COMPARATIVE ECOPHYSIOLOGY OF HYBRID WOOD FERN SPECIES

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Premise of research. Polyploidy and hybridization are prevalent and widespread phenomena in plants. The genetic consequences of genome duplication are numerous, but we understand very little about the comparative ecology of polyploids. In particular, the ways in which hybrid and polyploid offspring differ in ecology relative to their parents and relatives are poorly known. Among angiosperms, the triploid bridge is hypothesized to accrue some marginal fitness benefit to parent species from mostly sterile hybrid offspring. Because of the complexities of the fern life cycle, involving two free-living life stages, the ability to backcross via a triploid bridge should be more limited. If the triploid sporophyte offspring provide no fitness benefit to parents via subsequent generations, this raises the question about whether triploids further compete with parents by occupying similar ecological niches. We investigated comparative ecophysiology across light and wetness gradients of five members of the hybrid wood fern complex, Dryopteris.

Methodology. We selected two related triads. Each triad consisted of a triploid and its diploid and tetraploid progenitor species: the D. × triploidea (3n) triad, with the parents D. carthusiana (4n) and D. intermedia (2n), and the D. × bootii (3n) triad, with the parents D. cristata (4n) and D. intermedia (2n). We sampled the temperature and relative humidity, population abundance and relative frequency, photosynthetic parameters, and hydraulic conductivity of populations to examine the extent of shared ecological niche space.

Pivotal results. We found that triploids varied in habitat space relative to their parents. In the case of the triploid D. × bootii, this taxon occupied habitats similar to those of its parents at similar abundances and frequencies. In the case of D. × triploidea, the hybrid occurred across a more diverse range of habitats, which was more similar to its diploid parent. In both triads, the triploid’s photosynthetic rate was intermediate between those of its parents, and the majority of the photosynthetic parameters were similar to those of the parents. There were some differences in hydraulic conductivity: xylem-specific hydraulic conductivity of the triploid D. × bootii was significantly higher than that of its diploid parent.

Conclusions. Our results show that related triploids, diploids, and tetraploids substantially overlap in morphological and physiological niche space. The high relative frequency of triploids in habitats and the lack of clear niche partitioning within triads suggest that in addition to not contributing to parental reproductive fitness, the triploids could also negatively impact their progenitors by potentially competing for space and light resources in similar ways.

Keywords: polyploidy, triploid, physiology, ferns, Dryopteris.

Introduction

Polyploidy is a widespread and evolutionarily important phenomenon among plants (Otto and Whitton 2000; Levin 2002; Soltis et al. 2003; Soltis and Soltis 2009). Depending on the criteria used, approximately 40%–80% of angiosperm species display some signal of historic polyploidy (Soltis et al. 2003). Polyploidy is especially prevalent in pteridophytes, with anywhere from 43% to 95% of fern species showing evidence of additional sets of chromosomes (Haufler and Soltis 1986). Wood et al. (2009) estimated that 15% of speciation events in angiosperms and 31% in ferns are accompanied by a change in ploidal level. Whole-genome duplication events have now been identified in the evolutionary histories of most major lineages of land plants (Otto and Whitton 2000; Husband et al. 2013; Li et al. 2015). Research conducted with fertile polyploids indicates that there

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are many impacts from joining multiple sets of genomic material in one individual. Over evolutionary time, multiple genomes may provide more raw material for mutations, both advantageous and detrimental; diverse opportunities for gene expression and silencing; and buffering capacity against existing and de novo recessive mutations with negative outcomes (Soltis et al. 2003, 2014; Sessa 2019).

Polyploidy is predicted to correlate with both increased range size (Levin 2002) and more stressful habitats (Van de Peer et al. 2021; Naghiolo and Vamosi, forthcoming), but empirical support for this has been mixed (Lowry and Lester 2006; Te Beest et al. 2012; Johnson et al. 2014; Brittingham et al. 2018; Stevens et al. 2020; Granse et al. 2021). Among wild Solanum, diploids and tetraploids have similar range sizes, with other polyploids (3n, 5n, 6n) occupying much smaller ranges (Hijmans et al. 2007). Among other species, such as the allopolyploid Polystichum scopulinum, the range size of the hybrid exceeds that of the parents (Marchant et al. 2016). Research on allopolyploidy in Adiantum finds that edaphic characteristics are important for the partial niche partitioning seen between ploidal levels (Southgate et al. 2019). In some cases of fertile hybrids, such as in the Dryopteris triad of D. goldiana (2n), D. ludoviciana (2n), and their tetraploid hybrid offspring, D. celsa, the parents’ ranges do not overlap extensively with each other, although they each substantially overlap with that of the 4n hybrid (Marchant et al. 2016). Differences in physiology might also serve to partition niche space between progenitors and hybrid offspring. Previous research on the physiology of Dryopteris indicated that hydraulics might be responsible for the small-scale distribution of cytotypes (Sessa and Givnish 2014). Studies have sought to untangle the relationship of polyploidy with abiotic stress, with some studies showing greater tolerance of drought and salinity (Wang et al. 2013). Hao et al. (2013) reported that autotetraploid individuals of Atriplex canescens were more resistant to xylem cavitation than diploids. While the polyploids were not more stress tolerant, the study found that diploids and autopolyploids explored different niche space related to water availability. Diploids were found more frequently in sandy areas with higher water availability, and polyploids occupied broader niches (4n) and ones with more drought-prone clay soils (4n and 6n; Hao et al. 2013). However, other work has shown that diploids and polyploids may have similar water use efficiency, despite differences in morphology or physiology (Mtílení et al. 2021). Observations that diploid and fertile polyploids may occupy similar areas of climatic space (Glennon et al. 2014) imply that, at the local scale, individuals might often be coresidents in the same or in neighboring habitats with concomitant stressors. It may be that the ultimate benefit of polyploidy lies not in an increase in stress tolerance per se but rather in the increased flexibility demonstrated by most polyploids (Van de Peer et al. 2021).

