

Versatile Mating Systems and Mycorrhizal Associations Support the Remarkable Long-Time Evolutionary Success of the Early-Divergent Fern Genus *Botrychium*

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ABSTRACT.—We trace the development of our understanding regarding the mating systems of moonworts (*i.e.*, *Botrychium sensu stricto*), from the early recognition of inbreeding as the primary mode of sexual reproduction to the discovery of predominantly outcrossing populations in high mountain habitats. We propose an evolutionary trajectory in which both mating systems, in concert with mycorrhizal support, have been able to sustain *Botrychium* populations through changing environments. We present a depiction of the fern life cycle that portrays the positive role of inbreeding in speciation and evolution of *Botrychium* and its relevance to ferns and lycophytes in general.

KEY WORDS.—*Botrychium*, life cycle, speciation, dispersal, gametophytes, inbreeding

The fern genus species *Botrychium* s.s. and related species of the Ophioglossaceae have become prime examples of low genetic variability starting with the early analyses of allelic diversity revealed through enzyme electrophoresis (*i.e.*, for *Botrypus virginianus*; Soltis and Soltis, 1986). This reduced genetic diversity has generally been thought to result from the difficulty of sperm transfer between belowground gametophytes, *i.e.*, about 2 cm, as evidenced by studies of water movement through soil (Wagner *et al.*, 1985). Studies by Hauk and Haufler (1999) and Stensvold and Farrar (2016) have confirmed that low allelic variability and diminished locus heterozygosity characterize many populations of most *Botrychium* species (Table 1).

Contrary to what might be expected of plants with low genetic variability, species of *Botrychium*, although small and morphologically simple, continue to thrive in seed-dominated floras in temperate and boreal habitats throughout the northern hemisphere, with large disjunctions in similar habitats of the southern hemisphere (Farrar and Stensvold, 2017; Meza-Torres *et al.*, 2016).

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TABLE 1. Comparison of genetic diversity among vascular plants.

| Taxon group | Range %P ¹ | Mean %P ² | Range A/L ³ | Mean A/L ⁴ | Reference |
|---------------------------|-----------------------|----------------------|------------------------|-----------------------|--|
| Seed plants | | 58.90 | | 2.29 | Hamrick and Godt 1990 |
| Ferns | 0–80 | 36.00 | 1.00–2.8 | 1.65 | Li and Haufler 1999 (data for 32 taxa) |
| <i>Botrychium</i> genus | 0–17 | 7.44 | 1.00–1.2 | 1.10 | Farrar 1998 (data for nine diploid taxa) |
| | 0–21 | 7.30 | 1.00–1.21 | 1.07 | Hauk and Haufler 1999 (data for five diploid taxa) |
| <i>B. lunaria</i> complex | 10–45 | 25.80 | 1.10–1.60 | 1.32 | Stensvold and Farrar 2017 (data for six diploid taxa) |
| Swiss <i>B. lunaria</i> | 43–100 | 66.67 | 1.57–2.63 | 2.05 | Dauphin, Grant, and Farrar, 2020 |

Note: ¹Range of percent for polymorphic loci across groups; ²Mean of polymorphic loci per group; ³Range of number of alleles per all loci across groups; ⁴Mean number of alleles per locus of all members per group.

Despite their reduced allelic condition, the 36 currently described species maintain genetic distances similar to those documented between other closely related fern species (Stensvold and Farrar 2017; Williams, 2021) and have sufficient morphological distinctness to allow species recognition in a given area (Farrar *et al.*, 2017). These observations raise questions of how this degree of between-taxon differentiation occurred, and whether this condition influenced adaptation to environmental change over the course of their existence.

THE DISCOVERY OF GENETICALLY RICH MOONWORT POPULATIONS

Answers to these questions began to emerge when the Farrar laboratory received four morphologically similar plants of *B. lunaria* from Switzerland, collected by Benjamin Dauphin. Enzyme electrophoretic analysis of these plants revealed that each individual was unique in its allelic composition. Further analysis of hundreds of plants from sixteen sites across Switzerland demonstrated that these populations contained allelic diversity and locus heterozygosity consistent with what is expected from outcrossing populations (Dauphin, Grant, and Farrar, 2020). Farrar and Gilman (2017) found similar outcrossing in *B. campestre* var. *lineare* in the Rocky Mountains of western North America. In both cases, these outcrossing populations occurred on high, steep mountain meadows beneath areas of winter snow accumulation. How could these conditions promote outcrossing? We now postulate that sperm movement between belowground gametophytes may be facilitated by the flow of water through the gametophyte population provided by gradual melting of snowbanks positioned above the *Botrychium* populations.

