New Phytologist

Research

Polyploidy and environmental stress response: a comparative study of fern gametophytes

Jennifer Blake-Mahmud[1], Emily B. Sessa[2], Clayton J. Visger[3] and James E. Watkins Jr[4]

[1]Biology Department, Hope College, Holland, MI 49423, USA; [2]William & Lynda Steere Herbarium, New York Botanical Garden, Bronx, NY 10458, USA; [3]Department of Biological Sciences, California State University, Sacramento, CA 95819, USA; [4]Department of Biology, Colgate University, 13 Oak Drive, Hamilton, NY 13346, USA

Author for correspondence:
James E. Watkins Jr
Email: jwatkins@colgate.edu

Received: *28 March 2024*
Accepted: *24 June 2024*

New Phytologist (2024)
doi: 10.1111/nph.19969

Key words: climate change, drought, fern, gametophyte, polyploidy, temperature.

Summary

- Climate change is rapidly altering natural habitats and generating complex patterns of environmental stress. Ferns are major components of many forest understories and, given their independent gametophyte generation, may experience unique pressures in emerging temperature and drought regimes. Polyploidy is widespread in ferns and may provide a selective advantage in these rapidly changing environments. This work aimed to understand whether the gametophytes of allopolyploid ferns respond differently to climate-related physiological stress than their diploid parents.
- The experimental approach involved a multifactorial design with 27 treatment combinations including exposure to multiple levels of drought and temperature over three treatment durations, with recovery measured at multiple timepoints. We measured Chl fluorescence from over 2000 gametophytes to evaluate stress avoidance and tolerance in diploid and polyploid species.
- Polyploids generally showed a greater ability to avoid and/or tolerate a range of stress conditions compared with their diploid counterparts, suggesting that polyploidy may confer enhanced flexibility and resilience under climate stress.
- Overall, these results suggest that polyploidy may provide some resilience to climate change in mixed ploidy populations. However, all species remain susceptible to the impacts of extreme drought and heat stress.

Introduction

Polyploidy, or whole-genome duplication (WGD), is a major contributor to plant evolution and diversity (Bowers *et al*., 2003; Soltis *et al*., 2015; Ruprecht *et al*., 2017; One Thousand Plant Transcriptomes Initiative, 2019). Wood *et al*. (2009) estimated that 15% of speciation events in angiosperms, and 31% in ferns, are accompanied by a change in ploidal level. WGD events have now been identified in the evolutionary histories of most major lineages of land plants (Husband *et al*., 2013; Li *et al*., 2015; Escudero & Wendel, 2020; Pelosi *et al*., 2022). There is little doubt that polyploidy generates novel genetic combinations and is an important mechanism of speciation in plants (Soltis *et al*., 2015; Tossi *et al*., 2022). Yet by bringing together divergent genomes within a nucleus, polyploidy can also influence a range of genomic and functional attributes that dramatically alter ecology and potentially influence a species' ability to respond to environmental change (Levin, 1983, 2002; Soltis *et al*., 2014; Van de Peer *et al*., 2021). These ecological consequences of polyploidy are not well understood. Several studies have shown that allopolyploids can be intermediate in morphology, ecology, or niche relative to their diploid parents (Maherali *et al*., 2009; Chansler *et al*., 2016; Marchant *et al*., 2016), while others suggest that they frequently exhibit increased flexibility and/or transgressive ecology, physiology, and geographic ranges (Liu & Adams, 2007; Manzaneda *et al*., 2012, 2015; Coate *et al*., 2013; Hao *et al*., 2013; Sessa & Givnish, 2014; Baniaga *et al*., 2020; Blake-Mahmud & Watkins, 2022; Wefferling *et al*., 2024). The extent to which these differences might impact species' responses to climate change in natural environments, and whether ploidy-related differences in stress response might mitigate the effects of climate change on plant communities, is largely unknown.

By the end of the 21st Century, the Intergovernmental Panel on Climate Change (IPCC) predicts that global temperatures will increase from 1.1°C to 6.0°C and many regions will experience more extreme and/or more frequent drought (IPCC, 2023). Studies on angiosperm responses to climate indicate that polyploid taxa can respond differently compared with diploids, and that polyploids, in general, may be more robust and tolerant of environmental change relative to their diploid relatives (Leitch & Leitch, 2008; Van de Peer *et al*., 2021). The observation that polyploids may be better at invading newly available habitats (Trewick *et al*., 2002; Brochmann *et al*., 2004; te Beest *et al*., 2012; Baniaga *et al*., 2020; Folk *et al*., 2020) may be driven by increased ecological tolerance or plasticity (Levin, 1983;

Karunarathne *et al.*, 2018; Wei *et al.*, 2019; Moura *et al.*, 2021), and/or by new gene combinations or expression patterns that produce rapid changes in a few generations (Levin, 2002; Leitch & Leitch, 2008; Coate *et al.*, 2012). Either of these may confer fitness advantages that allow polyploids to outcompete diploid competitors and establish in novel habitats during or after environmental change. Phenotypic and ecological differentiation are frequently cited as potential means of promoting polyploid establishment, by facilitating niche partitioning and invasion of novel habitats or microclimates (Schwarzbach *et al.*, 2001; Maherali *et al.*, 2009). Nevertheless, the vast majority of research on polyploidy has concentrated on the genetic underpinnings and evolutionary consequences of extra genomes, leaving the phenotypic and ecological impacts under-researched in comparison.

Polyploidy is ubiquitous in ferns (Wood *et al.*, 2009), and ferns are known to play a number of important ecological roles, including shaping community assembly, diversity, and ecosystem processes in temperate and tropical forests (George & Bazzaz, 1999a,b; Allison & Vitousek, 2004; Ellwood & Foster, 2004; Watkins & Cardelús, 2012). Despite their importance, we have a poor understanding of factors that shape fern distribution patterns or influence their ecological success, and little of what we know involves comparative data between related diploid and polyploid taxa. This is significant given that polyploids can make up a major component of the fern flora of forests; for example, Pittermann *et al.* (2015) found that up to 80% of the fern community in some northeast temperate forests in the United States is comprised of polyploid and hybrid taxa. Our understanding of how ploidal level may shape fern communities, and especially their capacity to respond to environmental change, is severely hampered by the lack of integrated studies of ploidal level, ecology, and physiology in these plants.

Another missing piece in the puzzle to understand fern ecology and responses to environmental change is the gametophyte generation of the life cycle. Ferns are unusual among land plants in having independent, free-living sporophytes and gametophytes (Haufler *et al.*, 2016), and the haploid gametophyte plays a central role in species establishment: at some point in the past, a sporophyte's presence is dependent on the gametophytes that preceded it. Stress tolerance in the form of desiccation tolerance is particularly important for fern gametophytes as they lack vascular tissue, cuticles, and stomata and rely on water for fertilization (Watkins *et al.*, 2007). For this reason, gametophytes are in a constant state of equilibrium with their surrounding environment (Watkins *et al.*, 2007; Watkins & Cardelús, 2012), in conditions that are often deadly dry for the majority of vascular plants (Oliver *et al.*, 2000, 2020; Gaff & Oliver, 2013). The sole study of community phylogenetics in ferns that considered gametophytes and sporophytes found strong evidence that the two life stages are governed by different assembly rules (Nitta *et al.*, 2017). In previous work on the sporophyte physiology of North American *Dryopteris*, researchers found evidence for significant differences in photosynthetic capacity between polyploids and diploids (Blake-Mahmud & Watkins, 2022). Furthermore, while there was no evidence of transgressive physiological functioning in allotetraploids compared with their progenitors, several of the polyploids do have larger geographic ranges than their parent taxa (Sessa & Givnish, 2014). These findings, combined with expected strong selection at the sexual stage of the life cycle (Pelosi & Sessa, 2021), suggest that gametophyte biology is likely to play a critical role in niche partitioning, lineage establishment, and persistence, with strong potential effects of ploidal level that may translate to how diploids vs polyploids respond to climate change, as mediated by the gametophyte stage.

In order to understand better how fern gametophytes are likely to respond to global change, we investigated how species at different ploidal levels respond physiologically to increases in temperature and decreases in moisture (drought), two major aspects of climate that the IPCC has predicted will change in the coming decades and centuries (IPCC, 2023). We hypothesized that the gametophytes of diploid species and their allopolyploid progeny would differ in their physiological responses to, and ability to recover from, temperature and drought stress. To test our hypotheses, we used six eastern North American members of the genus *Dryopteris* that form two separate triads of diploid progenitors and allotetraploid hybrids.

Materials and Methods

Experimental design

Our study species included six species of *Dryopteris* present in eastern North America: two triads, each consisting of an allotetraploid and its two diploid progenitors (Fig. 1; Sessa *et al.*, 2012). We collected spores from wild populations of all six species. The first triad was composed of *Dryopteris campyloptera* (Kunze) Clarkson ($2n = 4x$) that was collected from Smuggler's Notch, Lamoille County, VT. This tetraploid's diploid father, *Dryopteris expansa* (C. Presl) Fraser Jenkins & Jermy ($2n = 2x$), was collected in northeastern MN and the diploid mother, *Dryopteris intermedia* (Muhlenberg ex Willdenow) A. Gray ($2n = 2x$), was collected in Madison County, NY. The second triad was composed of the tetraploid *Dryopteris celsa* (W. Palmer) Knowlton ($2n = 4x$), collected in northern Winston County, AL. Its diploid father, *Dryopteris goldiana* (Hooker ex Goldie) A. Gray ($2n = 2x$), was collected in Chenango County, NY, and its diploid mother, *Dryopteris ludoviciana* (Kunze) Small ($2n = 2x$), was collected in Alachua County, FL. Specimens are vouchered at Colgate University's Cooley Herbarium. Spore-bearing tissues were put in glassine envelopes and allowed to dry and release spores. Once dried, the envelopes were stored at 4°C.