Although research on the origin, evolution, and ecology of polyploids is rich and complex, less work has been done on the ecology and comparative physiology of parents and offspring (Soltis et al. 2010), especially coresident sterile hybrid offspring. Studies of angiosperms indicate that photosynthetic rates on a per cell basis may be higher in species with multiple genomic copies (Coate et al. 2012), such as grasses (reviewed in Te Beest et al. 2012) and Populus tremuloides (Greer et al. 2018). Because of increased cell size in polyploids, this signal sometimes disappears in the case of photosynthetic rate scaled by leaf area (Warner and Edwards 1989; Te Beest et al. 2012). Romero-Aranda et al. (1997) found that per cell photosynthetic rates were similar between 2n and 4n citrus but that overall, photosynthetic rates were lower for tetraploids. Studies from the 1940s and 1950s indicate that photosynthetic rates are lower in polyploids such as Hordeum vulgare, Galeopsis pubescens, and Ribes satigerum (reviewed in Te Beest et al. 2012). Work done at the organismal scale on the wood fern species complex, Dryopteris, across its range indicated that fertile tetraploid hybrids demonstrated introgressive photosynthetic physiology, relative to their diploid progenitors (Sessa and Givnish 2014).

In ferns, promiscuous mating systems often result in fertile and sterile hybrid lineages (Tate et al. 2005). Hybrid sporophytes have different levels of fertility (Barrington 2011), and allopolyploid individuals with uneven sets of chromosomes are often unable to produce fertile spores themselves, barring further mitotic errors (Otto and Whitton 2000). Because of their inability to create a self-sustaining lineage with a unique gene pool, allopolyploids have been considered evolutionary dead ends by some authors (Otto and Whitton 2000) and lack the necessary characteristics to be considered species (Barrington et al. 1989; Barrington 2011). Because of the complexities of the fern life cycle, mature triploid plants are unable to directly backcross with parent species and must first produce viable, unreduced gametophytes, likely a rare event. This might prevent fern plants from contributing to the triploid bridge, a model explaining the continued persistence of angiosperm polyploids (Ramsey and Schemske 1998; Soltis et al. 2003; Husband 2004). In spite of the limited evolutionary potential for triploids, they may play important roles in the ecology and thus evolution in the habitats in which they grow.

It remains unclear to what extent sterile hybrids compete with their parents or whether they exhibit unique physiological methods of partitioning niche space. While often rare, hybrids can become quite abundant in some habitats (Nesom 1983; Kim et al. 2006; Čertner et al. 2022), making their competitive presence nonnegligible. Triploids are recurrent hybrids and not self-sustaining lineages without asexual propagation. Because of this, the benefits associated with polyploidy (e.g., benefits allowing polyploids to replace their progenitors within a habitat) may be limited (Fowler and Levin 1984; Husband 2004; Basak 2005). Alternatively, the combination of multiple genomes may make them more flexible than their parents and potentially enhance their competitive advantage. The immediate consequences of polyploidization, such as phenological changes, larger body sizes, or increased sizes of stomata and cells, might allow polyploids to partition habitats more finely (Fowler and Levin 1984; Te Beest et al. 2012; Rodriguez 2019). The highly variable morphology of triploids and the resulting difficulties in distinguishing the triploid hybrids from their progenitors lend support to this second hypothesis.

