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Analysis of spore abortion in ferns

Master thesis

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Annotation:

Spore abortion rate was studied for a total of 109 samples belonging to 23 fern taxa. The resulting abortion rate was compared within several reproductive and phylogenetic groups. The method of spore abortion estimation was optimized by analyzing the amount of spores needed as well as the potential differences within frond.

Prohlášení

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1) 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 thesis 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* produced <10% of spores that appeared viable but did not germinate (Gomes et al. 2006). For *Cornopteris christenseniana*, 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 germination rates and inverted SAI are considered as comparable. However, germination rates may be affected by several factors (Kott and Peterson 1973; Kott and Britton 1982; Windham and Ranker 1986; Aragon and Pangua 2004). Because of these limitations, 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.

The thesis focuses on the largest European fern genera *Asplenium* and *Dryopteris*. Additionally, the genera *Athyrium*, *Gymnocarpium* and *Phegopteris* were represented by one taxon each. A total of 23 taxa including 14 sexual species, 5 apomictic species and 4 hybrids were selected. The selection includes 8 diploids, 6 triploids, 8 tetraploids and 1 pentaploid.

The main goals of the thesis are 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.

2) Literature review

2.1) Fern spores

2.1.1) Fern spores and abortion

Ferns reproduce via the formation of spores within sporangia. These spores take on a plethora of colors, shapes and sizes and detailed analysis of spores is a key part of pteridology (Tryon and Lugardon 1990). Since the pivotal research of Irene Manton (1950) was summarized in her book *Problems of cytology and evolution in the Pteridophyta*, traits of fern spores and sporangia have been used to determine various characteristics, ranging from ploidy levels to reproductive types. Assessing spores as either developed or aborted is also a key method of determining hybrid taxa as these taxa almost exclusively form aborted spores (Wagner and Chen 1965). Due to its simplicity and convenience, assessing spore abortion has become the standard tool for this task.

Additionally, the percentage of aborted spores can be viewed as a measure of reproductive fitness. For seed plants there is a great variance in seed size and weight among species, ranging from 2 μg in *Goodyera repens* to 27 kg in *Lodoicea maldivica* (Harper et al. 1970). However, the size and weight of fern spores among species is mostly uniform even among phylogenetically distant taxa (Tryon and Lugardon 1990, Gómez et al. 2016). As such, the traditional tradeoff between the amount of reproductive propagules and the level of investment into each propagule applies on a much smaller scale in ferns. Thus, the ability to reliably produce viable spores is a direct measurement of reproductive capabilities of the individual.

2.1.2) Germination

The assessment of germination capabilities also is being used as an alternative method for measuring fern spore fitness. This method has an undoubtable advantage of distinguishing potential viably looking spores that are for some reason incapable of germination. Gomes et al. (2006) report that less than 10% of spores appeared viable but did not germinate in *Dicksonia sellowiana*. For *Cornopteris christenseniana*, germination rate of viable spores is roughly proportional to their frequency among all spores (Park and Kato 2003). However, it is difficult to say, whether the non-germinating spores were incapable of germination

themselves or there was some sort of condition that prevented their germination specifically. It is well known that many factors influence the capability of fern spores to germinate and thus may considerably skew the assessment results. For example, germination capability may be affected by storage conditions (Kott and Britton 1982; Simabukuro et al. 1998; Aragon and Pangua 2004), the environment and substrate used for germination tests (Kott and Peterson 1973; Suo et al. 2015) and time passed since specimen collection (Windham and Ranker 1986). Alongside the potential problems mentioned above, germination assessment is also more time consuming. Therefore, visibly confirming spores as either viable or aborted may be a more practical and accurate approach.

2.1.3) Comparison with seed plants

Several comparisons can be drawn between measuring reproductive capabilities of ferns and seed bearing plants. As pollen grains are microspores, they present the closest parallel to fern spores. Assessing pollen viability is common practice when working with seed plants and there are several methods for doing so (Dafni and Firmage 2000). Among frequently used methods are vital tests, where the metabolic capability of pollen is used as a proxy for its capability to germinate (Rodriguez-Riano and Dafni 2000; Dafni and Firmage 2000). For example, pollen viability tests have been employed to study hybridization (Rieseberg 1997; Rieseberg et al. 1999; Bureš et al. 2010; Monteiro et al. 2011), the effects of environmental conditions (Herrero and Johnson 1980; Abdul-Baki and Stommel 1995; Srinivasan et al. 1999; Thakur et al. 2009) or apomixis (Nassar 2001; Podio et al. 2012; Caetano et al. 2013). Vital tests have been successfully performed for fern spores as a method of toxicity bioassay (Catala et al. 2009).

Seeds and fruits have no analogues in ferns; however, seed-set and fruit-set are commonly used as a metric to measure reproductive capabilities of seed plants. These metrics calculate the proportion of developed seeds or fruits, respectively, to all seeds or fruits initiated (Burd 1994). This method has been used for a plethora of topics ranging from the effect of environmental conditions (Prasad et al. 2002; Luo 2011; Madan et al. 2012) to pollination (Steffan-Dewenter and Tschardtke 1999; Taki et al. 2010; Garibaldi et al 2013).

2.2) Apomixis

2.2.1) Mechanisms of fern apomixis, a brief review

The majority of ferns reproduce sexually; however, Walker (1985) estimated that about 10% of all fern species reproduce asexually by apomixis. More recent studies have shifted the estimate to about 3%, highlighting *Dryopteridaceae* and *Pteridaceae* as the two families with most apomictic taxa (Liu et al. 2012). Nevertheless the number still considerably exceeds angiosperms with estimated less than 1% of species reproducing via apomixis (Mogie 1992).

Apomixis encompasses several terms and the processes accompanying them. Apospory is an uncommon type of apomixis where the gametophyte develops directly from sporophytic tissue, bypassing the need for meiosis and spore formation (Raghavan 1989). However, in most fern apomicts, two complementary mechanisms – agamospory and apogamy – play a key role in asexual reproduction.

Unreduced (diplo)spores are formed via agamospory a process differing from the production of spores by sexual species. Sporogenesis in sexual species occurs as follows: the spore mother cell undergoes four mitotic divisions forming a total of 16 diploid cells; consequently, these cells undergo meiosis and form 64 spores per sporangium (Raghavan 1989). In apomictic taxa, under the prevalent “Döpp-Manton” type of agamospory, the fourth mitotic division is completed but cytokinesis does not occur. As a result of this, 8 tetraploid cells undergo meiosis and thus form 32 unreduced (diploid) spores (Lovis, 1977; Walker 1979). Therefore, one of the key factors distinguishing fern and angiosperm apomicts is the presence of regular meiosis. The presence of meiosis allows for crossing-over leading to genetically different spores and a variable offspring population (Lin et al 1992; Ishikawa et al. 2003a, 2003b; Schneller and Krattinger 2010). An alternative type of fern agamospory was observed by Braithwaite (1964), where all four mitotic divisions happen regularly; however the meiosis is not completed and unreduced diplospores are produced in diads. Interestingly, it is also possible for apomicts to form spores via the standard sexual pathway but with irregular meiosis resulting in all spores aborted (Walker 1979). Finally, when viable unreduced spores germinate, sporophytes are formed via apogamy, the formation of the sporophyte from somatic cells of the gametophyte (Manton 1950; Walker 1979).

2.2.2) Apomixis and fern spore viability

The reproductive success of apomicts, when compared to sexual species, has been studied from several angles. For instance, fern apomicts are known to have faster gametophyte growth and sporophyte emergence (Whittier 1968, 1970; Kornaś and Jankun 1983; Huang et al. 2006). Sexual taxa also rely on the presence of a water film layer for fertilisation (Nayar and Kaur 1971). Nevertheless, one of the possible disadvantages of apomicts is the reduced capability to produce viable spores. No direct comparison of spore abortion among sexual and apogamous taxa exists. Therefore this thesis was realized. However, information on the subject may be drawn from studies of individual taxa depending on their reproductive mode.

Evidence exists for both minimal and considerable reduction of spore viability in apomictic taxa. Low spore abortion rates were reported directly or via germination rates for *Argyrochosma nivea* var. *tenera* (Hernández et al. 2015), *Cyrtogonellum* (Guo and Liu 2013), tetraploid *Pteris vittata* (Khare and Kaur 1983) and *Dryopteris affinis* (Quintanilla and Escudero 2006). Yet, some studies report higher and more varied incidence of increased spore abortion or germination failure. This decrease of reproductive fitness is reported for *Cornopteris christenseniana* (Park and Kato 2003), *Dryopteris affinis* complex (Eschelmüller 1988), *Dryopteris remota* (Eschelmüller 1993) and natural as well as synthetic *Pteris* apomicts (Walker 1962).

These results always have to be put in a perspective by drawing comparison to sexual taxa. Generally speaking, spore abortion rates of sexual taxa are below 10% as demonstrated in *Dryopteris* (Quintanilla and Escudero 2006), *Culcita macrocarpa*, *Woodwardia radicans* (Arosa et al. 2009) and *Dicksonia sellowiana* (Gomes et al. 2006) with some minor exceptions (Quintanilla and Escudero 2006).