We sowed spores directly on agar plates supplemented with Bold's basal medium (Bold, 1957), placed them in growth chambers (Percival, Perry, IA, USA), and allowed spores to germinate (Fig. 2). Chambers ran on a 12-h day : night cycle with daytime temperatures of 22°C and nighttime temperatures five degrees less. Once spores had germinated and gametophytes reached maturity (a minimum of 4 months after sowing), we removed groups of gametophytes *c.* 1 cm^2 in size and transferred them to small weigh boats filled with 5-ml moist soil that were then placed into one of several hydration boxes (Model 295c; Pioneer Plastics, Dixon, KY, USA) and kept at 100% relative humidity

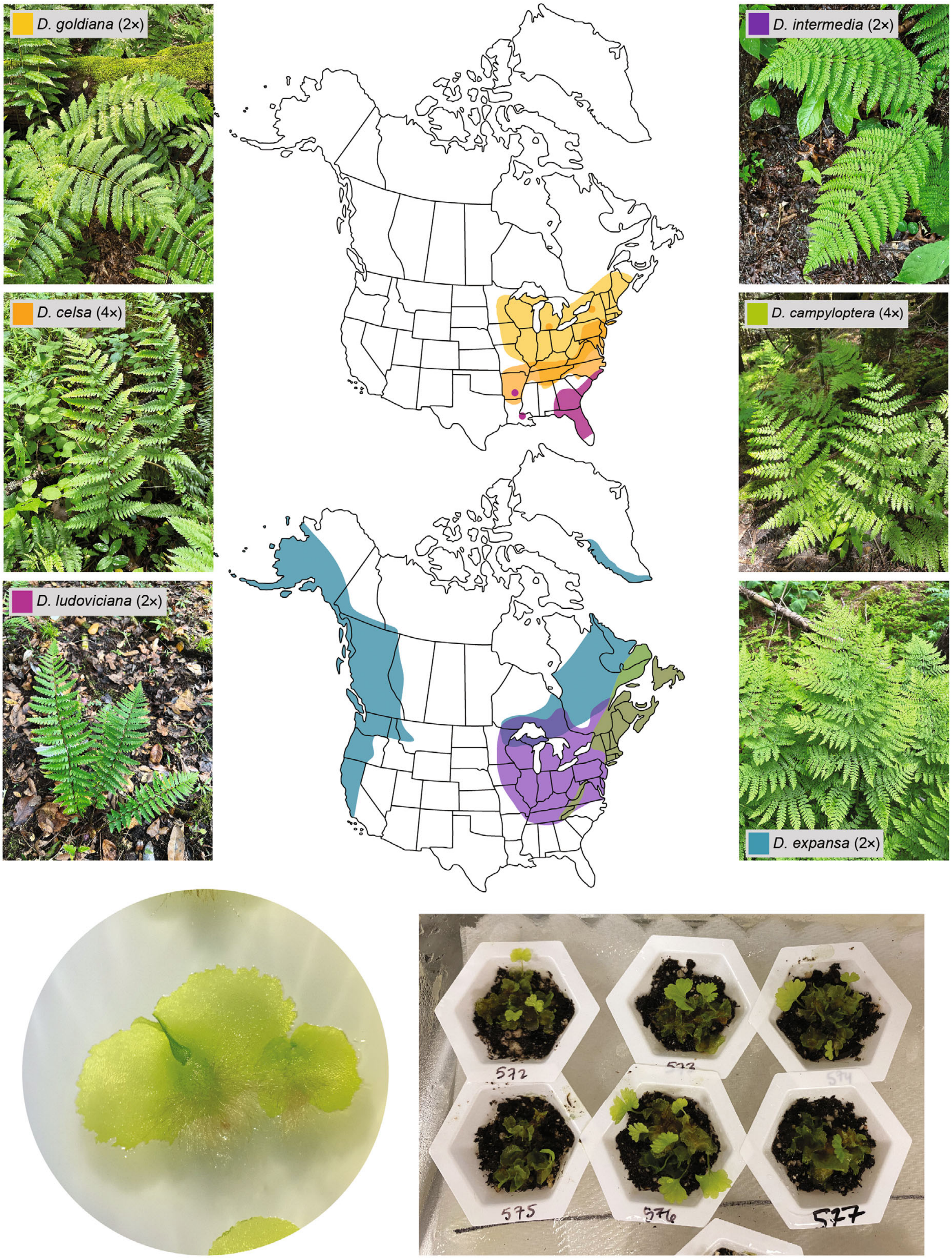


Fig. 1 Photographs and range maps of *Dryopteris* species included in this study. The *D. celsa* triad (left, and upper map) is composed of the tetraploid *D. celsa* (orange) and its diploid parent species, *D. goldiana* (yellow) and *D. ludoviciana* (magenta). The *D. campyloptera* triad (right, and lower map) is composed of the tetraploid *D. campyloptera* (green) and its diploid parent species, *D. intermedia* (purple) and *D. expansa* (teal). Lower photographs show two gametophytes (left) and several soil-filled weigh boats with gametophytes and small sporophytes (right).

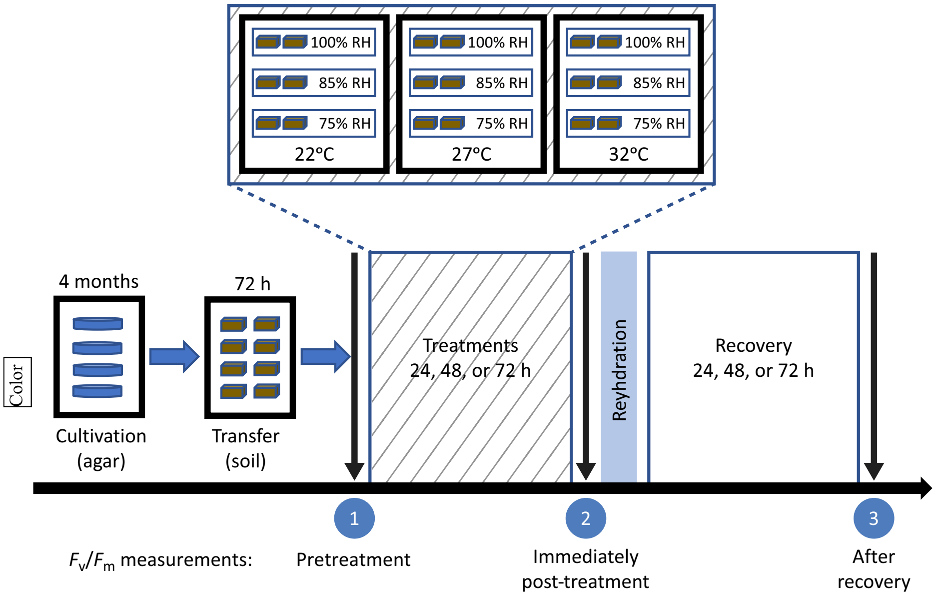


Fig. 2 Experimental design. After initial cultivation, gametophytes were transferred to soil and a baseline fluorescence (F_v/F_m) measurement taken (Timepoint 1), before any treatment. Gametophytes were then exposed to treatment conditions (at three temperatures and three levels of humidity; see text for description) for 24, 48, or 72 h. Immediately after the treatment period, another fluorescence measurement was taken (Timepoint 2). Gametophytes were then rehydrated and allowed to recover for 24, 48, or 72 h, and fluorescence measurements were again taken (Timepoint 3).

(RH). After 24 h in these conditions, we dark-adapted the gametophytes for a minimum of 20 min before taking an initial fluorescence (F_v/F_m) measurement using a Walz PAM 2000 (WALZ, Effeltrich, Germany; Fig. 2; Timepoint 1). Fluorescence is a recognized method by which the health of the photosystem can be assessed, and we used measurements of F_v/F_m at specific timepoints to assess the effects of the experimental treatments and gametophytes' ability to recover.

After taking dark-adapted F_v/F_m measurements, we transferred the weigh boats (8–10 replicates per species) into a set of experimental conditions that included a fully factorial design of three temperatures and three levels of RH, for either 24, 48, or 72 h. In total, this design had 27 combinations of experimental conditions. Temperature treatments were 22°C, 27°C, and 32°C, with temperatures set and maintained in three separate growth chambers. All growth chambers were kept on a 12-h temperature cycle, with nighttime temperatures dropping five degrees. Gametophytes were exposed to three different humidity levels: 100% RH, 85% RH, and 75% RH. These conditions were chosen to expose gametophytes to a realistic level of drought and temperature to approximate the IPCC's predictions for changes expected for eastern North America in the next century, over the range of these taxa.

We generated the drought stress treatments using the saturated salt procedure. The saturated salts keep the RH of each tub constant and this method has been used in several other studies examining gametophyte drought stress (Watkins *et al*., 2007; Testo & Watkins, 2013; Chambers & Emery, 2016). Briefly, gametophytes in weigh boats were removed from the initial high humidity boxes and placed into equilibrium dehydration tubs containing salts that correspond to a known humidity, as follows: 85% RH: potassium chloride, KCl, $\Psi = -21.5$ MPa at 22°C; 75% RH: sodium chloride, NaCl, $\Psi = -38.4$ MPa at 22°C, or a water control (100% RH). Each dehydration setup consisted of a 355-ml plastic tub (Model 156c; Pioneer Plastics) that contained a saturated salt solution in a large weigh boat. A piece of plastic window screen was placed on top of the large weigh boat, and the smaller gametophyte boats were placed on top of this screen. Salts were monitored to ensure that they remained saturated, with excess liquid removed or added to maintain a surface with both liquid and protruding salt crystals. We created control tubs (100% RH) by substituting a reservoir of water for the salt solution.

Following the treatment period, gametophytes were evaluated immediately to assess the impact of the treatment (Fig. 2; Timepoint 2). To assess recovery, we next rehydrated all gametophytes with 0.5 ml autoclaved DI water and placed them, still in their weigh boats, back into plastic hydration boxes with two layers of saturated paper towels. They were again kept in the dark at 22°C to recover. We then recorded fluorescence after 24, 48, and 72 h of recovery (Fig. 2; Timepoint 3); we report on the final 72 h recovery assessment here.