The pioneering work of Wagner, Wagner, and Beitel (1985) addressed the issue of sperm dispersal belowground by experimenting with rates of water flow through clumps of dry soil. They proposed that “the mass flow of water within the [soil] interstices might further disperse sperm into positions closer to other gametophytes. If one gametophyte lies somewhat above another, sperm can be carried from the upper to the lower”. In the Black Hills of South

TABLE 2. Allelic diversity of outbreeding populations of *Botrychium lunaria* and *Botrychium campestre* var. *lineare* relative to inbreeding taxa in the same molecular phylogenetic clade.

| Taxon group | Mean number of alleles per locus | Mean % of loci with allelic diversity | ¹ Mean % of heterozygous plants | ² Number of private alleles |
|-------------------------------|----------------------------------|---------------------------------------|--|--|
| Lunaria clade | | | | |
| *Swiss <i>B. lunaria</i> | 2.03 | 79.00 | 100.00 | – |
| Non-Swiss <i>B. lunaria</i> | 1.45 | 11.05 | 25.00 | 1 |
| <i>B. neolunaria</i> | 1.20 | 7.61 | 15.00 | 3 |
| <i>B. crenulatum</i> | 1.40 | 0.83 | 30.00 | 3 |
| <i>B. tunux</i> | 1.30 | 6.32 | 30.00 | 1 |
| <i>B. onondagense</i> | 1.25 | 25.00 | 00.00 | 1 |
| <i>B. nordicum</i> | 1.20 | 10.00 | 00.00 | 5 |
| Campestre clade | | | | |
| *Rocky Mt. var <i>lineare</i> | 1.77 | 84.01 | 54.50 | – |
| Eastern var. <i>campestre</i> | 1.21 | 00.00 | 00.00 | 0 |
| Eastern var. <i>lineare</i> | 1.23 | 15.00 | 13.60 | 1 |

Notes: ¹Mean percentage of plants heterozygous at one or more loci; ²Number of alleles that are not shared with outbreeding taxa. *Outbreeding populations.

Dakota an example of the importance of the steepness of the slope for sperm dispersal has been noted for *B. campestre* (Farrar and Gilman, 2017). Both varieties of *B. campestre*, var. *campestre* and var. *lineare*, are abundant in rolling prairie habitats, with each variety represented by only a single genotype—with one exception. This exception occurs at the base of a steep north-facing slope in where winter snow accumulations persist until early spring. These plants show allelic diversity and heterozygous loci indicative of outcrossing.

OUTCROSSING AND INBREEDING

Botrychium lunaria and *B. campestre* are the only two of 36 currently described *Botrychium* species known to harbor some outcrossing populations (Dauphin, Grant, and Farrar, 2020; Farrar and Gilman, 2017). Comparison of the allelic composition of outbreeding versus inbreeding populations in these two species and related taxa of their respective clades offers insight. Inbreeding populations of *B. lunaria* contain only a single allele that is not also present in the outcrossing Swiss populations (Table 2). In the non-Swiss populations of *B. lunaria* and in the five other species of the *Lunaria* clade, mainly found in North America, the lower mean number of alleles and the small number of private alleles suggest that these species primarily contain subsets of the alleles present in outcrossing Swiss populations.

Populations of *B. campestre* east of the Rocky Mountains show similar trends. The inbreeding populations contain a smaller total number of alleles, and these are primarily subsets of the alleles present in the outcrossing, high mountain populations (Table 2). Presence of heterozygous loci indicates that *B. campestre* var. *lineare* in the high Rocky Mountains is outcrossing, whereas the same variety found in lower, less steep habitats east of the mountains

expresses no heterozygosity, a characteristic of gametophytic selfing. It seems reasonable to assume that prevailing westerly winds could have dispersed individual spores to easterly habitats that were supportive of growth but not of outcrossing. No eastern populations of variety *campestre*, and relatively few of variety *lineare* display heterozygosity reflective of outcrossing. Populations in different eastern areas display distinct genotypes, possibly indicating that different spore genotypes established different populations.