2.3) Polyploidy

2.3.1) Polyploidy in ferns

Polyploidy is the genome-wide multiplication of chromosome number and it is an important factor in plant evolution (Grant 1981; Otto and Whitton 2000; Ramsey and Schemske 2002;

Soltis and Soltis 2009). It is assumed that all ferns as well as seed plants have undergone ancient polyploidy (paleopolyploidy) in their evolutionary history (Jiao et al. 2011; Clark et al. 2016). However, Wood et al. (2009) estimate the amount of recent polyploid (neopolyploid) ferns at 31%, more than double the percentage of angiosperms, 15%. Homosporous ferns also tend to have high chromosome numbers, mean haploid number of 54 (Klekowski 1973). In fact, the highest known chromosome count for any eukaryote is present in a homosporous fern, $n=720$ (*Ophioglossum reticulatum*, Abraham and Ninan 1954.) Additionally, there is a strong correlation between chromosome number and genome size in ferns (Nakazato et al. 2008; Clark et al. 2016). Klekowski and Baker (1966) hypothesized, that the large genome size is a key factor in maintaining genetic variability, as intragametophytic selfing of gametophytes produces completely homozygous sporophytes. As such, stored genetic variability may help mitigate this problem. Most ferns are known to exhibit mixed mating system, being capable of both gametophytic selfing and outcrossing (Schneller and Vogel 2000; de Groot et al. 2012; Sessa et al. 2016). However, evidence suggests that selfing is more common in neopolyploid taxa when compared to diploids (Soltis and Soltis 2000; Testo et al. 2015; Sessa et al. 2016). This is in congruence with the findings that diploids (even of high chromosome counts) have very few duplicated genes, while neopolyploids have considerable gene duplication (Haufler and Soltis 1986) and thus have greater inherent genetic variability.

2.3.2) Polyploidy and fern spore viability

In theory, newly formed polyploids must overcome several problems associated with reproduction (e.g. formation of univalents and multivalents during meiosis) and thus may have reduced reproduction capabilities (Ramsey and Schemske 2002). It is therefore of interest to researchers whether ploidy level affects metrics of reproductive fitness such as spore abortion or germination. The study on *Dryopteris* by Quintanilla and Escudero (2006) found no difference between diploids and tetraploids regarding both spore abortion and germination rates. These results are congruent with the results of Windham and Ranker (1986) studying the germination capabilities of *Pellaea*. However, some authors present contrasting results. Considerable differences in germination rates were found for the *Polystichum aculeatum* group (Pangua et al. 2003), *Polypodium virginianum* (Kott and Peterson 1973) and *Isoetes* (Kott and Britton 1982). In some cases the difference

in germination rates are as much as 60% between diploids and tetraploids (Kott and Peterson 1973; Pangua et al. 2003).

As mentioned above, the results of spore abortion and germination assessments generally correspond. Nevertheless, germination is affected by several factors that may skew the results. Due to the scarcity of literature comparing diploids and polyploids on spore abortion and the contrasting results of various germination tests, it remains uncertain whether differing ploidy levels influence both metrics or whether the discrepancy of results exists for germination tests only.

2.4) Hybridization

2.4.1) Hybridization in ferns

Hybridization is an important mechanism driving plant speciation (Rieseberg 1995; Arnold 1997; Soltis and Soltis 2009; Twyford and Ennos 2012) including ferns (Barrington et al. 1989; Otto and Whitton 2000). While varying degree of reproductive capabilities has been reported for angiosperms (Rieseberg 1997; Rieseberg et al. 1999; Campbell et al. 2003; Bureš et al. 2010; Monteiro et al. 2011), fern hybrids tend to have severely limited capability to reproduce due to the failure of meiosis resulting in the formation of aborted spores (Wagner and Chen 1965; Lubienski 2010; Zhang et al. 2013). However, a small amount of viable spores have been found in fern hybrid taxa (Pinter 1995; Kawakami et al. 2010; Regalado and Prada 2011; Yatabe et al. 2011).

2.4.2) Hybrids involving apomictic species

While gametophytes of apomictic species do not produce functioning archegonia, the production of spermatozoids is not precluded. This allows apomictic taxa to take part in hybridization events where the egg is provided by a gametophyte of a sexually reproducing taxon. In contrast to hybrids of sexual taxa, the partly apomictic hybrids are capable of reliably forming a small proportion of viable spores permitting further reproduction (Eschelmüller 1998; Fraser-Jenkins 2007; Regalado et al. 2010; Ekrt and Koutecký 2016). While this fact has already been described by Manton (1950), recently it has gotten increased attention (Regalado et al. 2010; Ekrt and Koutecký 2016).

2.5) Studied genera

Ferns are the second largest lineage of vascular plants with over ten thousand species in 319 genera (PPG I 2016). Over 80% of all fern species belong to the most derived order Polypodiales (PPG I 2016). From this order, a total of five genera have been used in this study (see Tab. 1). The majority of samples are within the genera *Asplenium* and *Dryopteris*. These genera form a major component of European pteridoflora and have a wealth of studies on apomixis, hybridization and polyploidy behind them, as mentioned below. Spores of all studied genera are described in detail by Tryon and Lugardon (1990).

The studied genera vary in worldwide species richness. Species richness of each genus worldwide, in Europe and in the Czech Republic is summarized in Tab 1. Of the studied taxa, *Asplenium* is undoubtedly the largest in all of the categories, while *Phegopteris* is the least abundant.

Tab. 1: A summary of taxa (hybrids excluded) richness worldwide (PPG I 2016), in Europe (Blockeel 2006) and the Czech Republic (Danilhelka et al. 2012) for each genus used in this study.

Genus	World	Europe	Czech Republic
<i>Asplenium</i>	700	54	13
<i>Athyrium</i>	230	2	2
<i>Dryopteris</i>	400	22	8
<i>Gymnocarpium</i>	8	3	2
<i>Phegopteris</i>	4	1	1

The following chapters present an overview of the genera under study. The species abundance as well as the origin of all polyploids used in this thesis is summarized. Various reproductive topics such as polyploidy, hybridization and apomixis are also reviewed.

2.5.1) *Asplenium*

Asplenium is potentially the largest fern genus comprising almost the entirety of the family *Aspleniaceae* (PPG I 2016). Several smaller genera (e.g. *Ceterach*, *Phyllitis*) were merged into *Asplenium* after a proper phylogenetic analysis (Schneider et al. 2004). Only *Hymenasplenium* may be separated as a minor sister genus. The genus has about 700 species worldwide (Christenhusz and Chase 2014; PPG I 2016), Blockeel (2006) lists 54 species and

subspecies of *Asplenium* in Europe, discussing additional subspecies. In the Czech Republic, 13 species and subspecies exist producing 8 hybrid taxa (Danihelka et al. 2012).

Asplenium is the largest Central European genus and as such is of great importance. Members of this genus, including apomictics (Dyer et al. 2012), are known to frequently hybridize as is exemplified by at least 40 distinct hybrids among European *Asplenium* (Reichstein 1981). Not only due to this fact, many polyploid complexes exist in Europe and around the world, for example *A. trichomanes* (Bennert and Fischer 1993; Ekrt and Štech 2008), *A. monanthes* (Dyer et al. 2012; 2013) or *A. normale* (Chang et al. 2013) complexes. In fact, the first reconstructed allopolyploid origin of a fern was for *A. adulterinum* (Lovis 1968). Additionally, the phenomenon of apomixis is well known in *Asplenium* (Manton 1950; Mehra and Bir 1960; Dyer et al. 2013) and about 10% of all apomicts belong to *Asplenium* (Liu et al. 2012). However, *Asplenium* apomicts are somewhat underrepresented in Europe (Reichstein 1981) and no apomict of this genus grows in the Czech Republic. Additionally, an uncommon type of apomixis was described using the *Asplenium aethiopicum complex* (Braithwaite 1964).

Autopolyploidy is widespread in *Asplenium* and autotetraploid origin is presumed for both *A. ruta-muraria* (Vida 1970; Bouharmont 1972a) and *A. trichomanes* subsp. *quadrivalens* (Bouharmont 1972b) from *A. ruta-muraria* subsp. *dolomiticum* and *A. trichomanes* subsp. *trichomanes*, respectively. However, *A. adiantum-nigrum* is of allotetraploid origin having *A. onopteris* and *A. cuneifolium* as parental species (Shivas 1970).

2.5.2) *Dryopteris*

Dryopteris is a major genus from the family *Dryopteridaceae* (PPG I 2016). The genus consists of 225 – 400 species worldwide (Fraser-Jenkins 1986; Zhang et al. 2013; PPG I 2016). Due to problems including species delimitation within the apomictic *D. affinis* complex, the number of species occurring in Europe varies by author. However, Blockeel (2006) lists 22 species and subspecies of *Dryopteris* in Europe. The Checklist of Czech Flora recognizes 8 species and four hybrids in the Czech Republic (Danihelka et al. 2012).

Alongside the aforementioned *Asplenium*, this genus is also a centerpiece of European pteridology. A large amount of research has been dedicated to elucidating polyploidy and

reticulate evolution within the genus as exemplified below. Apomixis is also of chief interest, as at least 58 species of *Dryopteris* are apomicts, potentially making it the genus with the most apomictic taxa among ferns (Liu et al. 2012). Consequently, several apomictic complexes have been thoroughly studied, for example the European *D. affinis* (Manton 1950; Widén et al. 1996; Fraser-Jenkins 2007; Ekrt et al. 2009; Schneller and Krattinger 2010) or the Asian *D. varia* (Lee et al. 2006; Lee and Park 2013; Hori et al. 2014) complexes. Coupled with frequent hybridization, even between sexual and apomictic taxa (Ekrt et al. 2009; Bär and Eschelmüller 2010; Ekrt and Koutecký 2016), this genus has been the source of much understanding we have about ferns. Recently, a review was published summarizing the progress made on the research of *Dryopteris* around the globe (Sessa et al. 2015).

