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Conserved thermal performance curves across the geographic range of a gametophytic fern

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Received: 25 August 2017 Editorial decision: 6 July 2018 Accepted: 10 September 2018 Published: 12 September 2018.

Associate Editor: Heidrun Huber
Citation: Chambers SM, Emery NC. 2018. Conserved thermal performance curves across the geographic range of a gametophytic fern. AoB PLANTS 10: ply050; doi: 10.1093/aobpla/ply050

Abstract. Species-level responses to environmental change depend on the collective responses of their constituent populations and the degree to which populations are specialized to local conditions. Manipulative experiments in common-garden settings make it possible to test for population variation in species’ responses to specific climate variables, including those projected to shift as the climate changes in the future. While this approach is being applied to a variety of plant taxa to evaluate their responses to climate change, these studies are heavily biased towards seed-bearing plant species. Given several unique morphological and physiological traits, fern species may exhibit very different responses from angiosperms and gymnosperms. Here, we tested the hypothesis that previously detected population differentiation in a fern species is due to differentiation in thermal performance curves among populations. We collected explants from six populations spanning the species’ geographic range and exposed them to 10 temperature treatments. Explant survival, lifespan and the change in photosynthetic area were analysed as a function of temperature, source population and their interaction. Overall results indicated that explants performed better at the lowest temperature examined, and the threshold for explant performance reflects maximum temperatures likely to be experienced in the field. Surprisingly, explant fitness did not differ among source populations, suggesting that temperature is not the driver behind previously detected patterns of population differentiation. These results highlight the importance of other environmental axes in driving population differentiation across a species range, and suggest that the perennial life history strategy, asexual mating system and limited dispersal potential of Vittaria appalachiana may restrict the rise and differentiation of adaptive genetic variation in thermal performance traits among populations.

Keywords: Climate change; ferns; gametophyte; geographic range; manipulative experiment; population differentiation; temperature; thermal performance curve; Vittaria appalachiana.

Introduction
Species’ responses to the environmental variation throughout their geographic ranges depend on the collective tolerances of the constituent populations. The extent to which populations evolve different tolerances is expected to depend on the spatial scale of gene flow relative to the grain of environmental heterogeneity (Van Tienderen 1991; Sultan and Spencer 2002). High rates of gene flow among populations experiencing different selective pressures should favour the
evolution of phenotypic plasticity, while asexual reproduction and restricted dispersal will favour specialization to local conditions (Bradshaw 1965; Kawecki and Ebert 2004; Sherman and Ayre 2008; but see Gray and Goddard 2012; Sexton et al. 2014). Gene flow among populations can be heavily influenced by the spatial distribution of habitat across a species’ range, as populations that are restricted to patchy or fragmented habitats will experience less interpopulation gene flow (Primack and Miao 1992; Thomas 2000). Furthermore, over evolutionary time, patchy habitat structure itself may generate selection for localized dispersal strategies due to the fitness consequences of dispersing propagules that land in unsuitable habitat between patches, generating a feedback between the evolution of environmental specialization and localized dispersal (Cody and Overton 1996; Cheptou et al. 2008; Schenk 2013; Van Den Elzen et al. 2016). In species where populations are locally specialized, the species’ geographic range as a whole may reflect a broader range of environmental conditions than each individual population can tolerate.