Despite sharing many alleles, it seems unlikely that the North America species of the *Lunaria* clade originated directly from spore dispersal out of Switzerland. More plausible is the former occurrence (or current but yet undetected) of outbreeding populations of ancestral “*lunaria*” in North America or eastern Asia. While we have determined that *Botrychium* species are not exclusively inbreeding, it remains true that most populations of most species are predominantly inbreeding, yet amazingly successful in terms of diversity, frequency of occurrence, ancient origins as evidenced by their early-divergent phylogenetic position (Rothfels *et al.*, 2015), and anatomical similarities to first land plants (Gerrienne and Gonez, 2011; Kerp, Trewin, and Hass, 2007). *Botrychium* anatomy and development reveals a possible resolution to this dilemma.

LONG-STANDING SYMBIOTIC INTERACTIONS

Botrychium spores germinate in darkness belowground, initially producing a single parenchymatous cell with a rhizoid (Whittier, 1981). They develop further after colonization by an arbuscular mycorrhizal fungus (Fig. 1A–B). Arbuscular mycorrhizal fungi are instrumental in terrestrial ecosystems due to their ability to form mutualistic associations with vascular plants, including more than 50% of fern species (Halliday, 2022; Lehnert, Krug, and Kessler, 2017). Arbuscular mycorrhizal fungi are obligate biotrophs that acquire fixed carbon from plants (*e.g.*, carbohydrates and lipids; Hodge, Helgason, and Fitter, 2010; Sheldrake, 2020), while providing nutrients and water to plants. These two-way trophic exchanges may be unbalanced during periods of host plant development (Sheldrake, 2020). Such is the case for *Botrychium* species in the belowground gametophyte and early sporophyte stage and thus there is no photosynthetic carbon production for several years. Are *Botrychium*’s arbuscular mycorrhizal fungi starving for so long? How then could long-term symbiotic interactions be possible?

Interestingly, it was shown that the same arbuscular mycorrhizal fungal species of the genus *Glomus* present in *Botrychium* sporophytes also colonize neighboring seed plants and photosynthetic products extracted from the seed plants are presumably transmitted to *Botrychium* through shared mycorrhizal networks (Winther and Friedman, 2007). Thus, these inter-kingdom symbiotic relationships are vital for establishing populations of *Botrychium* gametophytes, which can be traced back to the first divergent land plants (Fig. 1C; Sheldrake, 2020; Taylor *et al.*, 2005). Analysis of several *Glomus* taxa forming endomycorrhizal associations with *B. lunaria* in Switzerland suggests that

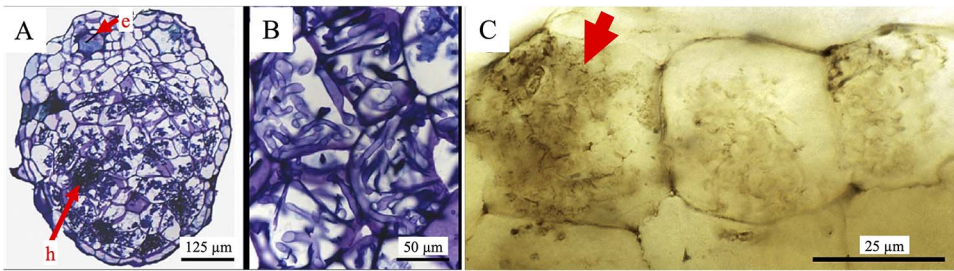


FIG. 1. Arbuscular mycorrhizal (AM) associations in *Botrychium crenulatum* gametophytes and its structural comparison with approximate 400 million-year old Rhynie chert mycorrhizas. (A) Longitudinal section of *B. crenulatum* gametophyte with AM hyphae (h) and egg cell (e). (B) Magnified view of AM hyphae. (A–B) were modified from Winther and Friedman (2007). (C) Arbuscules (arrow) in cells of early land plant gametophyte *Lyonophyton rhyniensis* (from Taylor *et al.*, 2005; Brundrett *et al.*, 2018). Structural comparison of arbuscular mycorrhizas seems to be highly similar between modern and ancient associations (Taylor *et al.*, 2005).