Here we investigate the prevalence, ecology, and ecophysiology of five Dryopteris species: the diploid D. intermedia and its two naturally occurring triploid hybrids, D. × triploidea and D. × boottii, formed from crosses with the tetraploids D. carthusiana and D. cristata, respectively (Sessa and Givnish 2014; Testo et al. 2015). Specifically, to understand the potential for intercytotype competition, we were interested in knowing whether the triploid hybrids occurred in habitats similar to those of either or both parents and to what extent their physiology
differed from that of these parents. On the basis of range maps and previous observations, we expected to find these five species occurring in the same general location, with some differentiation in habitat at the local scale. We hypothesized that the lack of marked differences in habitat could be the result of species-based partitioning of physiological niche space. If simply having more genetic variety was beneficial, the triploid $D. \times bootii$ might outperform both parents since it contains genetic material from three different genomes, $D. intermedia$, $D. ludoviciana$, and "D. semicristata" (Sessa et al. 2012). On the other hand, $D. \times triploidea$ would be more similar to its parents, especially $D. intermedia$, which makes up two-thirds of the genome in conjunction with $D. cristata$ (Sessa et al. 2012). Alternatively, if increased genomic content related directly to improved physiological functioning, we predicted that triploids would exhibit higher photosynthetic rates and related parameters compared with diploid parent species and would be more similar to their tetraploid parents because of the extra copy of that parent’s genome.

Material and Methods

Plants

In the summer of 2013, we collected data on the presence and physiology of five related $Dryopteris$ species with varying ploidy levels: $D. intermedia$ (2n), $D. \times triploidea$ (3n), $D. \times bootii$ (3n), $D. carthusiana$ (4n), and $D. cristata$ (4n). $Dryopteris \times triploidea$ is an infertile hybrid of $D. intermedia$ and $D. carthusiana$; $D. bootii$ is the infertile hybrid of $D. intermedia$ and $D. cristata$ (fig. 1). Ferns exhibit an alternation of generations characterized by free-living sporophyte and gametophyte phases. Ferns in the $Dryopteris$ genus are terrestrial and grow in rocky, wooded, or swampy areas. The species complex consists of varying ploidal levels that hybridize frequently. Sporophyte hybrids are identified by having unique combinations of characters from different parents (Montgomery and Wagner 1993). The study site was located at the Huyck Preserve (lat. 42.525275, long. −74.172492), in Rensselaerville, New York.

Fig. 1  Relationships between five related species in the $Dryopteris$ genus of wood ferns. Ploidal level is indicated in parentheses. Diploids and tetraploids are indicated by filled icons; triploids are indicated by open icons. Genome copies are italicized in a gray font and follow the notation given in Sessa et al. (2012) and Kinosian et al. (2019).
Habitat Data and Species Distribution

We established three 100-m transects that crossed three habitat types: swamp (fig. 2A), transition (fig. 2B), and forest (fig. 2C). Preexisting habitat maps and field experience were used to delineate habitat types, which were ground-truthed in the field. Swamp habitats were characterized by wet ground and sphagnum moss. Forests consisted of mixed, hardwood, and/or conifer, mainly hemlock, forests with well-drained soils. Transition areas were intermediate between the swamp and forest areas, with intermittently wet soils, shrubs, and marsh grasses.

We subdivided each transect into 2 × 10-m quadrants, and within each of these, we quantified the distribution of each taxon by calculating the relative species abundance and frequency across the transects. Our data were calculated relative to the three species and two hybrid ferns examined in the study. To calculate the relative abundance and frequency of each fern, we determined the number of plots with species i (F_i) and the number of individuals of species i in these plots (N_i). Relative abundance was then calculated by dividing N_i by the total number of ferns in the transect (N) using (N_i/N) × 100. Relative frequency was then calculated as (F_i/sum of all F_i) × 100. Next, we quantified the microenvironment of each habitat by collecting data on light transmittance, soil pH, temperature, and humidity. The light environment was determined by first taking hemispherical photos using a fish-eye lens on a Nikon D40 SLR camera in the center of every 2 × 10-m quadrant. We then used Gap Light Analyzer software (ver. 2.0; Cary Institute, Millbrook, NY) to characterize the percentage of canopy transmittance. We determined soil pH by taking a 20-g subsample of the top 10 cm of the soil core and thoroughly mixing this with distilled water for 5 min. The mixture was allowed to stand for 1 h, at which point we took a measurement of the solution pH using a Fisherbrand AE150 benchtop pH meter. Soil samples were taken at the same points where we took the hemispherical photos. Temperature and humidity data were collected using HOBO sensors (U23 Pro, Onset, Bourne, MA) in 15-min increments. Three sensors were placed along the transects at 0, 50, and 100 m, with three reference sensors placed in the center of an open field. All 12 sensors were programmed to run simultaneously, and all data were recorded over the summer of 2013.