Population-specific responses to environmental gradients can be examined using a variety of lab and field experiments (Lawlor and Mitchell 1991; Norby et al. 1999; Charles and Dukes 2009; Marsico and Hellmann 2009; Samis and Eckert 2009; Agren and Schemske 2012; Chambers and Emery 2016). Common garden experiments that include experimental manipulations have proven to be a powerful tool for isolating the effects of specific environmental variables (such as temperature) that are hypothesized to drive population-level differences in performance (Marion et al. 1997; McLeod and Long 1999; Grime et al. 2000) and the potential for populations to tolerate conditions that do not presently occur in their local environments. Reaction norms are a particularly useful way to quantify the effects of an environmental factor (e.g. temperature) on the phenotype (e.g. plant size) of genotypes from different populations. Reaction norms that represent fitness across temperature gradients, often called thermal performance curves (Kingsolver et al. 2004; Gilchrist 2015), can be compared among genotypes using a variety of techniques, ranging from relatively straightforward comparisons of the slopes and intercepts of the lines that represent phenotypic responses across two different environments (Bradshaw 1965, 1972; Schmitt 1993; Dorn et al. 2000), to more complex approaches that consider the shape of reaction norm curves across three or more environmental levels (Izem and Kingsolver 2005; Stinchcombe et al. 2012; Murren et al. 2014). Quantifying fitness and phenotypic responses of multiple populations across multiple levels of an environmental axis makes it possible to test for microevolutionary divergence among populations (Murren et al. 2014).

To date, the majority of experimental research that has evaluated plant responses to climate change has focused on seed-bearing plants, with comparatively less attention directed towards other major land plant lineages (Gignac 2001; Page 2002). Ferns are the second most diverse group of vascular plants on the planet, yet their ecology is severely understudied in comparison to seed-bearing plants. Ferns play significant ecological roles in their communities (George and Bazzaz 1999; Amatangelo and Vitousek 2008) and are often considered to be indicators of habitat quality and environmental change (Page 2002; Bassler et al. 2010; Bergeron and Pellerin 2014). Furthermore, a number of unique life history and physiological traits may cause ferns to exhibit different responses to climate change compared to angiosperms and gymnosperms (see Banks 1999 for a thorough review). One important difference between ferns and angiosperms is that both the gametophyte and the sporophyte are free-living and independent in ferns, while the gametophyte is highly reduced and dependent on the sporophyte in angiosperms and gymnosperms. Most fern gametophytes are one cell-layer thick, photosynthetic, lack cuticle or stomata and produce antheridia, archegonia and rhizoids, and thus are physiologically quite different from their sporophyte counterparts in ways that may have significant consequences for their responses to temperature variation. Despite their relatively small size and often delicate appearance, fern gametophytes are often more robust to environmental extremes than their respective sporophytes (Farrar 1978; Sato and Sakai 1981; Watkins et al. 2007; Pinson et al. 2017). Given the fact that fertilization, and even asexual reproduction in some species, occurs during the gametophyte portion of the life cycle, understanding how fern gametophytes respond to different temperatures is important for predicting the overall effects of climate change on fern lineages.

Throughout the Appalachian Mountains and Appalachian Plateau of eastern North America are a number of recesses in rock outcroppings called ‘rockhouses’ or ‘rockshelters’. These formations developed from the weathering of soft bedrock located below layers of sandstone, generating large sandstone overhangs. The recesses below these overhangs support a diverse flora (Walck et al. 1996, 1997; Oberle and Schaal 2011), including a variety of fern species, some of which are endemic to these unique habitats (Farrar 1967, 1998; Watkins and Farrar 2002, 2005; Testo and Watkins 2011). A handful of these endemics are temperate fern species that are phylogenetically nested within tropical clades that may have retreated to the buffered thermal
environments inside rockshelters during past glaciation events (Farrar 1990; Walck et al. 1996; Chambers and Emery 2016; Pinson and Schuettpelz 2016). \textit{Vittaria appalachiana} is one of the few species endemic to these rockshelters that never produces a viable sporophyte, but rather reproduces only asexually via gemmae and vegetative spread (Farrar 1967, 1978, 2016). Populations of \textit{V. appalachiana} occupy rockshelters from northern Alabama to south-western New York, spanning a total of 9° in latitude (Farrar and Mickel 1991). While rockshelters can buffer these populations from fluctuations in temperature (Farrar 1998; Chambers and Emery 2016), the latitudinal distribution exposes populations to different average thermal conditions (Table 1).