Botrychium is highly selective of its symbionts, and that in addition to transferring mineral and organic nutrients to *Botrychium*, the mycorrhizae favorably modify the physical and chemical properties of the soil around the *Botrychium* roots (Sandoz *et al.*, 2020).

FROM GAMETOPHYTE DEVELOPMENT TO SPOROPHYTE EMERGENCE

With carbon obtained via the fungus, the gametophyte grows to maturity and produces both male and female gametangia simultaneously on the same plant (Fig. 2A–C; Dauphin, Grant, and Farrar, 2020; Farrar and Johnson, 2022). This bisexual condition allows the production of a sporophyte through self-fertilization, also referred to as gametophytic selfing (Fig. 2D). The sporophyte differentiates over the course of several years into a stem with roots and a single apical meristem, all still belowground. This belowground development relies on carbon received from the arbuscular mycorrhizal fungi, which are found in all tissues except the apical bud throughout its lifetime (Winther and Friedman, 2007).

After several years of exclusively belowground growth (Bierhorst, 1971), the oldest of the leaf primordia in the apical bud elongates its common stalk and emerges aboveground where it differentiates dichotomously into two branches, one with a photosynthetic segment (*i.e.*, trophophore) and the other segment destined to produce sporangia (*i.e.*, sporophore; Fig. 2E–F). After spore release at maturity, the leaf withers and the belowground plant no longer obtains photosynthetic support from its trophophore that year. Hoefflerle (1999) has found that harvesting young leaves prior to spore release versus spore releasing leaves resulted in larger leaves the following year than do plants whose leaf is allowed to mature and release spores. This suggests that the production of spores is a net drain on the reserves of the belowground plant. Annual monitoring of marked leaf emergence has also revealed that individual plants may not produce aboveground leaves