Physiology

Physiological data were collected using the LiCor6400 (LiCor Biosciences, Lincoln, NE). For each of the five fern species, we generated 13 light curves on individuals along the transect across their range of habitat variation. We sampled at approximately 5-m intervals along transects so that sampling was not concentrated in one location or habitat type. For all five taxa across the three transects, we generated a total of 65 light response curves for 65 different fronds from 65 unique individuals. Measurements were taken on pinnae that were in the middle of each frond. We measured light responses using the standard 2 × 3 LED chamber with a flow rate of 400 mmol s⁻¹ and the CO₂ mixer set to 400 ppm. After running test curves, we established that all species reached their maximum photosynthetic rate and were uninhibited at 500 μmol m⁻² s⁻¹, and fronds needed 5–8 min to stabilize stomatal conductance at this light level. After this stabilization period, a custom auto program was run to develop the curves, backing light levels down from 500 μmol m⁻² s⁻¹. From these light curves, we derived the following parameters: quantum yield (QY; μmol m⁻² s⁻¹), light compensation point (LCP; μmol photosynthetically active radiation m⁻² s⁻¹), maximum photosynthetic rate (A_\text{max}; μmol m⁻² s⁻¹), stomatal conductance (mol H₂O m⁻² s⁻¹), and respiration (μmol m⁻² s⁻¹). All parameters were calculated using the program Photosyn Assistant (ver. 1.1.2; Dundee Scientific, Dundee, UK).

We collected morphological data on stomatal density and specific leaf area (SLA). We measured stomatal density by cutting pinnae from the middle of an individual frond, mounting a small section from the middle of the pinnae on a slide, and then counting stomata in three areas on each of five fresh leaves per individual per species using a compound light microscope at ×40. Mean stomatal densities were averaged for 7–12 individual plants per species. SLA was assessed by taking leaf punches from 20 leaves of separate individuals across their habitat range using a surgical 2.0-mm² biopsy punch (Miltex by Kai). These samples were dried for 72 h at 30°C and were then weighed using a Mettler Toledo five-place balance. We measured hydraulics using methods modified from Watkins et al. (2010). We collected 10 individual fronds from 10 different individuals per species between 0630 and 0730 hours. Fronds were double bagged and immediately brought to the laboratory. We then excised 5–10-cm stipe segments under deionized water and trimmed them with a fresh razor blade. These were then connected to a pressurized conductivity system connected to a digital pressure transducer. System pressure was created using an external air tank connected to a sealed plastic HPLC vessel partially filled with a perfusing solution of 10 mmol KCl and connected to a digital pressure transducer. Using this system, we took an initial survey of the hydraulic conductivity of freshly collected material and then pressurized the stem with three high-pressure (0.175–200 MPa) flushes to remove any air bubbles that might impede flow. Preliminary postflush conductivity was similar, indicating that the collected leaves were largely embolism-free. We then pushed the perfusing solution through the stems at an average pressure of 0.06 MPa (0.063–0.075 MPa) and into a small plastic beaker on a Mettler Toledo five-place balance. The increasing solution mass on the balance was recorded every 15 s for 2 min, and these data were used to calculate the slope (rate of flow) of a given stipe section length at a given pressure. Hydraulic conductivity measurements were initially made for segments by determining the flow rate of the KCl solution through segments of known length (data were normalized by the actual stipe length) and the known constant pressure gradient that was supplied by the pressure head. Thus, the length-normalized (I_{\text{seg}})-hydraulic conductance of the stipe (K_{\text{seg}}; kg m⁻¹ s⁻¹ MPa⁻¹) was calculated by incorporating length, time, pressure, and mass flow, as derived by the balance method. The xylem-specific conductivity (K_i) was expressed by dividing K_{\text{seg}} by the total vascular area in cross section; leaf-specific conductivity (K_{\text{leaf}}) was calculated by dividing the segment conductivity by the total leaf area supplied by the measured segment. Total vascular area was determined by making thin hand sections from the middle of each measured stipe. These sections were wet mounted on slides and observed under bright-field microscopy. Sections were photographed and analyzed using Image J software (ver. 1.37; National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/).
Fig. 2  *Dryopteris* species were sampled along transects in three habitats: swamp (A), transition zone (B), and forest (C). Over 72 h from July 9 through July 12, 2013, average temperature (D) and relative humidity (E) were recorded every 15 min. Measurements were taken in three habitats and an open field (as a reference).
Leaf-specific conductivity ($K_b$) was expressed by dividing the segment conductivity by the total frond area supplied by the measured segment from an individual frond. Total frond area was measured using a LiCor 3100C leaf area meter (LiCor Biosciences, Lincoln, NE).

**Statistical Methods**

We assessed differences among species using ANOVAs that were corrected for multiple comparisons using a Bonferroni correction. We tested for normality with the Shapiro-Wilk test. Data failing the test for normality were transformed; $QY$ was log 10 transformed, and respiration was exponentially transformed (base $e$). We then conducted ANOVAs on the differences in photosynthetic parameters ($A_{\text{max}}$, conductance, $QY$, LCP, and respiration) by species, using a Bonferroni-corrected $P$ value of 0.01 to demarcate statistical significance for the overall tests (Fig. 3). We tested for homogeneity of variances using Levene’s test. The assumed homogeneity of variances was generally met, but when it was violated (such as when comparing soil pH), we performed a Welch $F$-test. Tukey’s honestly significantly different (HSD) post hoc test was used to compare groups. We calculated Cohen’s $d$ to estimate effect size (Coe 2002; Vacha-Haase and Thompson 2004). All comparisons were conducted in JMP Pro 14; figures were produced using Sigma Plot (ver. 14) and with the ggplot package (ver. 2.2.1) in R (ver. 3.4.1).

