Understanding mechanisms of rarity in pteridophytes: Competition and climate change threaten the rare fern *Asplenium scolopendrium* var. *americanum* (Aspleniaceae)[1]

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- *Premise of the study:* Understanding the ecology of rare species can inform aspects of conservation strategies; however, the mechanisms of rarity remain elusive for most pteridophytes, which possess independent and ecologically distinct gametophyte and sporophyte generations. To elucidate factors contributing to recent declines of the rare fern *Asplenium scolopendrium* var. *americanum*, we studied the ecology and ecophysiology of its gametophyte generation, focusing on responses to competition, temperature, and water stress.
- *Methods:* Gametophytes of *A. scolopendrium* var. *americanum*, its widespread European relative *A. scolopendrium* var. *scolopendrium*, and five co-occurring fern species were grown from spores. Gametophytes were grown at 20°C and 25°C, and germination rates, intra- and interspecific competition, desiccation tolerance, and sporophyte production were determined for all species.
- *Key results:* Gametophytes of *A. scolopendrium* var. *americanum* had the lowest rates of germination and sporophyte production among all species studied and exhibited the greatest sensitivity to interspecific competition, temperature increases, and desiccation. Mature gametophytes of *A. scolopendrium* var. *americanum* grown at 25°C were 84.6% smaller than those grown at 20°C, and only 1.5% produced sporophytes after 200 d in culture. Similar responses were not observed in other species studied.
- *Conclusions:* The recent declines and current status of populations of *A. scolopendrium* var. *americanum* are linked to its gametophyte's limited capacity to tolerate competition and physiological stress linked to climate change. This is the first study to develop a mechanistic understanding of rarity and decline in a fern and demonstrates the importance of considering the ecology of the gametophyte in plants with independent sporophyte and gametophyte generations.

Key words: Aspleniaceae; *Asplenium scolopendrium* var. *americanum*; climate change; competition; conservation; fern; gametophyte; rare species.

The world's biomes are facing unprecedented anthropogenic pressures, and species rarity and extinction are only expected to rise as a result (Barnosky et al., 2011). Understanding the ecology of rare species can inform aspects of conservation strategies as well as population status and future growth trajectories. To date, many studies have attempted to quantify rarity and establish databases of the numbers and location of rare species (Cinquemani et al., 1988; Kelly, 1994; de Siqueira et al., 2009). Still others have searched for the best means of conserving such species (Miller et al., 2010; Barnicoat et al., 2011; Barrington, 2011).

While much work has examined the biology of rare plants (Cinquemani et al., 1988; Gundale, 2002; Kelsall et al., 2004; Wild and Gagnon, 2005), the mechanisms of rarity remain elusive for most taxa. Examining the ecological aspects of rarity is

of fundamental importance in our efforts to protect and develop rigorous restoration objectives for rare taxa. Such efforts on ferns are more complicated given their reliance on independent gametophyte and sporophyte stages that differ radically in their morphology, ecology, and physiology (Watkins et al., 2007a, b). Because of these differences, the biology of any fern species cannot be adequately understood without study of both the sporophyte and gametophyte life stages.

Fern gametophytes are often perceived as both prohibitively difficult to study on account of their diminutive size and of little ecological importance due to their simple body plan and purported ephemerality (e.g., Bower, 1923). These notions have perpetuated a significant gap in our understanding of fern gametophyte ecology that persists to this day. Although considerable advances in the study of fern sporophytes have been made in recent years (Watkins et al., 2010; Brodribb and McAdam, 2011; Pittermann et al., 2011), the ecology of these plants remains poorly understood due to the sustained lack of information on the gametophyte generation. Given this lack of knowledge and the central role of the free-living gametophyte in the fern life cycle (every fern sporophyte ultimately owes its existence to one or more gametophytes), it is unsurprising that we know little about the factors influencing the decline of rare fern species (Kelsall et al., 2004; Bucharová et al., 2010). To change this paradigm, a more balanced approach to the study of fern ecology is needed. In this study, we present the most comprehensive

examination to date on the gametophyte ecology of a rare fern, with the focus of developing a mechanistic understanding of the decline of the threatened hart's-tongue fern (*Asplenium scolopendrium* L.) by studying the development, reproductive biology, and stress ecophysiology of its gametophyte generation.

Asplenium scolopendrium (Aspleniaceae, Polypodiales) is an unusual temperate fern species that has drawn considerable scientific interest due to its uncertain taxonomic status, unusual distribution, dwindling numbers, and intriguing ecology (Cinquemani et al., 1988; Testo and Watkins, 2011). The species is subdivided into two varieties: the European hart's-tongue fern (*Asplenium scolopendrium* L. var. *scolopendrium*; EHTF), a widespread taxon distributed broadly throughout Europe and Asia, and the American hart's-tongue fern, *Asplenium scolopendrium* L. var. *americanum* (Fern.) Kartesz and Gandhi (AHTF), a rare tetraploid taxon found in the eastern United States, Mexico, and Canada, where it is restricted to edaphically unique sites with moderated microclimates (Fig. 1A–D; Kelsall et al., 2004). A recent assessment of the reproductive biology of this fern by Testo and Watkins (2011) has raised considerable doubts about the varietal level classification of this plant and the possibility that the New World representatives are a distinct species. Such taxonomic uncertainty only increases the need to better understand the ecology of this taxon.