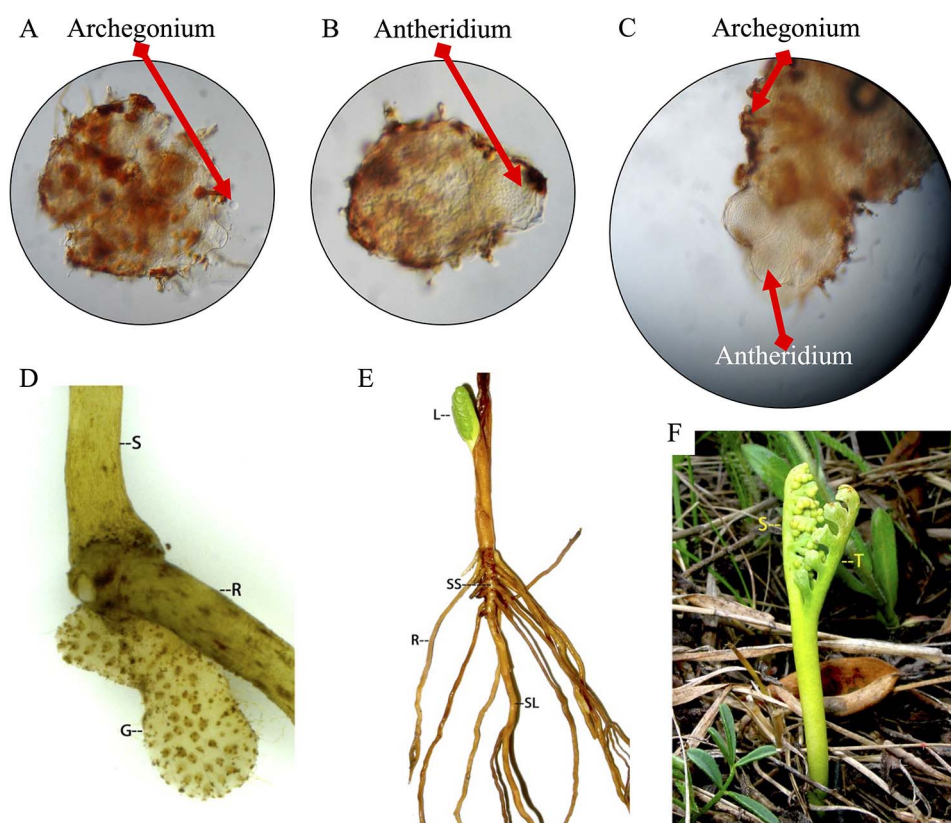


FIG. 2. Main sexual development stages and morphological structures displayed by *Botrychium* taxa. Initial first development of either female (A) or male (B) gametangia followed by a mix of both sexes (C) in *B. campestre* individuals. (D) Gametophyte supporting the young sporophyte stem and first root of *Botrychium minganense*. (E) Belowground roots, stem, and next year's leaf of *Botrychium pumicola*. (F) Immature leaf of *Botrychium campestre* with its aboveground leaf branches separated into a trophophore with pinnae and a sporophore with sporangia. (A–C) and (D–F) were modified from Dauphin, Grant, and Farrar (2020) and Farrar and Johnson (2022), respectively.

every year (Johnson-Groh and Lee, 2002), and in some cases (*e.g.*, extended drought) may persist indefinitely without production of aboveground leaves (Popovich, Farrar and Johnson, 2024).

AN ALTERNATIVE HYPOTHESIS FOR TAXON DIFFERENTIATION IN HOMOSPOROUS VASCULAR PLANTS

Isolated spores can form new populations sexually only if they are capable of gametophytic selfing, and if their resulting homozygous genotype is suited to the environment to which they have been transported. Since the capacity for gametophytic selfing is frequent in ferns (Sessa, Testo, and Watkins, 2016), and very common in *Botrychium* (Hauk and Haufler, 1999; Stensvold and

Farrar, 2016), it is thus probable that populations derived from single spores can be maintained through reiterative gametophytic selfing and can further disperse to suitable habitats by the same process (Fig. 3A). In homosporous vascular plants, the current view regarding taxon dispersal and differentiation via gametophytic selfing is *in isolation* where, after long-distance spore dispersal, a single isolated spore may establish a new sporophyte population instantly differentiated by its homozygous genotype and the low probability of contact with new migrants (interpreted in Fig. 3A; Haufler *et al.*, 2016).

We propose an alternative hypothesis in which the genetically depauperate taxa of *Botrychium* could have differentiated *in situ* from genetically rich populations through environmental disruption of sperm migration among gametophytes of a population (Fig. 3B). In habitats without seasonal mass water flow, reliance on gametophytic selfing is required for survival, while selection in concert with genetic drift shape homozygous genotypes. Subsequently, long-distance spore dispersal can generate additional new populations over broad geographical ranges. This dependence on gametophytic selfing leads to near-fixation of genotypes such as described in the worldwide *B. lunaria* complex (Stensvold and Farrar, 2016). Among these taxa, differences in genetic composition, morphology, ecological preferences, physiology (including drought tolerance and drought avoidance through phenology shifts), and distribution indicate that genotype selection has also occurred in the process of genotype fixation (Stensvold, Farrar and Johnson-Groh, 2002), with some evidence for adaptive forces taking place in particular habitats. For example, *B. crenulatum* exhibits a strong association with soils that are water-saturated for much of the growing season, and where *B. onondagense* and *B. neolunaria* grow in proximity, the former occupies well-drained grounds, whereas the latter occupies lower, flatter topography, often in stream bottomlands and beach meadows (Farrar, Gilman, and Moran, 2017; Mossion *et al.*, 2023).

Arbuscular mycorrhizal fungi have established mutualistic associations with more than half of modern ferns and lycopods (Lehnert, Krug, and Kessler, 2017). In genera of the Ophioglossales, in addition to *Botrychium*, they have been reported in *Botrypus* (Kovács, Balá, and Péntzes, 2007) and *Ophioglossum* (Field *et al.*, 2015). Similar fungal filaments have also been described as “filling most of the internal cells” of the underground gametophytes of *Psilotum* in the Psilotales, the closely related order to the Ophioglossales (Bierhorst, 1971). Arbuscular mycorrhizal associations have also been reported in Lycopodiaceae (Winther and Friedman, 2008), which are distantly related to ferns, but in *Lycopodium* species, similar to *Botrychium* in that they have a belowground gametophyte stage in their reproductive cycle. These observations suggest a spectrum of taxa to which our finding in *Botrychium* may be relevant. Studies of Devonian land plants have given rise to similar questions regarding dispersal and reproductive biology of the first land plants, including consequent loss of genetic variability and expression of deleterious alleles through obligatory gametophyte self-fertilization (Taylor, Kerp, and Hass, 2005). We invite specialists in this area to evaluate the differentiation and dispersal model we

