferns.

To understand the dynamics of hybridization in natural populations of ferns, we ask three main questions. First, what is the rate of formation of the three hybrid taxa within the *D. carthusiana* group in Europe? Second, does the relative abundance of parent species influence hybridization rates in natural populations? Finally, is there asymmetric hybrid formation among any of the three hybrid taxa?

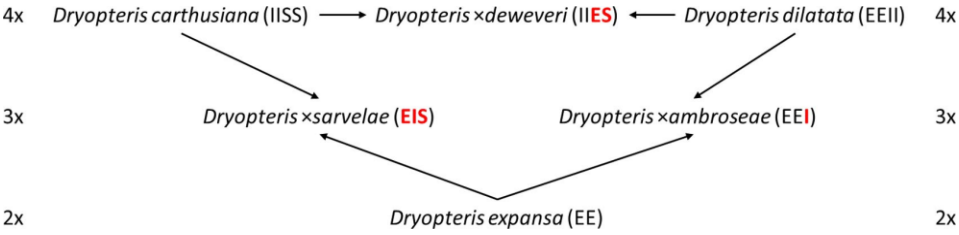

FIGURE 1. Overview of the three species and their hybrids in the *Dryopteris carthusiana* group. Letters in parentheses denote genomic composition of taxa (E = *D. expansa*, I = *D. intermedia*, S = *D. "semicristata";* Sessa et al., 2012b). Letters in red indicate chromosome sets without homologs present.

MATERIALS AND METHODS

Field Collection

A total of 40 mixed (i.e., at least two species present) populations of the *Dryopteris carthusiana* group were sampled during 2016 and 2017 in Austria, the Czech Republic, Germany, Slovakia, and Sweden (Appendix S1). All mature (i.e., bearing sporangia) plants in a continuous area containing ~100 individuals were collected from each population. Additional individuals were collected or obtained from the authors' herbaria for molecular analyses. Vouchers (Appendix S2) of all plants used for molecular analyses were deposited in herbarium CBFS (Thiers, 2019).

Flow cytometry

Genome size of all studied individuals was determined using flow cytometry. This method allows for the unambiguous identification of all the studied taxa, including hybrids, because even the two tetraploid species differ by ~21% in their genome size (Ekrt et al., 2010). The samples were measured using a Partec PA II flow cytometer equipped with a mercury arc lamp (Partec, now part of Sysmex, Münster, Germany) employing DAPI as a fluorescent stain (for details on methodology, see Ekrt et al., 2010). Very rarely, individuals had sample-to-standard ratio out of the norm for the taxa involved (based on Ekrt et al., 2010) and were excluded from further analyses. A single clone of *Chlorophytum comosum* (all-green-leaved cultivar; 2C = 24.14 pg) was used as an internal standard because it provides high-quality results and its genome size does not overlap with any of the studied plants. The genome size of *C. comosum* was determined by calibration with *Pisum sativum* 'Ctirad' (2C = 9.09 pg; Doležel et al., 1998) based on 10 measurements on five different days, using the same method of sample preparation described above except that propidium iodide staining was used (for details, see, e.g., Doležel et al., 2007), and the samples were measured using a Partec CyFlow SL flow cytometer equipped with a 100 mW 532 nm (green) solid state laser as a light source (Partec, now part of Sysmex, Münster, Germany).

In total, 3962 individuals of the *Dryopteris carthusiana* group were analyzed, pooling up to five individuals into one analysis (e.g., Ekrt and Koutecký, 2016; Hanušová et al., 2019). The fluorescence histograms were evaluated using FloMax version 2.6 (Partec, now part of Sysmex, Münster, Germany) and FlowJo version 10 (FlowJo, Ashland, Oregon, USA). Mean fluorescence, coefficient of variation, and number of nuclei were recorded for all fluorescent peaks. The relative genome size was then calculated as the ratio between the mean fluorescence of the sample and the internal standard.

Determining chloroplast origin in hybrids

To provide a reference, we sequenced 10 *D. carthusiana*, 13 *D. dilatata*, and 10 *D. expansa* samples. These sequences were then compared with those from 63 individuals of *D. ×ambroseae*, 35 *of D. ×deweveri*, and three of *D. ×sarvelae*. This method can demonstrate which species provided which gamete to the hybrids, because chloroplasts are maternally inherited in ferns (Vogel et al., 1998). The sequences are available in GenBank (accession nos. MK697576–MK697585). Total genomic DNA was extracted, using Nucleospin Plant II kit (Macherey-Nagel, Düren, Germany), from silica-dried plant material and herbaria specimens. The chloroplast region trnL–trnF was amplified using primers FernL (GGYAATCCTGAGCCAAATC; Li et al., 2009) and TabF (ATTTGAACTGGTGACACGAG; Taberlet et al., 1991). The polymerase chain reaction (PCR) mixture contained 1 μL genomic DNA, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.3 μM of each primer, 0.5 U Taq polymerase (Top-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 10 μL. Amplifications were performed with an initial denaturation of 3 min at 94°C; followed by 40 cycles of 1 min at 94°C, 30s at 51°C, and 1 min at 72°C; and a final extension of 10 min at 72°C. The PCR product was sequenced at Eurofins Genomics (Ebersberg, Germany).

Data analysis

The relationship between the abundance of the most common hybrid taxon (*D. ×ambroseae*) and the relative abundance of the parent species (*D. dilatata* and *D. expansa*) in a population was plotted and compared with a null model of completely random mating. Populations of the *D. carthusiana* group are often established after a disturbance in a single colonization event (O. Hornych et al., personal observation). Infrequent disturbances after the initial colonization event enable more individuals to establish. However, turnover is rather small, and the composition of a population remains relatively stable. Therefore, we assume that the current adult individual composition of a population reflects the proportion of colonizing spores and first-generation gametophytes of the parent species that gave rise to the hybrids. The model also presumes that hybridization is bidirectional—that is, each progenitor has an equal chance of providing either gamete to the hybrid. Finally, hybrid individuals are sterile (Hornych and Ekrt, 2017). Therefore, they do not form subsequent generations and their frequency depends solely on the frequencies of the parents. Presuming a complete lack of barriers to hybrid formation, the expected frequency of hybrids under random mating is 2*d*e, where d and e are frequencies of the parent species *D. dilatata* and *D. expansa*; and frequency of intraspecific offspring is d2 and e2, respectively. Our model is similar to the Hardy-Weinberg model of allele frequencies, with progeny substituted for alleles. The second-order polynomial model was used because it explained the most variation. The percentage

of *D.* ×*ambroseae* was arcsin transformed for the analysis, and only the populations containing at least one individual of *D.* ×*ambroseae* were used. This selection also leads to the inclusion of all populations with *D. expansa*. All statistical analyses were performed in R version 3.4.3 (R Core Team, 2017).

TABLE 1. Overview of the abundance of all studied taxa in the *Dryopteris carthusiana* group and the number of populations where the taxon was present. The abundance per parental population expresses the minimum, mean and maximum % of hybrid individuals present in a population, where both parental species are present.

Taxon	Total number of individuals	Total number of populations	Abundance per parental population (min)-mean-(max)
Dryopteris carthusiana	697 (17.59%)	30 (75%)	
Dryopteris dilatata	1722 (43.46%)	40 (100%)	
Dryopteris expansa	1102 (27.81%)	32 (80%)	
Dryopteris ×*ambroseae*	426 (10.75%)	33 (82.5%)	(0.0)-13.4-(51.2)%
Dryopteris ×*deweveri*	15 (0.38%)	5 (12.5%)	(0.0)-0.5-(5.3)%
Dryopteris ×*sarvelae*	0 (0%)	0 (0%)	0%
Total	3962	40	

RESULTS

Frequency of taxa in wild populations

All 40 studied populations (Table 1) included *D. dilatata*. The other two sexual species, *D. carthusiana* and *D. expansa*, were found in 75% and 80% of the populations, respectively, and all three species co-occurred in 55% of the populations. The three species were present relatively evenly in northern populations, *D. expansa* dominated the populations of the Alps and the Carpathians, and *D. carthusiana* was most abundant at low elevations (Fig. 2A).

Hybrids were collected in 34 (85%) populations with no geographic pattern (Fig. 2B). The highest hybridization rate was found in population 14 (for details, see Appendix S1), where 51.6% of plants were of hybrid origin. The most frequent hybrid was *D.* ×*ambroseae* (426 samples, 10.75%), which was found in all populations in which its parents co-occurred (on average, 13.7% individuals per population) and in a single population without *D. expansa* (Fig. 3). By contrast, only 15 (0.38%) individuals of *D.* ×*deweveri* were sampled from five populations despite a similar number of populations in which both parents were present for *D.* ×*ambroseae* and *D.* ×*deweveri* (Fig. 3). No individuals of *D.* ×*sarvelae* were found during population sampling.