Conceptual interpretation of results

Before analyzing the data, we conceptualized gametophyte stress response dynamics over two phases of post-experiment response (Fig. 3): (1) immediately post-treatment (Timepoint 2), capturing the treatment effect itself, and (2) after recovery (Timepoint 3). Immediately post-treatment, we predicted that species would be either: (1) stress *avoidant* (A in Fig. 3), with mean values similar to the initial value (i.e. at or above the relative zero line), or (2) stress *nonavoidant* (N in Fig. 3), with mean values lower than the initial value (i.e. below the relativized zero line), indicating a decrease in physiological functioning as a direct result of the treatment. In the recovery phase, stress-avoidant species were expected to remain close to their initial value, while stress-sensitive species were predicted to be either: (1) stress *tolerant* (T in Fig. 3), returning to a value near the initial value (i.e. at or above the relative zero line), or (2) stress *intolerant* (I in

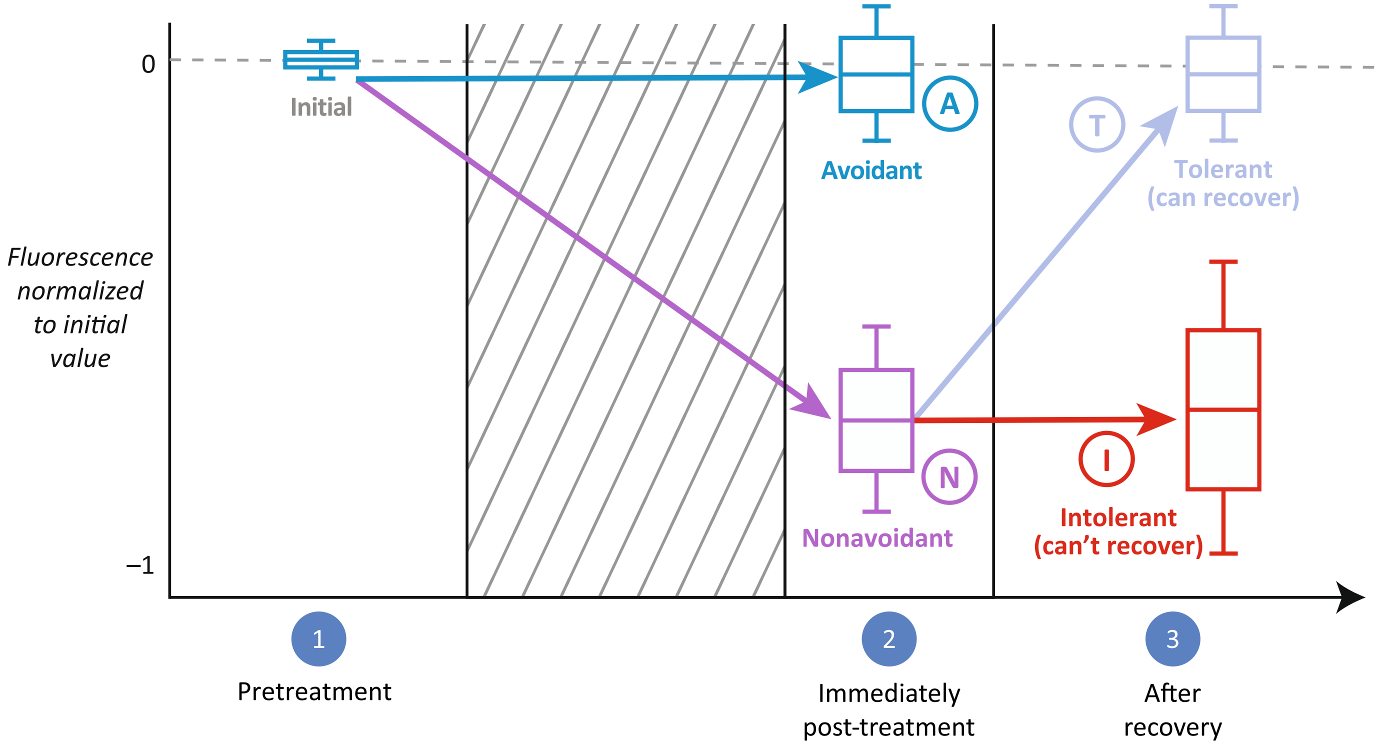


Fig. 3 Conceptual model of stress avoidance and tolerance. We conceptualized gametophyte stress response as having two stages: immediately post-treatment (Timepoint 2), when we could assess their capacity to avoid the effects of stress, and after a recovery period (Timepoint 3), when we could assess their capacity for recovery. Fluorescence measurements taken at these timepoints were relativized by the gametophyte's initial fluorescence reading, taken at Timepoint 1. If the average relativized F_v/F_m value for a species at Timepoint 2 returned to the zero line, in other words to match the initial value, we scored it as *avoidant* of stress (A); if not, we scored it as *nonavoidant* (N). At Timepoint 3, we made the same assessment – if gametophytes were not able to return to the zero line after the rehydration period, we scored them as *intolerant* of stress (I). If they were able to recover and return to the zero line, we scored them as *tolerant* of stress (T). The center line in each box represents the median, the upper and lower bounds of each box represent the 25th and 75th percentile and the whiskers represent the minimum and maximum values of the data set.

Fig. 3), remaining at a value lower than the initial value (i.e. not touching the zero line). There is substantial complexity involved with defining what stress is, how to quantify it, and how to define recovery (e.g. Stark *et al.*, 2022), and we therefore developed this conceptualization of stress avoidant vs nonavoidant and tolerant vs intolerant in order to provide a simple way to visualize and interpret our data.

Data analysis

For each species and treatment combination, we first relativized the post-treatment (Timepoint 2) and final recovery (Timepoint 3) fluorescence values against the initial, pretreatment value, in order to account for the fact that there was natural variation in the initial measurements. This was done by subtracting the initial measurement (Timepoint 1) from each of the subsequent timepoints (2 and 3) and then dividing by the initial measurement, for example: (measurement 3 − measurement 1)/measurement 1. This produced relative fluorescence values for each species and treatment combination, in which negative values indicated a decrease in F_v/F_m relative to the initial measurement, and therefore a decrease in physiological functioning, while positive values indicated an increase in F_v/F_m relative to the initial value and therefore an improvement in physiological functioning. A relative F_v/F_m measure of zero indicates that individuals fared no worse or better than their original measurement.

For statistical analyses of the fluorescence data, we treated the six species as two separate triads, one for each combination of *allopolyploid–diploid parent 1–diploid parent 2*, in order to determine how allopolyploid performance compared with the performance of closely related diploids. We developed two mixed models, one for each species triad. Each full mixed model contained the following variables: relative fluorescence as the outcome variable, with species, dehydration level, temperature, duration of treatment, measurement timepoint, and individual as predictors. We selected the initial random structure of the model using REML estimation. We then selected the fixed effects (both main and interactions) using maximum likelihood. We confirmed the random structure and final model statistics using REML. We used R (R Core Team, 2021) for statistical analyses and visualization, with packages EMMEANS (Lenth, 2021), LME4 (Bates *et al.*, 2015), CAR (Fox & Weisberg, 2019), and GGPLOT2 (Wickham, 2016).

We used a backward model selection procedure, starting with a full model including a random intercept for individual, a random slope for measurement timepoint, a five-way interaction of species, dehydration level, temperature, duration of treatment, and measurement timepoint and all the nested four-way, three-way, two-way interactions, and main effects. Using maximum likelihood, we then removed nonsignificant interactions, starting with the highest-level interaction first, and evaluated the change in Akaike information criterion (AIC). We discontinued removing interactions when the AIC did not improve with the removal of nonsignificant interactions. At this point, we also tested models with predictors viewed continuously instead of categorically and found that models with categorical predictors had substantially lower AICs. We reevaluated the final model using REML with and without the random slope.

Finally, we compared the estimated marginal means of treatment groups. A marginal mean provides the average relativized F_v/F_m for a specific species within a specific unique treatment combination, based on the model. For example, if the mean relative F_v/F_m for *D. intermedia* at Timepoint 3, at 22°, in water control, for 24 h was 0.007818 ± 0.0548 (SE); then, this would indicate that in this unique treatment combination, those gametophytes were functioning similarly to how they started off before the treatment, because the value is extremely close to zero. In order to assess pairwise differences in the means between closely related species, we next performed contrasts within each species triad. We corrected for multiple comparisons using the Tukey method for comparing a family of three estimates. We used these pairwise comparisons to ask whether there was a difference between any of the three species, for both of the species' triads. For the example of this exact treatment combination (i.e. at Timepoint 3, 22°C, in water control, for 24 h), if there was no statistically significant difference, with P-values for the pairwise comparisons ranging from 0.56 to 0.98, this would indicate that we cannot reject our null hypothesis that all three species respond the same to this unique combination of treatments.

Results

Overview of results

Our experimental approach was designed to reflect the complexities that these taxa face in field settings by combining temperature and drought stress over multiple durations. Our design of three temperatures, three levels of RH, three treatment durations, and F_v/F_m measurements taken at multiple timepoints, resulted in highly complex mixed models for each of the two triads. The structure of both models included a random intercept for individual and random slope for measurement timepoint. The final model for the *Dryopteris campyloptera* triad contained a statistically significant five-way interaction between species, dehydration level, temperature, duration of treatment, and measurement timepoint. Because of this we were unable to reduce the dimensionality of the model, as all lower-level interactions (even if not statistically significant) were included in the five-way interaction. The final model for the *D. celsa* triad contained a statistically significant four-way interaction of temperature, duration of treatment, measurement timepoint, and species, plus several additional statistically significant three-way interactions that were not fully nested within the four-way interaction. These three-way interactions included: dehydration, temperature, duration of treatment; dehydration, timepoint, species; and dehydration, duration of treatment, species. All lower-level interactions were included in the three- and/or four-way interactions.

