THE NATURAL HISTORY OF MODEL ORGANISMS

The biology of C. richardii as a tool to understand plant evolution

Abstract: The fern Ceratopteris richardii has been studied as a model organism for over 50 years because it is easy to grow and has a short life cycle. In particular, as the first homosporous vascular plant for which genomic resources were developed, C. richardii has been an important system for studying plant evolution. However, we know relatively little about the natural history of C. richardii. In this article, we summarize what is known about this aspect of C. richardii, and discuss how learning more about its natural history could greatly increase our understanding of the evolution of land plants.

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Introduction

The genus Ceratopteris has a long and complicated taxonomic history. It was first described by Linnaeus under the genus Acrostichum (Linnaeus, 1764), and the name Ceratopteris was later assigned by Brongniart (Brongniart, 1821). Since then, Ceratopteris has been placed in a number of different families, with the number of species within the genus ranging between one and twelve (Lloyd, 1974). Today it is placed within Pteridaceae, one of the largest and most diverse fern families (PPG, 2016; Figure 1).

There are about ten species within Ceratopteris, which can be found throughout the tropics (Figure 2; Masuyama and Watano, 2010; Zhang et al., 2020; Yu et al., 2021). The classification of these species was made difficult by their inconsistent morphologies, and molecular methods were needed to reconstruct a backbone phylogeny for the genus (Adjie et al., 2007; Kinosian et al., 2020a). Recent work has shown that cryptic and hybrid species may be quite common in Ceratopteris, warranting a more rigorous evaluation of the relationships between species in the genus (e.g., Kinosian et al., 2020b).

Ceratopteris richardii was first developed as a model system for ferns in the 1960s and 70s, primarily because it was easy to grow in the lab and had a short life cycle (Figure 2; Pal and Pal, 1962; Pal and Pal, 1963; Klekowski, 1970; Stein, 1971; Hickok, 1973; Hickok and Klekowski, 1973; Lloyd and Warne, 1978). Many studies used spores from a Cuban vouchered collection, now known as the Hnn strain or C-fern (Hickok, 1977). Additional strains of C. richardii and the species C. thalictroides and C. pteridoides have since been developed (Hickok and Klekowski, 1974; Nakazato et al., 2006; Muthukumar et al., 2013). In the past few decades, Ceratopteris has become an important model in the study of sex determination (Eberle et al., 1995; Ganger et al., 2019; Atallah et al., 2018; Banks, 1997), apogamy (Bui et al., 2017; Cordle et al., 2010), genome structure (Nakazato et al., 2006; Baniaga and Barker, 2019), hybridization (Hickok and Klekowski, 1974; Adjie et al., 2007), reproductive barriers (Nakazato et al., 2007), developmental biology (Hou and Hill, 2002; Conway and Di Stilio, 2020; Sun and Li, 2020; Aragón-Raygoza et al., 2020), and transgenic studies in ferns (Plackett et al., 2018; Bui et al., 2015; Cannon et al., 2018).
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*Ceratopteris* has a large genome (~11 GB) and high base chromosome number (n = 39), which has partly caused genetic resources for *C. richardii* to lag behind those of other plant model systems, delaying such comparative work.

Recently, however, the first draft genome sequence for *C. richardii* was published (Marchant, 2019a), which was the first for a homosporous fern. *Ceratopteris richardii* was chosen for sequencing because, although it has a large genome compared to other plants, it is relatively small for a homosporous fern (Sessa et al., 2014; Marchant et al., 2019b).

Having a reference genome for *C. richardii* expands its research potential and builds on decades of previous work. A homosporous plant genome provides the opportunity for exploring and comparing various aspects of plant biology such as the alternation of generations, sex determination, and reproductive modes between heterosporous and homosporous plants. In addition, a reference genome for *Ceratopteris* is beneficial for the development of new markers in targeted gene sequencing or whole-genome resequencing. In turn, this makes incorporating wild collections into genomic research much easier and will help us gain a more nuanced understanding of the biology, ecology, and evolutionary history of *Ceratopteris*.

**The variable natural history of *Ceratopteris***

The model species *Ceratopteris richardii* originates in the Caribbean and Western Africa, and grows rooted or floating in shallow water (Figure 2). Indeed, all species within *Ceratopteris* grow in or near areas in the tropics that become inundated seasonally (Figures 2 and 3), mostly growing in fresh water, though they can tolerate salt water (Lloyd, 1974; Warne and Hickok, 1987).

Its sister genus, *Acrostichum*, is well-known for being able to tolerate high levels of salt as it grows in tidal and intertidal habitats (Zhang et al., 2013; Medina et al., 1990). The extent of natural salt tolerance in *Ceratopteris* is not fully understood, but salt-tolerant mutants of *C. richardii* are easy to generate in the lab (Chasan, 1992; Warne et al., 1995). Continuing to study salt tolerance in *Ceratopteris* may be beneficial in understanding the genetic mechanisms of this trait, or applying such findings to crop systems in the future.
Through much of its range, Ceratopteris inhabits ephemeral water sources. To reproduce in this fleeting habitat C. richardii has a short life cycle of about 120 days (Stein, 1971), which is much shorter than almost all ferns’ annual or multi-year life cycles. The fern life cycle, like that of all land plants, is characterized by the alternation of generations between the diploid sporophyte and haploid gametophyte. Following fertilization, a diploid zygote is formed that is temporarily reliant on the gametophyte and becomes self-sufficient over time. In the fern life cycle, the gametophyte and sporophyte generations are completely separate at certain stages. Comparatively, in bryophytes, the sporophyte generation is entirely dependent on the gametophyte, and the opposite is true in seed plants. The fern life cycle provides an opportunity to study sporophyte and gametophyte generation separately, something that is not possible in other lineages of plants.

Because ferns have independent and free-living sporophytic and gametophytic phases, there are multiple ways in which the life cycle of Ceratopteris can proceed (Haufler et al., 2016). Fertilization can occur via gametes from different plants (sporophytic outcrossing), gametes from the same plant but different gametophytes (gametophytic outcrossing), and also gametes from the same gametophyte (gametophytic selfing). Studies show evidence of outcrossing within (Nakazato et al., 2007) and among species of Ceratopteris (i.e., hybridization, Adjie et al., 2007; Kinosian et al., 2020a; Hickok and Klekowski, 1974).