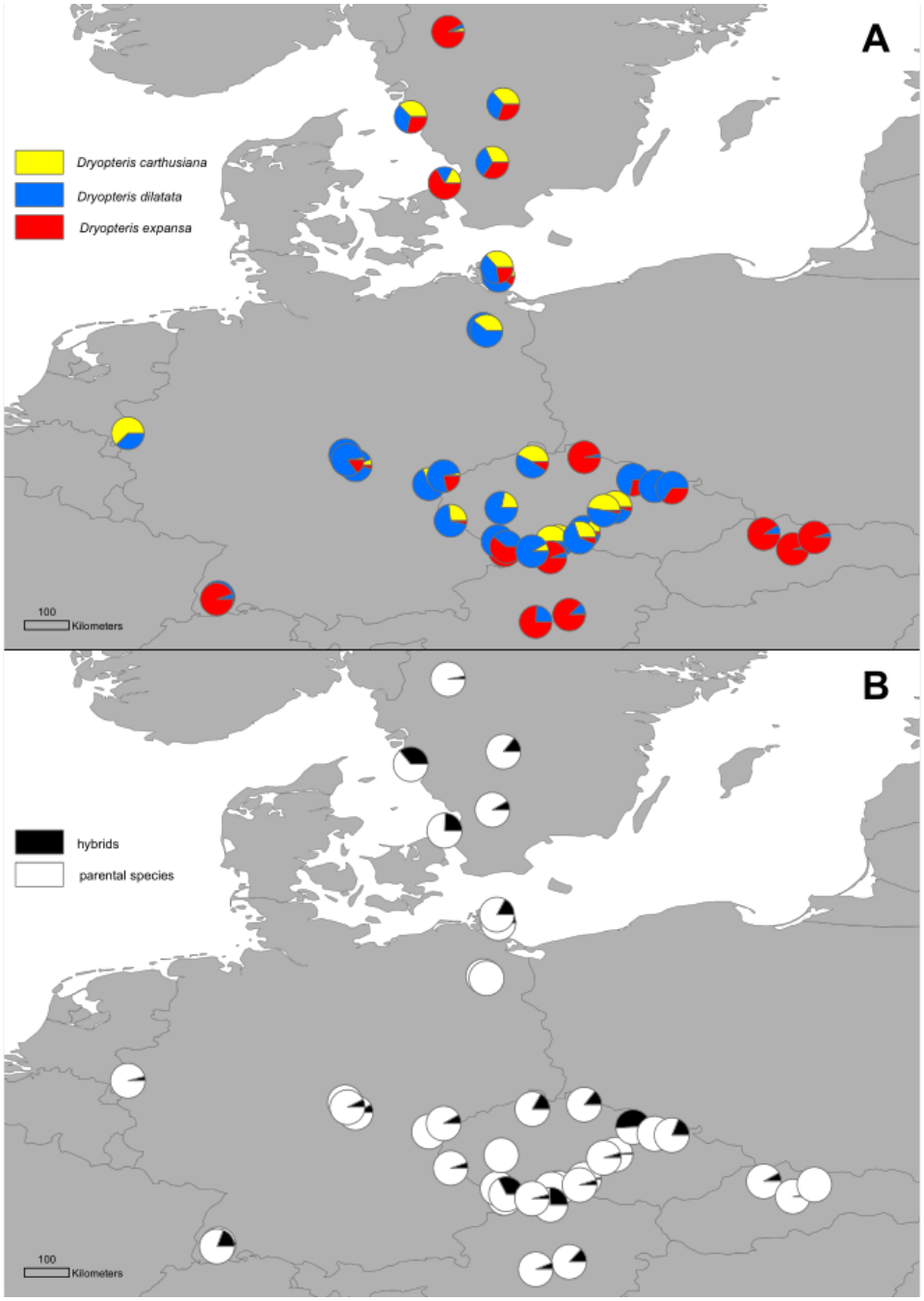

FIGURE 2. Distribution of (A) parent species and (B) hybrids collected in 40 sampled populations of the *Dryopteris carthusiana* group.

Frequency of *D. ×ambroseae* (the only hybrid for which enough data are available) depended on the frequencies of the parent taxa: this hybrid was more common in populations with a balanced proportion of the parents than in populations in which one of the parents dominated (modeled by the second order polynomial, $F_{2, 37} = 32.68$, $P < 0.001$, $R2 = 0.619$). However, compared to the null model of the random mating, the hybrid was less frequent than expected in balanced populations and populations in which *D. dilatata* dominated, whereas it was more frequent than expected in *D. expansa*-dominated populations.

Chloroplast DNA analyses

A single unique trnL–trnF sequence was found in each of the parent species. Among hybrid taxa, 52 of 63 (~83%) of the *D. ×ambroseae* samples received their chloroplast haplotype from *D. expansa* (Fig. 4). For *D. ×deweveri*, hybridization is almost unidirectional, with 33 of 35 (~94%) tested individuals obtaining the chloroplast haplotype from *D. carthusiana* (Fig. 4). The chloroplast haplotype of two of the three individuals of *D. ×sarvelae* match with *D. expansa*.

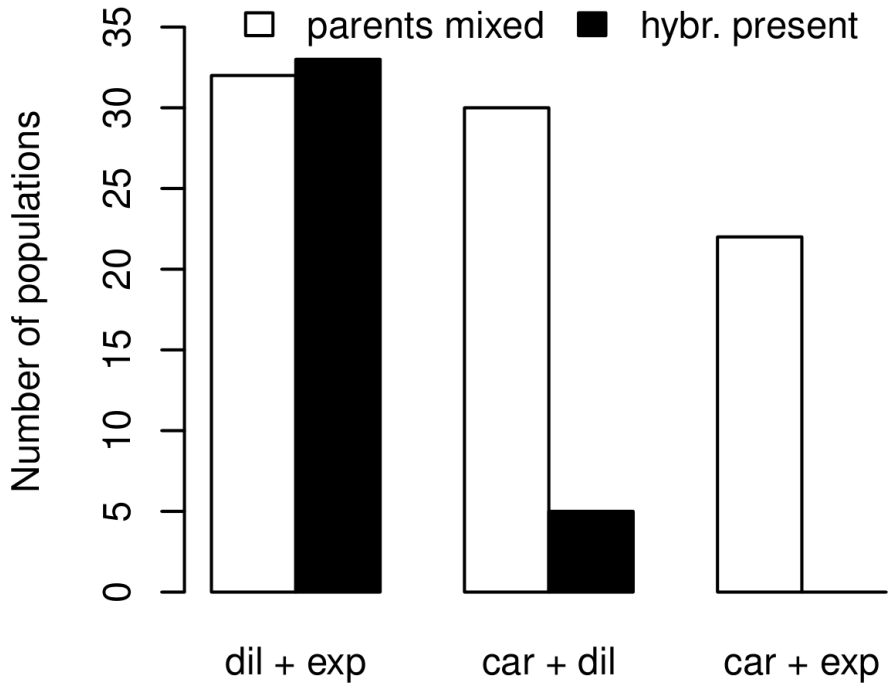

FIGURE 3. A comparison of the numbers of populations in which both parent species were present (mixed) and the numbers in which hybrids were present for all three parent combinations in the *Dryopteris carthusiana* group (car = *D. carthusiana*, dil = *D. dilatata*, exp = *D. expansa*).

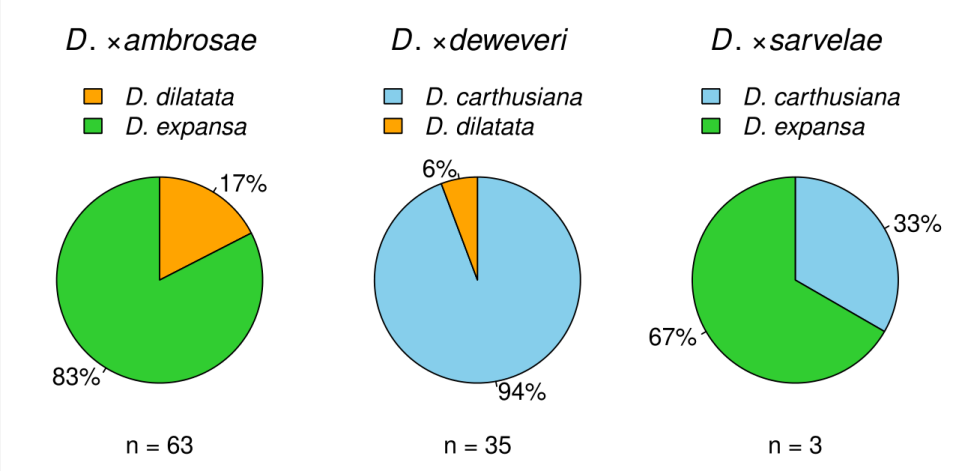

FIGURE 4. Distribution of chloroplast genome (trnL–trnF region) inheritance in the three hybrid taxa within the *Dryopteris carthusiana* group (n = number of individuals analyzed).