Conceptualization of each species as stress avoidant/nonavoidant and tolerant/intolerant

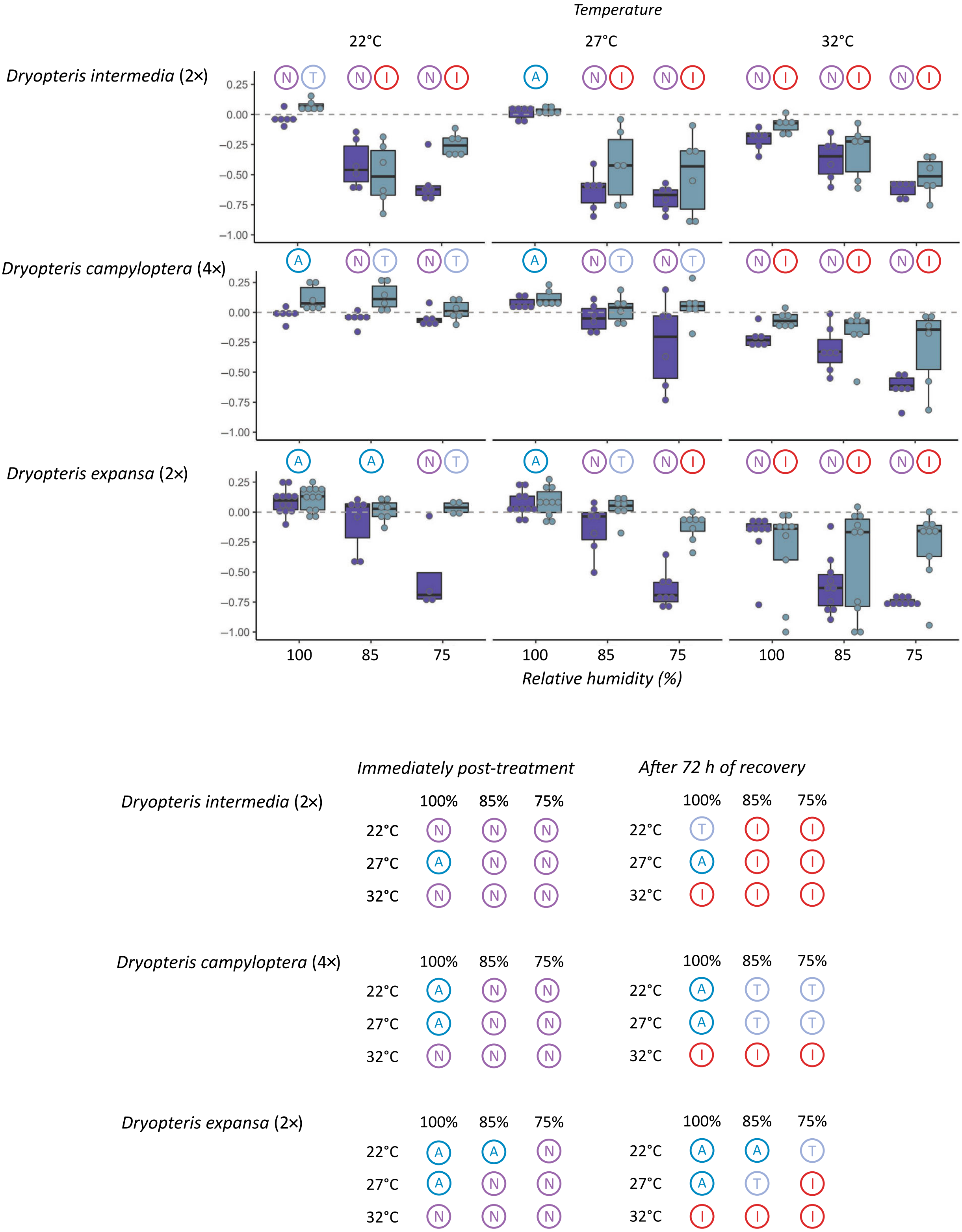
While our approach reflected a more realistic experiment, with three temperature and drought levels plus three treatment exposure durations (24, 48, and 72 h) and three recovery durations (24, 48, and 72 h), the complexity of this multifactorial design makes our results difficult to visualize. Therefore, while the Overview of results in the Results section above describes our analyses and model outputs for the complete dataset, for the remainder of the Results and Discussion sections, we will focus on interpreting results from the 72 h treatment and final 72 h recovery times, as these represent the most dramatic treatment duration and longest potential recovery time we tested.

In the *D. campyloptera* triad (Fig. 4), there were marked differences in avoidance and tolerance across the three taxa. *Dryopteris intermedia* (Fig. 4) was only able to avoid stress at 27°C and 100% RH. In all other treatment combinations, it was nonavoidant and intolerant, except for 22°C and 100% RH (essentially control conditions), where it was nonavoidant but was able to recover and so was considered tolerant. *Dryopteris campyloptera* (Fig. 4) was only able to avoid stress at 22°C and 27°C, at 100% RH. In all the other combinations, it was nonavoidant, but it was able to recover in the two less extreme temperatures (22°C and 27°C) and so was scored as tolerant for those treatments at both 85% and 75% RH; at 32°C, it was intolerant at all RH levels. Based on our definitions, *D. expansa* exhibited stress avoidance at 22°C and 27°C and 100% RH, and at 22°C and 85% RH. It was nonavoidant in the other combinations, and intolerant at the most extreme temperature (32°C and all RH levels) and at 27°C and 75% RH.

In the *D. celsa* triad (Fig. 5), *Dryopteris ludoviciana* was able to avoid stress at 22°C and 27°C at 100% RH, but it was nonavoidant and intolerant in all other treatment combinations. *Dryopteris celsa* was able to avoid stress at 22°C and 100% and 85% RH, but was nonavoidant in all other treatments. It was tolerant in all treatment combinations except 75% RH and 27°C and 32°C. *Dryopteris goldiana* exhibited nonavoidance and intolerance in all treatments.

Pairwise comparisons of species within each triad

We also examined pairwise comparisons between species in each triad for specific treatment combinations (temperature, drought, and duration of treatment). Here, we focus on similarities and differences between species, and between the initial measurement (pretreatment, at Timepoint 1), compared with Timepoint 2 (immediately following treatment, to test for stress avoidance) and to Timepoint 3 (after the 72 h rehydration period, to test for ability to recover). In the sections below, we describe significant



Immediately post-treatment / *After 72 h of recovery*

Dryopteris intermedia (2×)

	Immediately post-treatment			After 72 h of recovery		
	100%	85%	75%	100%	85%	75%
22°C	Ⓝ	Ⓝ	Ⓝ	Ⓣ	Ⓘ	Ⓘ
27°C	Ⓐ	Ⓝ	Ⓝ	Ⓐ	Ⓘ	Ⓘ
32°C	Ⓝ	Ⓝ	Ⓝ	Ⓘ	Ⓘ	Ⓘ

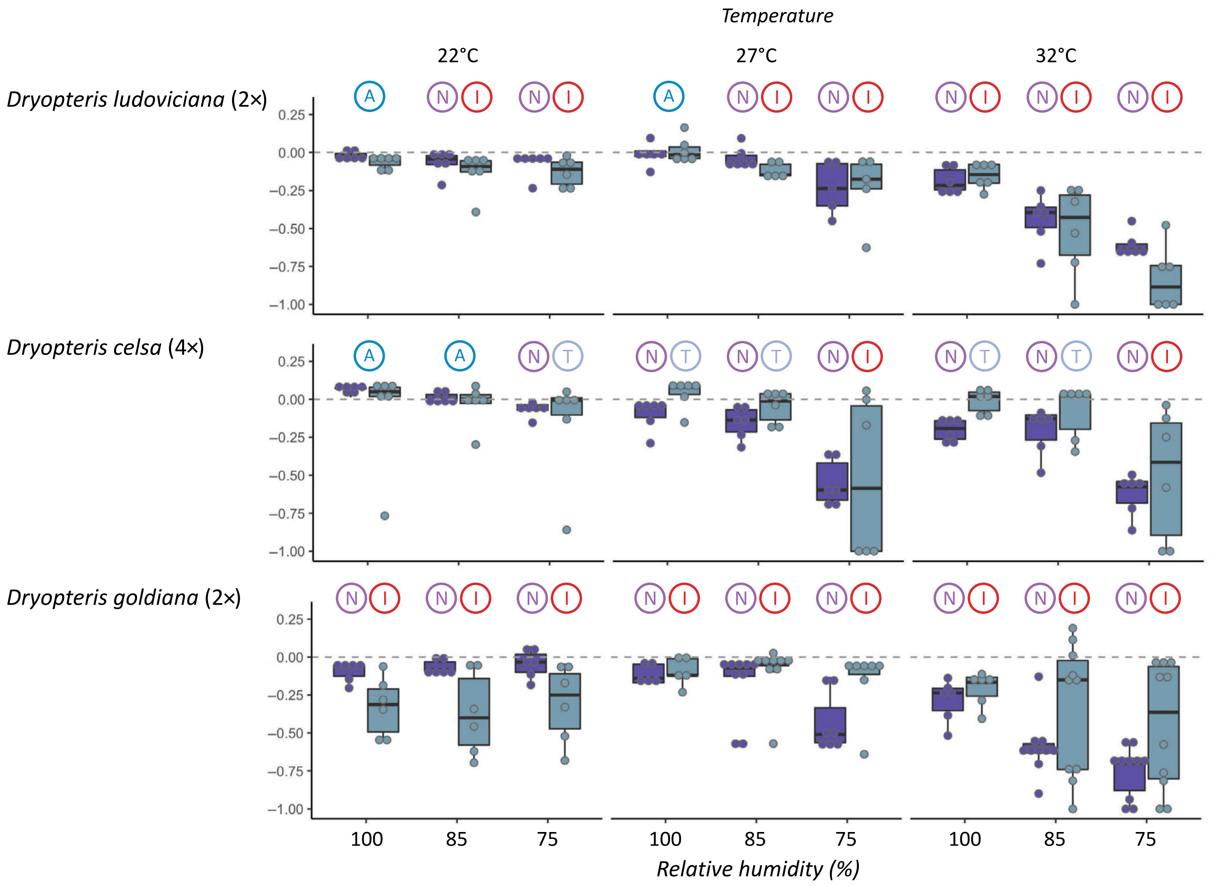
Dryopteris campyloptera (4×)

	Immediately post-treatment			After 72 h of recovery		
	100%	85%	75%	100%	85%	75%
22°C	Ⓐ	Ⓝ	Ⓝ	Ⓐ	Ⓣ	Ⓣ
27°C	Ⓐ	Ⓝ	Ⓝ	Ⓐ	Ⓣ	Ⓣ
32°C	Ⓝ	Ⓝ	Ⓝ	Ⓘ	Ⓘ	Ⓘ

Dryopteris expansa (2×)

	Immediately post-treatment			After 72 h of recovery		
	100%	85%	75%	100%	85%	75%
22°C	Ⓐ	Ⓐ	Ⓝ	Ⓐ	Ⓐ	Ⓣ
27°C	Ⓐ	Ⓝ	Ⓝ	Ⓐ	Ⓣ	Ⓘ
32°C	Ⓝ	Ⓝ	Ⓝ	Ⓘ	Ⓘ	Ⓘ

Fig. 4 Upper panel: Relativized F_v/F_m results for the *Dryopteris campyloptera* triad. Each row corresponds to one species (upper panel to lower panel: *Dryopteris intermedia*, *D. campyloptera*, and *Dryopteris expansa*) for all three temperatures (left to right: 22°C, 27°C, and 32°C), and with each temperature, all three drought levels (left to right: 100%, 85%, and 75% relative humidity (RH)). Within each treatment combination, the purple boxes and whiskers are relativized F_v/F_m values taken immediately after treatment (Timepoint 2), and the teal boxes and whiskers are these values taken after recovery (Timepoint 3). Data are only shown for the 72 h treatment duration and 72 recovery duration. All values are relativized to the initial measurement (taken at Timepoint 1). Circled letters above the data correspond to our conceptual model shown in Fig. 3. Lower panel: Conceptual results only, grouped by timepoint: immediately post-treatment (Timepoint 2), and after recovery (Timepoint 3).