The AHTF is rare throughout its range, federally listed as a threatened species in the United States, and listed as a species of special concern in Canada. Recent and ongoing demographic data show the species declining precipitously in the United States. The largest known populations of AHTF are found in upstate New York, where some populations have gone extinct and most have declined over 50% since 1993 (Kelsall et al., 2004). There are fewer than 4000 individuals remaining in scattered sites in the central part of the state (Cinquemani et al., 1988). The situation is more dire in Tennessee and Alabama. The combined size of the two extant Alabama populations dwindled from 117 to 43 individuals between 1980–1990 (United States Fish and Wildlife Service, 1993); in Tennessee, the only population in the state has decreased from 200 plants at the turn of the 20th century to 17 plants in 1980, and only two immature specimens persist today (Fig. 1A; Testo and Watkins, 2011).

Most reasons given for population declines of this species are largely speculative and relate to habitat disturbance. For example, one population reportedly declined in Michigan after an invasion of folivorous insects removed much of the forest's canopy cover (United States Fish and Wildlife Service, 1993). Similarly, vandals in Alabama removed tree cover above one sinkhole, resulting in rapid decline of that population (Fig. 1D). Such anecdotal reports are insightful but ultimately difficult to interpret from a conservation perspective and do little to increase our understanding of other concurrent declines over the species' range.

The goal of this research was 3-fold: (1) to improve understanding of the American hart's-tongue fern's recent decline by investigating the gametophyte generation's ecology and physiology, (2) to compare these aspects of its biology to those of associated fern species and potential competitors, and (3) to evaluate the importance of gametophyte ecology in studies of rare ferns.

MATERIALS AND METHODS

Study species—In the northern part of its range, *A. scolopendrium* var. *americanum* (AHTF) is closely associated with dolomitic limestone outcroppings of the Niagara Escarpment, which rises in central New York and runs through southeastern Ontario and Michigan's Upper Peninsula before terminating along the western margin of Lake Michigan (Kuehn and Leopold, 1993). Populations of AHTF in this region are restricted to glacial plunge ravines and similar habitats that provide a perpetually moist and cool microhabitat, a dense hardwood canopy, and calcareous substrate. Other important habitat characteristics include steep, north-facing slopes, and the presence of rock crevices and bryophyte mats for sporophyte and gametophyte establishment, respectively (Kuehn and Leopold, 1993).

Throughout the range of AHTF, habitats that support its populations also exhibit consistent assemblages of other fern species. In New York, the most prevalent of these associate species are *Asplenium trichomanes* (L.) subsp. *quadrivalens* D.E. Meyer, *Asplenium rhizophyllum* L., *Cystopteris bulbifera* (L.) Bernh., and *Dryopteris marginalis* (L.) Gray (Kuehn and Leopold, 1993). For comparative purposes, these four species and the European hart's-tongue fern, *A. scolopendrium* var. *scolopendrium* (EHTF), were included in this study in addition to AHTF.

Spore material and growth conditions—In total, six taxa were included in this study. Spore material from all taxa except for EHTF was collected at Clark Reservation and Chittenango Falls State Parks, New York. Spores from EHTF were collected from plants growing at the Colgate University greenhouse that were originally reared from wild European populations. Fragments of fertile fronds were put into glassine envelopes with tape-sealed seams and then stored in an air-conditioned laboratory and allowed to dry to permit the release of spores.

Except where noted otherwise, cultures were prepared using standard Bold's mix (Bold, 1949). Spores were sown at an approximate density of 25 spores per 100×25 mm Fisher Brand Petri plates (ThermoFisher Scientific, Waltham, Massachusetts, USA). Cultures were exposed to GE fluorescent plant and aquarium 40 watt grow bulbs (General Electric, Fairfield, Connecticut, USA) on a 12 h light/12 h dark cycle at 20°C.

Spore germination—Individual spores of each species were sown by hand onto the agar medium using a stereoscope and a microspatula to allow precise spore placement. One species was sown per plate, with three replicates per species. To control for spore density, spores were sown in a grid pattern using plastic screening as a template, allowing approximately 2.5 mm between spores and 90 spores per plate. Following sowing, spores were observed with a stereoscope for germination every 6 d for 120 d, and germination percentage was determined at each time interval.

Spore vernalization—Spores were collected from fertile fronds, placed in microcentrifuge tubes and placed in a growth chamber at 10°C with a 8 h light/16 h dark cycle for 20 d before being placed in a −15°C freezer for 90 d. Controls were established by placing spores in another set of tubes in a laboratory desk drawer at ~20°C during the freezing period. Following the freezing period, spores were sown on agar medium (3 plates per species in both treatments) and observed at 30 d to determine germination percentage.

Gametophyte competition—For gametophyte competition experiments, spores were sown using the method described previously for the spore germination experiments. Species pairs were formed between AHTF and each of the other study species, totaling five pairs. Spores of the two taxa in each pair were sown on the same plate in an alternating pattern with 45 spores per taxa for a total of 90 spores. Controls consisted of spores of a single species sown at the same density (90 per plate). Following sowing, spores were observed for germination under a stereoscope every 6 d for 60 d, and germination percentage was determined.