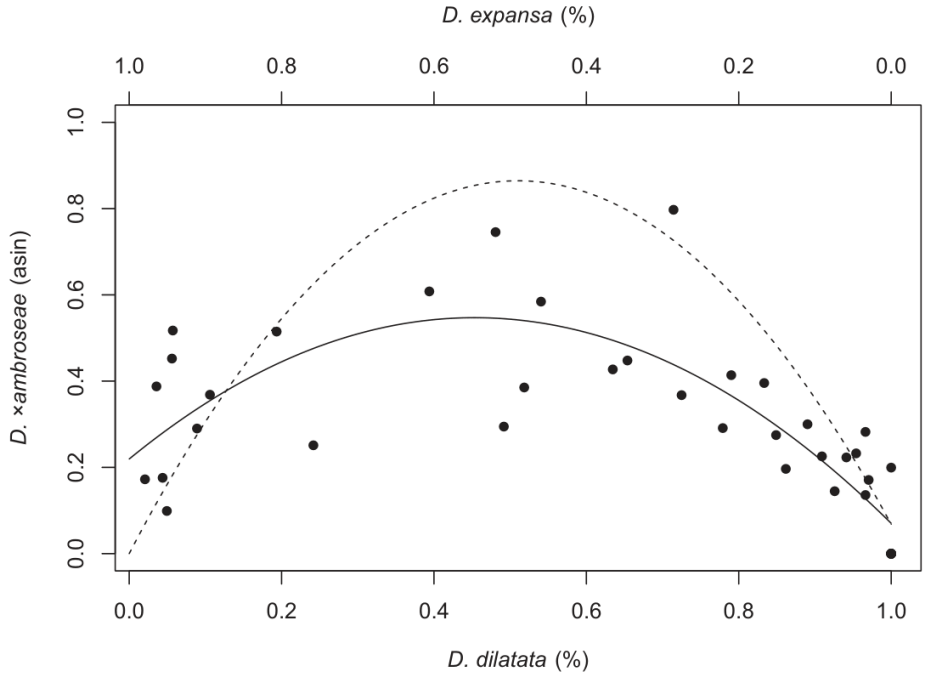

FIGURE 5. Correlation of abundance of *Dryopteris ×ambroseae* and the relative abundance of its parent species, which is expressed as the ratio of the parent to the sum of both parent taxa (bottom axis: *D. dilatata*, top axis: *D. expansa*). Solid line = second-order polynomial regression; dashed line = null model of random mating. Frequency of hybrids (y-axis) is arcsine transformed.

DISCUSSION

Fern hybridization rates in natural populations

Using flow cytometry, we determined 3962 individuals from 40 mixed populations of the three taxa of the *Dryopteris carthusiana* group, providing us with a perspective on past interactions leading to hybridization. We have found hybrids in 34 of the 40 (85%) sampled populations. However, we revealed a striking difference in abundance among the three hybrid taxa. No hybrids between *D. carthusiana* and *D. expansa* (*D. ×sarvelae*) were found in our sampled populations. Hybrids between *D. carthusiana* and D. *dilatata* (*D. ×deweveri*) occur rarely. Finally, hybrids between *D. expansa* and *D. dilatata* (*D. ×ambroseae*) are frequent. To our knowledge, this is the first quantitative record of fern hybridization rates in natural populations. In a preceding study of the *D. carthusiana* group (Ekrt et al., 2010), the authors sampled all taxa within a population nonrandomly and reported the frequency of populations containing hybrids but provided no information on frequencies of the taxa within populations.

Dryopteris ×ambroseae: the ever-present hybrid

The hybrid between *D. dilatata* and *D. expansa*, *D. ×ambroseae*, was found in all 32 populations with both parents and even in one population without *D. expansa*. These numbers are even higher than those described by Ekrt et al. (2010), who collected the hybrid in only 72% of populations that contained both parents. Averaging 13.4% of the individuals in populations in which it occurred, the barriers to forming this triploid hybrid appear to be extremely weak.

Chloroplast analyses of 63 individuals of the triploid *D. ×ambroseae* from 11 populations demonstrate strong asymmetry in hybridization. Asymmetry in chloroplast origin of heteroploid hybrids has been reported in seed plants (Buggs and Panell, 2006) as well as ferns (Vogel et al., 1998; Xiang et al., 2000; Testo et al., 2015). Based on these studies, the diploid ought to be the predominant paternal parent. Surprisingly, our results present the opposite trend, in that the tetraploid *D. dilatata* provided the sperm to 83% of tested hybrids.

The prevalence of *D. ×ambroseae* allows us to compare its frequency with the relative abundance of its parents (Fig. 5). Hybridization was most pronounced when both parents were equally abundant. In comparison, results from seed plants demonstrate that hybridization rates tend to increase as species frequency in mixed populations becomes more uneven (Arnold et al., 1993; Carney et al., 1994; Jorgensen and Andersen, 1994; Rieseberg et al., 1995; Emms et al., 1996; Prentis et al., 2007; Lepais et al., 2009; Koutecký et al., 2011; Ma et al., 2014). However, the curve estimated from our data is not symmetric and the hybridization rate is generally lower than would be expected under

the random-mating null model. Some differences between our data and our model might be attributed to deviations from our assumptions and the presence of hybridization barriers. For example, our model assumes bidirectional hybridization. However, the chloroplast analyses clearly show that hybridization is highly asymmetric, *D. expansa* being the female parent about four times more often than *D. dilatata*. Thus, offspring of a *D. dilatata* female and a *D. expansa* male are much less likely to occur within the populations. Frequency of hybridization might also be influenced by environment-related expression of sexes in ferns. It is known that fern gametophytes in suboptimal conditions produce mainly male structures (antheridia; Korpelainen, 1994; DeSoto et al., 2008). If a species is rare at a site because of suboptimal environmental conditions, it might produce more male and fewer female gametes, and thus more hybrids and fewer of its own offspring, than would be expected on the basis of sporophyte frequency. In case of asymmetric hybridization, this effect might lead to different hybrid frequencies in the two types of uneven populations. If the male parent is in the minority, hybridization can be enhanced by the production of mainly male gametophytes. In the opposite scenario, if the female parent is in the minority, production of mainly male gametophytes may suppress hybridization. Indeed, in the present study, the fitted curve of hybrid frequency (Fig. 5) is asymmetric, with more hybrids in populations in which *D. expansa* (mostly female parent) is dominant and *D. dilatata* (mostly male parent) is rare, compared to the opposite scenario.

Dryopteris ×deweveri: the rare hybrid

The hybrid between *D. carthusiana* and *D. dilatata*, *D. ×deweveri*, is rare. Although both of its parents grew together in 30 populations, the hybrid was found in just five of them (16.6%). Similarly, Ekrt et al. (2010) recorded this hybrid taxon in only about 5% of their populations. However, they sampled selectively and substantially fewer individuals per population than the present study. Despite having a similar number of populations in which parent species co-occurred, this hybrid was almost 30 times less abundant than *D. ×ambroseae*.

The vast majority (~94%) of *D. ×deweveri* samples tested for chloroplast origin received their chloroplast from *D. carthusiana*, suggesting an almost unidirectional pattern of hybridization. This striking asymmetry of chloroplast origin suggests that the hybrid combination of *D. dilatata* female and *D. carthusiana* male is either highly unlikely to originate or has low viability. Similar unidirectional hybridization patterns are well established in seed plants (Arnold and Bennett, 1993; Bacilieri, 1996; Peng and Chiang, 2000; Zhou et al., 2008; Beatty et al., 2009; Trucco et al., 2009; Ma et al., 2014) and have also been reported for homoploid hybrid mosses (van der Velde and Bijlsma, 2004) and ferns (Hunt et al., 2011; Zhang et al., 2013). By contrast, homoploid hybrids of lycopods

(*Diphasiastrum*) and of some ferns (*Polystichum*) are formed with no preferred direction of hybridization (Kentner and Mesler, 2000; Schnittler et al., 2018).

Dryopteris ×sarvelae: the absent hybrid

Interestingly, the hybrid between *D. carthusiana* and *D. expansa*, *D. ×sarvelae*, was not found in any of the 22 populations in which both of its parent species grew in sympatry. These results are congruent with Ekrt et al. (2010), who were also unable to find this hybrid. The absence of this elusive hybrid is even more surprising because our sampling covered general areas where it has previously been found, northern Germany (Jessen and Rasbach, 1987) and Sweden (L. Ekrt, personal observation).

Apart from the genetic dissimilarity (discussed below), the unusual rarity of *D. ×sarvelae* may be explained by microhabitat differences. Of the three *D. carthusiana* group species analyzed, *D. carthusiana* and *D. expansa* are the most ecologically distinct (Rünk et al., 2012). Although they can be present together in the same areas (Kaplan et al., 2016), they tend to occupy different microhabitats. Therefore, the two species might have limited opportunities to hybridize. This hypothesis is congruent with the fact that the hybrid has so far been observed only in parts of Northern Europe (Widén et al., 1967; Sorsa and Widén, 1968; Corley and Gibby, 1981; Jessen and Rasbach, 1987) where *D. expansa* grows more commonly together with *D. carthusiana* (Rünk et al., 2012). Nevertheless, even there, the hybrid seems to be extremely rare.

Given the low number of individuals of *D. ×sarvelae* tested for chloroplast origin and the fact that both types of chloroplast haplotypes were found, we can only conclude that hybridization is not unidirectional.

Factors explaining hybridization patterns

Numerous possible barriers may influence fern hybridization. Within our dataset, one of the three hybrids is extremely rare, perhaps because of (micro)habitat differentiation of the parent species. The other two hybrids have one parent species in common, *D. dilatata*. Interestingly, these two hybrids differ in ploidy level (*D. ×ambroseae* is a heteroploid triploid, whereas *D. ×deweveri* is a homoploid tetraploid). Comparing the hybrids gives us an opportunity to examine the role of prezygotic and postzygotic barriers.

Prezygotic barriers—The chance of a hybrid forming in the first place is limited by prezygotic barriers. Among the factors limiting fern hybrid formation are differences in mating strategies, gamete performance, and antheridiogen use.