Dryopteris ludoviciana (2×)

Immediately post-treatment	100%	85%	75%	After 72 h of recovery	100%	85%	75%
22°C	Ⓐ	Ⓝ	Ⓝ	22°C	Ⓐ	Ⓘ	Ⓘ
27°C	Ⓐ	Ⓝ	Ⓝ	27°C	Ⓐ	Ⓘ	Ⓘ
32°C	Ⓝ	Ⓝ	Ⓝ	32°C	Ⓘ	Ⓘ	Ⓘ

Dryopteris celsa (4×)

Immediately post-treatment	100%	85%	75%	After 72 h of recovery	100%	85%	75%
22°C	Ⓐ	Ⓐ	Ⓝ	22°C	Ⓐ	Ⓐ	Ⓣ
27°C	Ⓝ	Ⓝ	Ⓝ	27°C	Ⓣ	Ⓣ	Ⓘ
32°C	Ⓝ	Ⓝ	Ⓝ	32°C	Ⓣ	Ⓣ	Ⓘ

Dryopteris goldiana (2×)

Immediately post-treatment	100%	85%	75%	After 72 h of recovery	100%	85%	75%
22°C	Ⓝ	Ⓝ	Ⓝ	22°C	Ⓘ	Ⓘ	Ⓘ
27°C	Ⓝ	Ⓝ	Ⓝ	27°C	Ⓘ	Ⓘ	Ⓘ
32°C	Ⓝ	Ⓝ	Ⓝ	32°C	Ⓘ	Ⓘ	Ⓘ

Fig. 5 Upper panel: Relativized F_v/F_m results for the *Dryopteris celsa* triad. Each row corresponds to one species (upper panel to lower panel: *Dryopteris ludoviciana*, *D. celsa*, and *Dryopteris goldiana*) for all three temperatures (left to right: 22°C, 27°C, and 32°C), and with each temperature, all three drought levels (left to right: 100%, 85%, and 75% relative humidity (RH)). Within each treatment combination, the purple boxes and whiskers are relativized F_v/F_m values taken immediately after treatment (Timepoint 2), and the teal boxes and whiskers are these values taken after recovery (Timepoint 3). Data are only shown for the 72 h treatment duration and 72 recovery duration. All values are relativized to the initial measurement (taken at Timepoint 1). Circled letters above the data correspond to our conceptual model shown in Fig. 3. Lower panel: Conceptual results only, grouped by timepoint: immediately post-treatment (Timepoint 2), and after recovery (Timepoint 3).

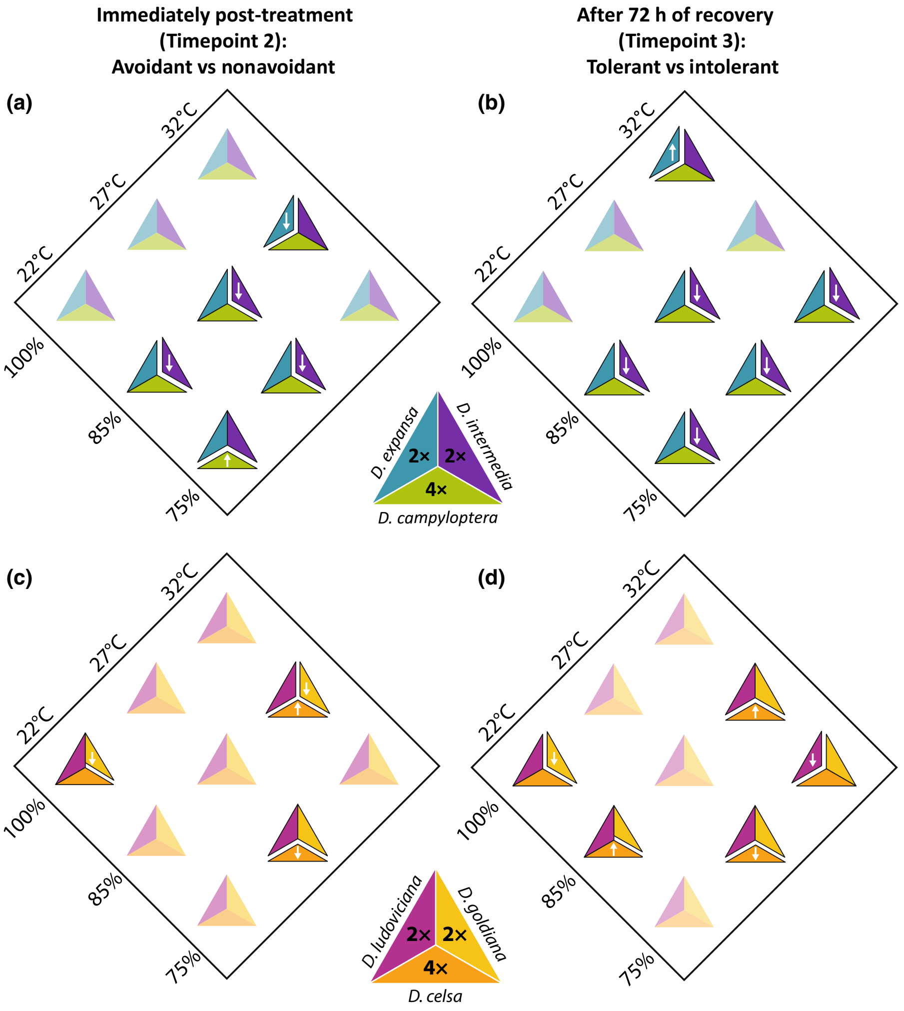


Fig. 6 Statistically significant differences between species in each triad, as predicted by estimated marginal means. (a, b) *Dryopteris campyloptera* triad; (c, d) *Dryopteris celsa* triad. Colors of triangles represent species as indicated by the respective legends in the center. (a, c) show comparisons in Timepoint 2 (immediately post-treatment) and (b, d) show comparisons at Timepoint 3 (after 72 h of recovery). Each box shows all nine combinations of temperature and drought treatments. For marginal mean predictions in which there was no statistically significant difference among species, the segments of the triangle are joined (e.g. in a, for all temperatures at 100% relative humidity (RH)). When there were no differences in the triad at all, the triangles are faded. For predictions in which one or more species was significantly different than the others at $P < 0.05$, those segments of the triangle are separated (e.g. in a, for all temperatures at 85% RH). If one segment is completely separated from the other two, that species was statistically unique, while the other species were not different from one another. If one segment is separated from one neighbor only (e.g. in c, at 22°C and 100% RH), this indicates that there are statistical differences between one pair of species, but the other two pairs responded similarly. Arrows indicate the direction of difference, that is a down arrow indicates that that species had a significantly lower relativized F_v/F_m than one or both of the others in its triad.

differences between species in relativized F_v/F_m; significant differences in these values indicate only a higher or lower level of stress, but not necessarily a label of 'avoidant' or 'tolerant' according to our definitions. For example, two species could be significantly different from one another, but both nonavoidant.

Differences in stress avoidance in the *D. campyloptera* triad To evaluate differences in the abilities of taxa to *avoid* stress, we compared the estimated marginal means of stress response (i.e. differences in relativized F_v/F_m) between Timepoints 1 (pre-treatment, initial measurement) and 2 (immediately post-treatment; Fig. 6a). In the *D. campyloptera* triad at 100% RH, there was no significant difference in the estimated marginal means of how species responded to increases in temperature. However, when exposed to dehydration levels of 85% RH, responses differed between species. At 22°C and 27°C, the relativized F_v/F_m for *D. intermedia* was significantly lower compared with *D. campyloptera* and *D. expansa* ($P < 0.0001$ for both comparisons). At 32°C and 85% RH, *D. campyloptera* and *D. intermedia* were not significantly different from one another, but the relativized F_v/F_m for *D. expansa* was significantly lower compared with the others ($P = 0.0005$ for *D. campyloptera* and $P = 0.0421$ for *D. intermedia*). At 75% RH and 22°C, the relativized F_v/F_m for *D. campyloptera* was significantly higher than the other two ($P = 0.0001$ for both comparisons), while at 75% RH and 27°C, the relativized F_v/F_m for *D. intermedia* was significantly lower than the other two ($P < 0.001$ for both comparisons).

Differences in recovery in the *D. campyloptera* triad In order to evaluate the ability to *recover*, we did the same as above, but comparing Timepoint 1 to Timepoint 3 (i.e. following the 72 h rehydration period; Fig. 6b). We found that at full hydration (100% RH), there was no significant difference between species at 22°C and 27°C. At 32°C and 100% RH, the estimated marginal mean (relativized F_v/F_m) for *D. expansa* was significantly higher compared with *D. campyloptera* ($P = 0.0047$) and *D. intermedia* ($P = 0.0170$). At 85% RH and the two lower temperatures (22°C and 27°C), the relativized F_v/F_m for *D. intermedia* was significantly lower compared with both *D. campyloptera* and *D. expansa* ($P < 0.0001$ for both comparisons), which were not different from each other. At 75% RH, the relativized F_v/F_m for *D. intermedia* was significantly lower than the other two at all temperatures (at 22°C, $P = 0.0453$ for the comparison with *D.*

campyloptera and $P = 0.0033$ for *D. expansa*; at 27°C, $P < 0.0001$ for both comparisons; at 32°C, $P = 0.0266$ for the comparison with *D. campyloptera* and $P = 0.0057$ for the comparison with *D. expansa*).