Outcrossing and gametophytic sex expression in ferns is often controlled by pheromones known as antheridiogens. These gibberellin-related chemicals are released by early-germinating archegoniate gametophytes to promote antheridiate gametophyte development in immature gametophytes. This pheromone system confers some of the benefits of heterospory to homosporous plants, namely outcrossing (Bateman and DiMichele, 1994; Hornych et al., 2021). In the case of Ceratopteris, however, solitary gametophytes can also become bisexual and self-fertilize; therefore, theoretically, only one spore can colonize new habitats (Schedlbauer and Klekowski, 1972). This is the case for many ferns and aids Ceratopteris as a model organism because studies can be designed around this flexible life history.

In addition, asexual reproduction (apomixis) can be induced in C. richardii in the lab (Cordle et al., 2007). The variation within the life cycle of Ceratopteris makes it a powerful system in which to study reproduction in ferns, as well as an evolutionary point of reference for understanding reproduction in seed plants.

Ceratopteris was developed as a model organism for many of the same reasons as other model systems. It is easy to grow in a lab setting, has a rapid life cycle that makes experiments tangible, and is tractable for genetic transformations (Eberle et al., 1995; Hickok et al., 1995). Model organisms are often chosen for convenience, but that can make them poor representative taxa (Alfred and Baldwin, 2015).

In the case of Ceratopteris, it has several traits that are very unusual among ferns. For example, it is one of only a handful of semi-aquatic species out of around 12,000 extant ferns (PPG, 2016). The life cycle of Ceratopteris, while beneficial...
for lab experiments, is incredibly short for a fern (Stein, 1971). Finally, it has half or a quarter of the number of spores typical for a leptosporangiate fern: most leptosporangiate ferns produce 64 spores per sporangium, whereas species in Ceratopteris produce 32 or 16 spores per sporangium (Lloyd, 1974). This is important because a spore number of 32 or 16 is often indicative of apogamy (Grusz, 2016), but no natural apogamous taxa have been described in Ceratopteris. These characteristics, among others, make Ceratopteris a good model species but not necessarily an accurate representation of all ferns.

A transgenic model for seed-free plants

Free-living generations and a flexible life cycle make Ceratopteris an important model for evolutionary developmental studies. A reference ontogenetic framework for the Hnn strain of C. richardii (C-fern; Hickok et al., 1995) was recently published, detailing the development of the gametophyte and sporophyte, providing an important reference for future work (Conway and Di Stilio, 2020). This reference, in combination with stable transformation techniques, plus a C. richardii transcriptome (Geng et al., 2021; Atallah et al., 2018) and genome (Marchant et al., 2022; Marchant et al., 2019b) now provide the necessary suite of tools for comparative work.

In the Hnn strain of Ceratopteris richardii stable transgenic lines have been established in both the gametophyte and sporophyte generations. Transformation of the tissue in these plants has been accomplished by bombardment of sporophytic tissue by tungsten microparticles (Plackett et al., 2014; Plackett et al., 2015a), Agrobacterium infection of haploid gametophyte tissue (Bui et al., 2015), and agrobacterium infection of haploid spores (Muthukumar et al., 2013).

Transformation on gametophytes provides an important perspective on gene function in the haploid generation, something that is not as easy in seed plants. Another benefit of working with transformed gametophytes is that they can self-fertilize to produce sporophytes that are stable homozygotes. However, this can also be accomplished if transformation is done on sporophytes. Spores can be collected and screening of resulting gametophytes can then be used to produce stable homozygous transgenic lines via gametophytic selfing.

Thus, transgenic lines of C. richardii have been developed, and allow for comparative studies of gene function and evolution across land plants in both the gametophyte and sporophyte generations. The ability to have transgenic lines in both generations provides a unique perspective for studying how genes, growth conditions, or other factors affect sporophytes and gametophytes differently.

A recent study using both transgenic gametophytes and sporophytes of Ceratopteris investigated the role of the LEAFY transcription factor (LFY) in development (Plackett et al., 2018). LFY is important for cell division in moss embryos (Tanahashi et al., 2005) and angiosperm floral meristem development (Carpenter and Coen, 1999). While it is known to be important in both of these lineages, there is no functional overlap between mosses and angiosperms, so understanding the evolutionary history of LFY has been challenging.

Ceratopteris provides an evolutionary and functional midpoint with which to study the role of the LFY gene in development. Using several transgenic lines of C. richardii, Plackett et al. evaluated the role of LFY in sporophyte development. They also reported, for the first time in any land plant, that LFY function is required for C. richardii gametophyte development (Plackett et al., 2018). This suggests that LFY was important for gametophyte development for the last common ancestor of ferns and seed plants, but this function was lost in seed plants where the gametophyte is greatly reduced (Plackett et al., 2018). Incorporating other vegetative developmental systems into further studies with C. richardii (e.g., Vasco et al., 2013; Vasco et al., 2016; Hernández-Hernández et al., 2021) will help us gain a more nuanced understanding of these processes across land plants.

Similarly, there is a growing body of work on the developmental patterns associated with reproduction using Ceratopteris. It is well-established that apogamy can be induced in C. richardii (Cordle et al., 2007), but the genetic mechanism responsible was unknown until recently. In flowering plants, BABY-BOOM (BBM) genes promote somatic embryogenesis (Boutilier et al., 2002; Soriano et al., 2013). These genes are absent in non-seed plants, but an ortholog of the BBM gene AINTEGUMENTA was identified in C. richardii (Bui et al., 2017). Using Agrobacterium-mediated transformations, Bui et al., created C. richardii gametophytes with both over- and knockdown-expression of the apogamy-inducing gene, CrANT. This was the first such study conducted in a non-seed plant and provides evidence for conserved gene
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The environmental influence on development

*Ceratopteris richardii* is a well-known model system for studying sex determination (*Banks, 1997*) and the alternation of generations in homosporous plants (*Eberle et al., 1995*). However, there are some steps in its life cycle that are poorly understood. Almost nothing is known about how *Ceratopteris* gametophytes are established in the wild. It is unknown if function in apomixis across land plant lineages (*Bui et al., 2017*).