Mating strategies impact hybridization and are, in turn, influenced by ploidy level. Polyploidization has been associated with a shift between monoecy and dioecy in mosses (Perley and Jesson, 2015) and seed plants (Buggs and Pannell, 2006; Njuguna et al., 2013)

altering the ability to form the two types of gametes on a single plant. However, sexual determination of homosporous ferns is environmental, and one gametophyte can form both types of gametes under the right conditions. Nevertheless, ploidy level may influence fern mating strategies. Most ferns studied to date employ a mixed mating system (Soltis and Soltis, 1987; Wubs et al., 2010; de Groot et al., 2012; Peredo et al., 2013; Sessa

et al., 2016). Under mixed mating, the gametophyte is capable of both selfing and outcrossing. In some cases, polyploid ferns have been demonstrated to better tolerate gametophytic selfing (Masuyama, 1979; Soltis and Soltis, 2000; Pangua et al., 2003; Flinn, 2006; Testo et al., 2015; Sessa et al., 2016). In theory, mating strategies of sexual fern species could influence hybridization in two opposing ways. First, predominant selfers may have fewer eggs available for hybridization. Apart from skewing the ratio of male parentage in favor of polyploids, this effect would also constitute a prezygotic barrier to hybridization. In our case, *D. ×deweveri* would be affected more by the barrier than the triploids, because both of its parents are polyploid and *D. carthusiana* is a known facultative selfer (Testo et al., 2015). Second, predominant outcrossers may have a greater proportion of sperm in the environment to facilitate outcrossing. These outcrossers could then swamp selfers with overabundant sperm. Although there are no data for ferns, an analogous process, pollen swamping, is well known in seed plants (Petit et al., 1997; Buggs and Pannell, 2006; Ouayjan and Hampe, 2018). The diploid would then be the predominant male parent. These two concepts directly contradict each other. Contrary to previously published results (Xiang et al., 2000; Testo et al., 2015), our results indicate that the former process may be influential, depending on the direction of asymmetry in the parentage of *D. ×ambroseae*.

Gamete performance plays a major role in plant reproduction and, consequently, hybridization. While pollen of seed plants use many vectors to move over long distances (Endress, 1994), sperm of mosses and ferns tend to reach archegonia by swimming in a film of water (Sharpe et al., 2010). This limitation greatly reduces the distance at which two gametophytes may interact (Schneller et al., 1990; van der Velde et al., 2001). In ferns, ploidy level may influence sperm motility. For example, haploid sperm (of diploid species) may swim up to three times farther than diploid sperm (from tetraploid species) in *Dryopteris* (Testo et al., 2015). This increased performance of haploid sperm may increase the likelihood of the diploid species being the paternal parent of hybrids. The formation of *D. ×ambroseae* involves a diploid parent (*D. expansa*) and is more common than that of the tetraploid hybrid. However, contradicting this hypothesized mechanism, the diploid species is less likely to be the paternal parent in our study.

Some ferns also possess a mechanism affecting mating strategies via the use of antheridiogens. Antheridiogens are pheromones that female or bisexual gametophytes

release to the environment, inducing development of antheridia in nearby gametophytes (Raghavan, 1989; Schneller, 2008). This system promotes outcrossing by reducing the amount of eggs and increasing the amount of sperm in proximity to female gametophytes. A system promoting outcrossing may also increase hybridization rates if the barriers are weak. However, not all species use antheridiogens and, should only one parent species be antheridiogen-sensitive, we can predict that many of its gametophytes will be male-only and that a relatively higher proportion of sperm will be formed by that parent in mixed populations of gametophytes (Testo et al., 2015). Therefore, the use of antheridiogens predisposes a species to be the paternal parent in this case. Reportedly, both *D. carthusiana* and *D. dilatata* do not react to congeneric antheridiogens (Barker, 1988; Testo et al., 2015). The third species, *D. expansa*, has not been tested yet; we are currently performing these tests. Antheridiogens could partly explain our results. For *D. ×deweveri*, the insensitivity of both parent species reduced the rates of their interactions and may effectively serve as a hybridization barrier. However, in the case of *D. ×ambroseae*, the insensitivity of *D. dilatata* precludes the use of antheridiogens as a viable explanation for the male parentage of the tetraploid. Nevertheless, future studies on dynamics of fern hybridization should take this pheromonal system into consideration.

Postzygotic barriers—Once the hybrid forms, it must overcome various postzygotic barriers. These often take the form of various genetic incompatibilities resulting in reduced viability and/or fertility. Our three studied hybrids differ markedly in abundance in nature. This difference is correlated with their genomic composition (Fig. 1). The most frequent hybrid, *D. ×ambroseae*, has as one of its parents *D. dilatata*, which originated by hybridization between the second parent, *D. expansa*, and *D. intermedia* (Fig. 1; Sessa et al., 2012b). Therefore, the formation of *D. ×ambroseae* involves merging two shared subgenomes with one different subgenome. Two of the four genomes involved in the formation of the intermediately abundant *D. ×deweveri* are probably shared by its parents, namely those of *D. intermedia* (Sessa et al., 2012b). Contrary to Juslén et al. (2011), the chloroplast haplotype detected in our *D. dilatata* samples differed from *D. expansa* and was identical with *D. intermedia* (AY268821, FR731994) and *D. azorica* (FR731969). Finally, the parents of the very rare *D. ×sarvelae* have no genomes in common: *D. carthusiana* has I and S subgenomes, and *D. expansa* has E genome (Fig. 1; Sessa et al., 2012b).

The disparity in hybrid abundance potentially based on genetic differences may be attributable to various genetic incompatibilities. To our knowledge, these incompatibilities have not been studied explicitly in ferns. Nevertheless, improper epistatic and cytonuclear interactions or the influence of the maternal effect may limit hybrid formation in general or asymmetrically (Turelli and Moyle, 2007; Maheshwari and Barbash, 2011). Rare

sex/parent combinations of *D. ×ambroseae* and *D. ×deweveri* may not be less likely to form (i.e., they may not be limited by prezygotic barriers) but simply less likely to survive. Fern sexual hybrids tend to form mostly aborted spores (Wagner and Chen, 1965; Hornych and Ekrt, 2017). So, hybrids may form and be viable, but their contribution to future generations is severely limited. Nevertheless, the presence of many polyploid taxa in ferns (Barrington et al., 1989; Wood et al., 2009; Schneider et al., 2017) indicates that hybrids occasionally regain fertility via polyploidy and form new species. However, some widely known incompatibilities do not affect ferns. For example, contrary to seed plants and some bryophytes, fern sexual determination is environmental, so incompatibilities involving sex chromosomes are inapplicable. Similarly, abnormal formation of triploid endosperm in hybrid angiosperms, limiting seed development (i.e., the "triploid block"; Köhler et al., 2010), has no analogy in ferns.

CONCLUSIONS

As in seed plants, hybridization rates in ferns may vary considerably even between closely related taxa. However, unlike in seed plants (Carney et al., 1994; Jorgensen and Andersen, 1994; Prentis et al., 2007; Lepais et al., 2009; Koutecký et al., 2011), formation of *D. ×ambroseae* is most frequent when both parents are equally abundant. Nevertheless, compared to the predictions of our random mating model, hybridization rate increases when *D. dilatata* is heavily outnumbered. This indicates that there are probably multiple factors affecting fern hybridization. In general, our results support the notion that some ferns may possess very weak barriers to hybridization.

We did not expect asymmetric hybridization in *D. ×deweveri*, and the expected direction of asymmetry in *D. ×ambroseae* was the reverse of our results. There seem to be one or more traits of *D. dilatata* that makes it the paternal parent. Some possibilities include unusual sperm motility, increased propensity toward outcrossing, or specific genetic incompatibilities. Nevertheless, the established correlation between ploidy levels and mating strategies expressed by asymmetric hybridization (e.g., Testo et al., 2015) is not universal. More research is required if we want to fully understand hybridization barriers in ferns and their effect on evolution.

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AUTHOR CONTRIBUTIONS

O.H. and L.E. conceived the study. O.H., F.R., and L.E. collected field data. All authors performed laboratory analyses, contributed to writing the manuscript, and gave final approval for publication.

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Appendix S1 List of locations of all populations of the *Dryopteris carthusiana* group, including date of sampling, collectors (OH – Ondřej Hornych, LE – Libor Ekrt, FR – Felix Riedel) and number of each taxon in a population.

Location No.	Date of sampling (DD.MM.YYYY)	GPS coordinates (WGS-84)	Location	Collector ID	*Dryopteris carthusiana*	*Dryopteris dilatata*	*Dryopteris expansa*	*Dryopteris ×ambroseae*	*Dryopteris ×deweveri*	*Dryopteris ×sarvelae*
1	6.7.2017	49°14'45.5"N 15°23'48.6"E	CZE - Řídelov: Spring alder forest with spruce ca 1 km NNW of Řídelov.; 625 m.a.s.l.	OH, LE, FR	18	86	3	2	0	0
2	17.7.2017	49°03'55.0"N 14°52'37.3"E	CZE - Stráž nad Nežárkou: Pine monoculture by a stream ca 2 km W of Stráž nad Nežárkou.; 425 m.a.s.l.	OH	81	14	0	0	0	0
3	17.7.2017	49°02'50.1"N, 14°44'26.6"E	CZE - Lužnice: Spring alder forest with spruce ca 1.2 km SSW of Lužnice.; 425 m.a.s.l.	OH	89	12	0	0	0	0
4	27.8.2016	48°52'29.7"N, 13°48'02.1"E	CZE - Stožec: Spruce monoculture with beech ca 2 km NW of Stožec.; 825 m.a.s.l.	OH, LE	2	25	27	46	0	0
5	27.8.2016	49°03'49.1"N, 13°39'38.5"E	CZE - Zdíkov: Spruce monoculture by a stream ca 2.5 km SW of Zdíkov.; 950 m.a.s.l.	OH, LE	1	58	22	12	0	0
6	22.7.2017	49°44'45.5"N, 16°01'58.1"E	CZE - Svratouch: Spring alder forest ca 1.5 km NNE of Svratouch.; 675 m.a.s.l.	OH, LE	40	50	4	2	0	0
7	22.7.2017	49°40'38.7"N, 15°47'51.6"E	CZE - Nové Ransko: Spruce monoculture with alder ca 1.2 km S of Nové Ransko.; 500 m.a.s.l.	OH, LE	46	48	3	5	0	0
8	21.7.2017	49°07'52.5"N, 15°18'29.1"E	CZE - Brandlín: Spring alder forest ca 1.2 km SSW of Brandlín.; 600 m.a.s.l.	OH, LE	29	60	6	5	0	0
9	28.8.2016	48°56'48.2"N, 13°50'01.4"E	CZE - Zátoň: Spring alder forest ca 2 km E of Zátoň.; 1000 m.a.s.l.	OH, LE	0	26	40	32	0	0
10	21.7.2016	50°44'51.2"N, 15°24'02.7"E	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.	OH	0	3	81	14	0	0