Differences in stress avoidance in the *D. celsa* triad For the *D. celsa* triad, we conducted the same analyses as above, comparing differences in relativized F_v/F_m between Timepoints 1 (pretreatment) and 2 (immediately post-treatment) in order to evaluate differences in the abilities of taxa to *avoid* stress. In this triad, there were few differences between species when comparing estimated marginal means for relativized F_v/F_m between these timepoints (Fig. 6c). At full hydration (100% RH), the relativized F_v/F_m of *D. goldiana* was significantly lower than *D. celsa* ($P = 0.0392$); neither *D. goldiana* nor *D. celsa* differed significantly from *D. ludoviciana*. At 85% RH and 32°C, all three species were significantly different from one another, with *D. celsa* having the highest relativized F_v/F_m and *D. goldiana* the lowest ($P < 0.0001$ for the *D. celsa* to *D. goldiana* comparison; $P = 0.0006$ for the *D. celsa* to *D. ludoviciana* comparison; $P = 0.0278$ for the *D. goldiana* to *D. ludoviciana* comparison). At 75% RH and 27°C, the relativized F_v/F_m value of *D. celsa* was significantly lower than the other two species ($P = 0.0012$ for the comparison with *D. goldiana*; $P < 0.0001$ for the comparison with *D. ludoviciana*).

Differences in recovery in the *D. celsa* triad For the *D. celsa* triad, between the initial measurement (Timepoint 1) and Timepoint 3 (following 72 h rehydration), there were a number of differences between species (Fig. 6d). At 100% RH and 22°C, the estimated marginal means of *D. goldiana* was significantly lower than both *D. celsa* ($P = 0.0002$) and *D. ludoviciana* ($P = 0.0015$). At 85% RH and 22°C, *D. ludoviciana* does not differ from *D. goldiana* or *D. celsa*, but the relativized F_v/F_m of *D. celsa* is significantly higher than that of *D. goldiana* ($P = 0.0118$). At 85% RH and 32°C, *D. celsa* is different from both *D. goldiana* ($P = 0.0001$) and *D. ludoviciana* ($P < 0.0001$), but those two are not different from each other. At 75% and 27°C, the relativized F_v/F_m of *D. celsa* was significantly lower than both *D. goldiana* ($P = 0.0011$) and *D. ludoviciana* ($P = 0.0151$), and those two again did not differ from one another. At 75% and 32°C, the relativized F_v/F_m of *D. ludoviciana* was significantly lower than both *D. celsa* ($P = 0.0038$) and *D. goldiana* ($P = 0.0002$).

Discussion

Climate change is expected to have myriad impacts on natural systems and the ecology of organisms living in those systems over the coming decades. The extent to which polyploidy may impact species' responses to these changes is unknown, but with its ability to alter a huge range of functional and genomic attributes (Levin, 1983, 2002; Soltis *et al*., 2014), WGD likely has enormous potential to differentially influence species' ability to respond to environmental change. Our ultimate goal was to test whether allopolyploid hybrid ferns exhibited different responses to increased climate stress relative to one or both of their diploid parent species. While studies have been done on comparative physiology of allopolyploid sporophytes and their parents (Sessa & Givnish, 2014; Blake-Mahmud & Watkins, 2022), the current work represents the most synthetic attempt to evaluate these responses in fern gametophytes to date. Our overall experimental approach included a complex multifactorial design with 27 total treatment combinations and >2000 gametophytes measured. The resulting statistical models were complex but with several consistent overall patterns, which we discuss below.

Under our conceptual model, in which each species was scored as either avoidant or nonavoidant of stress, and then either tolerant or intolerant based on its ability to recover from stress (Fig. 3), it is clear that there are differences between ploidal levels in their ability to avoid and tolerate various combinations of temperature and drought stress. In the *Dryopteris campyloptera* triad, diploid *D. intermedia*'s capacity for stress avoidance was limited to one set of environmental conditions (27°C and 100% RH), with nonavoidance in all other treatment combinations. It was also unable to recover in the majority of those treatment combinations, leading us to score it as intolerant in seven out of nine treatments, indicating substantial vulnerability to climate-related stress (Fig. 4). In the pairwise comparisons (Fig. 6a,b), in the majority of cases where we recovered significant interspecific differences, *D. intermedia* was the outlier, with significantly lower relativized F_v/F_m than the other two species, indicating a more profound impact of stress on this species than the others. The other diploid in this triad, *D. expansa*, performed slightly better, exhibiting avoidance in three treatment combinations at the lowest levels of drought and temperature. In the six treatments where *D. expansa* was nonavoidant, after recovery, we scored it as tolerant in two conditions, for a total of five treatment combinations in which it was either avoidant or tolerant (Fig. 4). The tetraploid, *D. campyloptera*, while avoidant in only two treatment combinations (at 100% RH and the two lowest temperatures), had more tolerance than either parent, as it was able to recover in all drought treatments for both 22°C and 27°C, and was therefore scored as avoidant or tolerant in six out of the nine treatments (Fig. 4). This polyploid was slightly more flexible than either parent, with tolerance in one more treatment combination than the diploid, *D. expansa*, that it was most similar to in performance (in terms of numbers of treatment combinations in which stress was avoided/tolerated). This ability to tolerate a slightly greater number of treatment combinations suggests somewhat increased flexibility in the polyploid relative to its parent species. For all species in this triad, the most nonavoidance and intolerance of stress occurred in the most extreme temperature treatments, with no species exhibiting avoidance or tolerance in any of the 32°C treatments. This may be expected based on the natural distributions of these taxa, which are more northerly and may not regularly experience temperatures of this magnitude (Fig. 1).

In the *D. celsa* triad, the difference between the polyploid and its diploid progenitors was even more apparent. Diploid *D. ludoviciana* demonstrated a capacity to avoid stress under two conditions (22°C and 27°C at 100% RH), but was nonavoidant in the seven other treatment combinations (Fig. 5); it was also intolerant in all of those other treatments, suggesting very limited flexibility in its stress response repertoire. The other diploid in the *D. celsa* triad, *D. goldiana*, had the most limited stress response of all the species in our study, with nonavoidance and intolerance across all tested conditions, suggesting significant constraints on its capacity for survival under changing climates (note that our scoring of nonavoidance was very strict, with any species whose mean relativized F_v/F_m did not return to or above the relative zero line considered nonavoidant; in the most benign conditions, 22°C and 100% RH, *D. goldiana* survived the treatment, but its mean values did not return close enough to the line to meet our strict definition of avoidance (Fig. 5)). Tetraploid *Dryopteris celsa* was only avoidant in two of the least stressful treatment combinations (100% and 85% RH at 22°C), but showed a much broader ability to tolerate stress, exhibiting this tolerance even at the highest temperature (32°C), the only species in the entire experiment to do so. It was scored as avoidant or tolerant in seven out of the nine treatment combinations, indicating a more robust mechanism for managing environmental stress than either of its parents. However, its tolerance was challenged under the most extreme conditions (75% RH at 27°C and 32°C), indicating potential vulnerabilities, and it had significantly lower relativized F_v/F_m compared with the other species in that treatment, both immediately post-treatment (Timepoint 1) and after rehydration (Timepoint 3; Fig. 6c,d).

In summary, according to our conceptual model, in both triads the polyploid was able to avoid and/or tolerate stress in a larger number of treatment combinations than either diploid parent, and this was clearer in the *D. celsa* triad (Fig. 5). Across all six species, it was difficult to avoid or tolerate stress at higher temperatures even at 100% RH, and increasing temperature seemed to have a larger impact than increasing drought (Figs 4, 5). *Dryopteris goldiana* had the poorest performance as it showed nonavoidance and intolerance in all conditions we exposed it to.

Polyploid biology

Much work has been done comparing the ecology of polypoid systems in seed plants. Many of these studies have approached their work from the perspective of a changing climate with the intent to test whether or not polyploids, with their multiple genomes and novel genetic traits, may be a lifeline for lineages as their habitats change. As expected, there is no clear and obvious pattern in these studies. Indeed, much confusion still exists. In one of the largest studies of its kind, Baniaga *et al.* (2020) modeled the distribution data of 52 polyploid species from 25 genera to test whether polyploid taxa have different niche space compared with diploids. They found that not only did polyploid taxa have unique niche space relative to diploids, but also that polyploids had faster rates of niche differentiation. Unfortunately, this study did not isolate ferns from other taxa. However, in a more fine-scaled modeling study with a greater focus on ferns, Marchant *et al.* (2016) showed that the majority of polyploid ferns examined (6 out of 11) exhibited niche intermediacy and only two had larger niche space compared to diploids. Niche space is intrinsically linked to ecophysiology and stress biology. Interestingly, in our study *D. celsa* was perhaps the most robust of all species examined, yet this allopolyploid species was included in the Marchant *et al.* (2016) study and exhibited strong niche intermediacy compared with its parents in the sporophyte stage. It is also one of the rarest species of *Dryopteris* in North America. In contrast to the rarity of *D. celsa*, diploid *D. intermedia* is one of the most common species in northeast temperate forests, yet this species was sensitive to most treatments in our experiments, only avoiding or tolerating temperature stress up to 27°C and only at 100% RH. This apparent contrast between commonness or rarity of sporophytes and gametophyte performance in our experiments may have to do with differences in climatic preferences between the life stages.

While limited in scope, some studies have suggested that polyploid fern sporophytes can occupy an intermediate niche space relative to diploids. In a study on diploid and triploid *Dryopteris* sporophyte physiology, Blake-Mahmud & Watkins (2022) showed that polyploid hybrids were intermediate in almost all measured physiological variables. In a larger study on the genus that included many allotetraploid taxa, Sessa & Givnish (2014) similarly found that while polyploid taxa grow in habitats outside the range of the diploids, the polyploids had intermediate ecophysiological traits. Similar patterns of intermediacy have also been found in the xeric resurrection fern genus *Oeosporangium*, where intermediacy may allow for coexistence of polyploids and their putative parents (Quintanilla *et al.*, 2023). Far from showing broader physiological niche space, de Groot *et al.* (2012) have shown that the tetraploid *Polystichum aculeatum* may in fact be more sensitive to extreme cold stress than at least one of its parents, though they also found that *P. aculeatum* had higher recruitment than the diploids due to significantly better performance in all recruitment-related metrics (spore germination, gametophyte survival, and fertilization). The degree to which lineage-specific patterns are at play must also be considered, however; in contrast to de Groot *et al.* (2012)'s findings for *Polystichum*, Pangua *et al.* (2019) found that a tetraploid *Cheilanthes* had no fitness advantage over its diploid progenitors in demography-related metrics of gametophyte performance.