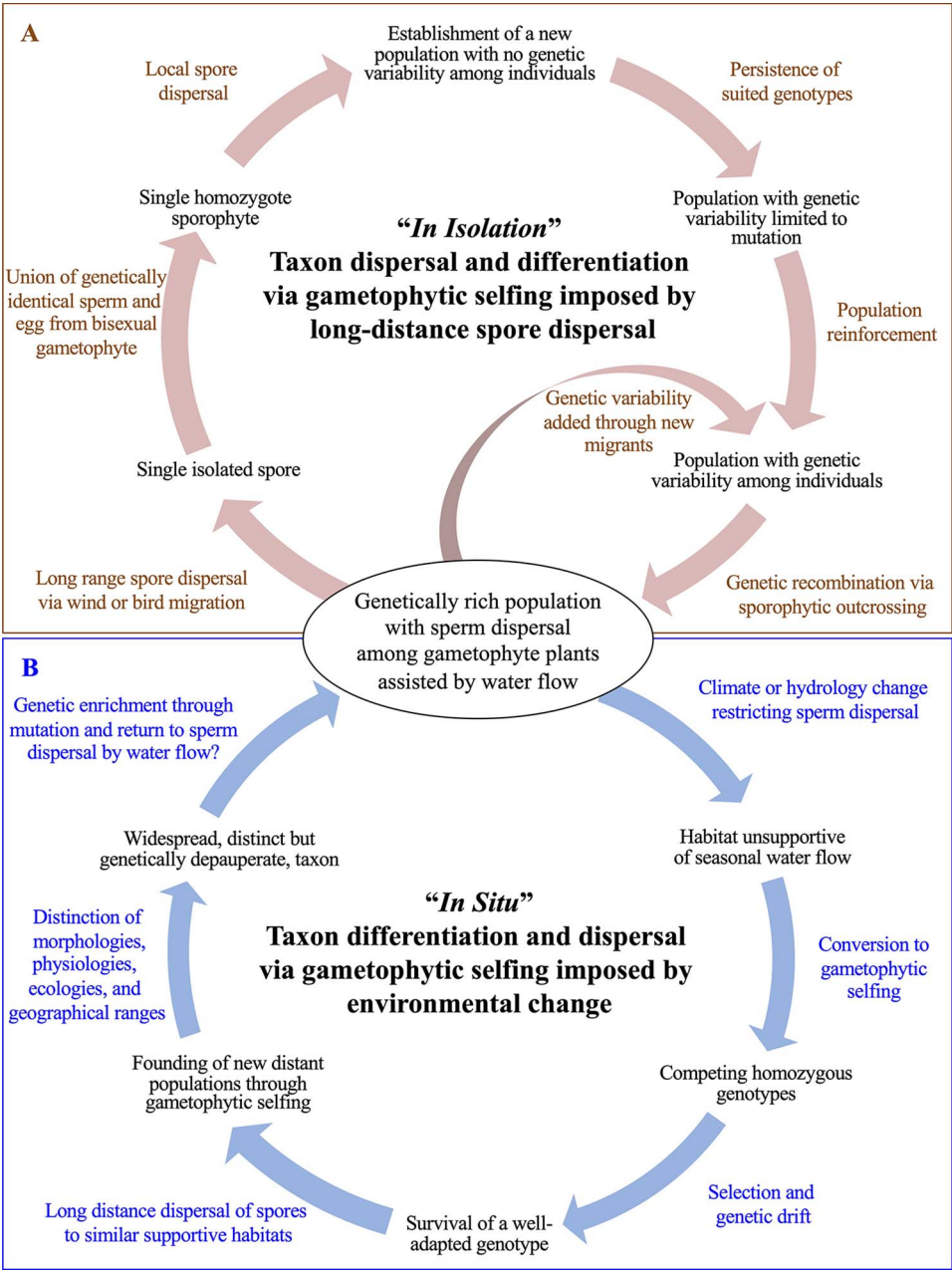


FIG. 3. Two models for differentiation and dispersal in homosporous vascular plants facilitated by gametophytic selfing. (A) Authors’ interpretation of hypothesis presented by Haufler *et al.* (2016) emphasizing spore isolation following long distance spore dispersal. (B) An alternative hypothesis of *in situ* differentiation of genetically depauperate *Botrychium* taxa described in this study.

present for *Botrychium* as to its potential applicability in accommodating constraints imposed by gametophytic selfing.