Location No.	Date of sampling (DD.MM.YYYY)	GPS coordinates (WGS-84)	Location	Collector ID	*Dryopteris carthusiana*	*Dryopteris dilatata*	*Dryopteris expansa*	*Dryopteris ×ambroseae*	*Dryopteris ×deweveri*	*Dryopteris ×sarvelae*
11	10.7.2016	48°43'54.6"N, 14°43'13.0"E	CZE - Hojná Voda: Spruce scree forest ca 0.6 km NW of Hojná Voda.; 925 m.a.s.l.	OH	0	4	66	23	1	0
12	4.7.2016	50°39'26.3"N, 14°21'28.4"E	CZE - Verneřice: Beech scree forest by stream ca 2.5 km E of Verneřice.; 400 m.a.s.l.	OH	36	40	8	15	2	0
13	10.7.2016	48°51'23.7"N, 14°21'9.6"E	CZE - Plešovice: Spruce scree forest ca 0.6 km S of Plešovice.; 500 m.a.s.l.	OH	8	90	0	4	0	0
14	19.7.2016	50°17'52.4"N, 16°23'05.6"E	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.	OH	0	30	12	44	0	0
15	8.8.2016	50°09'47.5"N, 16°49'03.7"E	CZE - Dolní Morava: Spruce monoculture with beech ca 1 km N of Dolní Morava.; 725 m.a.s.l.	OH, LE	7	81	13	4	0	0
16	9.8.2016	50°07'58.4"N, 17°09'51.1"E	CZE - Bělá pod Pradědem: Spruce-beech forest by stream ca 3 km SW of Bělá pod Pradědem.; 800 m.a.s.l.	OH, LE	0	51	27	18	0	0
17	2.8.2016	49°43'48.4"N, 13°43'59.2"E	CZE - Strašice: Water-logged edge of forest path in spruce forest ca 1.5 km W of Strašice.; 475 m.a.s.l.	OH	23	83	0	0	0	0
18	25.8.2016	47°35'05.5"N, 15°05'58.8"E	AUT - Thörl: Spruce forest with beech ca 4 km SW of Hochschwab mountain.; 1000 m.a.s.l.	LE	1	7	59	10	0	0
19	27.6.2017	47°55'56.7"N, 8°02'31.8"E	DEU - Breitnau: Spruce clearing on scree ca 2.5 km WSW of Breitnau.; 900 m.a.s.l.	OH, LE	0	98	3	3	0	0
20	28.6.2017	47°53'38.2"N, 8°00'48.0"E	DEU - Oberried: Fir-beech forest on stony scree ca 6.2 km SE of Oberried.; 950 m.a.s.l.	OH, LE	0	5	84	21	0	0
21	29.6.2017	51°13'42.4"N, 6°13'17.2"E	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.	OH, LE	60	36	0	0	4	0
22	5.7.2017	50°12'18.5"N, 12°16'44.5"E	CZE - Výhledy: Spruce monoculture ca 1.5 km NNE of Výhledy by Aš.; 675 m.a.s.l.	OH	31	68	0	0	0	0

Location No.	Date of sampling (DD.MM.YYYY)	GPS coordinates (WGS-84)	Location	Collector ID	*Dryopteris carthusiana*	*Dryopteris dilatata*	*Dryopteris expansa*	*Dryopteris ×ambroseae*	*Dryopteris ×deweveri*	*Dryopteris ×sarvelae*
23	6.7.2017	50°22'15.6"N, 12°34'22.9"E	CZE - Stříbrná: Spruce monoculture by stream ca 2.5 km NE of Stříbrná.; 900 m.a.s.l.	OH	3	67	19	8	0	0
24	29.7.2017	49°28'14.5"N, 12°42'48.2"E	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.	OH	24	62	3	5	0	0
25	16.8.2017	49°12'20.9"N, 19°0'52.5"E	SVK - Terchová: Beech scree forest ca 6 km S-SSW of Terchová; 1200 m.a.s.l.	OH, LE	0	9	92	9	0	0
26	17.8.2017	48°53'56.0"N, 19°35'58.7"E	SVK - Horná Lehota: Spruce-beech forest with fir ca 4.9 km S-SSE of Chopok mountain, 850 m.a.s.l.	OH, LE	3	2	94	3	0	0
27	18.8.2017	49°8'19.4"N, 20°2'4.1"E	SVK - Štrbské Pleso: Mountain spruce forest by stream ca 3.1 km NW of Štrbské Pleso train station, 1550 m.a.s.l.	OH, LE	0	5	96	1	0	0
28	23.8.2017	47°26'13.0"N, 14°25'40.7"E	AUT - Hohentauern: Pinus cembra forest east of Grosser Scheibelsee lake, 1750 m.a.s.l.	LE	0	22	68	6	0	0
29	1.7.2017	53°19'55.1"N, 13°22'24.2"E	DEU - Lüttenhagen: Douglas fir stand within a beech forest near a small lake ca 900 SW of Lüttenhagen, 125 m.a.s.l.	FR	2	64	17	16	0	0
30	13.7.2017	54°23'44.0"N, 13°40'25.3"E	DEU - Sellin: Larch stand within a beech forest ca 0.3 km NE of Schwarzer See lake, 75 m.a.s.l.	FR	9	73	9	9	3	0
31	14.7.2017	54° 34'7.3"N, 13° 38'38.8"E	DEU - Sassnitz: Transition from a beech forest to an open and wet meadow 1.6 km NEE of Hagen village, 125 m.a.s.l.	FR	30	33	19	17	0	0
32	26.7.2017	50°47'34.4"N, 10°35'32.6"E	DEU - Tambach-Dietharz: Montane spruce and beech forest ca 1.6 km W of center of Tambach-Deitharz, 525 m.a.s.l.	FR	5	91	0	0	0	0
33	8.8.2017	53°17'22.3"N, 13°26'9.7"E	DEU - Carwitz: Pine stand 50 east of Dreetzsee lake ca 1.4 km S of Carwitz, 100 m.a.s.l.	FR	43	67	0	0	0	0
34	12.8.2017	50°35'8.8"N, 10°48'8.5"E	DEU - Vasser: Slope above a stream surrounded by a beech-spruce forest ca 1.3 km SSE of Vasser, 600 m.a.s.l.	FR	6	86	3	8	0	0
35	14.8.2017	50°41'48.5"N, 10°38'22.1"E	DEU - Oberschönau: Scree under a single beech tree ca 0.4 km E of Hoherstein hill SE of Oberschönau, 750 m.a.s.l.	FR	2	73	13	7	0	0

Location No.	Date of sampling (DD.MM.YYYY)	GPS coordinates (WGS-84)	Location	Collector ID	*Dryopteris carthusiana*	*Dryopteris dilatata*	*Dryopteris expansa*	*Dryopteris ×ambroseae*	*Dryopteris ×deweveri*	*Dryopteris ×sarvelae*
36	22.8.2017	56°15'29.0"N, 12°35'30.8"E	SWE - Höganäs: Moist broadleaved forest ca 1.1 km E of Brunnby settlement near Arild, 50 m.a.s.l.	FR	13	12	50	24	0	0
37	22.8.2017	56°40'36.9"N, 13°33'17.2"E	SWE - Ljungby: Moist depression in spruce-brich forest ca 1.7 km S of the center of Mäen lake SW of Ljungby, 175 m.a.s.l.	FR	28	29	30	8	0	0
38	24.8.2017	57°50'55.7"N, 13°46'26.0"E	SWE - Mullsjö: Moint mixed broadleaves forest 0.4 km from the point of entry of Tidon river into Nässjön lake, 275 m.a.s.l.	FR	31	28	26	14	0	0
39	25.8.2017	59°18'19.3"N, 12°39'46.3"E	SWE - Karlstad: Moist stream valley within a spruce-birch forest ca 1.5 km NW of the center of Aspen lake W of Nysäter settlement, 100 m.a.s.l.	FR	4	4	87	3	0	0
40	27.8.2017	57°35'37.4"N, 11°54'40.9"E	SWE - Lindome: Oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome, 5 m.a.s.l.	FR	22	20	17	28	5	0

Appendix S2 List of all samples of the *Dryopteris carthusiana* group used in chloroplast (trnL–trnF region) analyses, including haplotype and collection information.