Understanding niche space in ferns is complex, and it is important to consider that gametophytes and sporophytes have markedly unique biology. Unlike sporophytes, gametophytes lack cuticles, stomata, and vascular tissue, and are in a constant state of equilibrium with their external environment. This lends itself to unique, and at times opposing, selective pressures compared with sporophytes, and it is not surprising that the preferences of these life stages do not always line up (see Pinson *et al.*, 2017 for a review of fern species in which the gametophytes have separate distributions from their sporophyte counterparts). We know essentially nothing about gametophyte distributions in *Dryopteris*, but in our experiments, the gametophytes of both tetraploids were more flexible in their overall stress response relative to their diploid parents. A great deal of work has shown that far from being a delicate and ephemeral stage of the life cycle, fern gametophytes can exhibit remarkable degrees of drought and desiccation tolerance (Ong & Ng, 1998; Watkins *et al.*, 2007; Testo & Watkins, 2013;

Pittermann *et al.*, 2013; Nitta *et al.*, 2021; Schneller & Farrar, 2022). Stress-tolerant taxa that invest heavily in functional mechanisms to manage stress are often slower-growing and poor competitors (Liancourt *et al.*, 2005; Alpert, 2006). In some cases, this may push stress-tolerant taxa into intermediate habitats where neither extreme can prosper. The apparent differences in stress tolerance and avoidance between the diploids and tetraploids in our study may allow for coexistence at the gametophyte level. In spite of the relatively few studies that have been done, we see a pattern emerging between common and rare sporophyte populations, in which species with common sporophytes seem not to rely on broadly stress-tolerant gametophytes, whereas rare taxa seem to exhibit more flexible or robust gametophyte performance. Clearly, more work is needed to assess gametophyte distributions in the field and to understand the relationship between the climatic preferences of the two generations, especially when they appear to differ.

Autecology of *Dryopteris*

The species that make up these two triads include some of the most common and the most rare species of *Dryopteris* in eastern North America. While there is significant range overlap across these six species (Fig. 1), locally they generally occupy different ecological niches. In the *D. campyloptera* triad, sporophytes of all three species are common and found over a wide range of elevational gradients in cool, mesic forests. Tetraploid *D. campyloptera* is typically restricted to cooler, higher-elevation montane forests in the Appalachian mountains, while diploid *D. intermedia* grows across a diverse array of habitats from lowland wet forests to dry upland forests (Wagner, 1971; Nickrent *et al.*, 1978). Diploid *D. expansa*'s habitat is similar to that of *D. intermedia*, but it is widely distributed into northeastern Canada (as well as occurring along the Pacific coast; Sessa, 2024). The members of the *D. celsa* triad, instead, are typically limited to low elevations in wet and swampy areas, with these species rarely found above 1000 m. Diploid *D. ludoviciana* is a southern endemic found along the southeast Coastal Plain (Fig. 1), and while it can be locally abundant, it is relatively rare. Diploid *D. goldiana* is similar in that it prefers wet forested habitats and is generally uncommon but can become locally abundant, but it occurs in the northeast and midwest. Tetraploid *Dryopteris celsa* is one of the rarest *Dryopteris* species in eastern North America, and it is always found in wet streamside habitats or lowland swamps (Nickrent *et al.*, 1978).

Taken together, our experiments included one set of common, widespread species and a second set of locally abundant but generally rare species. We found no clear signal across these two triads, based on gametophyte stress response, that might explain these current distributions; in some ways, we found opposing patterns. For example, the sporophytes of *D. intermedia* occupy a seemingly broad ecological niche, but their gametophytes, when exposed to a range of stress combinations, were able to avoid or tolerate the lowest number of combinations in their triad of species. A potential strategy in this species may be to rely on a long-lived, robust sporophyte stage that can wait for more environmentally suitable local conditions for sexual reproduction. While we do not have data on sporophyte demography of this species, it is not uncommon for individual ferns to live for several decades (Sharpe, 1997; Mehltreter *et al.*, 2010). Gametophytes of *Dryopteris goldiana* were also remarkably sensitive to any type of stress; this species is almost always limited to cool mesic habitats, where the sporophytes can become very robust. It is possible that this species is limited to these habitats given the overall sensitivity of their gametophyte phase. The reverse pattern may be playing out in tetraploid *D. celsa*. Its gametophytes had the highest avoidance and tolerance of stress of any species we tested, yet it occurs only rarely in the landscape, suggesting a potential mismatch between its gametophytic and sporophytic capacities for tolerating climatic heterogeneity. The contrast between physiological performance of *Dryopteris* sporophytes and the breadth of their ranges (i.e. polyploid hybrids being intermediate in their physiology yet occupying, overall, larger geographic areas than their diploid relatives (Sessa & Givnish, 2014; Blake-Mahmud & Watkins, 2022)), further speaks to the importance of the gametophyte as the establishment phase of the fern life cycle, and the need for an improved understanding of how differences in the ecology of the life stages, especially at different ploidal levels, can affect establishment, niche, and survival in a changing world.

Conclusion

The past decade has seen a great deal of work focused on understanding the impacts of abiotic stress on plant growth and survival. These studies are all the more important now given our immediate need to understand how plants respond to a rapidly changing world, driven by climate change. We have shown here that gametophytes of several fern species are able to avoid or tolerate brief periods of intense drought and temperature stress. Furthermore, the polyploids we evaluated demonstrated increased capacity for avoiding and tolerating stress in at least some combinations of high temperature and drought, in comparison with their diploid progenitors. This stress recovery is not unlimited; at higher levels of stress, gametophytes of all species are less able to recover to baseline levels of physiological functioning. These results suggest that as we see the rising frequency of extreme weather events such as droughts and heat waves, polyploids might initially offer some resilience in mixed ploidy populations. However, even polyploids will not be exempt from the impacts of extreme drought and heat waves in the long term, and further investigation of the apparently contrasting patterns of physiological performance, gametophyte performance, and distribution in these and other fern species is urgently needed.

Acknowledgements

We thank Colgate University undergraduates Taylor Atkin, Luke Calderaro, Cameron Campbell, Kiersten Daly, Aidan Harrington, Evelyn Lawson, Asiana Sample, Jacob Watts, Justice Wysinger, and Aziz Zafar; and Hamilton Junior-Senior High School Student Solomon Beeching for their help with data collection. We thank Donald R. Farrar and two anonymous reviewers for helpful suggestions on the manuscript. This research was supported by NSF IOS grants 1754911 and 2310485 (EBS), 1754864 (JEW), and 1754072 (CJV).

Competing interests

None declared.

Author contributions

EBS, CJV and JEW secured funding for the project. EBS, CJV, JB-M and JEW designed the experiments. JB-M and JEW conducted the experiments and analyzed the data. EBS, JB-M and JEW wrote the manuscript. All authors contributed to the final draft.

ORCID

Jennifer Blake-Mahmud https://orcid.org/0000-0002-6737-2824
Emily B. Sessa https://orcid.org/0000-0002-6496-5536
Clayton J. Visger https://orcid.org/0000-0001-9306-772X
James E. Watkins Jr https://orcid.org/0000-0002-4789-2056

Data availability

The data that support the findings of this study are available in the supplementary material of this article (Supporting Information Dataset S1).

References

Allison SD, Vitousek PM. 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawaii. *Oecologia* **141**: 612–619.

Alpert P. 2006. Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? *Journal of Experimental Biology* **209**: 1575–1584.

Baniaga AE, Marx HE, Arrigo N, Barker MS. 2020. Polyploid plants have faster rates of multivariate niche differentiation than their diploid relatives. *Ecology Letters* **23**: 68–78.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using LME4. *Journal of Statistical Software* **67**: 1–48.

Blake-Mahmud J, Watkins JE. 2022. Comparative ecophysiology of hybrid wood fern species. *International Journal of Plant Sciences* **183**: 707–718.

Bold HC. 1957. *Morphology of plants*. New York, NY, USA: Harper & Row.

Bowers JE, Chapman BA, Rong J, Paterson AH. 2003. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422**: 433–438.

Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, Scheen AC, Elven R. 2004. Polyploidy in Arctic plants. *Biological Journal of the Linnean Society* **82**: 521–536.

Chambers SM, Emery NC. 2016. Population differentiation and countergradient variation throughout the geographic range in the fern gametophyte *Vittaria appalachiana*. *American Journal of Botany* **103**: 86–98.

Chansler MT, Ferguson CJ, Fehlberg SD, Prather LA. 2016. The role of polyploidy in shaping morphological diversity in natural populations of *Phlox amabilis*. *American Journal of Botany* **103**: 1546–1558.

Coate JE, Luciano AK, Seralathan V, Minchew KJ, Owens TG, Doyle JJ. 2012. Anatomical, biochemical, and photosynthetic responses to recent allopolyploidy in *Glycine dolichocarpa* (Fabaceae). *American Journal of Botany* **99**: 55–67.

Coate JE, Powell AF, Owens TG, Doyle JJ. 2013. Transgressive physiological and transcriptomic responses to light stress in allopolyploid *Glycine dolichocarpa* (Leguminosae). *Heredity* **110**: 160–170.

de Groot GA, Zuidema PA, de Groot H, During HJ. 2012. Variation in ploidy level and phenology can result in large and unexpected differences in demography and climatic sensitivity between closely related ferns. *American Journal of Botany* **99**: 1375–1387.

Ellwood MDF, Foster WA. 2004. Doubling the estimate of invertebrate biomass in a rainforest canopy. *Nature* **429**: 549–551.

Escudero M, Wendel JF. 2020. The grand sweep of chromosomal evolution in angiosperms. *New Phytologist* **228**: 805–808.

Folk RA, Siniscalchi CM, Soltis DE. 2020. Angiosperms at the edge: extremity, diversity, and phylogeny. *Plant, Cell & Environment* **43**: 2871–2893.

Fox J, Weisberg S. 2019. *An R companion to applied regression*. Thousand Oaks, CA, USA: Sage.

Gaff DF, Oliver M. 2013. The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. *Functional Plant Biology* **40**: 315–328.