Future work using transgenic lines of *Ceratopteris richardii* has the potential to connect gene expression and function across land plants. Work has already been done on many gene families in model bryophytes, the lycophyte *Selaginella*, and seed plants. As mentioned above, however, there is not always functional overlap between these lineages. Including *Ceratopteris* may provide such a functional or developmental link. It is important to note that ferns like *Ceratopteris* are an independent lineage with unique development characteristics that have continued to evolve since diverging from other land plants, as are bryophytes and seed plants (*Plackett et al., 2015b; McDaniel, 2021*). However, *Ceratopteris* does share many characteristics with bryophytes (e.g., spores, independent gametophyte generation) and seed plants (e.g., vasculature, independent sporophyte generation), which make it a key lineage to include in comparative work.

Many model and non-model plants have recently established CRISPR/Cas9 gene-editing systems. Developing CRISPR in *Ceratopteris* is promising because *C. richardii* (and the Hnn strain) is diploid with a short life cycle and homozygotes can be easily created in one generation of gametophytic selfing (*Shan et al., 2020*). One potential avenue of study with CRISPR could be the apogamy pathway in *C. richardii* (*Bui et al., 2017*), investigating the connection between apogamy and spore number in *C. richardii*. This species produces 16 spores per sporangium, a number often indicative of apogamy in ferns (*Grusz, 2016*); *C. richardii* reproduces sexually but apogamy can be easily induced. If a CRISPR system could be established in *Ceratopteris*, one might be able to extend such technology to other members of the Pteridaceae known for apogamy (*Grusz et al., 2021; Grusz, 2016; Grusz et al., 2009*), or for application in other non-model ferns.

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**Box 1. Glossary**

Homosporous: Plants that produce one type of spore, which germinates into a gametophyte capable of producing both eggs and sperm. This group comprises most ferns, and some lycophytes, and all non-vascular plants.

Heterosporous: Plants that produce separate spores, which produce sperm and eggs respectively. This includes all seed plants, as well as a few lineages of ferns and lycophytes.

Apomixis: A form of asexual reproduction in plants. It can proceed by ‘apogamy’, where unreduced spores germinate into gametophytes from which sporophytic tissue can grow without fertilization; the alternative is ‘apospory’, where no spore is produced and a gametophyte grows directly from the parent sporophytic tissue.

Sporophyte: The diploid generation of plants that produce spores. In mosses, this generation is dependent on the gametophyte. In ferns, lycophytes, and seed plants this generation is independent.

Gametophyte: The haploid generation in plants that produce gametes. They can be ‘archegoniate’ (having archegonia that produce eggs) or ‘antheridiate’ (having antheridia that produce sperm), or be ‘hermaphroditic’ (producing both egg and sperm). Gametophytes are free-living in mosses, ferns, and lycophytes, but dependent on the sporophyte generation in seed plants.

Sporangium: The structure in plants that create a spore. In ferns, these are found on the underside of a leaf, often grouped in small clusters called sori.

Leptosporangiate fern: One of the major lineages of ferns, in the subclass Polypodiidae. These ferns have sporangia with long stalks that produce (typically) 64 spores, all derived from a single initial cell (*PPG, 2016*).
Ceratopteris spores must germinate on soil, or if they can germinate and establish gametophytes in standing or slow-moving water.

Recently the effect of soil bacteria on sex determination in *C. richardii* was investigated for the first time (Ganger et al., 2019). In the presence of a soil bacterium, there were more hermaphroditic (compared to antheridiate) gametophytes as well as increased growth (Ganger et al., 2019). *Ceratopteris* uses an antheridiogen pheromone system to control sex determination (Scott and Hickok, 1987; Banks, 1997), and soil bacteria may be influencing sex determination in a similar way. Additional experiments would benefit our understanding of how natural conditions might affect gametophyte establishment and sex determination in *Ceratopteris*, outside of the known role of antheridiogens. The establishment of new plants is particularly important as climate change is a threat to the current habitat of *Ceratopteris*, both as sea levels rise and rainfall becomes less predictable.

In addition to the establishment of new plants, climate change may influence the morphology, ecology, and physiology of *Ceratopteris*. There is dramatic variation in frond morphology within Japanese *C. thalictroides* based on the growing season length (Masuyama, 1992); such intra-species variety has not been systematically characterized in any other species in the genus. This is important to understand because leaves have been used by some authors as the primary method of identification in *Ceratopteris* (Benedict, 1909; Lloyd, 1974), despite this being one of the most variable traits. Understanding the model species *C. richardii* and its relatives in the wild is important for conservation efforts, as well as to understand what natural variation exists in these species that may be informative to future work.

**Systematics and hybridization**

Hybridization among *Ceratopteris* species is well-documented (Hickok and Klekowski, 1974; Nakazato et al., 2007; Hickok, 1977; Hickok, 1973), and these hybrid taxa as well as progenitor species can be morphologically cryptic (Adjie et al., 2007). Lloyd predicted the presence of multiple cryptic lineages in *C. thalictroides*, but detecting these taxa was not possible at the time without genetic analysis (Lloyd, 1974).

During the 1990s and early 2000s, Masuyama and colleagues examined cryptic variation within *C. thalictroides* from Asia and Oceania. They used a combination of work on allozymes and cross-breeding experiments (Masuyama et al., 2002), chromosome counts (Masuyama and Watano, 2005), morphology of wild and cultivated plants (Masuyama and Adjie, 2008; Masuyama, 1992), along with plastid and nuclear markers (Adjie et al., 2007) to describe three cryptic species (Masuyama and Watano, 2010). More recently, Zhang et al. described another cryptic species of *C. thalictroides* endemic to Hainan Province in China. This taxon, *Ceratopteris shingii*, has some unique characteristics in the genus: a creeping rhizome, terrestrial growth on volcanic rock, and is sister to all other species in the genus (Zhang et al., 2020). Its phylogenetic placement and unique characteristics could provide some new hypotheses for trait evolution and an updated perspective on the life history and ecology of the genus.