**Results**

All three habitats differed from the standard open field in mean temperature, with the open field displaying the highest temperatures during the daylight hours and sometimes overnight (Fig. 2D). Mean relative humidity was also similar among habitats, with swamps and transition zones demonstrating slightly higher mean relative humidity. All three habitats had higher relative humidity than the open field (Fig. 2E).

When comparing soil pH, we found that the forest was significantly more acidic (mean pH = 4.86) than the swamp (mean pH = 5.94); pairwise comparison using Wilcoxon: $P = 0.0004$) and the transition zone (mean pH = 5.49); pairwise comparison using Wilcoxon: $P = 0.0148$; Welch $F$-test: $F_{2,28.136} = 9.1502$, $P = 0.0009$).

*Dryopteris intermedia* and *D. × triплоidea* were the only taxa found to grow in the forest and across all three habitat types, with *D. intermedia* most abundant and frequently found in the forest (Fig. 4). The tetraploids *D. cristata* and *D. carthusiana* and the triploid *D. × bootii* were limited to the swamp and transition zone habitats. *Dryopteris carthusiana* was the most abundant species in the transition zone and swamp and was encountered more frequently in the swamp than other species. The triploid *D. × bootii* was equally relatively abundant in the swamp and transition zones and showed similar relative frequencies in both habitats, a pattern it shared with *D. cristata*. *Dryopteris × triплоidea* was equally abundant in swamps and forests but was more frequently encountered in the forested plots. *Dryopteris × bootii* was completely absent from the forested plots (Fig. 4). Canopies averaged between 15% and 20% open for all areas in which study species were naturally found. Although *D. cristata* tended to be found in slightly more open habitats, the difference was not statistically significant (Fig. 3; $F_{4,68} = 2.1815$, $P = 0.0803$, $N = 73$).

We constructed light response curves for each species triad and found that both hybrids demonstrated introgression with their respective parents (Fig. 5). ANOVAs (Fig. 3) showed that respiration differed significantly ($F_{4,50} = 4.2081$, $P = 0.0052$, $N = 55$) among groups, with *D. × bootii* having significantly higher respiration than *D. carthusiana* (Tukey’s HSD: $P = 0.0272$; Cohen’s $d = 1.23$), *D. × triпоidea* ($P = 0.0296$, Cohen’s $d = 1.22$), and *D. × bootii’s* diploid parent, *D. intermedia* ($P = 0.0155$, Cohen’s $d = 1.24$). Maximum photosynthetic rate differed significantly ($F_{4,55} = 7.2290$, $P < 0.0001$, $N = 60$) among groups; $A_{\text{max}}$ for *D. cristata* was significantly higher than $A_{\text{max}}$ for *D. × triпоidea* ($P = 0.0041$, Cohen’s $d = 1.52$) and *D. intermedia* ($P < 0.0001$, Cohen’s $d = 2.08$).

We found differences in LCP among species to be of borderline statistical significance ($F_{4,55} = 3.4309$, $P = 0.0142$, $N = 60$), with *D. × bootii’s* LCP trending higher than that of its diploid parent, *D. intermedia* ($P = 0.0152$, Cohen’s $d = 1.3411$). We found no statistically significant difference in $QY$ ($F_{4,55} = 0.9698$, $P = 0.4315$, $N = 60$) or in conductance ($F_{4,54} = 1.8850$, $P = 0.1264$, $N = 59$) among taxa.

We investigated hydraulic conductivity scaled by total leaf area ($K_b$) and scaled by xylem ($K_t$). We found no statistically significant differences in $K_b$ among species ($F_{4,38} = 1.8283$, $P = 0.1435$, $N = 43$). We found differences among species in stem-specific conductivity ($K_s$, $F_{4,42} = 4.1819$, $P = 0.0061$, $N = 47$), with *D. × bootii* having higher $K_s$ values than its tetraploid parent, *D. cristata* ($P = 0.0023$, Cohen’s $d = 1.8776$). There were no differences among species in SLA ($F_{4,64} = 1.6828$, $P = 0.1649$, $N = 69$) or mean stomatal density ($F_{4,55} = 1.4212$, $P = 0.2392$, $N = 60$).

**Discussion**

The North American *Dryopteris* complex represents a promiscuous array of taxa capable of remarkable examples of hybridization and polyploidy. Understanding the ecology of this group has been hampered by the difficulty of identifying discrete taxa. To deal with the former, we selected an assemblage of five taxa that are closely related and hybridize frequently (Fig. 1). We initially predicted that triploids would be more similar to their tetraploid parents, compared with their diploid parents, given the probable variation in genomic dosage. This was rarely the case, and taxa differed in surprising ways. Triploids were generally intermediate between both parents in physiological traits, with the exceptions of respiration rate and hydraulic conductivity. From a physiological and ecological perspective, the spatial overlap between triads suggests opportunities for interactions between parents and sterile offspring.