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
1	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Plešovice: Spruce scree forest ca 0.6 km S of Plešovice.; 500 m.a.s.l.; Hornych, O.; herbCBFS.	48°51'23.7"N, 14°21'9.6"E	10.7.2016
2	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
3	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
4	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
5	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
6	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
7	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
8	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
9	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	DEU - Oberried: Fir-beech forest on stony scree ca 6.2 km SE of Oberried.; 950 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	47°53'38.2"N, 8°00'48.0"E	28.6.2017
10	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
11	*Dryopteris ×ambroseae*	*Dryopteris dilatata*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
12	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
13	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
14	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
15	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
16	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
17	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
18	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
19	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
20	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016
21	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Plešovice: Spruce scree forest ca 0.6 km S of Plešovice.; 500 m.a.s.l.; Hornych, O.; herbCBFS.	48°51'23.7"N, 14°21'9.6"E	10.7.2016
22	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
23	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
24	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
25	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
26	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
27	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
28	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
29	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
30	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
31	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
32	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
33	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
34	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Deštné v Orlických Horách: Spruce scree forest by stream ca 0.5 km E of Zákoutí by Deštné v Orlických Horách.; 850 m.a.s.l.; Hornych, O.; herbCBFS.	50°17'52.4"N, 16°23'05.6"E	19.7.2016
35	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Verneřice: Beech scree forest by stream ca 2.5 km E of Verneřice.; 400 m.a.s.l.; Hornych, O.; herbCBFS.	50°39'26.3"N, 14°21'28.4"E	4.7.2016
36	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Čeladná: Along red tourist track 0.5 km of Kněhyně mountain, ca. 2,2 km E of Tanečnice peak.; 1125 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°29'43.3"N, 18°18'16.6"E	3.8.2015
37	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Protivanov: Spruce forest "Prášilka", ca. 2.75 km SW of the church in Protivanov.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'6.9"N, 16°48'22.0"E	4.8.2015
38	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Čeladná: Along red tourist track 0.5 km of Kněhyně mountain, ca. 2,2 km E of Tanečnice peak.; 1125 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°29'43.3"N, 18°18'16.6"E	3.8.2015
39	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Čeladná: Along red tourist track 0.5 km of Kněhyně mountain, ca. 2,2 km E of Tanečnice peak.; 1125 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°29'43.3"N, 18°18'16.6"E	3.8.2015
40	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Rudník: Forest between Bolkovský and Janovický streams ca 0.9 km W of Zlatá vyhlídka peak.; 700 m.a.s.l.; Hornych, O.; herbCBFS.	50°37'30.2"N, 15°44'48.6"E	17.7.2015
41	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Zátoň: Spring alder forest ca 2 km E of Zátoň.; 1000 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	48°56'48.2"N, 13°50'01.4"E	26.8.2016
42	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Paseky nad Jizerou: Spruce-beech scree forest ca 2 km N-NNE of Paseky nad Jizerou.; 625 m.a.s.l.; Hornych, O.; herbCBFS.	50°44'51.2"N, 15°24'02.7"E	21.7.2016

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
43	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Zátoň: Spring alder forest ca 2 km E of Zátoň.; 1000 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	48°56'48.2"N, 13°50'01.4"E	26.8.2016
44	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Zátoň: Spring alder forest ca 2 km E of Zátoň.; 1000 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	48°56'48.2"N, 13°50'01.4"E	26.8.2016
45	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Zátoň: Spring alder forest ca 2 km E of Zátoň.; 1000 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	48°56'48.2"N, 13°50'01.4"E	26.8.2016
46	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Bělá pod Pradědem: Spruce-beech forest by stream ca 3 km SW of Bělá pod Pradědem.; 800 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°07'58.4"N, 17°09'51.1"E	9.8.2016
47	*Dryopteris ×ambroseae*	*Dryopteris expansa*	DEU - Oberried: Fir-beech forest on stony scree ca 6.2 km SE of Oberried.; 950 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	47°53'38.2"N, 8°00'48.0"E	28.6.2017
48	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
49	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
50	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
51	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
52	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
53	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
54	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
55	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
56	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
57	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
58	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
59	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
60	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
61	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
62	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
63	*Dryopteris ×ambroseae*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
64	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
65	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
66	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Brandlín: Spring alder forest ca 1.2 km SSW of Brandlín.; 600 m.a.s.l.; Hornych, O.; herbCBFS.	49°07'52.5"N, 15°18'29.1"E	21.7.2017
67	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Brandlín: Spring alder forest ca 1.2 km SSW of Brandlín.; 600 m.a.s.l.; Hornych, O.; herbCBFS.	49°07'52.5"N, 15°18'29.1"E	21.7.2017
68	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Nové Ransko: Spruce monoculture with alder ca 1.2 km S of Nové Ransko.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°40'38.7"N, 15°47'51.6"E	22.7.2017
69	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Nové Ransko: Spruce monoculture with alder ca 1.2 km S of Nové Ransko.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°40'38.7"N, 15°47'51.6"E	22.7.2017
70	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Nové Hrady: Terčino údolí valley 2.7 km SW-WSW of Nové Hrady chruch.; 525 m.a.s.l.; Hornych, O.; herbCBFS.	48°46'40.1"N, 14°44'53.5"E	17.6.2018
71	*Dryopteris carthusiana*	*Dryopteris carthusiana*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
72	*Dryopteris carthusiana*	*Dryopteris carthusiana*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
73	*Dryopteris carthusiana*	*Dryopteris carthusiana*	CZE - Slavný village: beech forest near the Zaječí rokle gorge ca 1 km ENE of the centrum of village.; 600 m.a.s.l.; Ekrt, L.; herbCBFS.	50°32'16.0"N, 16°17'57.0"E	6.9.2005
74	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: Spring alder forest with spruce ca 1 km NNW of Řídelov.; 625 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°14'45.5"N 15°23'48.6"E	6.7.2017
75	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
76	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
77	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
78	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
79	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
80	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
81	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
82	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
83	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
84	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Waldniel: Alder forest ca 2.5 km NW of Waldniel.; 50 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	51°13'42.4"N, 6°13'17.2"E	29.6.2017
85	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
86	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Jestřebí: at the bottom of a slope near read tourist track by Metuje river ca 1 km W of Jestřebí bus stop; 325 m.a.s.l.; Ekrt, L.; herbCBFS.	50°21'41.0"N, 16°10'7.0"E	6.8.2018
87	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	SWE - Lindome: oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome.; 5 m.a.s.l.; Riedel, F.; herbCBFS.	57°35'37.4"N, 11°54'40.9"E	27.8.2017

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
88	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	SWE - Lindome: oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome.; 5 m.a.s.l.; Riedel, F.; herbCBFS.	57°35'37.4"N, 11°54'40.9"E	27.8.2017
89	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	SWE - Lindome: oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome.; 5 m.a.s.l.; Riedel, F.; herbCBFS.	57°35'37.4"N, 11°54'40.9"E	27.8.2017
90	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	SWE - Lindome: oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome.; 5 m.a.s.l.; Riedel, F.; herbCBFS.	57°35'37.4"N, 11°54'40.9"E	27.8.2017
91	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	SWE - Lindome: oak forest nead a rock face near the coast on the Stora Amundön isle NWW of Lindome.; 5 m.a.s.l.; Riedel, F.; herbCBFS.	57°35'37.4"N, 11°54'40.9"E	27.8.2017
92	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Moravské Budějovice: alder forest on Jevišovka stream 0.3 km NW of Hrachovec pond.; 475 m.a.s.l.; Ekrt, L.; herbCBFS.	49°2'56.2"N, 15°43'47.0"E	7.7.2018
93	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Moravské Budějovice: alder forest on Jevišovka stream 0.3 km NW of Hrachovec pond.; 475 m.a.s.l.; Ekrt, L.; herbCBFS.	49°2'56.2"N, 15°43'47.0"E	7.7.2018
94	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	DEU - Sassnitz: By Herthasee lake.; in cultivation; 125 m.a.s.l.; Ekrt, L.; herbCBFS.	54°34'10.2"N, 13°38'57.0"E	26.6.2018
95	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Stonařov: alder forest ca 2 km W of the village.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°17'1.9"N, 15°33'8.9"E	13.7.2006
96	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: alder forest by forest track ca 1.4 km NW of town center.; 625 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°14'45.5"N, 15°23'48.0"E	15.7.2014
97	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Svratouch: spruce and alder forest near a stream ca 2.5 km.; 650 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°44'46.0"N, 16°1'58.0"E	10.7.2015
98	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Kostelní Myslová: south edge of the Velký Hulišťský rybník pond ca 1.6 km WNW of the centrum of the village.; 525 m.a.s.l.; Ekrt, L.; herbCBFS.	49°9'7.4"N, 15°24'27.2"E	8.8.2005
99	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Olší u Telče: alder forest behind Olešský pond ca 0.85 km NW of town center.; 600 m.a.s.l.; Ekrt, L.; herbCBFS.	49°9'41.9"N, 15°22'3.6"E	15.7.2009
100	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Polesí u Počátek: alder forest ca 1 km SE of the village.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°17'16.0"N, 15°15'24.0"E	11.7.2006
101	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Stonařov: alder forest ca 2 km W of the village.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°17'1.9"N, 15°33'8.9"E	13.7.2006
102	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: alder forest Lukšovská ca 1.3 km NNW of town center.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°14'43.0"N, 15°23'50.0"E	20.9.2010