Gametophyte desiccation experiments—For the initial survey experiment, 30 mature gametophytes of all seven species were transferred from growth chambers to a greenhouse room at ~90% RH for 3 wk to acclimate to conditions similar to those experienced in the field. Dark-adapted (30 min) photochemical efficiency (F_v/F_m) was measured using a portable fluorometer (Mini-PAM, Walz Gmbh, Effeltrich, Germany) to determine pretreatment photosystem II efficiency values in unstressed plants. Gametophytes were then transferred to humidity-controlled desiccation chambers, each regulated at one of five drying intensities ($\Psi = -4.1$ MPa to -282.1 MPa) by saturated salt solutions. Salts used and drying intensities attained were as follows: KNO_3 ($\Psi = -4.1$ MPa), $MgSO_4$ ($\Psi = -8.4$ MPa), $NaCl$ (($\Psi = -38.4$ MPa), NH_4NO_3 ($\Psi = -57.5$ MPa), and $LiCl$ ($\Psi = -282.1$ MPa). A Hobo Pro RH/Temp Data Logger (Onset Computer, Bourne, Massachusetts, USA) was used to verify that each chamber attained the desired drying intensity. Five gametophytes per species were placed above the

Fig. 1.   Life history stages and habitat of *Asplenium scolopendrium* var. *americanum* (AHTF). (A) One of two young sporophytes remaining at last remaining station in Tennessee; (B) mature fertile plant growing in sinkhole in Morgan County, Alabama; (C) population of mature sporophytes from one of the largest colonies in New York (Clark Reservation, Onondaga County); (D) large limestone sinkhole in Morgan County, Alabama, one of only two locations for this species in the stage; (E) young sporophyte and (F) mature gametophytes demonstrating occasional recruitment in the New York populations.

saturated salt solutions on a fine plastic mesh screen and allowed to equilibrate with the atmosphere created by the salts, which in all cases happened within 72 h. To verify that equilibrium had been reached, we individually weighed gametophytes at 2-h intervals during the drying period until no further mass was lost for three intervals. After 72 h, posttreatment F_v/F_m values were recorded, and samples were then rehydrated with deionized water. To evaluate postdehydration recovery, we measured F_v/F_m again at 5, 15, 30, 45, and 1440 min (1 d) postrehydration. These data were compared to controls that were watered with deionized water but not dried. Following the desiccation treatments, all samples and controls were dried for 72 h in a drying oven at 70°C to determine gametophyte dry mass.

Gametophyte thermotolerance—To determine the effects of growing temperature on gametophyte development for each species, we cultured ~50 gametophytes on each of three Petri dishes per species at 20°C and 25°C, with all other growth conditions as described previously. The lower temperature represents average summertime high temperatures in climatically buffered rock-house-like habitats (Farrar, 1998) to which AHTF is restricted, and the higher represents the 5°C temperature increase consistent with current climate predictions for the range of the species (IPCC, 2007).

At 100 d postsowing, 10 gametophytes per species were selected at random from each temperature treatment and measured across their widest axis using digital calipers. At 200 d, cultures were observed for sporophyte formation, and sporophyte formation percentages for all taxa were determined. Gametophytes with bulges indicating early stages of sporophyte formation were considered to have formed sporophytes. Cultures were observed with a stereoscope to identify these early stages of sporophyte development and to accurately determine the total number of gametophytes in culture.

Statistical analyses—All statistical analyses were run using the program SPSS 19.0 (SPSS Science, Chicago, Illinois, USA). Spore germination rates were expressed as a percentage of the total number of spores sown. Final germination and sporophyte recruitment percentages were tested for normality and then compared across species using ANOVA and posthoc Tukey tests. For gametophyte competition experiments, the effect of interspecific competition was expressed for each time interval as (treatment % germination − control % germination). Two-way *t* tests were used for within-species comparisons of vernalization and temperature effects. For all analyses, a significance level of α = 0.05 was used.

RESULTS

Spore germination—Spore germination rates varied considerably across species, though not significantly at all time intervals (Fig. 2). Generally, *A. trichomanes* subsp. *quadrivalens* exhibited the highest mean germination percentage at each time interval, while *A. scolopendrium* var. *americanum* (AHTF) consistently had the lowest. Maximal germination percentage varied significantly across study species (one-way ANOVA, $F_{5,17} = 5.92$, $P = 0.006$) and ranged from 71.9% for AHTF to 95.8% for *A. trichomanes* subsp. *quadrivalens* (Fig. 2).

Spore vernalization—Exposure to prolonged freezing did not significantly influence spore germination percentages in any taxa except AHTF, which exhibited a mean germination increase of 15.6 ± 3.3% following the vernalization treatment relative to the control.

Gametophyte competition—Competitive effects on spore germination were observed in all species pairs, though the intensity of these effects produced by the gametophytes of AHTF on other species were variable, ranging from extreme inhibition to moderate facilitation (Fig. 3). The presence of AHTF gametophytes induced a strong, long-lasting inhibitory effect on *D. marginalis* spore germination and weakly repressed spore germination in *A. scolopendrium* var. *scolopendrium*, *A. trichomanes* subsp. *quadrivalens*, and *A. rhizophyllum* at some, but not all, time intervals. Germination of AHTF spores was repressed strongly by both *C. bulbifera* and *A. trichomanes* at all time intervals and to a lesser extent by all other taxa. The presence of AHTF spores increased germination of *C. bulbifera* relative to the control at all time intervals except 12, 18, and 30 d. Competitive effects (±SE) exerted by AHTF at 60 d ranged from moderate facilitation (+5.7 ± 1.5%, *C. bulbifera*) to strong inhibition (−25.2 ± 3.4%, *D. marginalis*). Competitive effects exerted on AHTF at 60 d ranged from neutral (−1.8 ± 2.1%, *A. rhizophyllum*) to strong inhibition (−23.0 ± 4.3%, *A. trichomanes* subsp. *quadrivalens*). *Asplenium scolopendrium* var. *americanum* did not experience facilitation when grown with any species.