Given the temperature variation encompassed within the species’ range, and results of previous studies documenting dispersal limitation (Stevens and Emery 2015), and population differentiation (Chambers and Emery 2016; Chambers et al. 2017) among \textit{V. appalachiana} populations, we predicted that \textit{V. appalachiana} would exhibit population differentiation in their responses to a thermal gradient (i.e. their thermal tolerance curves). We also predicted that all \textit{V. appalachiana} populations would have a relatively narrow range of temperature tolerances along a temperature gradient, as \textit{V. appalachiana} is restricted to relatively climatically buffered microhabitats and therefore would not have experienced selection to tolerate a broad range of temperature conditions.

**Methods**

**Sample collection**

We identified six populations from different locations across the geographic range of \textit{V. appalachiana} using occurrence information obtained from non-profit organizations (New York Natural Heritage Program and North Carolina Natural Heritage Program), state botanists and previously published locality data (Farrar and Mickel 1991; Table 1). Populations included in this study were selected based on site accessibility and our ability to secure permission to collect samples from the resident populations. In October of 2012, 30 gametophyte explants were collected from each of the six source populations over an 8-day period (N = 180). Based on the results of genetic studies that evaluated the distribution of genetic variation within and among populations of \textit{V. appalachiana} (Farrar 1990), it is most likely that explants from a single site represented replicates of the same vegetative clone, but that clones differed between sites.

Within each rockshelter, samples were collected from random positions along a horizontal transect that spanned the length of each population. A blade was used to carefully detach gametophytes that were directly attached to rock surfaces or sandy substrates within rockshelters. After removal, each sample was trimmed to a standardized circle with a 4.5-mm diameter (which contained roughly 10–20 individual thalli), and placed on an agar medium containing half-strength Murashige and Skoog Basal Salt Mixture (Sigma, St. Louis, MO, USA) supplemented with 0.5 mL/L plant vitamins, 1.0 mL/L Benomyl, titrated to a pH of 6.5 using potassium hydroxide, and solidified with 0.65 % agar (Sigma, St. Louis, MO, USA).

After samples were collected from all populations, each explant was transferred to a fresh agar plate to minimize growth of contaminants. The samples were then placed in a Revco RI-50-555-A growth chamber at 20 °C, under 0.8 μmol light levels following an 8-h light:16-h dark cycle, for seven months to establish on the agar medium and recover from any stressors associated with

<table>
<thead>
<tr>
<th>Population location</th>
<th>Range location</th>
<th>Coordinates</th>
<th>Daily average (°C)</th>
<th>Daily minimum (°C)</th>
<th>Daily maximum (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cane Creek Nature Preserve (Colbert Co., AL)</td>
<td>Southern</td>
<td>34 37.27N 87 47.88W</td>
<td>15.38</td>
<td>14.06</td>
<td>16.77</td>
</tr>
<tr>
<td>Jones Property (Transylvania Co., NC)</td>
<td>Eastern</td>
<td>35 11.44N 82 42.88W</td>
<td>12.40</td>
<td>11.08</td>
<td>14.17</td>
</tr>
<tr>
<td>Pennyrile State Park (Christian Co., KY)</td>
<td>Western</td>
<td>37 04.54N 87 39.95W</td>
<td>14.77</td>
<td>13.61</td>
<td>15.99</td>
</tr>
<tr>
<td>Hemlock Cliffs (Crawford Co., IN)</td>
<td>Central</td>
<td>38 16.38N 86 32.20W</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Deep Woods (Hocking Co., OH)</td>
<td>Central</td>
<td>39 24.49N 82 34.60W</td>
<td>11.51</td>
<td>10.20</td>
<td>12.78</td>
</tr>
<tr>
<td>Rock City Park (Cattaraugus Co., NY)</td>
<td>Northern</td>
<td>42 04.79N 78 28.62W</td>
<td>7.74</td>
<td>6.92</td>
<td>8.70</td>
</tr>
<tr>
<td>Temperature grand average</td>
<td></td>
<td></td>
<td>11.97</td>
<td>10.81</td>
<td>13.25</td>
</tr>
</tbody>
</table>
collection. Field measurements indicated that populations experience light levels between 0 and 5.99 μmol m$^{-2}$ s$^{-1}$, averaging 0.5 μmol m$^{-2}$ s$^{-1}$, and temperatures between −3.70 and 27.60 °C (S. M. Chambers, unpubl. data); thus, the growth chamber conditions in which explants were maintained fell within the conditions they experience in their natural habitat.