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103	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: alder forest by forest track ca 1.4 km NW of town center.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°14'45.5"N, 15°23'48.0"E	15.7.2014
104	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Velké Poříčí: Lokvencův Důl valley ca 1.5 l, ESE of Hronov train stop.; 375 m.a.s.l.; Ekrt, L.; herbCBFS.	50°28'14.7"N, 16°12'0.2"E	5.8.1995
105	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: alder forest by forest track ca 1.4 km NW of town center.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°14'35.0"N, 15°23'36.0"E	13.9.2010
106	*Dryopteris ×deweveri*	*Dryopteris carthusiana*	CZE - Řídelov: alder forest by forest track ca 1.4 km NW of town center.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	49°14'35.0"N, 15°23'36.0"E	13.9.2010
107	*Dryopteris ×deweveri*	*Dryopteris dilatata*	DEU - Münchenbernsdorf: By Wolfsgefärth.; in cultivation; 250 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°48'47.9"N, 12°4'0.4"E	26.8.2018
108	*Dryopteris ×deweveri*	*Dryopteris dilatata*	CZE - Božanov: mixed forest ca 1.3 km SSE of the Červený vršek colony.; 625 m.a.s.l.; Ekrt, L.; herbCBFS.	50°29'59.0"N, 16°20'43.0"E	17.8.2008
109	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
110	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
111	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
112	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
113	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Svratouch: Spring alder forest ca 1.5 km NNE of Svratouch.; 675 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°44'45.5"N, 16°01'58.1"E	22.7.2017
114	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Brandlín: Spring alder forest ca 1.2 km SSW of Brandlín.; 600 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°07'52.5"N, 15°18'29.1"E	21.7.2017
115	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Nové Ransko: Spruce monoculture with alder ca 1.2 km S of Nové Ransko.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°40'38.7"N, 15°47'51.6"E	22.7.2017
116	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Nové Ransko: Spruce monoculture with alder ca 1.2 km S of Nové Ransko.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°40'38.7"N, 15°47'51.6"E	22.7.2017
117	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Nové Hrady: Terčino údolí valley 2.7 km SW-WSW of Nové Hrady chruch.; 525 m.a.s.l.; Hornych, O.; herbCBFS.	48°46'40.1"N, 14°44'53.5"E	17.6.2018

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
118	*Dryopteris dilatata*	*Dryopteris dilatata*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
119	*Dryopteris dilatata*	*Dryopteris dilatata*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
120	*Dryopteris dilatata*	*Dryopteris dilatata*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
121	*Dryopteris dilatata*	*Dryopteris dilatata*	CZE - Kostelní Myslová: south edge of the Velký Hulišťský rybník pond ca 1.6 km WNW of the centrum of the village.; 525 m.a.s.l.; Ekrt, L.; herbCBFS.	49°9'7.4"N, 15°24'27.2"E	8.8.2005
122	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Postřekov: Beech scree forest ca 5 km WNW of Postřekov.; 800 m.a.s.l.; Hornych, O.; herbCBFS.	49°28'14.5"N, 12°42'48.2"E	29.7.2017
123	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Svratouch: Spring alder forest ca 1.5 km NNE of Svratouch.; 675 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°44'45.5"N, 16°01'58.1"E	22.7.2017
124	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Svratouch: Spring alder forest ca 1.5 km NNE of Svratouch.; 675 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°44'45.5"N, 16°01'58.1"E	22.7.2017
125	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
126	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
127	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Cikháj: Spruce-alder forest near a road under Žákova hora hill ca 0.6 km ESE of the peak.; 775 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°39'19.1"N, 16°0'0.1"E	22.7.2017
128	*Dryopteris expansa*	*Dryopteris expansa*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
129	*Dryopteris expansa*	*Dryopteris expansa*	DEU - Dreba: mixed wetland forest between ponds south of the bird observation tower.; 500 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°39'20.7"N, 11°45'41.7"E	25.6.2018
130	*Dryopteris expansa*	*Dryopteris expansa*	DEU - Oberschönau: Scree under a single beech tree ca 0.4 km E of Hoherstein hill SE of Oberschönau.; 750 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	50°41'48.5"N, 10°38'22.1"E	14.8.2017
131	*Dryopteris expansa*	*Dryopteris expansa*	CZE - Čeladná: Along red tourist track 0.5 km of Kněhyně mountain, ca. 2,2 km E of Tanečnice peak.; 1125 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	49°29'43.3"N, 18°18'16.6"E	3.8.2015
132	*Dryopteris ×sarvelae*	*Dryopteris carthusiana*	DEU - Sassnitz: By Herthasee lake.; in cultivation; 125 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	54°34'10.2"N, 13°38'57.0"E	26.6.2018

ID	Taxon	Haplotype (trnL–trnF)	Location; collector; herbarium	GPS coordinates (WGS-84)	Date of sampling (DD.MM.YYYY)
133	*Dryopteris ×sarvelae*	*Dryopteris expansa*	DEU - Sassnitz: "Kranichbruch" SW of Neu Mukran.; in cultivation; 5 m.a.s.l.; Hornych, O.; Ekrt, L.; herbCBFS.	54°28'33.5"N, 13°32'44.7"E	26.6.2018
134	*Dryopteris ×sarvelae*	*Dryopteris expansa*	NOR - Sel: near Selsverket town. ; Ekrt, L.; herbCBFS.	61°48'2.9"N, 9°32'19.6"E	21.2.2007

General conclusions

The life cycle of ferns involves two independent phases, gametophytic and sporophytic, each living quite a different experience. Thus, in order to understand fern reproduction, a multipronged approach is needed. So, the connecting link between the presented research is not a taxon, or a single process, but rather the entirety of fern reproduction. Due to the overall rarity of ferns and a general hesitance by researchers to study them, many concepts explored in angiosperms have barely been touched in ferns. Much is still there to be revealed.

A key transitional stage between generations is the spore. A tiny but highly consequential object, just a couple tens of millimeters in size. It carries multiple important properties when formed properly, but the aim of the first paper was to study spores not forming properly, spore abortion. The paper focused on comparing the prevalence of spore abortion between genera and reproductive groups. Using a comprehensive comparative set of 109 samples from 23 taxa, this paper robustly demonstrates the general assumption, that apomicts have higher spore abortion and sexually reproducing species. Hybrid taxa were also agreed to abort the vast majority of their spores, with the notable exception of apomictic hybrids. Additionally, a general set of guidelines, such as using 1000 spores, from two different parts of the frond, were offered, based on empirical research.

Once, and if, a spore germinates, its role is to produce gametes. To that end, fern gametophytes possess the ability to choose their sex based on environmental cues, called environmental sex determination. An important cue is the presence of a pheromone called antheridiogen. Antheridiogens are produced by female gametophytes and elicit a premature formation of antheridia in surrounding asexuals. This simple mechanism fundamentally affects the relative proportions of both gamete types in fern gametophyte populations, improving the odds of outcrossing. Yet many of its aspects were not properly explored. The second paper of this thesis combined available literature with the results of cultivation experiments using various species. Many of the cultivated species were from the tropics, addressing the general lack of data from this biotope. The combined dataset includes 498 interactions between 208 species (ca. 2% of fern diversity). About 65% of fern species seem to respond to antheridiogens, making this pheromone a key driver of sexual reproduction. There are several antheridiogen types, which were also assessed in the paper. A new nomenclature was proposed. The dataset allowed for a thorough discussion of the evolution of antheridiogens, including some implication for the evolution of heterospory, or rather lack thereof in ferns. Polyploids and apomicts were found to respond just as frequently as diploids and sexually reproducing species, most likely because antheridiogen sensitivity is inherited and conserved. However, several tantalizing hypotheses were presented to show why polyploids and apomicts may benefit from,

abandon, or abuse the antheridiogen system. Finally, germination in darkness caused by antheridiogens correlates with its regular effect in light. While this correlation was rarely doubted, a quantitative assessment lays ground for an expedient method of antheridiogen sensitivity testing.

A successful sexual reproduction hopefully results in a viable sporophytic plant. These plants carry the legacy of the turbulent gametophytic stage in their genomes. This legacy has dire consequences in fern hybrids, which are mostly sterile, due to their imbalanced genomes. Once again studying things not going according to plan, the third paper of the thesis focuses on hybridization patterns in Central European buckler ferns (*Dryopteris carthusiana* group). The paper is based on a sampling of 40 mixed populations, with ca 100 individuals each. Each plant was identified by flow cytometry. This approach allowed for the first quantitative assessment of hybridization rates in ferns, something that was conspicuous in its absence in a group notoriously known for seemingly unrestricted hybridization. Hybrids were found in 85% of populations. The three hybrid taxa differed considerably in their abundance, ranging from abundant to absent. The most common hybrid *D. ×ambroseae* was most abundant in populations, where both parents were equal in number, which runs contrary to evidence from angiosperms. Additionally, the level of asymmetric hybridization was assessed using chloroplast sequencing. The results revealed that factors influencing hybridization are even more complex than was previously assumed.

In summary, this thesis explores fern reproduction from several angles. From spore to sporophyte, each aspect of fern life cycle provides valuable information. By combining all this information, we can create a more complete picture of what affects and is affected by reproduction in ferns.