Our autecological surveys revealed that while all taxa occurred in swamp and transitional habitats, the diploid *D. intermedia* and the triploid *D. × triплоidea* (*D. intermedia × D. carthusiana*) were the only taxa found in upland forest habitats. In fact, in abundance, *D. × triплоidea* was intermediate between both parents: *D. intermedia* was most abundant in forests, *D. carthusiana* in swamps. *Dryopteris × triплоidea* was abundant...
### Physiological and Ecological Parameters

|                     | Mean respiration (μmol m⁻² s⁻¹) | Mean $A_{\text{max}}$ (μmol m⁻² s⁻¹) | Mean LCP (μmol PAR m⁻² s⁻¹) | Mean QY 0-30 (μmol m⁻² s⁻¹) | Mean conductance (mol H₂O m⁻² s⁻¹) |
|---------------------|----------------------------------|--------------------------------------|-----------------------------|-----------------------------|-----------------------------------|
| $D. \text{ carthusiana}$ | -0.330 ± 0.082                   | 4.319 ± 0.245                        | 4.973 ± 1.204               | 0.066 ± 2.90 x 10⁻³         | 0.069 ± 5.28 x 10⁻³               |
| $D. \text{ x triploidea}$ | -0.306 ± 0.082                   | 3.897 ± 0.245                        | 5.101 ± 1.204               | 0.061 ± 2.90 x 10⁻³         | 0.074 ± 5.28 x 10⁻³               |
| $D. \text{ intermedia}$ | -0.313 ± 0.072                   | 3.421 ± 0.236                        | 4.211 ± 1.156               | 0.061 ± 2.79 x 10⁻³         | 0.064 ± 5.08 x 10⁻³               |
| $D. \text{ x bootii}$    | -0.673E ± 0.082                  | 4.232 ± 0.256                        | 9.803 ± 1.257               | 0.066 ± 3.03 x 10⁻³         | 0.082 ± 5.52 x 10⁻³               |
| $D. \text{ cristata}$    | -0.519 ± 0.075                   | 5.187 ± 0.245                        | 7.196 ± 1.204               | 0.067 ± 2.90 x 10⁻³         | 0.077 ± 5.52 x 10⁻³               |

|                     | Mean % of canopy open | Mean SLA (m⁻² kg⁻¹) | Mean stomatal density ( stomata/0.126 mm⁻²) | Mean $K_s$ (mol m⁻³ s⁻¹ MPa⁻¹) | Mean $K_l$ (mmol m⁻³ s⁻¹ MPa⁻¹) |
|---------------------|-----------------------|---------------------|---------------------------------------------|-------------------------------|-----------------------------------|
| $D. \text{ carthusiana}$ | 15.96 ± 0.930         | 4.8 x 10⁻⁵          | 5.243 ± 0.387                               | 8.411 ± 2.29                  | 3.252 x 10⁻⁶ ± 6.46 x 10⁻⁶       |
| $D. \text{ x triploidea}$ | 16.24 ± 0.960         | 5.3 x 10⁻⁵          | 6.143 ± 0.387                               | 10.982 ± 3.24                 | 4.560 x 10⁻⁵ ± 1.20 x 10⁻⁵       |
| $D. \text{ intermedia}$ | 15.97 ± 0.960         | 5.1 x 10⁻⁵          | 5.743 ± 0.547                               | 11.260 ± 1.67                 | 5.662 x 10⁻⁵ ± 9.19 x 10⁻⁶       |
| $D. \text{ x bootii}$    | 15.91 ± 1.12          | 6.0 x 10⁻⁵          | 6.533 ± 0.418                               | 19.654 ± 4.58                 | 7.733 x 10⁻⁵ ± 1.90 x 10⁻⁵       |
| $D. \text{ cristata}$    | 19.12 ± 0.930         | 6.0 x 10⁻⁵          | 6.000 ± 0.402                               | 3.462 ± 0.725                 | 5.340 x 10⁻⁵ ± 8.32 x 10⁻⁶       |

Fig. 3  Physiological and ecological parameters from the study, showing untransformed values for ecological parameters plus or minus the standard error. Significance levels (with Bonferroni correction) for entire tests are indicated by asterisks in column headers: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Significance levels for post hoc Tukey’s highly significantly different tests are indicated next to brackets for significant overall tests: *$P < 0.01$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$. $A_{\text{max}}$ = maximum photosynthetic rate; $K_s$ = leaf-specific hydraulic conductivity; $K_l$ = xylem-specific hydraulic conductivity; LCP = light compensation point; PAR = photosynthetically active radiation; QY = quantum yield; SLA = specific leaf area.
and frequently encountered in all three habitats. Interestingly, while not as abundant in the forest as *D. intermedia*, the sterile *D. × triploidea* was more frequently found (i.e., widely dispersed along the transect) in forest habitats than its forest-dwelling parent. A different story emerged for *D. × bootii* (*D. intermedia × D. cristata*). This taxon was one of the least frequently encountered in the study, and it showed locally low relative abundances in swamp and transition zone habitats. It was wholly absent from the forest habitat of its putative diploid, *D. intermedia*. In this case, *D. × bootii* shared more ecological similarities with its tetraploid parent, *D. cristata*, which was rare and infrequent at this site. Even within the closely related species of a single genus, there are nuanced responses to habitat differences that may be obscured by broad generalizations about diploids or polyploids.