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LITERATURE CITED

- BIERHORST, D. W. 1971. *Morphology of vascular plants*. Macmillan.
- BRUNDRETT, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* 154: 275–304.
- BRUNDRETT, M. C., C. WALKER, C. J. HARPER, and M. KRINGS. 2018. Fossils of arbuscular mycorrhizal fungi give insights into the history of a successful partnership with plants. Pp. 461–480, in M. KRINGS, C. J. HARPER, N. R. CUNEO, and G. ROTHWELL (eds.), *Transformative Paleobotany*. Academic Press, New York.
- DAUPHIN, B., J. R. GRANT, and D. R. FARRAR. 2020. Outcrossing mating system of the early-divergent moonwort fern (*Botrychium lunaria*, Ophioglossaceae) revealed in the European Alps. *International Journal of Plant Sciences* 181:926–936.
- FARRAR, D. R., and A. V. GILMAN. 2017. Relationships in the *Botrychium campestre* (Ophioglossaceae) complex. *Brittonia* 69:265–275.
- FARRAR, D. R., A. V. GILMAN, and R. C. MORAN. 2017. Ophioglossales. In R. F. C. NACZI, and J. R. ABBOTT (eds.), *New manual of vascular plants of northeastern United States and adjacent Canada*. NYBG Press, New York.
- FARRAR, D. R., and C. L. JOHNSON. 2022. Methodologies for soil extraction and conservation analysis of ferns and lycophytes with belowground gametophytes. *Applications in Plant Sciences* 10(2):e11469.
- FARRAR, D. R., and M. C. STENSVOID. 2017. Observations on bipolar disjunctions of moonwort ferns (*Botrychium*, Ophioglossaceae). *American Journal of Botany* 104:1675–1679.
- FIELD, K. J., J. R. LEAKE, S. TILLE, K. E. ALLINSON, W. R. RIMINGTON, M. I. BIDARTONDO, D. J. BEERLING, and D. CAMERON. 2015. From mycoheterotrophy to mutualism: mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytologist* 205:1492–1502.
- GERRIENNE, P., and P. GONEZ. 2011. Early evolution of life cycles in embryophytes: A focus on the fossil evidence of gametophyte/sporophyte size and morphological complexity. *Journal of Systematics and Evolution* 49:1–16.
- HALLIDAY, T. 2022. *Otherlands: A Journey Through Earth's Extinct Worlds*. Random House.
- HAUFLER, C. H., K. M. PRYER, E. SCHUETTPELZ, E. B. SESSA, D. R. FARRAR, R. C. MORAN, J. J. SCHNELLER, J. E. WATKINS, and M. D. WINDHAM. 2016. Sex and the single gametophyte: Revising the homosporous vascular plant life cycle in light of contemporary research. *BioScience* 66:928–937.
- HAUK, W. D., and C. H. HAUFLER. 1999. Isozyme variability among cryptic species of *Botrychium* subgenus *Botrychium* (Ophioglossaceae). *American Journal of Botany* 86:614–633.
- HODGE, A., T. HELGASON, and A. H. FITTER. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* 3:267–273.
- HOEFFERLE, A. M. 1999. *Impacts of aerial leaf removal on leaf size of the daisy leaf moonwort (Botrychium matricariifolium) and the triangle moonwort (Botrychium lanceolatum var. angustisegmentum) in the subsequent year* Michigan Technological University. Houghton, MI, U.S.A.
- JOHNSON-GROH, C. L., and J. M. LEE. 2002. Phenology and demography of two species of *Botrychium* (Ophioglossaceae). *American Journal of Botany* 89:1624–1633.
- KERP, H., N. H. TREWIN, and H. HASS. 2007. New gametophytes from the Early Devonian Rhynie chert. *Transactions of the Royal Society of Edinburgh* 94:411–428.