Perhaps one of the most studied fern polyploid complexes is the *D. carthusiana* complex. In the Czech Republic the complex includes two tetraploids, *D. carthusiana*, *D. dilatata*, and one diploid, *D. expansa*. Several hypotheses have been raised on the origin of *D. carthusiana*, however, the “semicristata” hypothesis is currently the most supported (Stein et al. 2010; Sessa et al. 2012a). Walker (1955) recognized that *D. carthusiana* and a related tetraploid *D. cristata* share a common ancestral diploid. This diploid was later determined as an extinct or yet undiscovered taxon named “*D. semicristata*” (Wagner 1971). For *D. carthusiana*, the other diploid ancestor is known to be *D. intermedia* (Stein et al. 2010; Sessa et al. 2012a). *D. intermedia* is also one of the parents of the tetraploid *D. dilatata*, the other parent being *D. expansa* (Sessa et al. 2012a).

Dryopteris filix-mas is the type species of the genus, nevertheless, the origin of this tetraploid is not clear. While autoploidy was suggested (Wagner 1971), the allopolyploid origin is widely accepted. The prevailing hypothesis lists *D. oreades* and *D. caucasica* as the two parents (Fraser-Jenkins 1976; Fraser-Jenkins and Widén 2006; Sessa et al. 2012a).

Closely related to *D. filix-mas*, is the apomictic *Dryopteris affinis* complex. *D. affinis* itself is hypothesized to be an allodiploid with *D. oreades* and an unknown sexual diploid close to *D. wallichiana* being the parental taxa (Widén et al. 1996). In turn *D. affinis* possibly plays a role in the origin of the triploid *D. borrieri* and *D. cambrensis*, the other parents being *D. caucasica* and *D. oreades*, respectively (Widén et al. 1996). The origin of *D. remota* is yet

to be thoroughly studied; however, allopolyploid origin is hypothesized as a result of hybridization between *D. affinis* and *D. expansa* (Krause 1998). This hypothesis failed to receive support by Sessa et al. (2012a), suggesting instead “*D. semicristata*” as one of the parents.

2.5.3) *Athyrium*

Athyrium is a major genus belonging to the family *Athyriaceae* (PPG I 2016). Traditionally, this genus has been merged together with several other genera into *Woodsiaceae* where it formed a major component (Smith et al. 2006). The genus is composed of 180-230 species worldwide (Kramer et Kato 1990; Wang et Kato 2013; PPG I 2016). Blockeel (2006) lists three European taxa – *A. distentifolium*, *A. filix-femina*, *A. flexile*. However, *A. flexile* is considered a variant of *A. distentifolium* (McHaffie et al. 2001). Apart from the aforementioned *A. flexile*, the Czech flora contains the remaining European species of *Athyrium* (Daníhelka et al. 2012), both diploid. *A. filix-femina* and *A. distentifolium* are known to produce a hybrid, *A. ×reichsteinii* (Schneller and Rasbach 1984).

2.5.4) *Gymnocarpium*

Gymnocarpium is a small genus belonging to the family *Cystopteridaceae* (PPG I 2016). The genus consists of seven to eight species depending on the delimitation of *G. jessoense* (Rothfels 2012; PPG I 2016). Two subspecies are recognized for this taxon. However, based on differences in ploidy levels, the two subspecies should be treated as distinct species (Sarvela et al 1981; Rothfels et al. 2012). Three tetraploid species of *Gymnocarpium* grow in Europe - *G. continentale* (*G. jessoense* subsp. *parvulum*), *G. dryopteris* and *G. robertianum* (Pellinen et al. 1999; Blockeel 2006). The latter two are also present in the Czech Republic (Daníhelka et al. 2012). Two European species, *G. continentale* and *G. dryopteris* hybridize to produce *G. ×intermedium* (Pryer et al. 1984).

The origin of *G. dryopteris* is well understood. This allotetraploid arose from *G. disjunctum* and the North American endemic *G. appalachianum* (Rothfels et al. 2014).

2.5.5) *Phegopteris*

Phegopteris is a small genus belonging to the family *Thelypteridaceae* (PPG I 2016). Some authors have suggested merging *Phegopteris* with its paleotropical sister genus *Pseudophegopteris* (Christenhusz and Chase 2014), however, most authors maintain the distinction. While several species of *Phegopteris* were described, mainly from Asia (e.g. *P. koreana* - Kim et al. 2004), three to four main species are recognized (Driscoll et al. 2003; Schneider et al. 2013; PPG I 2016). *P. connectilis* is the only species occurring in Europe (Blockeel 2006) and the Czech Republic (Danihelka et al. 2012).

While in Europe *P. connectilis* has only been found as an apomictic triploid (Manton 1950; Ivanova and Piekos-Mirkowa 2003), apomictic tetraploid and sexual diploid races are also known (Hirabayashi 1969; Mulligan et al. 1972). Nevertheless, diploid and tetraploid plants are quite rare even in their areas of occurrence, in Japan and North America respectively (Mulligan et al. 1979; Matsumoto 1982).

The origin of the triploid is unknown. On the other hand, as per its Northern American distribution, the tetraploid was hypothesized to have arisen from an unreduced spore of triploid *P. connectilis* and a reduced spore of sexual diploid *P. hexagonoptera* (Mulligan et al. 1972). However, when tested, this hypothesis failed to gain support (Driscoll et al. 2013).

3) Materials and methods

3.1) 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 (Tab. 2).

Tab. 2: A list of taxa under study with characteristics described (ploidy level, mode of reproduction, hybrid status).

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

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 Kouřecký 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. Studied taxa were determined using flow cytometry (for the methods see Ekrt and Štech 2008; Ekrt et al. 2009, 2010) or morphological characters. 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 (Appendix 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.

3.2) 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 microscope (LABO COMFORT 1502, Arsenal) was used to determine the viability of spores under 400x 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.

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.

3.3) 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 arcsin transformed for every performed Nested ANOVA and no samples were excluded from their respective analyses. All abovementioned analyses were performed using Statistica 13 (Statsoft 2016).

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 calculated. This pair of selections was performed 9999 times for a total of 10000 numbers. The sample difference between two parts of the frond was then compared with the modelled 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).

4) Results

4.1) 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-d) aborted spores are completely black and vary greatly in size sometimes being much larger than developed spores. These spores are 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*.

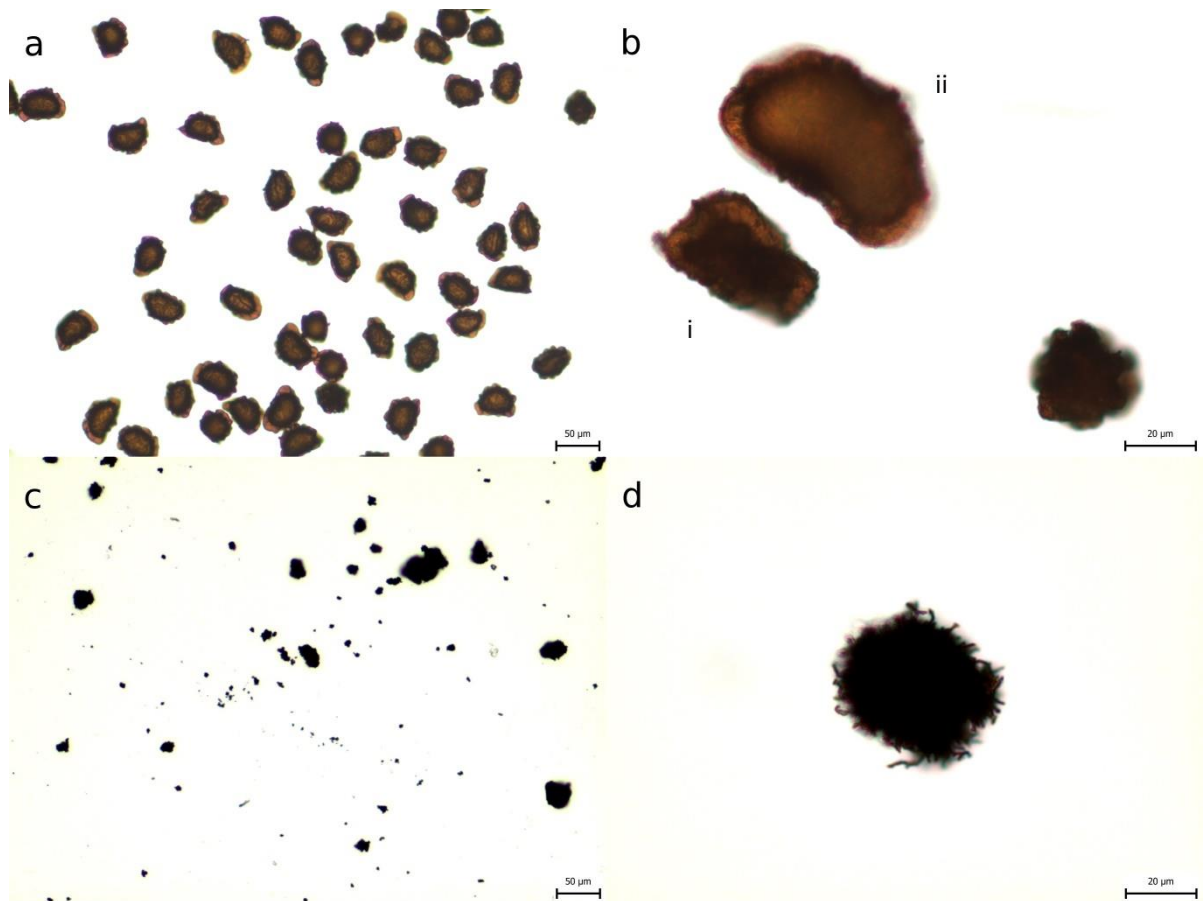


Fig. 1: Different types of spores and its variability in plants under study a: *Dryopteris filix-mas*: Developed light brown spores observed in most non-hybrid sexual taxa. b: *D. borrieri*: i) aborted spore present in darker colors in apomictic taxa ii) well developed spore, typical for non-hybrid taxa c: *D. ×ambroseae*: Black irregularly shaped and sized aborted spores typical for hybrids with debris scattered around. d: *D. ×deweveri*: a close up on an aborted spore of a hybrid taxon.