But the search is not over. Sadly, much of the topic is still unknown, or we just have glimpses into a previously unexpected complexity. As mentioned above, antheridiogen sensitivity was studied in only ca 2% of species, more work is to be done on that front, possibly through a more expedient method of germination assessment. The fact, that the chemical structure of the most prominent antheridiogen is unknown, shows a glaring hole in our knowledge. The third paper also revealed how little we still know about hybridization of a group, in which hybridization is a dominant aspect of evolution. As hinted at throughout the thesis introduction and the associated papers, studying apomictic hybrids may explore many of the topics of fern reproduction in a condensed format. Apomictic hybrids have an unusually high proportion of viable spores. They seem to be capable of both the sexual and apomictic pathways of sporophyte formation, in some way. They may also be the outcome of antheridiogen interactions, and likely inherit the pheromone system. In nature, some possibly unique barriers and promoters of their

origin, hybridization, may exist. The study of apomictic hybrids should be multifaceted and will likely yield fascinating results.

Shrnutí (Summary in Czech)

Životní cyklus kapradin je složen ze dvou nezávislých fází, gametofytu a sporofytu. Každá fáze prožívá úplně jiné zkušenosti. Proto je pro pochopení rozmnožování kapradin potřeba použít více přístupů. Tuto práci tedy nespojuje taxon, nebo jediný proces, ale celý fenomén rozmnožování kapradin. Kvůli celkové vzácnosti kapradin a obecnému nezájmu vědců je mnoho konceptů, dobře známých u krytosemenných rostlin, u kapradin málo prozkoumaných.

Klíčová přechodová fáze mezi generacemi je spora (výtrus). Tento malý, ale významný objekt je jen desítky milimetrů velký. Nese několik důležitých vlastností, pokud je vytvořen správně. Cílem prvního článku ale bylo studovat spory nesprávně vytvořené, abortované. Článek byl zaměřen na srovnání výskytu abortovaných spor mezi rody a reprodukčními skupinami. Zahrnujíc komplexní sadu 109 vzorků z 23 taxonů, tento článek jasně prokazuje obecně předpokládanou představu, že apomikti (nepohlavně se rozmnožující druhy) mají vyšší míru abortace než sexuální druhy. Hybridní taxony (kříženci) také abortují většinu spor, dle předpokladu. Významnou výjimku ale tvoří apomiktičtí hybridi. V druhé části tohoto článku byly navrženy obecné směrnice na základě empirického výzkumu, například použití 1000 spor z dvou částí listu.

Když, a pokud, spora vyklíčí, jejím cílem je tvorba gamet (pohlavních buněk). Pro tento účel má gametofyt (prokel) kapradin schopnost vybrat své pohlaví na základě prostředí. Tato schopnost se nazývá určení pohlaví prostředím. Významným podnětem je přítomnost antheridiogenů. Antheridiogeny jsou tvořeny samičími gametofyty a vyvolávají předčasnou tvorbu antheridií (pelatek) u blízkých asexuálů. Tento jednoduchý mechanismus zásadně ovlivňuje relativní poměr obou typů gamet v populacích gametofytů kapradin, podporuje tak páření mezi jedinci. Přesto mnoho aspektů antheridiogenů nebylo řádně zkoumáno. Druhý článek této dizertace kombinuje dostupnou literaturu s výsledky kultivačních pokusů různých druhů kapradin. Ve snaze podchytit celkový nedostatek dat z tropů bylo pěstováno mnoho druhů právě z těchto oblastí. Výsledný dataset obsahuje 498 interakcí mezi 208 druhy (zhruba 2% druhů kapradin). Okolo 65% druhů kapradin reaguje na antheridiogen, tento feromon je proto klíčovým hnacím mechanismem sexuálního rozmnožování. Článek také rozebírá různé typy antheridiogenů a navrhuje pro ně nové názvosloví. Dataset také umožnil detailní diskuzi evoluce antheridiogenů, včetně dopadů na evoluci heterosporie a její nevelké zastoupení mezi kapradinami. Ukázalo se také, že polyploidní a apomiktické druhy reagují na antheridiogeny stejně často jako diploidní a sexuální druhy, nejspíše protože citlivost na antheridiogeny se dědí a je konzervována. I přesto byly navrženy některé hypotézy, proč by polyploidní a apomiktické druhy měly použít, zneužít, nebo opustit antheridiogenový systém. Nakonec byla ukázána korelace mezi klíčením spor ve tmě a

běžným vlivem antheridiogenů. I když málokdo tuto korelaci zpochybňoval, toto kvantitativní srovnání klade základy pro rychlou metodu testování reakce na antheridiogeny.

Úspěšné pohlavní rozmnožení umožňuje vznik úspěšného sporofytu. Takový sporofyt v sobě nese dědictví bouřlivé gametofytické fáze ve formě jeho genomu. Toto dědictví ale může mít zoufalé následky v hybridech, kteří jsou většinou neplodní, a to právě kvůli jejich nevyváženým genomům. Třetí článek dizertace se soustředí na vzorec rozmnožování u středoevropských kapradí (*Dryopteris carthusiana* agg.). Článek je postaven na analýze 40 smíšených populací, zhruba po 100 jedincích v každé. Každá rostlina byla určená pomocí průtokové cytometrie. Tímto způsobem vznikl u kapradin první kvantitativní odhad míry hybridizace, což u skupiny známé svým prakticky neomezeným křížením citelně chybělo. Kříženci byly nalezeni v 85% populací. Všechny tři hybridní taxony se podstatně lišily v početnosti, od častého po nenalezený. Nejběžnější kříženec *D. ×ambroseae* byl nejčastější v populacích, kde byly oba rodiče podobně zastoupeny, což je opačný trend, než známe u krytosemenných rostlin. Stupeň asymetrické hybridizace byl určen pomocí sekvenace chloroplastu. Tyto výsledky ukazují, že faktory ovlivňující křížení kapradin jsou složitější, než se předpokládalo.

Tato dizertační práce zkoumá rozmnožování kapradin z mnoha úhlů pohledu. Od spory po sporofyt, každý aspekt života kapradin nám poskytuje cenné informace. Spojení těchto znalostí nám umožňuje získat ucelenější náhled na to, co ovlivňuje rozmnožování kapradin.

Ale nejsme u konce. Bohužel se stále o tomto tématu mnoho neví, nebo máme jen zběžný pohled na dříve nečekanou složitost. Jak bylo zmíněno, reakce na antheridiogeny byla zatím zkoumána u asi jen 2% druhů. Zbývá tedy mnoho práce, třeba pomocí rychlejší metody kontroly klíčivosti. To, že stále neznáme chemickou strukturu nejčastějšího antheridiogenu, ukazuje do očí bijící díru v našich znalostech. Třetí článek také ukazuje, jak málo zatím víme o křížení ve skupině, kde je křížení klíčovým aspektem evoluce. Jak bylo naznačeno, mnoho témat rozmnožování kapradin by se mohlo studovat v uceleném formátu u apomiktických hybridů. Apomiktičtí hybridi tvoří neobvykle vysoké množství funkčních spor. Vypadá to, že jsou schopni nějakým způsobem tvořit sporofyty jak pohlavně, tak nepohlavně. Apomiktičtí hybridi také možná vznikají v důsledku antheridiogenových interakcí a antheridiogeny dědí. Některé možná unikátní jevy potlačují nebo podporují vznik těchto hybridů v přírodě. Mnohostranné studium apomiktických hybridů jistě přinese fascinující výsledky.

Curriculum Vitae

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Education:

- 2017 – 2021: **Doctoral studies**: Faculty of Science at the University of South Bohemia in České Budějovice, Czech Republic.
  - Thesis topic: **Reproduction and hybridization in ferns.** (supervisor: RNDr. Libor Ekrt, Ph.D.)
- 2014 – 2017: **Master studies**: Faculty of Science at the University of South Bohemia in České Budějovice, Czech Republic.
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- 2007 – 2010: Gymnázium Jiřího Ortena, Kutná Hora, Czech Republic.

Internships:

- 09 – 12/2018: **Visiting scholar**: Biology department at the University of Florida, FL, USA (sponsor: Dr. Emily B. Sessa).
  - Purpose: Learn methodology and perform cultivation experiments for antheridiogen research with the help of Dr. Weston L. Testo.

Conferences:

- 07/2020: **Botany 2020 Virtual** (oral, online).
- Presentation: **Hornych O.**, Testo W., Sessa E., Watkins J., Campany C., Pittermann J., Ekrt L. **Pheromones largely control sex expression in ferns: a meta-analysis of fern mating via antheridiogens.**

Work experience, grants:

- 2019 – 2021**: Laboratory assistant** (Faculty of Science at the University of South Bohemia in České Budějovice, Czech Republic)
- 2018 – 2021: **Floristic and phytocoenological investigations, review of rare fern species** (Agency for Nature Conservation and Landscape Protection of the Czech Republic)
- 2018: **Private tutoring of English** (Sophia language school, České Budějovice, Czech Republic)
- 2014 – 2019: **Herbaria digitalization** (project PLADIAS)
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Publications in journals with IF:

- **Hornych O**, Ekrt L. 2017. Spore abortion index (SAI) as a perspective tool of evaluation of spore fitness in ferns: An insight into sexual and apomictic species. *Plant Systematics and Evolution* 303: 497–507.
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- Ekrt L, Podroužek J, **Hornych O**, Košnar J, Koutecký P. 2020. Cytotypes of bracken (*Pteridium aquilinum*) in Europe: widespread diploids and scattered triploids of likely multiple origin. *Flora* Accepted.

Floristic publications:

- **Hornych O.** 2020 Floristická inventarizace lokality NPP Kaňkovy Hory.

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