These observations are intriguing, as they demonstrate that, at least at this site, a hybrid (*D. × triploidea*) can be relatively common, can outnumber its progenitors, and may gain flexibility in habitat preferences from both parents. On the other hand, a hybrid might ecologically align more strongly with a single parent, showing little influence from the other progenitor, as in the case of *D. × bootii*. In this *D. cristata–D. intermedia* complex, *D. cristata* is largely limited to wet, swampy habitats, and *D. intermedia*, while clearly widespread, prefers more well-drained forest soils. Their triploid hybrid, *D. × bootii*, shares nearly identical habitat preferences with *D. cristata*. Interestingly, the hybrid *D. × triploidea* exhibits less stringent ecological requirements relative to its tetraploid parent, *D. carthusiana*. The genetic contribution from *D. intermedia* increases the niche space of *D. × triploidea*, allowing it to become a frequently encountered wood fern in the forest habitats. On the other hand, *D. intermedia*’s contribution seems to have little impact on the niche space of *D. × bootii*.

Given the ecology of the hybrids and parents, one might predict that the physiology of *D. × bootii* would more closely resemble that of *D. cristata* and that *D. × triploidea* would be transgressive when compared with *D. carthusiana*. However, for many of the variables measured (i.e., SLA, stomatal density, QY, conductance), both hybrids either exhibited no difference or were intermediate relative to both parents (fig. 3). We found that, in the *D. × bootii* triad, xylem-specific hydraulic conductivity (K_{x}) was higher for the hybrid offspring than the tetraploid parent. The *D. × bootii–D. cristata–D. intermedia* triad showed the most within-triad differences in respiration and LCP. However, in general, triploid hybrids occupied physiological niche spaces similar to those of both of their parents.

In one of the few studies of its kind on the physiology and polyploidy of *Dryopteris*, Sessa and Givnish (2014) failed to find evidence of transgressive physiology in fertile polyploids relative to diploid taxa. While their work specifically avoided sterile triploid hybrids, we largely found similar results for our carbon-fixation measurements. An important take-home point from Sessa and Givnish (2014) was the close positive relationship between light availability and maximum photosynthetic rates. When we compared the results from our diploid and tetraploid taxa, our rates of photosynthesis were consistently lower than those reported by Sessa and Givnish. Interestingly, their study sites averaged approximately 5% more canopy openness relative to our site (Sessa and Givnish 2014). Not surprisingly, because of increased shade, our study demonstrated lower maximum photosynthetic rates. Rates of stomatal conductance were commensurate between the two studies. In our investigation, both triads demonstrated substantial within-triad overlap in the measured photosynthetic parameters QY and conductance. In both cases, hybrids exhibited photosynthetic maxima and light response curves that were intermediate between those of the parents. This was not the case in previously investigated diploid-tetraploid triads, where *D. celsa* (4n) and *D. campyloptera* (4n) had lower rates of A_{max} than either diploid parent (Sessa and Givnish 2014). Overall, the light environment experienced by *D. cristata* was brighter than that of all other taxa, and this species exhibited the highest maximum photosynthetic rates. These results are similar to those reported by Sessa and Givnish (2014). The light...
environments of the other taxa in this study were similar, yet photosynthetic parameters varied widely. For example, the tetraploid *Dryopteris carthusiana* had higher photosynthetic values than *D. intermedia*, yet the difference was never significant.

We did not observe a universal increase in physiological functioning with increased ploidal levels. In some situations, such as conductance and LCP, the triploid hybrid *D. × bootii* tended to transgress both parents, while *D. × triplodia* consistently
demonstrated intermediacy. Our results suggest that, when it comes to photosynthesis, the rates for hybrid offspring are not always higher than those of their parents in any substantial way. While previous work suggested that hydraulics could play a large role in habitat selection (Pittermann et al. 2013), we found significant differences in xylem-specific hydraulic conductivity ($K_x$) only between $D. \times bootii$ and its tetraploid parent, $D. cristata$. Hydraulic conductivity may be scaled by different aspects, such as total leaf area ($K_y$) to assess how sufficient the water flow is to the leaves or xylem ($K_x$) to measure how conductive the stem is for water (Tyree and Ewers 1991). Our results indicate that $D. \times bootii$ stipes are significantly more conductive than those of $D. cristata$, which is surprising since they share a more similar ecological niche than do $D. \times bootii$ and $D. intermedia$. This difference does not appear to be related to tracheid length, which increases with ploidy (Pittermann et al. 2013). It has been well established that $K_y$ decreases with smaller tracheid diameter (Tyree and Ewers 1991); unfortunately, we did not collect tracheid diameter for any of the species in this study. While smaller tracheid diameter has been linked to reduced cavitation in some species, pit membrane density and anatomy play a more significant role in limiting the spread of runaway cavitation (Choat et al. 2008). Yet we know of no studies that have examined the relationship of pit membrane anatomy and cavitation in ferns.