4.2) Spore abortion index

A variety of SAI values were obtained from the 109 samples tested, ranging from less than 1% to 100% (Tab. 3).

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

Of the total 23 taxa sampled, the diploid sexual *Athyrium filix-femina* has the lowest mean SAI, with all samples having less than 1% of aborted spores (mean SAI = 0.76%). The sexual tetraploid *Gymnocarpium dryopteris* has mean abortion rate of 1.04 %, whilst 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 less than 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. borneri*, sample 5). The values of SAI of the apomictic *P. connectilis* are similar to SAI of studied *Dryopteris* apomictic taxa (Nested ANOVA: 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 of SAI between various modes of reproduction is shown in Fig. 2.

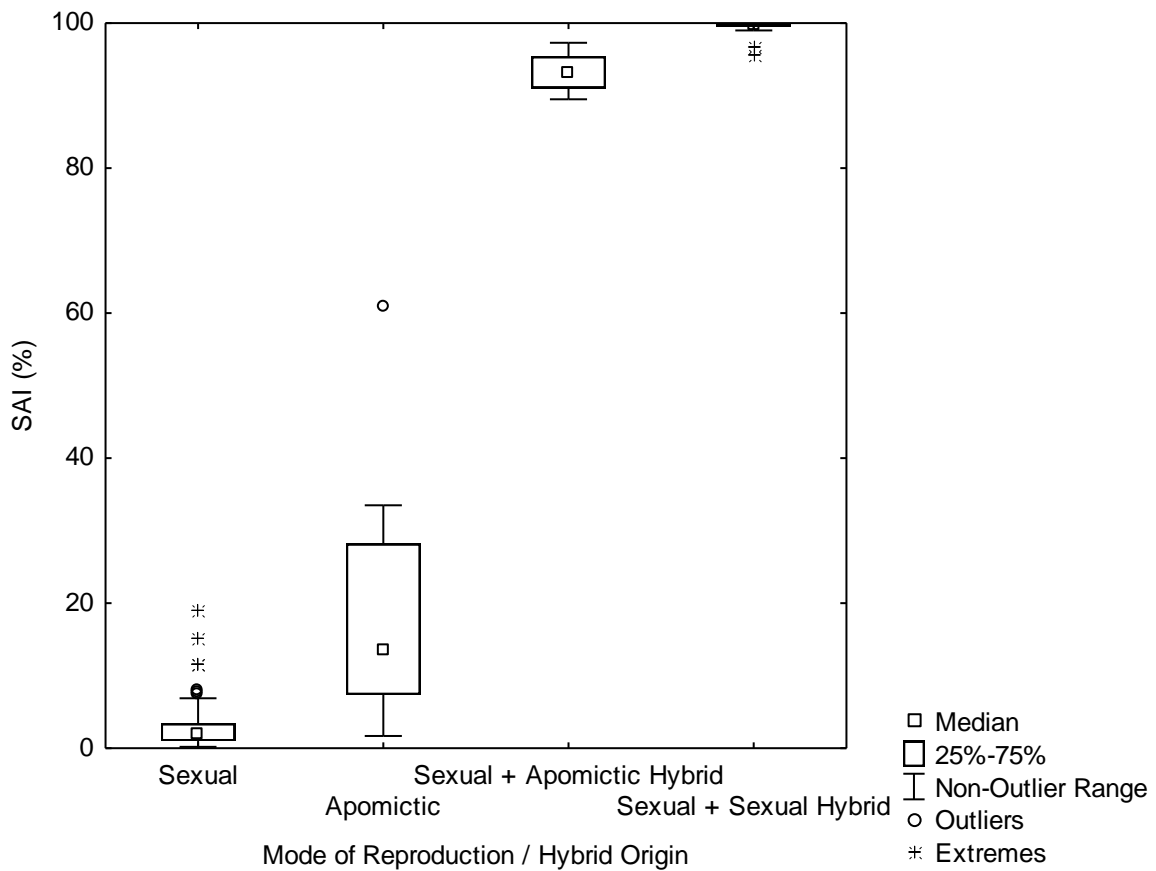


Fig. 2: Spore abortion index (SAI) for all reproduction modes using all samples.

4.3) Comparing SAI of different groups

Highly significant differences in SAI exist between sexual and apomictic taxa of the genus *Dryopteris* (Nested ANOVA: 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.

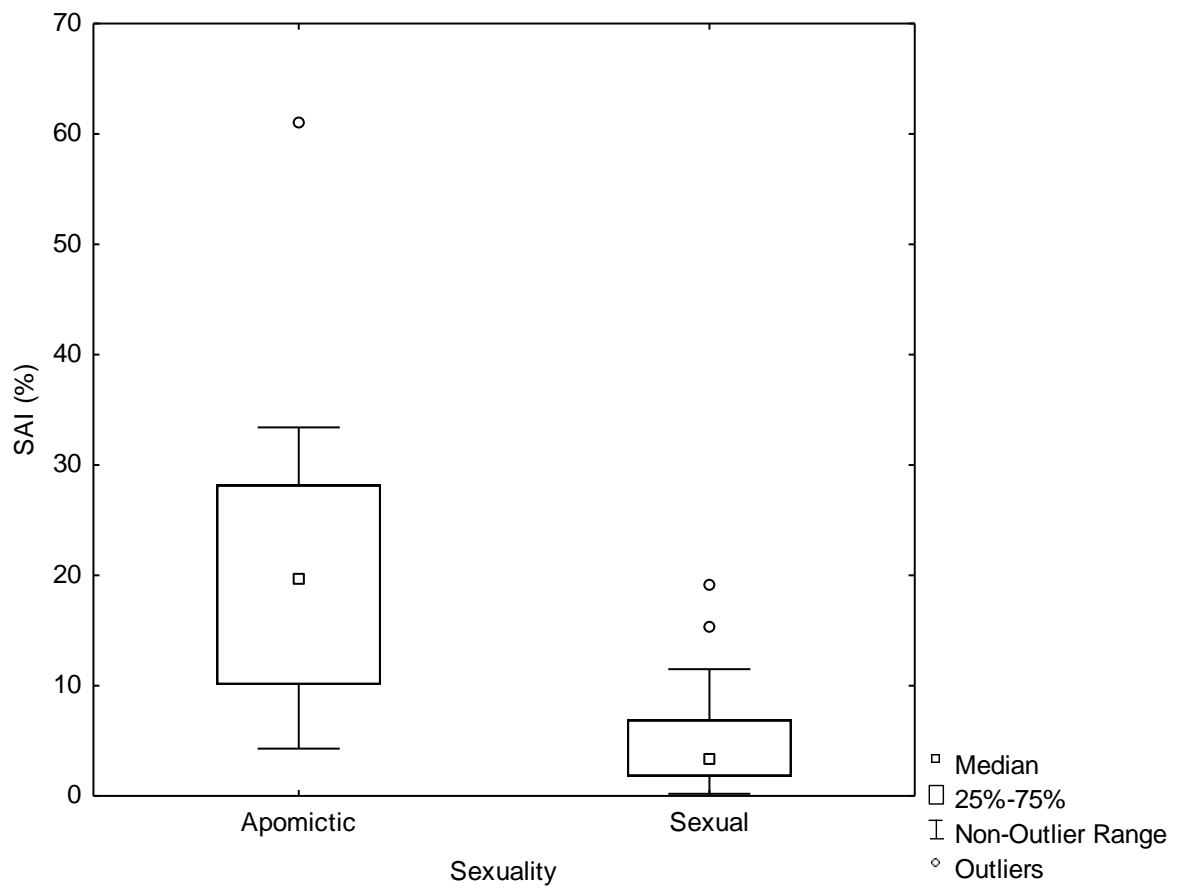


Fig. 3: Spore abortion index (SAI) of sexual and apomictic taxa of the genus *Dryopteris*.

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 (Nested ANOVA: 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 (Fig 4).