Desiccation experiments—Gametophytes of different species displayed broadly disparate tolerance of drying, with some taxa failing to recover from exposure to water potentials more negative than −8.4 MPa, while others recovered completely
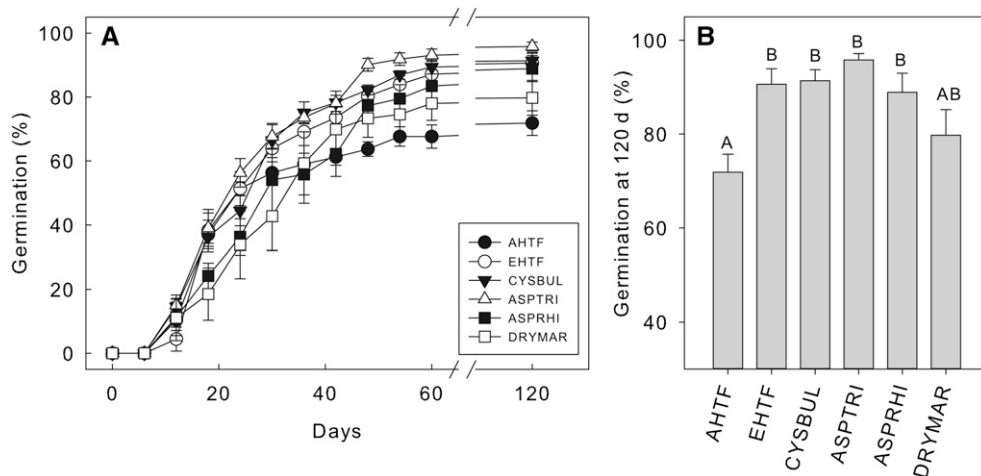

Fig. 2. Spore germination rates for AHTF and associate fern taxa. (A) Mean germination percentages for each species determined at 6-d intervals from 0–120 d. (B) Mean maximal (120 d) germination percentage for all taxa. Error bars represent ±1 SE. One-way ANOVA $F_{5,17} = 5.92$, $P = 0.006$; letters above bars designate homogenous subsets recovered by a posthoc Tukey test. *Asplenium scolopendrium* var. *americanum* (AHTF); *Asplenium scolopendrium* var. *scolopendrium* (EHTF); *Cystopteris bulbifera* (CYSBUL), *Asplenium trichomanes* (ASPTRI), *Asplenium rhizophyllum* (ASPRHI), *Dryopteris marginalis* (DRYMAR).
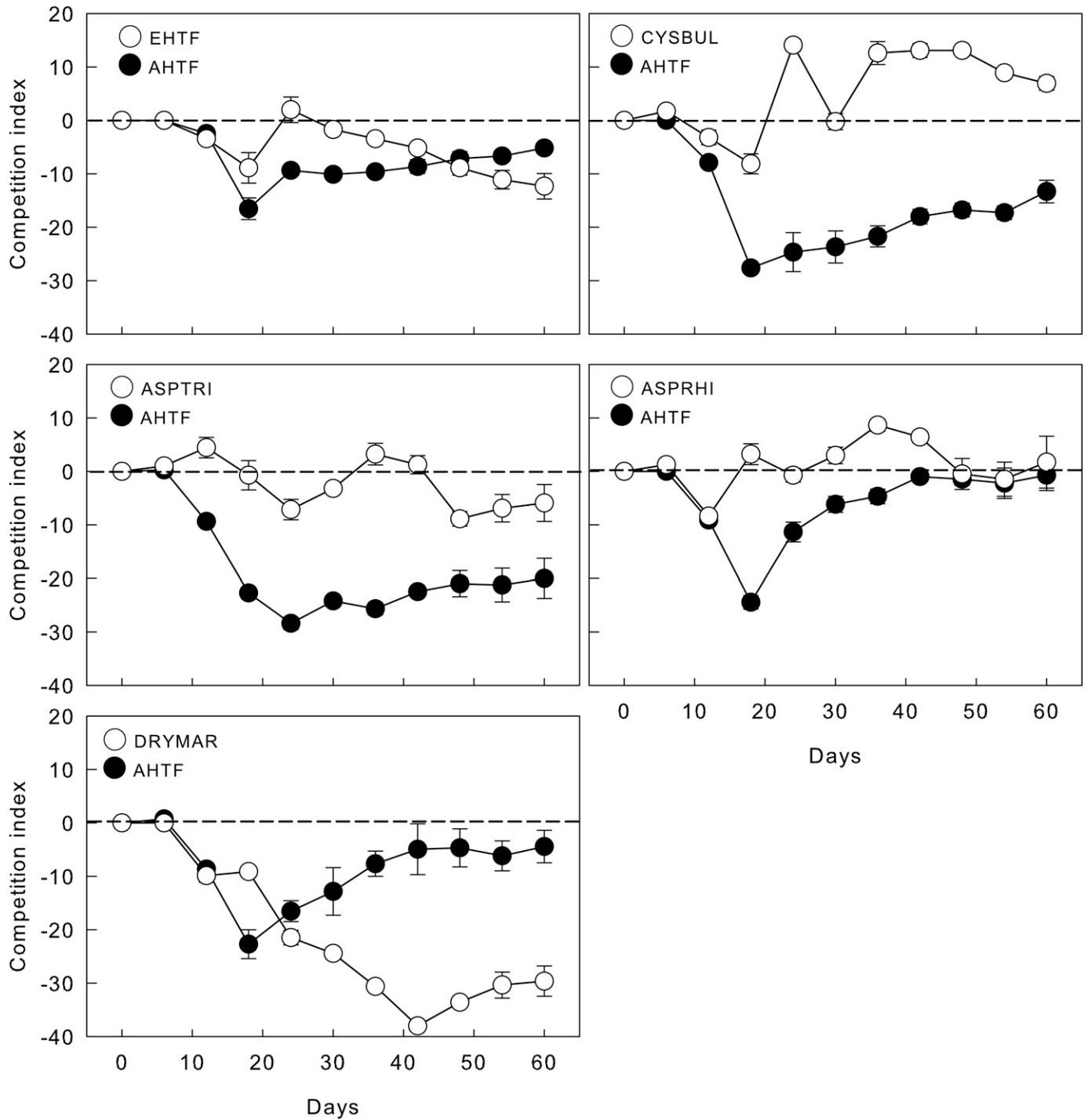
Fig. 3.   Germination competition between AHTF and associate fern taxa. Competition index for each species was determined for each time interval as percentage germination of spores sown with competing species' spores minus percentage germination of spores sown with conspecific spores (CI = % germination under competition −% germination in control). Closed circles indicate AHTF, open circles indicate associate species. Values above the dotted line indicate a facilitative effect; values below the dotted line represent an inhibitory effect. Error bars represent ±1 SE.