George LO, Bazzaz FA. 1999a. The fern understory as an ecological filter: emergence and establishment of canopy-tree seedlings. *Ecology* **80**: 833–845.

George LO, Bazzaz FA. 1999b. The fern understory as an ecological filter: growth and survival of canopy-tree seedlings. *Ecology* **80**: 846–856.

Hao G-Y, Lucero ME, Sanderson SC, Zacharias EH, Holbrook NM. 2013. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytologist* **197**: 970–978.

Haufler CH, Pryer KM, Schuettpelz 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.

Husband BC, Baldwin SJ, Suda J. 2013. The incidence of polyploidy in natural plant populations: major patterns and evolutionary processes. In: Greilhuber J, Dolezel J, Wendel JF, eds. *Plant genome diversity*, vol. **2**. Vienna, Austria: Springer, 255–276.

IPCC. 2023. Section 2: Current status and trends. In: Arias P, Bustamante M, Elgizouli I, Flato G, Howden M, Méndez-Vallejo C, Pereira JJ, Pichs-Madruga R, Rose SK, Saheb Y *et al.*, eds. *Climate change 2023: synthesis report*. Chapter 1. Geneva, Switzerland: IPCC.

Karunarathne P, Schedler M, Martínez EJ, Honfi AI, Novichkova A, Hojsgaard D. 2018. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. *Annals of Botany* **121**: 1183–1196.

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481–483.

Lenth R. 2021. *EMMEANS: estimated marginal means, aka least-squares means*. [WWW document] URL https://cran.r-project.org/package=emmeans [accessed 1 November 2023].

Levin DA. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* **122**: 1–25.

Levin DA. 2002. *The role of chromosomal change in plant evolution*. Oxford, UK: Oxford University Press.

Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS. 2015. Early genome duplications in conifers and other seed plants. *Science Advances* **1**: e1501084.

Liancourt P, Callaway RM, Michalet R. 2005. Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology* **86**: 1611–1618.

Liu Z, Adams KL. 2007. Expression partitioning between genes duplicated by polyploidy under abiotic stress and during organ development. *Current Biology* **17**: 1669–1674.

Maherali H, Walden AE, Husband BC. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* **184**: 721–731.

Manzaneda AJ, Rey PJ, Anderson JT, Raskin E, Weiss-Lehman C, Mitchell-Olds T. 2015. Natural variation, differentiation, and genetic trade-offs of ecophysiological traits in response to water limitation in *Brachypodium distachyon* and its descendent allotetraploid *B. hybridum* (Poaceae). *Evolution* **69**: 2689–2704.

Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, Mitchell-Olds T. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* **193**: 797–805.

Marchant DB, Soltis DE, Soltis PS. 2016. Patterns of abiotic niche shifts in allopolyploids relative to their progenitors. *New Phytologist* **212**: 708–718.

Mehltreter K, Walker LR, Sharpe JM, eds. 2010. *Fern ecology.* Cambridge, UK: Cambridge University Press.

Moura RF, Queiroga D, Vilela E, Moraes AP. 2021. Polyploidy and high environmental tolerance increase the invasive success of plants. *Journal of Plant Research* **134**: 105–114.

Nickrent DL, Musselman LJ, Pitchford LA, Sampson DW. 1978. The distribution and ecology of Dryopteris in Southeastern Virginia and adjacent North Carolina. *American Fern Journal* **68**: 45–51.

Nitta JH, Meyer J-Y, Taputuarai R, Davis CC. 2017. Life cycle matters: DNA barcoding reveals contrasting community structure between fern sporophytes and gametophytes. *Ecological Monographs* **87**: 278–296.

Nitta JH, Watkins JE, Holbrook NM, Wang TW, Davis CC. 2021. Ecophysiological differentiation between life stages in filmy ferns (Hymenophyllaceae). *Journal of Plant Research* **134**: 971–988.

Oliver MJ, Farrant JM, Hilhorst HWM, Mundree S, Williams B, Bewley JD. 2020. Desiccation tolerance: avoiding cellular damage during drying and rehydration. *Annual Review of Plant Biology* **71**: 435–460.

Oliver MJ, Tuba Z, Mishler BD, Oliver M, Tuba Z, Mishler B. 2000. The evolution of vegetative desiccation tolerance in land plants. *Plant Ecology* **151**: 85–100.

One Thousand Plant Transcriptomes Initiative. 2019. One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **574**: 679–685.

Ong BL, Ng ML. 1998. Regeneration of drought-stressed gametophytes of the epiphytic fern, *Pyrrosia pilosellodes* (L.) Price. *Plant Cell Reports* **18**: 225–228.

Pangua E, Pajarón S, Quintanilla LG. 2019. Fitness of an allopolyploid rupicolous fern compared with its diploid progenitors: from sporogenesis to sporophyte formation. *American Journal of Botany* **106**: 984–995.

Pelosi JA, Kim EH, Barbazuk WB, Sessa EB. 2022. Phylotranscriptomics illuminates the placement of whole genome duplications and gene retention in ferns. *Frontiers in Plant Science* **13**: 882441.

Pelosi JA, Sessa EB. 2021. From genomes to populations: a meta-analysis and review of fern population genetics. *International Journal of Plant Sciences* **182**: 325–343.

Pinson JB, Chambers SM, Nitta JH, Kuo L-Y, Sessa EB. 2017. The separation of generations: biology and biogeography of long-lived sporophyteless fern gametophytes. *International Journal of Plant Sciences* **178**: 1–18.

Pittermann J, Brodersen C, Watkins JE. 2013. The physiological resilience of fern sporophytes and gametophytes: advances in water relations offer new insights into an old lineage. *Frontiers in Plant Science* **4**: 1–10.

Pittermann J, Watkins JE, Cary KL, Schuettpelz E, Brodersen C, Smith AR, Baer A. 2015. The structure and function of xylem in seed-free vascular plants: an evolutionary perspective. In: Hacke U, ed. *Functional and ecological xylem anatomy.* Cham, Switzerland: Springer International Publishing, 1–37.

Quintanilla LG, Aranda I, Clemente-Moreno MJ, Pons-Perpinyà J, Gago J. 2023. Ecophysiological differentiation among two resurrection ferns and their allopolyploid derivative. *Plants* **12**: 1–15.

R Core Team. 2021. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Ruprecht C, Lohaus R, Vanneste K, Mutwil M, Nikoloski Z, Van de Peer Y, Persson S. 2017. Revisiting ancestral polyploidy in plants. *Science Advances* **3**: e1603195.

Schneller JJ, Farrar DR. 2022. Photographic analysis of field-monitored fern gametophyte development and response to environmental stress. *Applications in Plant Sciences* **10**: e11470.

Schwarzbach AE, Donovan LA, Rieseberg LH. 2001. Transgressive character expression in a hybrid sunflower species. *American Journal of Botany* **88**: 270–277.

Sessa E. 2024. *Ferns, Spikemosses, Clubmosses, and Quillworts of Eastern North America.* Princeton, NJ, USA: Princeton University Press.

Sessa EB, Givnish TJ. 2014. Leaf form and photosynthetic physiology of *Dryopteris* species distributed along light gradients in eastern North America. *Functional Ecology* **28**: 108–123.

Sessa EB, Zimmer EA, Givnish TJ. 2012. Unraveling reticulate evolution in North American *Dryopteris* (Dryopteridaceae). *BMC Evolutionary Biology* **12**: 104.

Sharpe JM. 1997. Leaf growth and demography of the rheophytic fern *Thelypteris angustifolia* (Willdenow) Proctor in a Puerto Rican rainforest. *Plant Ecology* **130**: 203–212.

Soltis PS, Liu X, Marchant DB, Visger CJ, Soltis DE. 2014. Polyploidy and novelty: Gottlieb's legacy. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **369**: 1–12.

Soltis PS, Marchant DB, Van de Peer Y, Soltis DE. 2015. Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development* **35**: 119–125.

Stark LR, Greenwood JL, Brinda JC. 2022. How to dry a bryophyte: A review and experimental test of four methods to induce desiccation tolerance. *The Bryologist* **125**: 1–22.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesová M, Pysek P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* **109**: 19–45.

Testo WL, Watkins JE. 2013. Understanding mechanisms of rarity in pteridophytes: competition and climate change threaten the rare fern *Asplenium scolopendrium* var. *americanum* (Aspleniaceae). *American Journal of Botany* **100**: 2261–2270.

Tossi VE, Martínez Tosar LJ, Laino LE, Iannicelli J, Regalado JJ, Escandón AS, Baroli I, Causin HF, Pitta-Álvarez SI. 2022. Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in Plant Science* **13**: 869423.

Trewick SA, Morgan-Richards M, Russell SJ, Henderson S, Rumsey FJ, Pintér I, Barrett JA, Gibby M, Vogel JC. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Molecular Ecology* **11**: 2003–2012.

Van de Peer Y, Ashman T-L, Soltis PS, Soltis DE. 2021. Polyploidy: an evolutionary and ecological force in stressful times. *Plant Cell* **33**: 11–26.

Wagner WH. 1971. Evolution of *Dryopteris* in relation to the Appalachians. In: *The distributional history of the biota of the Southern Appalachians, Part II: Flora.* Virginia Polytechnic: Blacksburg, VA, USA, 147–192.

Watkins JE, Cardelús CL. 2012. Ferns in an angiosperm world: cretaceous radiation into the epiphytic niche and diversification on the forest floor. *International Journal of Plant Sciences* **173**: 695–710.

Watkins JE, Mack M, Sinclair TR, Mulkey SS. 2007. Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. *New Phytologist* **176**: 708–717.

Wefferling KM, Castro M, Castro S, Holmlund H, Loureiro J, Rothfels CJ, Schuettpelz E. 2024. Polyploid goldback and silverback ferns (*Pentagramma*) occupy a wider, colder, and wetter bioclimatic niche than diploid counterparts. *American Journal of Botany* **111**: e16305.

Wei N, Cronn R, Liston A, Ashman T-L. 2019. Functional trait divergence and trait plasticity confer polyploid advantage in heterogeneous environments. *New Phytologist* **221**: 2286–2297.

Wickham H. 2016. *GGPLOT2: elegant graphics for data analysis.* New York, NY, USA: Springer-Verlag.

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences, USA* **106**: 13875–13879.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Chlorophyll fluorescence data and results for all estimated marginal means and pairwise comparisons.