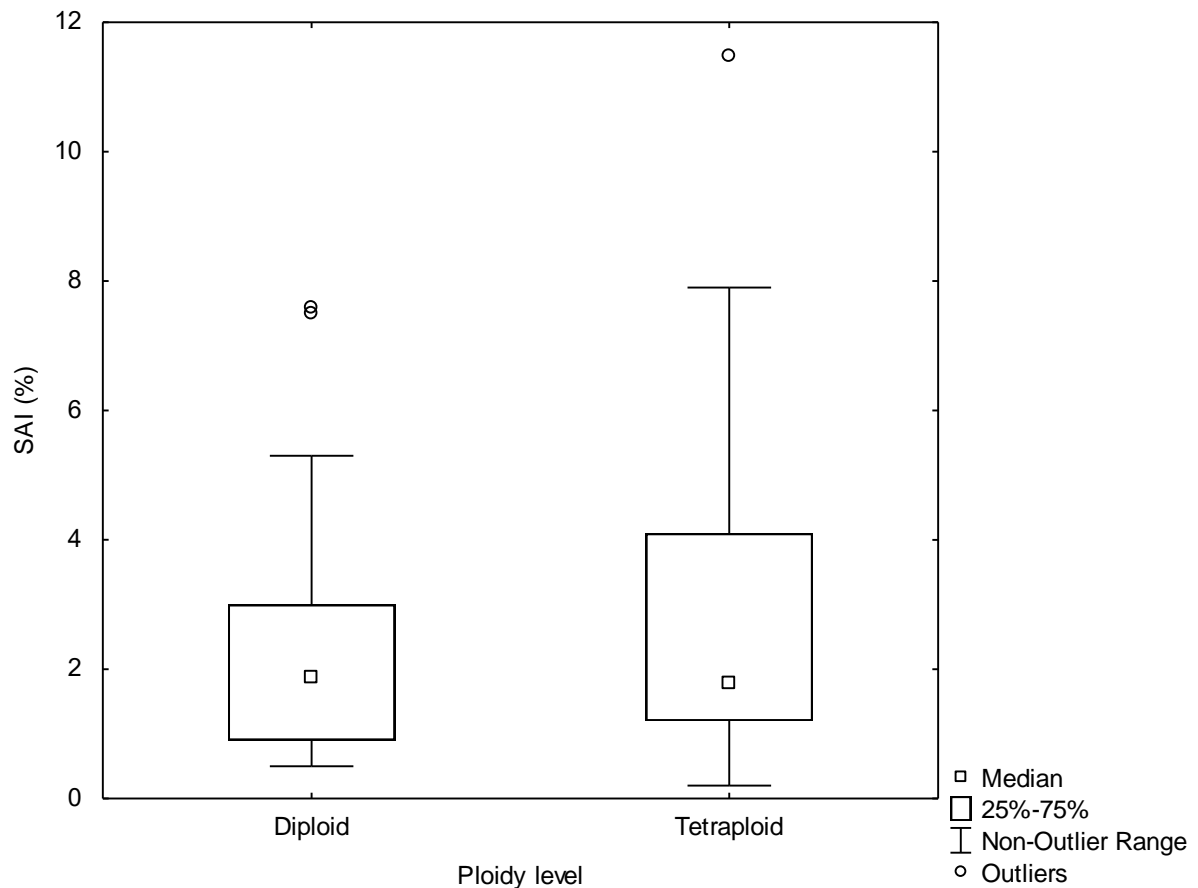


Fig. 4: Spore abortion index (SAI) of diploid and tetraploid sexual taxa.

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 (Nested ANOVA: species: $p=0.3357$, hybrid origin: $p=0.0012$). Median SAI value for *Dryopteris ×critica* is 93.05% while other hybrids (with both progenitors sexually reproducing) 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. ×deweeveri* having SAI of 95.6% and 96.6%, respectively (Fig. 5).

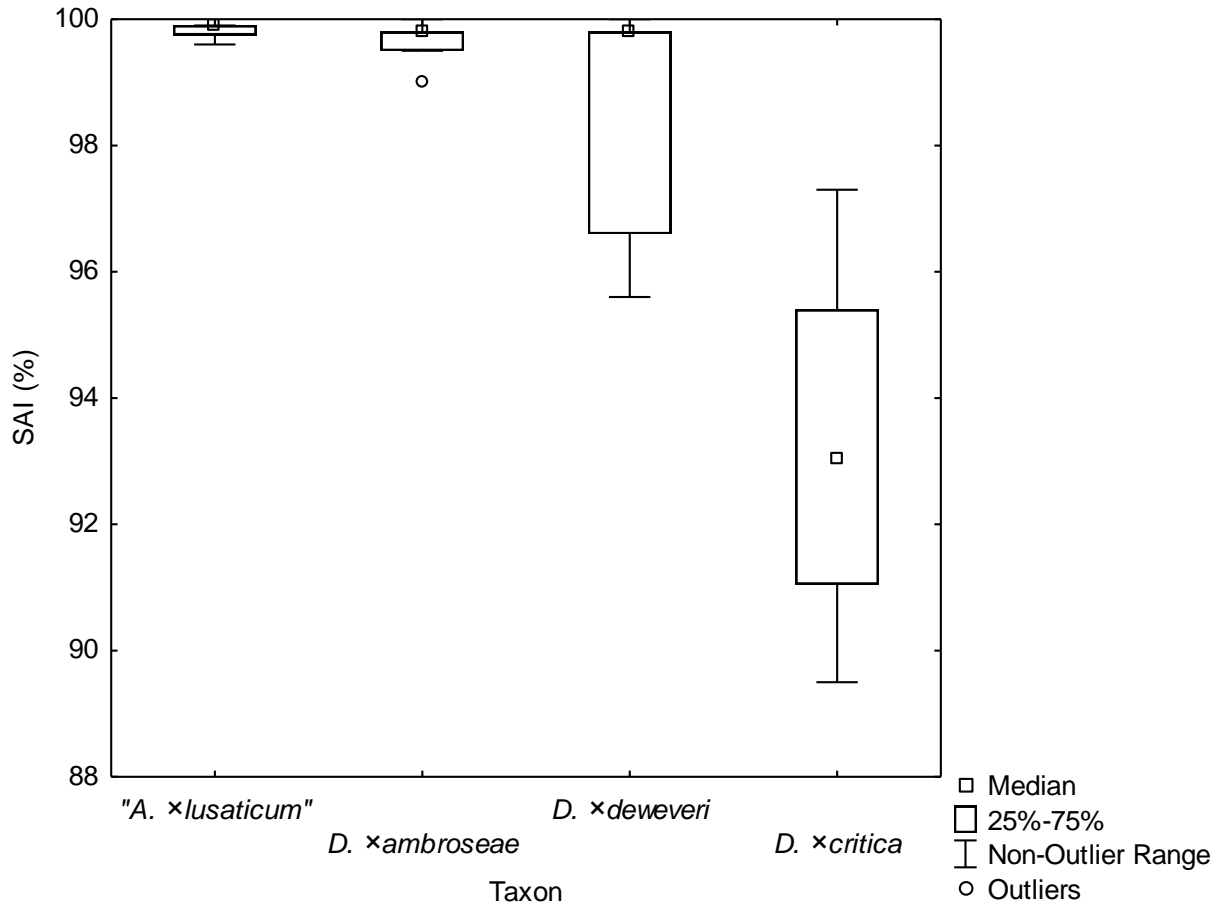


Fig. 5: Spore abortion index (SAI) of hybrid taxa under study, the name “*A. x lusaticum*” refers to the hybrid *Asplenium trichomanes* nothosubsp. *lusaticum*.

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 (Fig. 6).

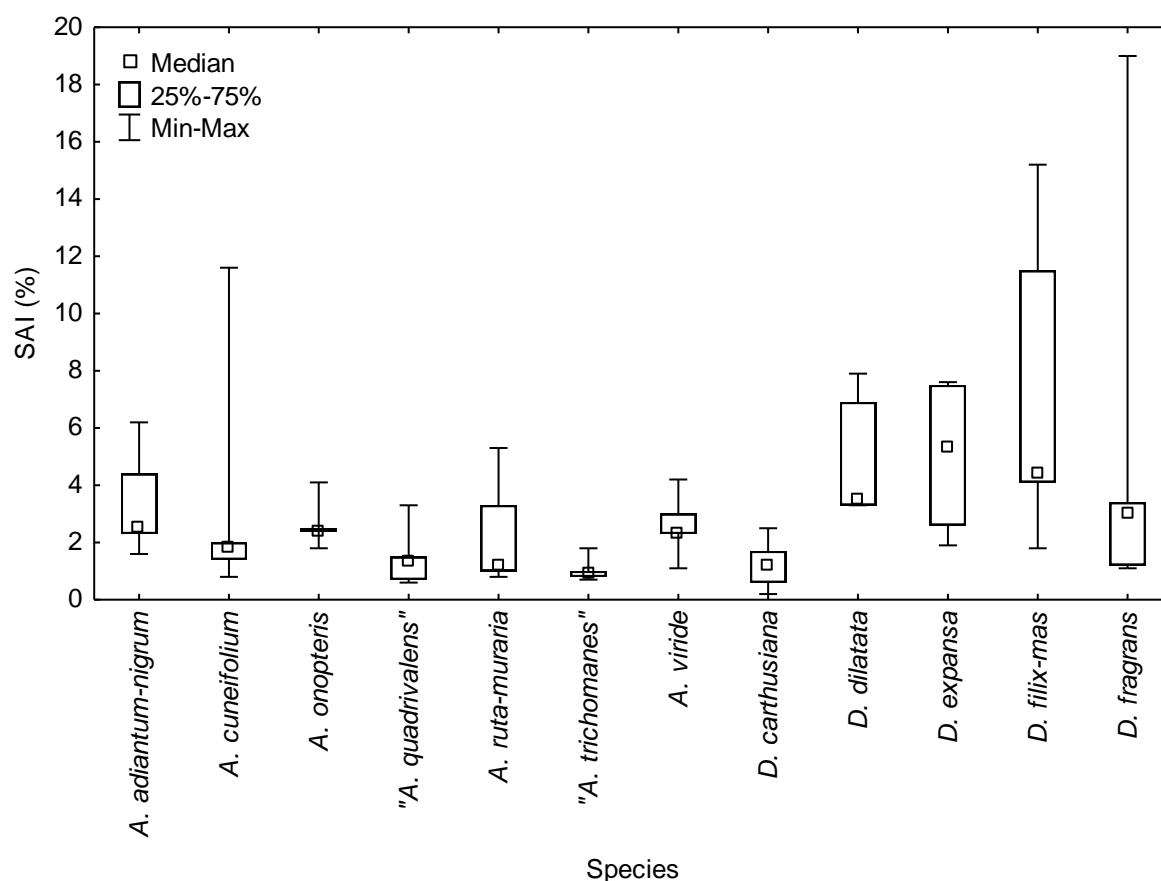


Fig. 6: Spore abortion index (SAI) of individual sexual species of *Asplenium* and *Dryopteris*. “*A. quadrivalens*” and “*A. trichomanes*” refer to *A. trichomanes* subsp. *quadrivalens* and *A. trichomanes* subsp. *trichomanes*, respectively.

4.5): Optimization of SAI assessment method

Standard error of estimate was calculated (Tab. 4). 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.