after drying to equilibrium at a water potential of −57.5 MPa (Fig. 4). AHTF displayed the greatest sensitivity to drying, recovering completely from only the mildest treatment ($\Psi = -4.2$ MPa) and exhibiting no recovery following exposure to water potentials more negative than −8.4 MPa. Both *A. trichomanes* subsp. *quadrivalens* and *A. rhizophyllum* recovered from all but

the driest ($\Psi = -282.1$ MPa) treatment; the remaining three species had intermediate responses.

Temperature effects—For all species studied, gametophytes grown at 25°C were on average smaller than those grown at 20°C at 100 d; however, this difference was not significant for
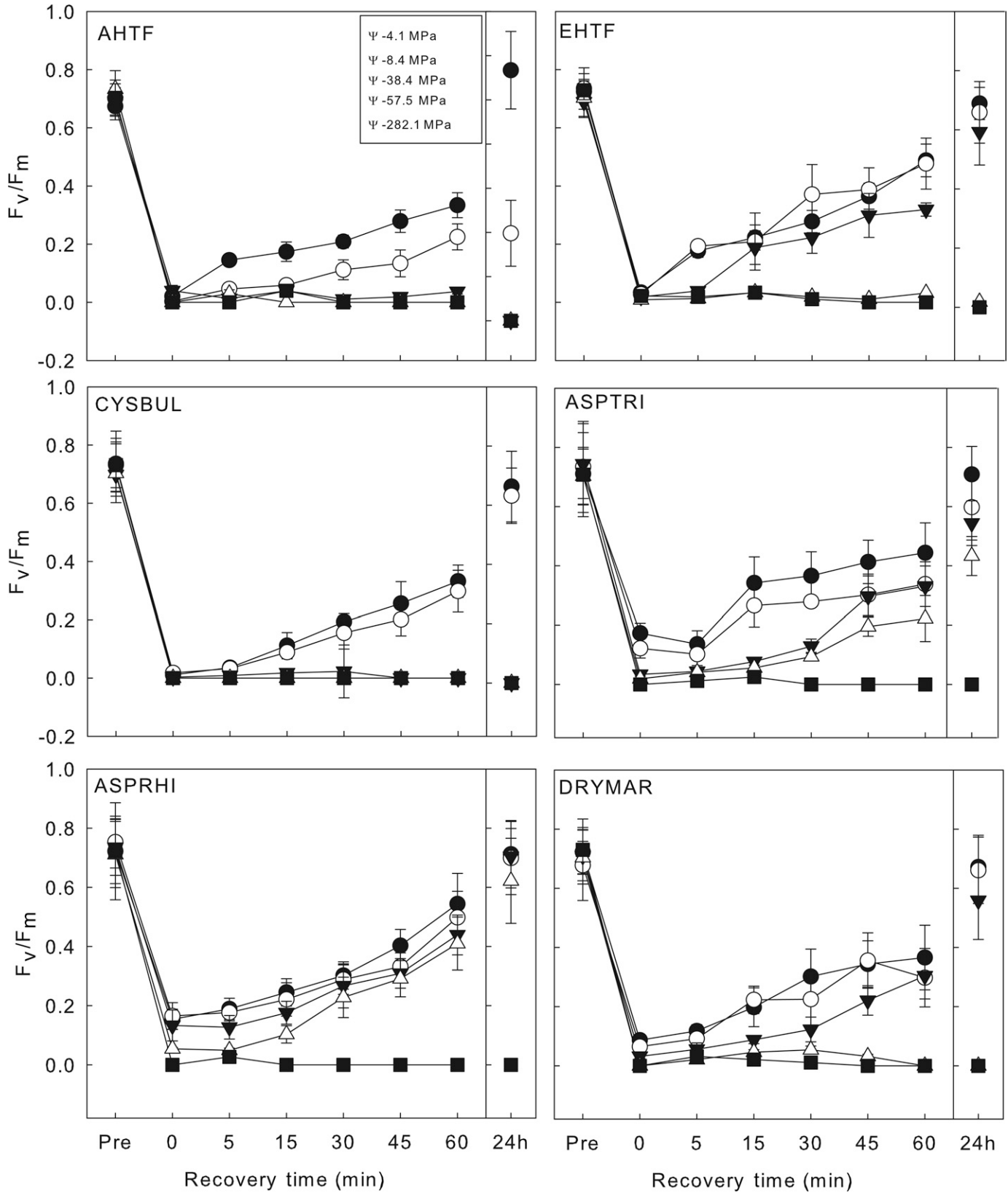
Fig. 4.   Recovery curves from desiccation experiments. Gametophytes were exposed to five desiccation intensities controlled by saturated salt solutions: KNO_3 ($\Psi = -4.1$ MPa; filled circles), $MgSO_4$ ($\Psi = -8.4$ MPa; unfilled circles), NaCl (-38.4 MPa; filled triangles), NH_4NO_3 ($\Psi = -57.5$ MPa; unfilled triangles), and LiCl ($\Psi = -282.1$ MPa; filled squares). Plants were dried to equilibrium and maintained for 72 h. Graphs depict recovery of F_v/F_m following rehydration. Error bars represent ± 1 SE.