Across almost all measured parameters, $D. \times bootii$ was less similar to its parents than was $D. \times triploidea$, although the differences were only sometimes significant. In $K_y$, $D. \times bootii$ was most similar to its diploid parent, $D. intermedia$. In other parameters, such as respiration rate and LCP, $D. bootii$ was more similar to its tetraploid parent, $D. cristata$. $Dryopteris \times bootii$ also showed habitat preferences more similar to those of $D. cristata$. The similarity between the triploid and tetraploid could be due to asymmetrical parentage. Previous work indicates that the tetraploid usually serves as the egg donor for the hybrid triploid offspring (Testo et al. 2015), which makes $D. cristata$ the likely mother of $D. \times bootii$. In addition to providing the larger portion of nuclear genes, $D. cristata$ would also provide the mitochondrial and chloroplast genomes, resulting in a substantial genetic contribution of the tetraploids to the triploid offspring. While this abundance of triploid genetic material is in line with the physiological and ecological patterns we see with the $D. \times bootii$ triad, the patterns of affinity were less evident in the $D. \times triploidea$ triad, suggesting that parentage is not wholly responsible.

Although both triads contain a diploid, tetraploid, and sterile triploid, there is a key difference in the two groups: the triad $D. intermedia$–$D. triploidea$–$D. carthusiana$ holds more of its genome in common. Sessa et al. reconstructed the genome of an extinct $Dryopteris$ progenitor. The genomic vestiges of the extinct parent, named “semi-cristata” and whose genome is indicated by the letters SS, are found in several extant wood ferns, including the tetraploids $D. carthusiana$ (IISS) and $D. cristata$ (SSLL; Sessa et al. 2012). Both resulting triploids also contain one copy of the “semi-cristata” genome (fig. 1). Within the $D. \times triploidea$ triad, each species contains two copies of the $D. intermedia$ (II) genome. The tetraploid parent, $D. carthusiana$, is half $D. intermedia$ (II), half “$D. semi-cristata$” (SS). The triploid genome is two-thirds $D. intermedia$ (II), one-third “$D. semi-cristata$” (SS). In contrast to $D. \times triploidea$, $D. \times bootii$’s genome is evenly distributed: one-third $D. intermedia$ (II), one-third “$D. semi-cristata$” (SS), and one-third $D. ludoviciana$ (LL). $Dryopteris cristata$ is one-half “$D. semi-cristata$” (SS) and one-half $D. ludoviciana$ (LL; Sessa et al. 2015; Testo et al. 2015; Kinosian et al. 2019). The differences in the proportions of shared genomes might be partially responsible for the different patterns of responses in the two triads, with $D. \times bootii$ more closely resembling its putative mother, $D. cristata$. In the $D. \times triploidea$ triad, each species has at least two copies of the $D. intermedia$ genome, and the triploid is more intermediate between the two parents (fig. 1). Even then, it appears that the relative proportions of genomes may be key. The $D. intermedia$ half of $D. carthusiana$ (IISS) is apparently insufficient to alter its habitat preference for wet soils. However, the two-thirds contribution of $D. intermedia$ may be responsible for broadening $D. \times triploidea$’s niche space into forest habitats.

Conclusions

In summary, we find that habitats differ in soil pH and humidity but maintain generally similar temperature regimes and canopy cover and that morphological data reveal substantial overlap between species and their hybrids. Physiological parameters are also similar within parent-offspring triads, with some differences in photosynthesis and hydraulic functioning arising in the $D. \times bootii$ group. This lack of substantial morphological, ecological, or physiological differentiation supports the hypothesis that triploids are occupying a niche space similar to that of their parents. This overlap may be a consequence of a relatively benign environment in which there is enough space, water, or nutrients to go around. Future work may investigate whether that lack of niche partitioning persists in more harsh environments. Alternatively, there may be other untested physiological mechanisms responsible for supporting the co-occurrence we observe in these species complexes. Our work suggests that hydraulics in sporophyte generation may be partly responsible for this co-occurrence, a hypothesis that could be investigated via morphological studies of tracheary elements and pit membranes. Given the observed general similarity of sporophyte physiology, exploring gametophyte physiological functioning could provide important insight into parent-hybrid dynamics.

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