In addition to the diversity of *Ceratopteris* in Asia, the Americas may have novel cryptic species. Natural hybrids between *C. thalictroides* and *C. pteridoides* have been described in South
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America (Hickok and Klekowski, 1974), as well as synthesized hybrids between several New World species (Hickok, 1973; Hickok, 1978). Kinosian et al., found several hybrid individuals and potentially a cryptic species of *C. thalictroides* in the Americas (Kinosian et al., 2020a). Interestingly, the same study did not find distinct wild populations of *C. richardii*. Future work on systematics in the group should focus on detangling cryptic species and identifying the extant range and phylogenetic placement of *C. richardii*.

A robust evolutionary tree is particularly important for *Ceratopteris* following the publication of the *C. richardii* genome. The taxonomy of model organisms is not always fully understood until after they become model systems (e.g., *Arabidopsis*, Al-Shehbaz and O’Kane, 2002; *Rattus norvegicus*, Musser et al., 2005 and *Caenorhabditis elegans*, Denver et al., 2003; De Ley, 2006), and *C. richardii* is no exception.

Despite having unique characteristics like a distinct deltoid leaf shape and only 16 spores per sporangium (Lloyd, 1974), *C. richardii* is not often identified correctly. For example, specimens identified as *C. richardii* from Central and South America, as well as western Africa, are each more genomically similar to other species than they are to one another (Kinosian et al., 2020b). This could be due to misidentification of collections, a poor understanding of its native range, or the extinction of *C. richardii* in the wild. This last possible explanation is troubling because, as we discuss above, it is important to have wild populations to best understand the potential of model organisms. Revisiting the localities of known *C. richardii* collections (detailed in Lloyd, 1974) should be a goal for future fieldwork. New wild collections will help elucidate the outstanding questions about the taxonomy and natural history of *C. richardii*, but may also provide novel populations or strains to include in lab studies.

**Box 2. Outstanding questions about the natural history of *Ceratopteris***

- What is the evolutionary history of *C. richardii*? Kinosian et al., 2020b were unable to find a consistent genetic identity for *C. richardii*; is this due to poor sampling, extirpation of *C. richardii* from its native range, and/or misidentification of specimens?
- Why do some species of *Ceratopteris* produce 32 spores per sporangium, and *C. richardii* produces only 16? This is substantially less than the typical leptosporangiate fern which produces 64 spores per sporangium.
- What is the genetic population structure of *Ceratopteris* species? Plants are typically locally abundant but regionally rare; is this due to environmental conditions, spore dispersal, or other factors? How does it affect genetic diversity across a landscape?
- How are *Ceratopteris* gametophytes established in the wild?
- What is the biogeographic history of the genus? How might that be influencing current species distributions and hybridization?
- How does the habit (aquatic vs. semi-aquatic) of different *Ceratopteris* species influence population structure, breeding system, or genetic structure and function?

**Plant genome structure and evolution**

On average, heterosporous plants have fewer chromosomes and smaller genomes than homosporous plants. *Ceratopteris richardii* is the first homosporous fern with the genomic resources to address why these differences between heterosporous and homosporous genomes exist. Nakazato et al. generated a genetic linkage map for *C. richardii* which showed that it is likely not repeated rounds of polyploidization that leads to larger genomes in ferns, but rather small-scale gene duplications (Nakazato et al., 2006). More recently, Marchant et al. published the first draft genome assembly for *C. richardii* and found additional support for genetic diploidy and limited rounds of polyploidization (Marchant et al., 2019b). These data further support the theory that homosporous fern genomes are large not because of whole-genome duplication, but because they do not have the same mechanisms for genome
downsizing as heterosporous plants (Clark et al., 2016; Szövényi et al., 2021). The draft genome of Ceratopteris richardii is an important stepping stone for studying land plant evolution (Marchant et al., 2019b); a more complete genome is on the way that will be a better resource for genomic work (Marchant et al., 2022). A high-quality genome for C. richardii will also aid in the development of targeted enrichment or whole-genome resequencing. This latter advancement in sequencing resources for ferns will help us understand reticulate evolution and polyploidy in ferns, as phylogenies can be estimated with hundreds of genes.

In addition to the Ceratopteris richardii genome, there are many other fern genomes that have been recently published or will be available soon. Several heterosporous fern and lycophyte genomes have been published in the last few years, including the heterosporous ferns Azolla filiculoides and Salvinia cucullata (Li et al., 2018), and the heterosporous lycophytes Selaginella moellendorfii (Banks et al., 2011), S. lepidophylla (VanBuren et al., 2018), and Isoëtes taiwanensis (Wickell et al., 2021). In the near future, several additional homosporous fern genomes will be available, including Adiantum capillus-veneris (Polypodiales), Alsophila spinulosa (Cyatheales), Dipteris conjugata (Gleicheniales), Ptisana robusta (Marattiales), Huperzia asiatica and Diphiasastrum complanata (Lycopodiales; Drs. F.-W. Li and M. Barker, personal communication). As more homosporous fern genomes become available, the preliminary work with the C. richardii genome will be tested in a more rigorous phylogenetic context, hopefully leading to a clearer picture of land plant genome evolution.

**Conclusion**

Although Ceratopteris richardii has been used as a model for decades, fundamental aspects of its natural history are still unknown. A few examples include basic taxonomy, origins of spore number, salt tolerance, origins of polyploids, phenotypic plasticity, and intraspecies morphological variation (see Box 2). Many of these topics are ripe for undergraduate or graduate student projects and could be integrated into existing research programs to answer fundamental aspects of fern biology.

Additionally, C. richardii is a useful tool for teaching students at all grade levels about plant biology (https://www.c-fern.org/). As detailed by Marchant, the C-fern can be incorporated into curriculum topics ranging from basic plant biology to evolution and development to bioinformatics. In the lab, field, or classroom, the recently published C. richardii genome provides a new window into the study of this fern (Marchant et al., 2019b). As more fern genomic resources become available, having Ceratopteris as a well-established model system will only become more important to test novel hypotheses about land plant evolution.