A. scolopendrium var. *scolopendrium* or *A. rhizophyllum* (Fig. 5A). Gametophytes of AHTF exhibited the greatest difference between treatments; those grown at 25°C were on average 84.6% smaller than those grown at 20°C (Fig. 5B, $t_{14} = 20.864$, $P < 0.0001$).

Sporophyte formation varied significantly across species at both 20°C ($F_{5,17} = 68.416$, $P < 0.000$) and 25°C ($F_{5,17} = 27.466$, $P < 0.000$) (Fig. 5). In both treatments, AHTF exhibited the lowest sporophyte formation and differed statistically from all other taxa. Increased temperature reduced sporophyte formation in AHTF but no other species; just $1.5 \pm 0.6\%$ of gametophytes of AHTF cultured at 25°C produced sporophytes, compared to $9.0 \pm 3.2\%$ at 20°C (Fig. 5).
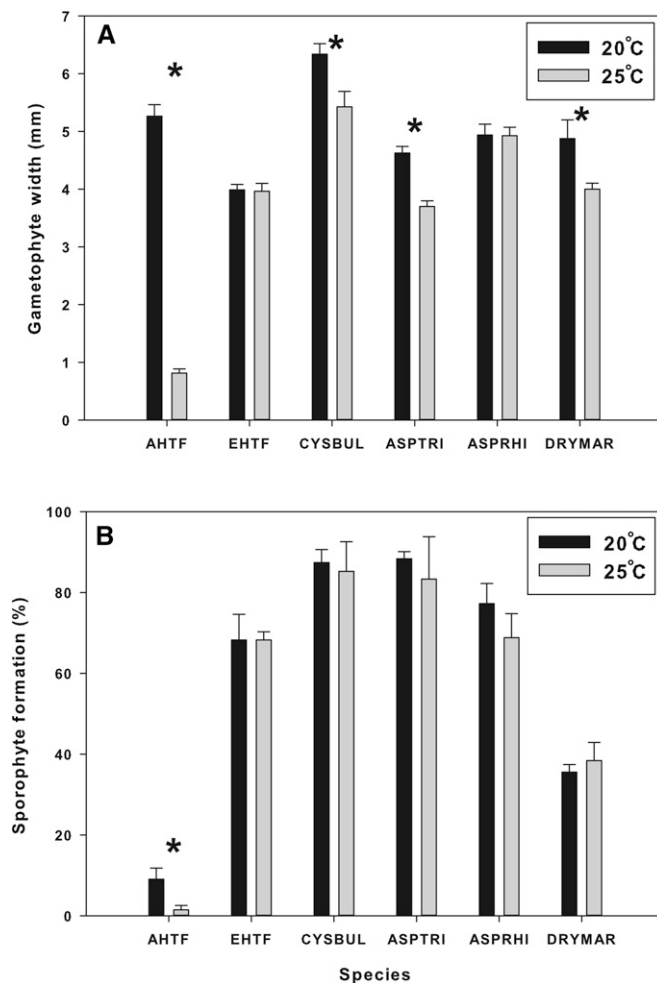

Fig. 5. Temperature effects on (A) gametophyte size at 100 d and (B) sporophyte formation at 200 d of *Asplenium scolopendrium* var. *americanum* (AHTF) and associate fern taxa. *Asplenium scolopendrium* var. *americanum* (A) $t = -20.864$, $P > 0.0001$; (B) $t = -2.56$ $P = 0.096$; *Asplenium scolopendrium* var. *scolopendrium* (EHTF), (A) $t = -0.151$, $P = 0.882$; IB) $t = 0.0820$ $P = 0.941$; *Cystopteris bulbifera* (CYSBUL), (A) $t = -2.813$, $P = 0.0153$; (B) $t = -0.2101$ $P = 0.8484$; *Asplenium trichomanes* (ASPTRI), (A) $t = -6.179$, $P < 0.0001$; (B) $t = -0.4732$. $P = 0.6804$; *Asplenium rhizophyllum* (ASPRHI), (A) $t = -0.0525$, $P = 0.9589$; (B) $t = -1.092$. $P = 0.3380$; *Dryopteris marginalis* (DRYMAR), (A) $t = -5.667$, $P < 0.0001$; (B) $t = 0.5818$ $P = 0.6061$. Error bars represent ±1 SE; asterisks designate statistically significant differences at $\alpha = 0.05$.