Tab. 4: 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%

The change of the difference between cumulatively calculated partial SAI and total SAI demonstrates the variance in data (Fig. 7). The following taxa represent the different levels of mean deviation from total SAI in increasing order. The hybrid *D. ×ambroseae* (Fig 7a) is very uniform and SAI never differs more than 1% from total SAI. The example of *A. ruta-muraria* (Fig 7b) shows little change of 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 7c) and *D. cambrensis* (Fig 7d), respectively.

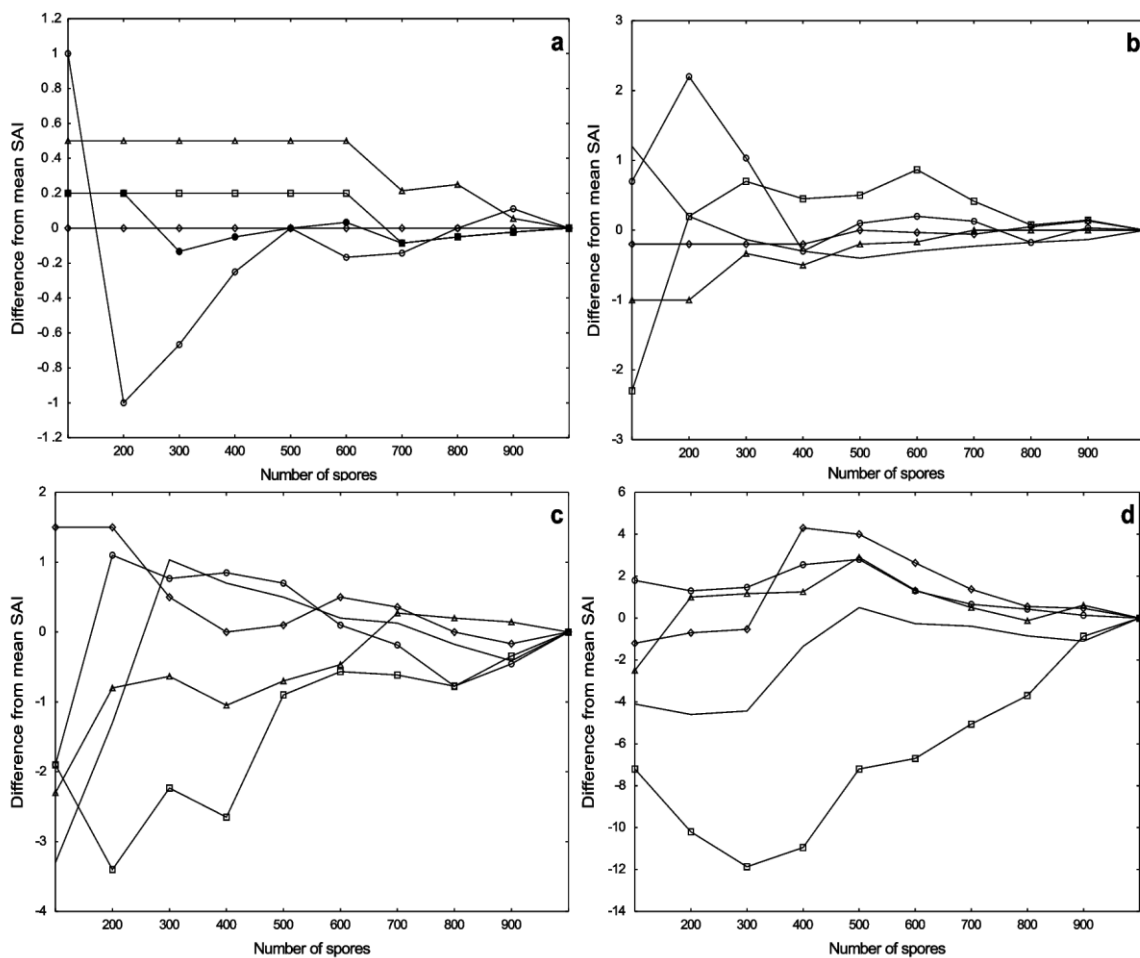


Fig. 7: The change of the difference between partial and total SAI (%) with the increasing amount of spores used to calculate partial SAI. The change is presented for each sample in *Dryopteris ×ambroseae* (a), *Asplenium ruta-muraria* (b), *D. dilatata* (c) and *D. cambrensis* (d).

After p-value adjustment, 12 of 109 (11%) samples significantly differed ($p < 0.05$) between the two sets of 500 spores, each estimated from a distinct part of the frond, hence the SAI value of these plants varies within the frond. Furthermore, 10 plants are marginally significantly different ($0.05 < p < 0.1$). Of the significantly differing plants, eight were apomicts, three 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 Appendix 2 for the results of individual tests alongside other measures of variation within sample.

5) Discussion

5.1) Effects of reproduction mode on spore abortion

The 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 a 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 multiple 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). The thesis results with a robust 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* (Guo and Liu 2013), tetraploid *Pteris vittata* with stated 100% germination rate, and therefore supposed 0% SAI (Khare and Kaur 1983). High germination rates and less than 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* (Hernández et al. 2015).

In the thesis, 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 reported to have comparable SAI (mostly around or below 5%) and germination rates to sexual *Dryopteris* species (Quintanilla and Escudero 2006). To my 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* and *Woodwardia radicans*. 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, the thesis 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 *Asplenium aethiopicum*, 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, the thesis 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. borrieri*. 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* (Pinter 1995), *Osmunda* (Yatabe et al. 2011) and *Cystopteris* (Kawakami et al. 2010; Hanušová and Ekrt, unpublished). Further detailed reproductive

studies are needed to fully understand this problem and a standardization of the SAI estimate method may help in future endeavors.

5.2) Effects of ploidy levels on spore abortion

No difference in SAI was observed in the thesis between sexual diploids and tetraploids. Similar results were reached in several other studies. This factor had no effect on germination rates of herbaria specimens of *Pellaea* (Windham and Ranker 1986). In *Psilotum nudum*, 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* presuming that the increase in SAI is a result of a relatively recent origin of the not yet stabilized allotetraploid. Some of my 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* (Kott and Peterson 1973) and *Isoëtes*, 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.

5.3) Different rates of spore abortion among genera

The thesis 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. 2012b). The marginally significant differences could reflect different habitat preferences or different position on phylogeny tree. Spore retention during the season may also be reflected in SAI estimates. Parts of the frond that have already shed the majority of spores appear to have proportionally higher SAI (pers. obs.). Misshapen aborted spores may be retained more within the sporangial mass thus artificially inflate SAI estimates to some degree.

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

Estimation of SAI was employed 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 the results I 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 many 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 my knowledge, common practice. Therefore, using at least two distinct parts of the frond is suitable, at least for apomictic taxa. I also recommend avoiding fronds or parts of fronds, that have already shed a majority of spores as well 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.

6) Conclusions

I have conducted the first major comparative study featuring a total of 23 fern taxa in order to compare spore abortion rates among several reproductive and phylogenetic groups. The collected data also allowed me to optimize the method for future use.

The key results of this thesis are:

- 1) Apomictic taxa form aborted spores with a higher frequency (18%) than sexual taxa (3%).
- 2) Ploidy level does not affect spore abortion among sexual taxa.
- 3) The hybrid of apomictic and sexual taxa forms significantly less aborted spores (93.1%) than hybrids of sexual taxa only (99.8%).
- 4) The sexual taxa of genera *Asplenium* and *Dryopteris* marginally significantly differ in spore abortion, forming 1.8% and 3.3% of aborted spores, respectively.
- 5) At least 1000 spores per sample are needed for proper spore abortion assessment and two distinct parts of the frond should be used, if possible.

In this thesis the method was applied to compare various reproductive and phylogenetic groups. Apomicts form a considerably higher proportion of aborted spores. This fact sparks a range of further questions on advantages and disadvantages of apomixis in ferns and the resulting trade-off. Diploid and polyploid taxa do not seem to differ in spore abortion; however, the results of various published germination test do not always reflect this. Additionally, hybrids of apomictic and sexual taxa produce a considerable proportion of developed spores. This fact opens up a plethora of topics for further research, such as the possibility of backcrosses and diploidization, which so far received little attention in ferns. Finally, there may be differences in spore abortion among genera. While the causal factors behind this difference remain unclear, future studies may help elucidate this problem.

This thesis demonstrates that working with spores can be a convenient and relatively expedient method of fern study and spore abortion assessment can surely find a suitable application in further studies. However, prior to this study, there was no unified approach and this made it difficult to properly compare the results by different authors. The thesis provides several key recommendations on the proper assessment of SAI and will hopefully help with future research in fern biology.