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**Data availability**

Source data for Figure 2 (Range map of Ceratopteris) can be found in the file cer_locations.csv in https://github.com/sylviakinosian/ceratopteris-map (copy archived at swh:1:rev:02f4523dc32b20cb18b17e226eb6f2ff-b60cb05a) (previously published in Kinosian et al., 2016).
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Kinosian and Wolf. eLife 2022;11:e75019. DOI: https://doi.org/10.7554/eLife.75019

2020a, https://doi.org/10.1016/j.ympev.2020.106938, and in Ceratopteris Brongn. in GBIF Secretariat (2021). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei (accessed via GBIF.org on 2021-10-4).

The following previously published datasets were used:

| Author(s)          | Year | Dataset URL                   | Database and Identifier |
|--------------------|------|-------------------------------|-------------------------|
| GBIF Secretariat   | 2021 | https://doi.org/10.15468/39omei | GBIF, 10.15468/39omei   |
| Kinosian, SP, Pearse, WD, Wolf | 2020 | https://github.com/sylvia.kinosian/germer/ | GitHub, 02f4523 |

References

Adjie B, Masuyama S, Ishikawa H, Watano Y. 2007. Independent origins of tetraploid cryptic species in the fern Ceratopteris thalictroides. Journal of Plant Research 120:129–138. DOI: https://doi.org/10.1007/s10265-006-0032-5, PMID: 16955374

Al-Shehbaz IA, O’Kane SL. 2002. Taxonomy and Applications. In Working with Ferns: Issues and Strategies. British Columbia Ministry of Forests, p. 1105/tpc.4.2.113, PMID: 12297641

Antirrhinum majus, Carpintero R, Coen ES. 1990. Floral homeotic mutations produced by transposon-mutagenesis in Antirrhinum majus. Genes & Development 4:285–303. DOI: https://doi.org/10.1016/j.ydbio.2019.08.017, PMID: 31470018

Banks JA, Nishiyama T, Hasebe M, Bowman JL, Gribskov M, dePamphilis C, Albert VA, Aono N, Aoyama T, Ambrose BA, Ashton NW, Axtell MJ, Barker E, Barker MS, Bennetzen JL, Bonavitz ND, Chapple C, Cheng C, Correa LGG, Dacre M, et al. 2011. The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science332:960–963. DOI: https://doi.org/10.1126/science.1203810, PMID: 21551031

Bateman RM, DiMichele WA. 1994. Heterospor: the most iterative key innovation in the evolutionary history of the plant kingdom. Biological Reviews 69:345–417. DOI: https://doi.org/10.1111/j.1469-185X.1994.tb01276.x

Benedict RC. 1909. The genus Ceratopteris: a preliminary revision. Bulletin of the Torrey Botanical Club 36:463. DOI: https://doi.org/10.2307/2479023

Boutilier K, Ofringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, Hattori J, Liu CM, van Lammeren AAM, Miki BLA, Custers JBM, van Lookeren Campagne MM. 2002. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. The Plant Cell 14:1737–1749. DOI: https://doi.org/10.1105/tpc.011941, PMID: 12172019

Brongniart A. 1821. Description d’un nouveau genre de fougere, nomme Ceratopteris. Bull. Sci. Soc. Philom. Paris Sér 3:184–187.

Bui LT, Cordle AR, Irish EE, Cheng CL. 2015. Transient and stable transformation of Ceratopteris richardii gametophytes. BMC Research Notes 8:214. DOI: https://doi.org/10.1186/s13104-015-1193-x, PMID: 26040630

Bui LT, Pandzic D, Youngstrom CE, Wallace S, Irish EE, Szövényi P, Cheng CL. 2017. A fern AINTEGUMENTA gene mirrors BABY BOOM in promoting apogamy in Ceratopteris richardii. The Plant Journal 90:122–132. DOI: https://doi.org/10.1111/tpj.13479, PMID: 28078730

Cannon AE, Salmi ML, Cantero A, Roux SJ. 2018. Generation of trasgenic spores of the fern Ceratopteris richardii to analyze Ca2+ transport dynamics during gravity-directed polarization. Fernández Helena (Ed). In Current Advances in Fern Research. Cham: Springer International Publishing. p. 285–303. DOI: https://doi.org/10.1007/978-3-319-75103-0_14

Carpenter R, Coen ES. 1990. Floral homeotic mutations produced by transposon-mutagenesis in Antirrhinum majus. Genes & Development 4:1483–1493. DOI: https://doi.org/10.1101/gad.4.9.1483, PMID: 1979295

Chasan R. 1992. Ceratopteris: a model plant for the 90s. The Plant Cell 4:113–115. DOI: https://doi.org/10.1105/tpc.4.2.113, PMID: 12297641

Clark J, Hidalgo O, Pellicer J, Liu H, Marquardt J, Robert Y, Christenhusz M, Zhang S, Gibby M, Leitch IJ, Schneider H. 2016. Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny. The New Phytologist 210:1072–1082. DOI: https://doi.org/10.1111/nph.13833, PMID: 26756823

Conway SJ, Di Stilio VS. 2020. An ontogenetic framework for functional studies in the model fern Ceratopteris richardii. Developmental Biology 457:20–29. DOI: https://doi.org/10.1016/j.ydbio.2019.08.017, PMID: 31470018

Cordle AR, Irish EE, Cheng CL. 2007. Apogamy induction in Ceratopteris richardii. International Journal of Plant Sciences 168:361–369. DOI: https://doi.org/10.1086/511049

Cordle AR, Bui LT, Irish EE, Cheng CL. 2010. Laboratory-induced apogamy and apospory in Ceratopteris richardii. Kumar A, Fernández H, Revilla MA (Eds). In Working with Ferns: Issues and Applications. New York, NY: Springer New. p. 25–36. DOI: https://doi.org/10.1007/978-1-4419-7162-3

Cove D. 2005. The moss Physcomitrella patens. Annual Review of Genetics 39:339–358. DOI: https://doi.org/10.1146/annurev.genetics.39.052004.143609

Kinosian and Wolf. eLife 2022;11:e75019. DOI: https://doi.org/10.7554/eLife.75019
The Natural History of Model Organisms | The biology of *C. richardii* as a tool to understand plant evolution

doi.org/10.1146/annurev.genet.39.073003.110214, PMID: 16285864

De Ley P. 2006. A quick tour of nematode diversity and the backbone of nematode phylogeny. *WormBook: The Online Review of C. Elegans Biology* 1:1–8. DOI: https://doi.org/10.1895/wormbook.1.11.1, PMID: 18050465