DISCUSSION

Understanding the ecology of rare species is important for developing effective conservation strategies and determining both current and future population status. Detailed understanding of the ecological and physiological underpinnings of a given taxon's rarity can help pinpoint specific threats to its survival (Rumsey et al., 1999; Bucharová et al., 2010); however, such information is lacking for most at-risk taxa and does not exist for ferns. Whereas many studies have examined reasons behind rarity and population declines, few have helped to develop a mechanistic understanding of how these drivers of rarity impact the species. In cases where such data are available, research has generally focused on one age class or life stage (Bucharová et al., 2010). Such studies necessarily overlook aspects of the species' biology and likely provide skewed insight into causes of rarity as well as the current and future status of populations (Grillas et al., 1991; Rumsey et al., 1999). Nowhere is this problem more acute than for the ferns given their reliance on two independent life stages.

While a number of studies have explicitly focused on the ecology of rare or declining fern species (Futyma, 1980; Kuehn and Leopold, 1993; Kelly, 1994; Gundale, 2002; Sessions and Kelly, 2002; Bucharová et al., 2010), the gametophyte generation has been completely ignored on account of the perceived difficulty of working with this life stage. This paradigm must be changed to properly understand the ecology of these plants and to develop effective plans for their conservation. Our study is the first to thoroughly examine the role of the gametophyte generation in the ecology of rare ferns and presents new insight into the importance of gametophyte-based ecological inquiry to the conservation of these taxa.

Rare species often exhibit lower germination rates than common ones (Grillas et al., 1991), and low germination has been identified as an important factor contributing to rarity and decline in many species of angiosperms (Menges, 1995; Quintana-Ascencio and Menges, 2000). *Asplenium scolopendrium* var. *americanum* exhibited the lowest germination rate at all intervals beyond 36 d, whereas the species with broader distributions (*A. trichomanes*, *C. bulbifera*, and *A. scolopendrium* var. *scolopendrium*) had the highest maximal germination percentages (Fig. 2). Though a number of variables are known to impact spore germination in ferns, including temperature, light, age, and hormonal cues (Lloyd and Klekowski, 1970), it is unclear why widespread fern species exhibit more plastic responses to germination cues. In this situation, the AHTF would be at a disadvantage at the most basic level of establishment. This response may begin to explain the general rarity; however, we did observe a small number of gametophytes and young sporophytes in the field (Fig. 1E, F).

The positive effect of prolonged freezing on spore germination in AHTF (an increase of ~15% relative to no effect on the other taxa) suggests that extensive freezing of the soil may be important to its developmental success. This effect likely explains, in part, the positive relationship previously demonstrated between population growth and the number of days below freezing annually (Kelsall et al., 2004). Similar vernalization requirements have been reported for some temperate plant species, particularly among angiosperms from northern latitudes (Lunnan, 1989; Thompson and Leege, 2011). All species included in this study release their spores in mid to late summer, and it is thought that these spores overwinter, forming spore banks that later germinate under favorable environmental conditions. All

species other than AHTF showed no significant response to the freezing treatment, which may help explain the success of these species in warmer habitats where AHTF is not found. It also demonstrates a decided lack of plasticity in this species, which may have a significant impact on its long-term survival. For example, ongoing and future warming are predicted to reduce the number of freezing days experienced annually in the geographic range of AHTF (IPCC, 2007), potentially resulting in further disparity between the germination rates of AHTF and other fern species present in its habitats.

In addition to temperature effects on spore germination, the gametophytes of AHTF were less tolerant of increased temperatures in both growth and sporophyte formation (Fig. 5). The extreme temperature sensitivity in this species suggests that this taxon has reached its temperature maximum, a concern that is forecasted for many other taxa across a diverse array of habitats (Colwell et al., 2008; Laurance et al., 2011). Clearly, conservation efforts of this species must carefully monitor and control temperature extremes in its native habitat. Especially important in this work will be future studies that include individuals from the southern range of this fern. If individuals from these populations are more tolerant of warm temperatures, ex situ conservation of their germplasm may be needed to extend the taxon's survival.

At both 20°C and 25°C, AHTF exhibited far lower levels of sporophyte formation than the other study species. While it is difficult to determine gametophyte breeding systems in multispore cultures and such was not the intent of this work, these results strongly suggest that reproductive strategies are not constant across the species studied. Although most fern species are capable of producing bisexual gametophytes, such gametophytes are rarely functionally bisexual, and the majority of species are outcrossing (Soltis and Soltis, 1990). One reasonable explanation for the low sporophyte formation in AHTF is that this taxon maintains significant genetic load that reduces selfing capacity. Recent work by the authors has demonstrated that AHTF gametophytes are incapable of producing sporophytes in isolated culture (Testo and Watkins, 2011) supporting this hypothesis. In contrast, the more widespread *A. scolopendrium* var. *scolopendrium* is capable of both outcrossing and selfing (Wubs et al., 2010); this mixed mating system is almost certainly linked to the differential sporophyte formation observed between these conspecific taxa. *Asplenium rhizophyllum, Dryopteris marginalis*, and *Cystopteris bulbifera* have been shown to maintain high levels of heterozygosity, which suggests that all three are outcrossing (Werth et al., 1985; Haufler, 1987). Whereas *D. marginalis* had the second lowest sporophyte formation, *C. bulbifera* and *A. rhizophyllum* had some of the highest. Clearly, a great deal more needs to be known about breeding systems in ferns to adequately understand how they relate to population dynamics and species rarity. Regardless, for an apparent obligate outcrosser that may have reached its temperature maximum, the future appears bleak for AHTF under current and forecasted ecosystem dynamics.