7) References

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8) Supplementary material

Appendix 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
<i>Asplenium adiantum-nigrum</i>	1	0.025	2014-09-11	Ekrt, L.	GBR: Wales, Abergwynnregyn - 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
<i>Asplenium adiantum-nigrum</i>	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
<i>Asplenium adiantum-nigrum</i>	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
<i>Asplenium adiantum-nigrum</i>	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
<i>Asplenium adiantum-nigrum</i>	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
<i>Asplenium cuneifolium</i>	1	0.02	1927-10-09	Suza, J.	CZE: Moravia occid. Tišnov: in valle rivi Libochovka prope p. Rojetín in fissuris rupium serpentinacearum.; OP
<i>Asplenium cuneifolium</i>	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
<i>Asplenium cuneifolium</i>	3	0.008	1937-08	Laus, H.	CZE: M. Schönberg auf Serpentin b. Nikles.; OP
<i>Asplenium cuneifolium</i>	4	0.014	1906-06	Servit, M.	CZE: Moravia austro-occidentalis: montes Českomoravská vysočina, in rupibus et lapidibus serpentinicis prope Rožná.; OP
<i>Asplenium cuneifolium</i>	5	0.116	1929-09	Wihan, R.	CZE: Bohemia septentrionali-occidentalis: montes Čísařský Les (Kaiserwald), in monte Wolfstein prope thermas Mariánské Lázně (Marienbad), solo serpentinico, 900 m n.m.; OP
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	1	0.996	1949-09-05	Medlinová, M.	BGR: Vápencové skály - záp. Rhodopy; PR
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	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
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	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
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	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
<i>Asplenium onopteris</i>	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
<i>Asplenium onopteris</i>	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

Taxon	ID	SAI	Date	Collector	Location
					Cueva del Viento, 890 m s.m.; 28°20'16"N, 016°42'19"E; CBFS
<i>Asplenium onopteris</i>	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
<i>Asplenium onopteris</i>	4	0.041	s. d.	Fernandes, A.; Fernandes, R.; Mates, J.	PRT: Estrada de Saboia, Barrance de Pisees (Algarve); PR
<i>Asplenium onopteris</i>	5	0.018	1949-09-05	Medlinová, M.	BGR: Vápencové skály - záp. Rhodopy; PR
<i>Asplenium ruta-muraria</i>	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
<i>Asplenium ruta-muraria</i>	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
<i>Asplenium ruta-muraria</i>	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
<i>Asplenium ruta-muraria</i>	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
<i>Asplenium ruta-muraria</i>	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
<i>Asplenium trichomanes</i> subsp. <i>quadrivalens</i>	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
<i>Asplenium trichomanes</i> subsp. <i>quadrivalens</i>	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
<i>Asplenium trichomanes</i> subsp. <i>quadrivalens</i>	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
<i>Asplenium trichomanes</i> subsp. <i>quadrivalens</i>	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
<i>Asplenium trichomanes</i> subsp. <i>quadrivalens</i>	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
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	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
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	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

Taxon	ID	SAI	Date	Collector	Location
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	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
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	4	0.007	s. d.	Deyl, M.	CZE:Bohemia occidentalis: In rupibus serpentinicis Schwarzholz et Steinhügel dictis inter vicos Drahotín et Poběžovice.; CBFS
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	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
<i>Asplenium viride</i>	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
<i>Asplenium viride</i>	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
<i>Asplenium viride</i>	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
<i>Asplenium viride</i>	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
<i>Asplenium viride</i>	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
<i>Athyrium filix-femina</i>	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
<i>Athyrium filix-femina</i>	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
<i>Athyrium filix-femina</i>	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
<i>Athyrium filix-femina</i>	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
<i>Athyrium filix-femina</i>	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
<i>Dryopteris affinis</i>	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
<i>Dryopteris affinis</i>	2	0.335	1869-09-01	Meandon, G.	PRT: Pl. Maderenses In umborsis S. Antioio das Serrera.; PR
<i>Dryopteris affinis</i>	3	0.017	1899-08	Lösch, A.	DEU: Flora von Baden. In silvis umborsis et humosis montis nigri meridionalis prope Zastler.; PR
<i>Dryopteris borrieri</i>	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

Taxon	ID	SAI	Date	Collector	Location
<i>Dryopteris borrieri</i>	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
<i>Dryopteris borrieri</i>	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
<i>Dryopteris borrieri</i>	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
<i>Dryopteris borrieri</i>	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
<i>Dryopteris cambrensis</i>	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
<i>Dryopteris cambrensis</i>	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
<i>Dryopteris cambrensis</i>	3	0.072	2008-10-15	Ekrt, L.	CZE: 33. Branžovský hvozď: 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
<i>Dryopteris cambrensis</i>	4	0.135	2007-09-17	Ekrt, L.	CZE: Krnov, Brantice - ca 1,5 km SE of the church in the village of Brantice; 50°03'09"N, 017°38'16"E; CBFS
<i>Dryopteris cambrensis</i>	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
<i>Dryopteris carthusiana</i>	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
<i>Dryopteris carthusiana</i>	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
<i>Dryopteris carthusiana</i>	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
<i>Dryopteris carthusiana</i>	4	0.025	1939-07-07	Deyl, M.	CZE: Bohemia australis; In silvis turfosis Blata dictis prope vicum Mažice procul oppidum Soběslav.; PR
<i>Dryopteris carthusiana</i>	5	0.002	1954-07-31	Klásterský, I.	CZE: Bohemia meridionalis: circulus Sušice: in declivibus saxosis in valle fluvii Otava (Vydra) ad Turnerova chata, 790 m s.m.; PR
<i>Dryopteris dilatata</i>	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
<i>Dryopteris dilatata</i>	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
<i>Dryopteris dilatata</i>	3	0.069	2005-08-08	Ekrt, L.	CZE: Kostelní Myslová - souh edge of the Velký Hulišský 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
<i>Dryopteris dilatata</i>	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

Taxon	ID	SAI	Date	Collector	Location
<i>Dryopteris dilatata</i>	5	0.035	1975-07-07	Hájková, A.	CZE: Podbeskydská pahorkatina, Vyšní Lhoty Prašivá, údolí potoka Hliseník; 6376d; CBFS
<i>Dryopteris expansa</i>	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
<i>Dryopteris expansa</i>	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
<i>Dryopteris expansa</i>	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
<i>Dryopteris expansa</i>	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
<i>Dryopteris expansa</i>	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
<i>Dryopteris filix-mas</i>	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
<i>Dryopteris filix-mas</i>	2	0.044	2014-08-21	Hornych, O.	CZE: Rejstejn: 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
<i>Dryopteris filix-mas</i>	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
<i>Dryopteris filix-mas</i>	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
<i>Dryopteris filix-mas</i>	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
<i>Dryopteris fragrans</i>	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
<i>Dryopteris fragrans</i>	2	0.03	1968-10-05	Vašák, V.	RUS: Buriatia: in locis humidis apud rivulum 15 km versus septentr. Ab oppido Ulan Ude.; PR
<i>Dryopteris fragrans</i>	3	0.011	1913-06-08	Gorbovetz, I.	RUS: Prov. Krasnojarsk. prope pag. Ossipovo. In rupium umbrosorum fissuris ad ripam meridionalen fl. Ossipovka.; PR
<i>Dryopteris fragrans</i>	4	0.19	1867	Fowler	CAN: Canada: Mount Prospect Resteonche.; PR
<i>Dryopteris fragrans</i>	5	0.034	1967-09-04	Egorova, Alekseeva	RUS: Sachalin skály v lesu na sopke blíž Gomona.; PR
<i>Dryopteris remota</i>	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

Taxon	ID	SAI	Date	Collector	Location
<i>Dryopteris remota</i>	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
<i>Dryopteris remota</i>	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
<i>Dryopteris ×ambroseae</i>	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
<i>Dryopteris ×ambroseae</i>	2	0.998	2015-07-17	Hornych, O.	CZE: Strážné: Lesní potok 1,1 km VSV od rozcestí "Hřiběcí boudy", 900 m n.m.; 50°40'55"N, 015°38'27"E; CBFS
<i>Dryopteris ×ambroseae</i>	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
<i>Dryopteris ×ambroseae</i>	4	0.995	2005-09-06	Ekrt, L.	CZE: Teplice nad Metují town - NPR Teplické skály rocks, Teplické skalní město, Sibíř 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
<i>Dryopteris ×ambroseae</i>	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
<i>Dryopteris ×critica</i>	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
<i>Dryopteris ×critica</i>	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
<i>Dryopteris ×critica</i>	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
<i>Dryopteris ×critica</i>	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
<i>Dryopteris ×deweveri</i>	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
<i>Dryopteris ×deweveri</i>	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
<i>Dryopteris ×deweveri</i>	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
<i>Dryopteris ×deweveri</i>	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
<i>Dryopteris ×deweveri</i>	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
<i>Gymnocarpium dryopteris</i>	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

Taxon	ID	SAI	Date	Collector	Location
<i>Gymnocarpium dryopteris</i>	2	0.015	2015-07-17	Hornych, O.	CZE: Strážné: Lesní potok 1,1 km SV od rozcestí "Hřiběcí boudy", 925 m n.m.; 50°40'57"N, 015°38'26"E; CBFS
<i>Gymnocarpium dryopteris</i>	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
<i>Gymnocarpium dryopteris</i>	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
<i>Gymnocarpium dryopteris</i>	5	0.003	1998-08-14	Ekrt, L.	NOR: Rondane Rondane National Park, area near Selsverket town; CBFS
<i>Phegopteris connectilis</i>	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 Skrychově, 700 m n.m.; 49°09'42"N, 015°19'30"E; CBFS
<i>Phegopteris connectilis</i>	2	0.074	2014-07-24	Hornych, O.	CZE: České Budějovice: Podmáčená smrčina u PP Kaliště mezi Kalištěmi a Zalinami, 1 km ZJZ od kostela v obci Zaliny, 500 m n.m.; 48°57'26"N, 014°35'28"E; CBFS
<i>Phegopteris connectilis</i>	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
<i>Phegopteris connectilis</i>	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
<i>Phegopteris connectilis</i>	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

Appendix 2 Total SAI (%) and two measures of variation (%) for all samples used in the study. Range of SAI within 100s represents the difference between the highest and the lowest SAI value for individual sets of 100 spores within a sample. Difference between 500s expresses the difference between two distinct parts of the frond, this difference is tested and adjusted p value presented.