Denver DR, Morris K, Thomas WK. 2003. Phylogenetics in Caenorhabditis elegans: an analysis of divergence and outcrossing. *Molecular Biology and Evolution* 20:393–400. DOI: https://doi.org/10.1093/molbev/msg044, PMID: 12644560

Eberle J, Nemacheck J, Wen CK, Hasebe M, Banks JA. 1995. *Ceratopteris*: a model system for studying sex-determining mechanisms in plants. *International Journal of Plant Sciences* 156:359–366. DOI: https://doi.org/10.1086/297257

Ganger MT, Hiles R, Hallowell H, Cooper L, McAllister N, Youngdahl D, Alfieri J, Ewing SJ. 2019. A soil bacterium alters sex determination and rhizoid development in gametophytes of the fern *Ceratopteris richardii*. *AoB PLANTS* 11:p1012. DOI: https://doi.org/10.1093/aobpla/plz012, PMID: 31019671

Geng Y, Cai C, McAdam SAM, Banks JA, Wisecaver JH, Zhou Y. 2021. A de novo transcriptome assembly of *Ceratopteris richardii* provides insights into the evolutionary dynamics of complex gene families in land plants. *Genome Biology and Evolution* 13:3. DOI: https://doi.org/10.1093/gbe/evab042, PMID: 33681974

Grusz AL, Windham MD, Pryer KM. 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes* yavapensis complex (*Pteridaceae*). *American Journal of Botany* 96:1636–1645. DOI: https://doi.org/10.3732/ajb.0900019, PMID: 21622350

Grusz AL. 2016. A current perspective on apomixis in ferns. *Journal of Systematics and Evolution* 54:656–665. DOI: https://doi.org/10.1111/jse.12228

Grusz AL, Windham MD, Picard KT, Pryer KM, Haufler CH. 2017. A drought-driven model for the evolution of obligate apomixis in ferns: evidence from pellaeids (*Pteridaceae*). *American Journal of Botany* 108:263–283. DOI: https://doi.org/10.1002/jxb.21611, PMID: 33624306

Haufler CH, Pryer KM, Schuettelpe E, Sessa EB, Farrar DR, Moran R, Schneller JJ, Watkins JE, Windham MD. 2016. Sex and the single gametophyte: revising the homosporous vascular plant life cycle in light of contemporary research. *Bioscience* 66:928–937. DOI: https://doi.org/10.1093/biosci/biw108

Hernández-Hernández B, Tapia-López R, Ambrose BA, Vaso A. 2021. R2R3-MYB gene evolution in plants, incorporating ferns into the story. *International Journal of Plant Sciences* 182:1–8. DOI: https://doi.org/10.1086/710579

Hickok LG. 1973. Karyotype evolution in *Ceratopteris*. An analysis of the synthesized hybrid *C. pteridoides* x *C. richardii*. *American Journal of Botany* 60:1010–1022.

Hickok LG, Klekowski EJ. 1973. Abnormal reduction and non-reductional meiosis in *Ceratopteris*: alternatives to homoyogosity and hybrid sterility in homosporous ferns. *American Journal of Botany* 60:1010–1022. DOI: https://doi.org/10.1002/j.1537-2197.1973.tb06002.x

Hickok LG, Klekowski EJ. 1974. Inchoate speciation in *Ceratopteris*: an analysis of the synthesized hybrid *C. richardii*. *Evolution; International Journal of Organic Evolution* 28:439–446. DOI: https://doi.org/10.1111/j.1558-5646.1974.tb00755.x, PMID: 28564836

Hickok LG. 1977. Cytological relationships between three diploid species of the fern genus *Ceratopteris*. *Canadian Journal of Botany* 55:1660–1667. DOI: https://doi.org/10.1139/b77-194

Hickok LG. 1978. Homoeologous chromosome pairing: frequency differences in inbred and intraspecific hybrid polyplod ferns. *Science* (New York, N.Y.) 202:982–984. DOI: https://doi.org/10.1126/science.202.4371.982, PMID: 17798797

Hickok LG, Warne TR, Fribourg RS. 1995. The biology of the fern *Ceratopteris* and its use as a model system. *International Journal of Plant Sciences* 156:332–345. DOI: https://doi.org/10.1086/297255

Hornych O, Testo WL, Sessa EB, Watkins JE, Campmy CE, Pittermann J, Ekrt L. 2021. Insights into the evolutionary history and widespread occurrence of antheridiogen systems in ferns. *The New Phytologist* 229:607–624. DOI: https://doi.org/10.1111/nph.16836, PMID: 32740926

Hou GC, Hill JP. 2002. Heteroblastic root development in *Ceratopteris richardii* (Parkeriaceae). *International Journal of Plant Sciences* 163:341–351. DOI: https://doi.org/10.1086/339156

Kinosian SP, Pease WD, Wolf PG. 2020a. Cryptic diversity in the model fern genus *Ceratopteris* (*Pteridaceae*). *Molecular Phylogenetics and Evolution* 152:106938. DOI: https://doi.org/10.1016/j.ympev.2020.106938, PMID: 32791300

Kinosian SP, Pease WD, Wolf PG. 2020b. There and back again: reticulate evolution in *Ceratopteris* (*Pteridaceae*). *American Fern Journal* 110:193–210. DOI: https://doi.org/10.1640/0002-8444-110.4.193

Klekowski EJ. 1970. Reproductive biology of the *Pteridophyta IV*. An experimental study of mating systems in *Ceratopteris thalictroides* (L.) Brongn. *Botanical Journal of the Linnean Society* 63:153–169. DOI: https://doi.org/10.1111/j.1095-8339.1970.tb02547.x

Li F-W, Brouwer P, Carretero-Paulet L, Cheng S, de Vries J, Delaux P-M, Eily A, Koppers N, Kuo L-Y, Li Z, Simenc M, Small I, Wafula E, Angarita S, Barker MS, Bräutigam A, dePamphilis C, Gould S, Hosmani PS, Huang Y-M, et al. 2018. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. *Nature Plants* 4:460–472. DOI: https://doi.org/10.1038/s41477-018-0188-8, PMID: 29967517

Linnaeus C. 1764. *Species Plantarum*: Volume 2. Imprimerie Laurentii Salvii.