- KOVÁCS, G. M., T. BALÁZS, and Z. PÉNZES. 2007. Molecular study of arbuscular mycorrhizal fungi colonizing the sporophyte of the eusporangiate rattlesnake fern (*Botrychium virginianum*, Ophioglossaceae). *Mycorrhiza* 17:597–605.
- LEHNERT, M., M. KRUG, and M. KESSLER. 2017. A review of symbiotic fungal endophytes in lycophytes and ferns – A global phylogenetic and ecological perspective. *Symbiosis* 71:77–89.
- MEZA-TORRES, E. I., M. C. STENSVOLD, D. R. FARRAR, and M. S. FERRUCCI. 2016. Circumscription of the South American moonwort *Botrychium* (Ophioglossaceae). *Plant Biosystems* 151:258–268.
- MOSSION, V., E. KOENEN, J. GRANT, D. CROLL, D. R. FARRAR, and M. KESSLER. 2023. Global diversification of the common moonwort ferns (*Botrychium lunaria* group, Ophioglossaceae) was mainly driven by Pleistocene climatic shifts. *bioRxiv*. <https://doi.org/10.1101/2022.09.28.509846>
- POPOVICH, S. J., D. R. FARRAR, and C. L. JOHNSON. 2024. Population persistence of *Botrychium* (Ophioglossaceae) without production of aboveground leaves. *American Fern Journal* 114:84–93.
- ROTHFELS, C. J., F. W. LI, E. M. SIGEL, L. HUIET, A. LARSSON, D. O. BURGE, M. RUHSAM, M. DEYHOLOS, D. E. SOLTIS, C. N. STEWART, S. W. SHAW, *et al.* 2015. The evolutionary history of ferns inferred from 25 low-copy nuclear genes. *American Journal of Botany* 102:1089–1107.
- SANDOZ, F. A., S. BINDSCHEDLER, B. DAUPHIN, L. FARINELLI, J. R. GRANT, and V. HERVE. 2020. Biotic and abiotic factors shape arbuscular mycorrhizal fungal communities associated with the roots of the widespread fern *Botrychium lunaria* (Ophioglossaceae). *Environmental Microbiology Reports* 12:342–354.
- SESSA, E. B., W. L. TESTO, and J. E. WATKINS. 2016. On the widespread capacity for, and functional significance of, extreme inbreeding in ferns. *New Phytologist* 211:1108–1119.
- SOLTIS, D. E., and P. S. SOLTIS. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). *American Journal of Botany* 73:588–592.
- STENSVOLD, M. C., and D. R. FARRAR. 2017. Genetic diversity in the worldwide *Botrychium lunaria* (Ophioglossaceae) complex, with new species and new combinations. *Brittonia* 69:148–175.
- STENSVOLD, M. C., D. R. FARRAR, and C. JOHNSON-GROH. 2002. Two new species of moonworts (*Botrychium* subg. *Botrychium*) from Alaska. *American Fern Journal* 92:150–160.
- TAYLOR, T. N., H. KERP, and H. HASS. 2005. Life history biology of early land plants: deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences* 102:5892–5897.
- WAGNER, W. H., F. S. WAGNER, and J. M. BEITEL. 1985. Evidence for interspecific hybridisation in pteridophytes with subterranean mycoparasitic gametophytes. *Proceedings of the Royal Society of Edinburgh* 86:273–281.
- WHITTIER, P. 1981. Spore germination and young gametophyte development of *Botrychium* and *Ophioglossum* in axenic culture. *American Fern Journal* 71:13–19.
- WILLIAMS, E. W. 2021. Population genetics of species in the genera *Botrychium* and *Botrypus* (Ophioglossaceae). *American Fern Journal* 111:129–146.
- WINTHER, J. L., and W. E. FRIEDMAN. 2007. Arbuscular mycorrhizal symbionts in *Botrychium* (Ophioglossaceae). *American Journal of Botany* 94:1248–1255.
- WINTHER, J. L., and W. E. FRIEDMAN. 2008. Arbuscular mycorrhizal associations in Lycopodiaceae. *New Phytologist* 177:790–801.