Taxon	ID within taxon	Total SAI	Range of SAI within 100s	Difference between 500s	Adjusted p-value
<i>Asplenium adiantum-nigrum</i>	1	2.5	0-5	0.6	0.7815
<i>Asplenium adiantum-nigrum</i>	2	1.6	0-4	0.4	0.9079
<i>Asplenium adiantum-nigrum</i>	3	4.4	2-7	0.8	0.8122
<i>Asplenium adiantum-nigrum</i>	4	6.2	4-9	0.4	0.9871
<i>Asplenium adiantum-nigrum</i>	5	2.3	0-5	0.2	0.9834
<i>Asplenium cuneifolium</i>	1	2	1-4	0	1
<i>Asplenium cuneifolium</i>	2	1.8	0-5	0.8	0.5522
<i>Asplenium cuneifolium</i>	3	0.8	0-2	0	1
<i>Asplenium cuneifolium</i>	4	1.4	0-3	2	0.0559
<i>Asplenium cuneifolium</i>	5	11.6	5-16	3.6	0.2510
<i>Asplenium onopteris</i>	1	2.4	0-4	0.4	0.8382
<i>Asplenium onopteris</i>	2	2.4	1-5	2	0.1662
<i>Asplenium onopteris</i>	3	2.5	0-6	1.8	0.2449
<i>Asplenium onopteris</i>	4	4.1	0-9	2.2	0.2619
<i>Asplenium onopteris</i>	5	1.8	0-4	1.6	0.2449
<i>Asplenium ruta-muraria</i>	1	3.3	1-6	1	0.7159
<i>Asplenium ruta-muraria</i>	2	0.8	0-2	0.8	0.5038
<i>Asplenium ruta-muraria</i>	3	1	0-2	0.4	0.8382
<i>Asplenium ruta-muraria</i>	4	1.2	0-2	0	1
<i>Asplenium ruta-muraria</i>	5	5.3	1-9	0.2	1
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	1	99.6	98-100	0.8	0.2510
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	2	99.9	99-100	0.2	0.7815
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	3	99.9	99-100	0.2	0.7815
<i>Asplenium trichomanes</i> nothosubsp. <i>lusaticum</i>	4	99.9	99-100	0.2	0.7815
<i>Asplenium trichomanes</i> subsp. <i>quadri-valens</i>	1	3.3	1-5	0.2	0.9871
<i>Asplenium trichomanes</i> subsp. <i>quadri-valens</i>	2	0.6	0-2	0.8	0.3802
<i>Asplenium trichomanes</i> subsp. <i>quadri-valens</i>	3	1.5	0-5	1.4	0.1923
<i>Asplenium trichomanes</i> subsp. <i>quadri-valens</i>	4	0.7	0-2	0.6	0.6187
<i>Asplenium trichomanes</i> subsp. <i>quadri-valens</i>	5	1.3	0-4	1.4	0.2000
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	1	0.9	0-2	0.2	0.9871
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	2	1	0-4	0.4	0.8382
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	3	0.8	0-3	0.4	0.8122
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	4	0.7	0-2	1	0.2574

Taxon	ID within taxon	Total SAI	Range of SAI within 100s	Difference between 500s	Adjusted p-value
<i>Asplenium trichomanes</i> subsp. <i>trichomanes</i>	5	1.8	0-4	1.6	0.2449
<i>Asplenium viride</i>	1	4.2	0-10	0.4	0.9834
<i>Asplenium viride</i>	2	3	1-6	1.6	0.3872
<i>Asplenium viride</i>	3	1.1	0-3	0.2	0.9871
<i>Asplenium viride</i>	4	2.3	0-5	2.2	0.0946
<i>Asplenium viride</i>	5	2.3	0-5	2.2	0.0946
<i>Athyrium filix-femina</i>	1	0.9	0-2	0.2	0.9871
<i>Athyrium filix-femina</i>	2	0.6	0-2	0	1
<i>Athyrium filix-femina</i>	3	0.5	0-3	1	0.1809
<i>Athyrium filix-femina</i>	4	0.9	0-3	1	0.3229
<i>Athyrium filix-femina</i>	5	0.7	0-3	1	0.2544
<i>Dryopteris</i> × <i>ambroseae</i>	1	99	96-100	0	1
<i>Dryopteris</i> × <i>ambroseae</i>	2	99.8	98-100	0.4	0.5522
<i>Dryopteris</i> × <i>ambroseae</i>	3	100	100-100	0	1
<i>Dryopteris</i> × <i>ambroseae</i>	4	99.5	98-100	1	0.1809
<i>Dryopteris</i> × <i>ambroseae</i>	5	99.8	99-100	0	1
<i>Dryopteris</i> × <i>critica</i>	1	92.6	85-96	2	0.5282
<i>Dryopteris</i> × <i>critica</i>	2	97.3	92-100	2.6	0.0780
<i>Dryopteris</i> × <i>critica</i>	3	89.5	83-93	2.2	0.5522
<i>Dryopteris</i> × <i>critica</i>	4	93.5	90-98	1.4	0.7159
<i>Dryopteris</i> × <i>deweveri</i>	1	95.6	90-98	4	0.0248
<i>Dryopteris</i> × <i>deweveri</i>	2	100	100-100	0	1
<i>Dryopteris</i> × <i>deweveri</i>	3	99.8	98-100	0.4	0.5522
<i>Dryopteris</i> × <i>deweveri</i>	4	99.8	99-100	0.4	0.5522
<i>Dryopteris</i> × <i>deweveri</i>	5	96.6	93-99	1.6	0.4875
<i>Dryopteris affinis</i>	1	5	3-8	1.2	0.7080
<i>Dryopteris affinis</i>	2	33.5	21-44	1.4	0.8688
<i>Dryopteris affinis</i>	3	1.7	0-4	1.4	0.2616
<i>Dryopteris borrieri</i>	1	4.3	2-7	2.6	0.1809
<i>Dryopteris borrieri</i>	2	10.2	2-16	2	0.6167
<i>Dryopteris borrieri</i>	3	33.4	20-45	7.6	0.0680
<i>Dryopteris borrieri</i>	4	21.1	14-34	7	0.0559
<i>Dryopteris borrieri</i>	5	60.9	37-75	1	0.9667
<i>Dryopteris cambrensis</i>	1	28.2	13-50	14.4	0.0014
<i>Dryopteris cambrensis</i>	2	10.1	5-20	1	0.8440
<i>Dryopteris cambrensis</i>	3	7.2	1-13	5.6	0.0085
<i>Dryopteris cambrensis</i>	4	13.5	7-23	5.8	0.0559
<i>Dryopteris cambrensis</i>	5	8.2	2-27	8	0.0014
<i>Dryopteris carthusiana</i>	1	0.6	0-2	0.4	0.7815
<i>Dryopteris carthusiana</i>	2	1.2	0-3	0.8	0.5522
<i>Dryopteris carthusiana</i>	3	1.7	0-4	0.6	0.7421
<i>Dryopteris carthusiana</i>	4	2.5	1-4	0.6	0.7816
<i>Dryopteris carthusiana</i>	5	0.2	0-1	0	1
<i>Dryopteris dilatata</i>	1	3.3	0-9	1	0.7159

Taxon	ID within taxon	Total SAI	Range of SAI within 100s	Difference between 500s	Adjusted p-value
<i>Dryopteris dilatata</i>	2	3.3	1-8	1.4	0.5038
<i>Dryopteris dilatata</i>	3	6.9	2-13	1.8	0.5522
<i>Dryopteris dilatata</i>	4	7.9	3-12	1.4	0.7240
<i>Dryopteris dilatata</i>	5	3.5	1-6	0.2	0.9871
<i>Dryopteris expansa</i>	1	2.6	1-4	0.8	0.7421
<i>Dryopteris expansa</i>	2	7.6	2-13	2.8	0.2809
<i>Dryopteris expansa</i>	3	1.9	0-6	1	0.5522
<i>Dryopteris expansa</i>	4	5.3	1-14	3.4	0.0684
<i>Dryopteris expansa</i>	5	7.5	2-14	7	0.0014
<i>Dryopteris filix-mas</i>	1	11.5	8-17	0.6	0.9834
<i>Dryopteris filix-mas</i>	2	4.4	1-7	0.4	0.9834
<i>Dryopteris filix-mas</i>	3	1.8	0-5	2.4	0.0520
<i>Dryopteris filix-mas</i>	4	4.1	2-7	3	0.0839
<i>Dryopteris filix-mas</i>	5	15.2	5-23	5.2	0.1261
<i>Dryopteris fragrans</i>	1	1.2	0-5	0.8	0.5522
<i>Dryopteris fragrans</i>	2	3	1-4	1.2	0.5522
<i>Dryopteris fragrans</i>	3	1.1	0-3	0.6	0.7159
<i>Dryopteris fragrans</i>	4	19	7-41	16	0.0014
<i>Dryopteris fragrans</i>	5	3.4	0-8	4	0.0087
<i>Dryopteris remota</i>	1	30.2	13-52	12	0.0014
<i>Dryopteris remota</i>	2	20.9	7-46	18.2	0.0014
<i>Dryopteris remota</i>	3	19.6	12-28	0.4	0.9871
<i>Gymnocarpium dryopteris</i>	1	1.8	1-4	0.4	0.8848
<i>Gymnocarpium dryopteris</i>	2	1.5	0-3	0.6	0.6769
<i>Gymnocarpium dryopteris</i>	3	1.3	0-3	0.2	0.9871
<i>Gymnocarpium dryopteris</i>	4	0.4	0-2	0.4	0.7159
<i>Gymnocarpium dryopteris</i>	5	0.3	0-1	0.2	0.9554
<i>Phegopteris connectilis</i>	1	14.5	7-22	1.8	0.7379
<i>Phegopteris connectilis</i>	2	7.4	2-11	1.2	0.7815
<i>Phegopteris connectilis</i>	3	32.2	24-40	8.4	0.0463
<i>Phegopteris connectilis</i>	4	5.4	0-13	6.8	0.0014
<i>Phegopteris connectilis</i>	5	12.4	5-27	11.6	0.0014