Lloyd RM. 1974. Systematics of the genus *Ceratopteris* Brongn. (*Parkeriaceae*) II. Taxonomy. *Botrittonia* 26:139. DOI: https://doi.org/10.2307/2805883

Lloyd RM, Warne TR. 1978. The absence of genetic load in a morphologically variable sexual species, *Ceratopteris thalictroides* (Parkeriaceae). *Systematic Botany* 3:20. DOI: https://doi.org/10.2307/2418530

Marchant DB. 2019a. Ferns with benefits: revising the homosporous vascular plant life cycle in *Ceratopteris thalictroides* (Parkeriaceae). *Journal of Organic Evolution* 28:439–446. DOI: https://doi.org/10.1111/j.1558-5646.1974.tb00755.x, PMID: 28564836

Marchant DB, Sessa EB, Wolf PG, Heo K, Barbazuk WB, Solits PS, Solits DE. 2019b. The C-Fern (*Ceratopteris richardii*) genome: insights into plant evolution
genome evolution with the first partial homosporous fern genome assembly. *Scientific Reports* 9:18181. DOI: https://doi.org/10.1038/s41598-019-53968-8, PMID: 31796775

Marchant DB, Chen G, Cai S, Chen F, Scafran P, Jenkins J, Shu S. 2022. Ancient yet dynamic: the evolution of a fern genome. *Nature Plants* 4:460. DOI: https://doi.org/10.1038/s41477-018-0188-8

Masuyama S. 1992. Clinal variation of frond morphology and its adaptive implication in the fern *Ceratopteris thalictroides* in Japan. *Plant Species Biology* 7:87–96. DOI: https://doi.org/10.1111/j.1442-8941.1992.tb00222.x

Masuyama S, Yatabe Y, Murakami N, Watano Y. 2002. Cryptic species in the fern *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae). I. Molecular analyses and crossing tests. *Journal of Plant Research* 115:87–97. DOI: https://doi.org/10.1007/s102650200013, PMID: 12884131

Masuyama S, Watano Y. 2005. Cryptic species in the fern *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae). IV. Taxonomic revision. Acta Phytotaxonomica et Geobotanica 56:231–240. DOI: https://doi.org/10.18942/apg.KJ0004623524

Masuyama S, Adjie B. 2008. Three forms of *Ceratopteris thalictroides* in Guam. *American Fern Journal* 98:104–107. DOI: https://doi.org/10.1640/0002-8444(2008)98(104:TF0CTI)2.0.CO;2

Masuyama S, Watano Y. 2010. Cryptic species in the fern *Ceratopteris thalictroides* (L.) Brongn. Parkeriaceae. IV. Taxonomic revision. *Acta Phytotax.* 61:75–86. DOI: https://doi.org/10.18942/apg.KJ00009281704

McDaniel SF. 2021. Bryophytes are not early diverging land plants. *The New Phytologist* 230:1300–1304. DOI: https://doi.org/10.1111/nph.17241, PMID: 33521973

Medina E, Cuevas E, Popp M, Lugo AE. 1990. Soil salinity, sun exposure, and growth of *Acrostichum aureum*, the mangrove fern. *Botanical Gazette* 151:41–49. DOI: https://doi.org/10.1086/337803

Musser GG, Carleton MD, Wilson DE, Reeder DM. 2005. Mammal species of the world: A taxonomic and geographic reference. *Wilson DE, Reeder DM (Eds).* (7th edition). Oxford University Press. p. 824–830. DOI: https://doi.org/10.1111/j.1442-8941.1992.tb00222.x

Muthukumar B, Joyce BL, Elless MP, Stewart CN. 2013. Stable transformation of ferns using spores as targets: *Pteris vittata* and *Ceratopteris thalictroides*. *Plant Physiology* 163:648–658. DOI: https://doi.org/10.1104/pp.112.224675, PMID: 2393990

Nakazato T, Jung MK, Housworth EA, Rieseberg LH, Nakazato T. 2007. A genomewide study of reproductive barriers between allopatric populations of a homosporous fern, *Ceratopteris richardii*. *Genetics* 173:1585–1597. DOI: https://doi.org/10.1534/genetics.106.055624, PMID: 16648591

Nakazato T, Jung MK, Housworth EA, Rieseberg LH, Nakazato T. 2007. A genomewide study of reproductive barriers between allopatric populations of a homosporous fern, *Ceratopteris richardii*. *Genetics* 173:1141–1150. DOI: https://doi.org/10.1534/genetics.107.076851, PMID: 17720917

Pal N, Pal S. 1962. Studies on morphology and affinity of the Parkeriaceae I. Morphological observations of *Ceratopteris thalictroides*. *Botanical Gazette* 124:132–143. DOI: https://doi.org/10.1086/336183

Pal N, Pal S. 1963. Studies on morphology and affinity of the Parkeriaceae II. Sporogenesis, development of the gametophyte, and cytology of *Ceratopteris thalictroides*. *Botanical Gazette* 124:405–412. DOI: https://doi.org/10.1086/336226

Plackett ARG, Huang L, Sanders HL, Langdale JA. 2014. High-efficiency stable transformation of the model fern species *Ceratopteris richardii* via microparticle bombardment. *Plant Physiology* 165:3–14. DOI: https://doi.org/10.1104/pp.113.231357, PMID: 24623851

Plackett ARG, Di Stilio VS, Langdale JA. 2015a. Ferns: the missing link in shoot evolution and development. *Frontiers in Plant Science* 6:972. DOI: https://doi.org/10.3389/fpls.2015.00972, PMID: 26594222

Plackett ARG, Rabbinowitsch EH, Langdale JA. 2015b. Protocol: genetic transformation of the fern *Ceratopteris richardii* through microparticle bombardment. *Plant Methods* 11:37. DOI: https://doi.org/10.1186/s13007-015-0080-8, PMID: 26146510