In culture, *Asplenium scolopendrium* var. *americanum* exhibited reduced germination when grown in the presence of other species that frequently occur with it in the wild. Our findings indicate both that interspecific competitive interactions occur and that these effects range broadly across species, from facilitation to germination repression. Germination of AHTF was repressed by the presence of all other taxa included in the study, though the effect exerted by other species on AHTF was either minimal (e.g., *A. scolopendrium* var. *scolopendrium*) or

present only in the initial portion of the germination period (*A. rhizophyllum* and *D. marginalis*). In contrast, the competitive effects exerted on AHTF by other species were strong and long lasting. Germination of AHTF was strongly repressed by the presence of both *C. bulbifera* and *A. trichomanes*, with both species reducing spore germination of AHTF by greater than 10% at all time intervals past 12 d. Haufler and Ranker (1985) reported that *C. bulbifera* lacks a response to antheridiogens, which are hormones known to induce spore germination as well as influence the sexuality and size of some fern species' gametophytes. They postulated that this lack of a response played an important role in the interactions between the gametophytes of *C. bulbifera* and other species of *Cystopteris*. It is unknown if *C. bulbifera* produces antheridiogens; if so, the combination of antheridiogen production and a lack of a response to their presence by other species may be driving the competitive interactions described here. A similar system is known to exist in the widely distributed bracken fern, *Pteridium aquilinum* (L.) Kuhn, in which antheridiogen effects are thought to facilitate that species' worldwide success (Wolf et al., 1988). Our study presents the first experimental evidence of interspecific competition in fern gametophytes and suggests that such interactions play an important role in determining the relative success of fern species present in these habitats.

It can be difficult to assess the impact of competition on species interactions and coexistence. In this case, some insight may be gained by considering our data in light of existing information on the structure of fern communities associated with AHTF. In the best-studied New York populations, Kuehn and Leopold (1993) reported a negative correlation between percentage cover of AHTF and both *C. bulbifera* and *D. marginalis*. These taxa experienced the strongest facilitation and repression effects, respectively, when grown with AHTF in our study. In these populations, *C. bulbifera* is frequently the dominant fern species present, often forming extensive colonies and attaining a mean density of >4 plants/m^2. Conversely, *D. marginalis*, though present in all sites where AHTF is found, is a relatively minor representative of these fern communities, with a mean density of only 1.1 plants/m^2 (Kuehn and Leopold, 1993). This pattern of community composition may be impacted by the gametophytic interactions we describe. Additionally, *C. bulbifera* produces asexual sporophytic bulbils that, in combination with the facilitative influence of AHTF gametophytes, set up a highly competitive matrix for this rare species. Future studies should include detailed field surveys of the density and richness of gametophyte communities and examine the relative success of sexual and asexual reproduction in *C. bulbifera*.

In addition to disadvantages at the level of spore germination and competitive interactions, the gametophytes of AHTF were significantly less tolerant to drought and desiccation relative to the other taxa in the study. Unlike previous studies on gametophyte water stress that demonstrate a remarkable degree of desiccation tolerance in the gametophyte generation (Watkins et al., 2007a), the gametophytes of this taxon are considerably sensitive to even moderate drying (Fig. 4). All other species exhibited near complete recovery from considerably drier conditions, and both *Asplenium trichomanes* and *A. rhizophyllum* tolerated complete desiccation, recovering from exposure to water potentials of greater than −55 mPa. These results may help explain why AHTF is limited to protected environments throughout its range and demonstrates the critical need for long-term microclimatic monitoring of populations.

Global temperatures are expected to rise over the course of the upcoming century, and the resulting climatic changes have, and will, continue to impact the distribution and survival of many of the world's species (Lenoir et al., 2008; Miller-Rushing and Primack, 2008). Species that exhibit little physiological tolerance to changing temperatures, drought, or those already at their thermal maxima are anticipated to be the most strongly affected (Pörtner and Knust, 2007; Møller et al., 2008). *Asplenium scolopendrium* var. *americanum* is among this imperiled group. The physiological limitations of the gametophytes of this species are likely linked to the recent and rapid decline of most populations and suggest that current demographic trends will increase in severity the future. With reduced sporophyte recruitment resulting from higher rates of gametophyte mortality, young plants will likely be increasingly underrepresented in AHTF populations in the future. Demographic data taken from populations in New York demonstrate this pattern—as of 2001, the four largest populations of AHTF in the state were composed of 62–70% mature ferns (Kelsall et al., 2004). Without sufficient recruitment of young sporophytes, these populations are likely to experience further declines.

The findings presented here have implications that extend well beyond the conservation and ecology of the hart's tongue fern. Numerous studies have identified ferns as crucial components of both temperate and tropical forest ecosystems. For example, George and Bazzaz (1999a, b) have shown that ferns play a major role in temperate forest regeneration and growth, acting as a powerful filter which controls seed germination and seedling establishment. Similarly, ferns have been shown to regulate nutrient cycling (Cardelús et al., 2009; Cardelús and Mack, 2010; Chau et al., 2012) and maintain high levels of biodiversity (Ellwood and Foster, 2004) in tropical rain forests. As our world's climate changes, ferns are bound to be impacted, and the expansion or contraction of fern populations is certain to have downstream impacts on forest ecology. Understanding such impacts where ferns are involved is complex, as they possess independent sporophyte and gametophyte generations with fundamentally different ecologies. The extreme functional dissimilarity between these life stages results in each experiencing different selective pressures, and this reality must be considered in future studies of fern ecology. Fern conservation biologists and ecologists alike must carefully consider ferns as a unique system in which studies of organismal ecology as presented in this paper are the new norm; in short, the biology of the fern gametophyte cannot be ignored. Such examinations are important in our quest to accurately understand a myriad of ecological interactions, from rare species biology to ecosystem ecology.

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