Plackett AR, Conway SJ, Hewett Hazelton KD, Rabbinowitsch EH, Langdale JA, Di Stilio VS. 2018. LEAFY maintains apical stem cell activity during shoot development in the fern *Ceratopteris richardii*. *eLife* 7:e39625. DOI: https://doi.org/10.7554/eLife.39625, PMID: 30355440

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54:563–603. DOI: https://doi.org/10.1111/jse.12229

Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, Tanahashi T, Sakakibara K, Fujita T, Oishi K, Shin-I T, Kuroki Y, Toyoda A, Suzuki Y, Hashimoto SI, Yamaguchi K, et al. 2008. The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. *Science (New York, N.Y.)* 319:64–69. DOI: https://doi.org/10.1126/science.1150646, PMID: 18079367

Schedlbauer MD, Klekowski EJ. 1972. Antheridogen activity in the fern *Ceratopteris thalictroides* (L.) Brongn. *Botanical Journal of the Linnean Society* 65:399–413. DOI: https://doi.org/10.1111/j.1095-8339.1972.tb02280.x

Scott RJ, Hickog LG. 1987. Genetic analysis of antheridogen sensitivity in *Ceratopteris richardii*. *American Journal of Botany* 74:1872–1877. DOI: https://doi.org/10.1002/0002-0952(1987)74:7<1872::AID-AJB1872>3.0.CO;2-O

Sepp S,libraries DB, Pryer KM, Rothfels CJ, Roux SJ, Marchant DB, Prapaik K, Rossell CA, Roux SJ, Salmin KL, Sigel EM, Soltis DE, Soltis PS, Stevenson DW, Wolf PG. 2014. Between two fern genomes. *GigaScience* 3:15. DOI: https://doi.org/10.1186/2047-217X-3-15, PMID: 25324956

Shan S, Soltis DE, Soltis PS, Stevenson DW, Wolf PG. 2014. Between two fern genomes. *GigaScience* 3:15. DOI: https://doi.org/10.1186/2047-217X-3-15, PMID: 25324956

Soriano M, Li H, Boutiller K. 2013. Microspore embryogenesis: establishment of embryo identity and pattern in culture. *Plant Reproduction* 26:181–196. DOI: https://doi.org/10.1007/s00497-013-0226-7, PMID: 23852380
Stein DB. 1971. Gibberellin-induced fertility in the fern Ceratopteris thalictroides (L.) Brongn. Plant Physiology 48:416–418. DOI: https://doi.org/10.1104/pp.48.4.416, PMID: 16657811

Sun J, Li GS. 2020. Leaf dorsoventrality candidate gene CpARF4 has conserved expression pattern but divergent tasiR-ARF regulation in the water fern Ceratopteris pteridoides. American Journal of Botany 107:1470–1480. DOI: https://doi.org/10.1002/ajb2.1570, PMID: 33216953

Szövényi P, Gunadi A, Li FW. 2021. Charting the genomic landscape of seed-free plants. Nature Plants 7:554–565. DOI: https://doi.org/10.1038/s41477-021-00888-z, PMID: 33820965

Tanahashi T, Sumikawa N, Kato M, Hasebe M. 2005. Diversification of gene function: homologs of the floral regulator FLO/LFY control the first zygotic cell division in the moss Physcomitrella patens. Development (Cambridge, England) 132:1727–1736. DOI: https://doi.org/10.1242/dev.01709, PMID: 15743879

VanBuren R, Wai CM, Ou S, Pardo J, Bryant D, Jiang N, Mockler TC, Edger P, Michael TP. 2018. Extreme haplotype variation in the desiccation-tolerant clubmoss Selaginella lepidophylla. Nature Communications 9:13. DOI: https://doi.org/10.1038/s41467-017-02546-5, PMID: 29296019

Vasco A, Moran RC, Ambrose BA. 2013. The evolution, morphology, and development of fern leaves. Frontiers in Plant Science 4:345. DOI: https://doi.org/10.3389/fpls.2013.00345, PMID: 24027574

Vasco A, Smalls TL, Graham SW, Cooper ED, Wong GKS, Stevenson DW, Moran RC, Ambrose BA. 2016. Challenging the paradigms of leaf evolution: Class III HD-Zips in ferns and lycophytes. The New Phytologist 212:745–758. DOI: https://doi.org/10.1111/nph.14075, PMID: 27385116

Warne TR, Hickok LG. 1987. Single gene mutants tolerant to NaCl in the fern Ceratopteris: characterization and genetic analysis. Plant Science 52:49–55. DOI: https://doi.org/10.1016/0168-9452(87)90104-X

Warne TR, Vogelien DL, Hickok LG. 1995. The analysis of genetically and physiologically complex traits using Ceratopteris: a case study of NaCl-tolerant mutants. International Journal of Plant Sciences 156:374–384. DOI: https://doi.org/10.1086/297259

Wickell D, Kuo LY, Yang HP, Dhabalia Ashok A, Irisari I, Dadras A, de Vries S, de Vries J, Huang YM, Li Z, Barker MS, Hartwick NT, Michael TP, Li FW. 2021. Underwater CAM photosynthesis elucidated by Isoetes genome. Nature Communications 12:6348. DOI: https://doi.org/10.1038/s41467-021-26644-7, PMID: 34732722

Yu JH, Zhang R, Liu QL, Wang FG, Yu XL, Dai XL, Liu YB, Yan YH. 2021. Ceratopteris chunii and Ceratopteris chingii (Pteridaceae), two new diploid species from China, based on morphological, cytological, and molecular data. Plant Diversity 1:2. DOI: https://doi.org/10.1016/j.pld.2021.10.002

Zhang R, Liu T, Wu W, Li Y, Chao L, Huang L, Huang Y, Shi S, Zhou R. 2013. Molecular evidence for natural hybridization in the mangrove fern genus Acrostichum. BMC Plant Biology 13:74. DOI: https://doi.org/10.1186/1471-2229-13-74, PMID: 23634934

Zhang R, Yu JH, Shao W, Wang WQ, Yan YH. 2020. Ceratopteris shingii, a new species of Ceratopteris with creeping rhizomes from Hainan, China. Phytotaxa 449:23–30. DOI: https://doi.org/10.11646/phytotaxa.449.1.3