**Temperature treatments**

After transplants had established, we exposed each explant to 1 of 10 different temperature treatments and measured its response over 22 weeks. Temperatures were selected to span the average and maximum temperatures that we had previously measured in each of the sampled populations over a 3-year period prior to this experiment (6–18 °C, measured between 2010 and 2013), as well as the elevated temperature levels projected to occur by the end of the 21st century due to global climate change (21–30 °C; IPCC 2013; Table 1). Specific temperature level treatments were 6, 9, 12, 15, 18, 21, 24, 26, 27 and 30 °C. The entire temperature gradient was replicated in three different growth chambers. Temperature was manipulated within each chamber placing explants from each population inside heated and insulated containers that increased temperature above the base growth chamber temperature of 6 °C using seedling heating mats (Fig. 1). One explant from every population was exposed to each temperature treatment in each growth chamber, resulting in three replicates of the entire temperature gradient per population.

In May of 2013, each explant was transferred from the agar medium to a 2.5 × 1.25 × 0.5 cm section of rockwool, a suitable substrate for propagating *V. appalachiana* because it resembles the porous sandstone that is the natural substrate for most *V. appalachiana* populations. Prior to transfer, the rockwool was moistened with a liquid nutrient medium (created as above without agar; see ‘Sample collection’) to facilitate establishment. A pilot experiment indicated that explant performance was greatest when we minimized the fluctuations in relative humidity experienced each time a container was opened to collect data. Consequently, we standardized the relative humidity...
for all explants to 75 %, which was the level maintained in the laboratory during the course of the experiment, by placing explants on wire trays over a sodium chloride (NaCl) salt solution inside their container (Fig. 1). The 75 % humidity level is slightly lower than levels we have measured in the field during daylight hours in the summer months, which ranged between 85 and 95 %, but are likely within levels experienced over the course of daily and annual temperature cycles (S. M. Chambers, unpubl. data).

One explant from each population was placed in each polystyrene container that was wrapped with parafilm and placed on a seedling heating mat inside a seedling tray. Each seedling tray was placed inside a large insulated polystyrene box and covered with a clear humidity dome to help maintain temperature and humidity levels within each treatment (Fig. 1). Sodium chloride salt solutions were replaced every 2 weeks to ensure relative humidity was kept at a constant level. To prevent explant dehydration, 500 μL of deionized water was added directly to the rockwool substrate every 2 weeks for the duration of the experiment.

We measured explant fitness as (i) explant survival (binary yes/no), (ii) lifespan (days) and (iii) changes in the area of visible photosynthetically active tissue. Given that V. appalachiana only reproduces asexually via the production of gemmae along the margins of the gametophyte thalli, the length of time an explant survives and thalli surface area together provide an estimate of the number of gemmae it produces and therefore serve as proxies for fitness. Explant survival and lifespan were monitored every 2–3 days for the first 6 weeks of the experiment, and once per week for the final 16 weeks. During each census date (i.e. the dates when data were collected), an explant was recorded as ‘alive’ if any photosynthetically active (green) tissue was visible to the naked eye. The amount of surface area occupied by photosynthetically active tissue was determined from digital photographs that were taken of each explant at the midpoint (week 9) and end (week 22) of the experiment, thus capturing the change in photosynthetic area over two consecutive time periods. Photographs were taken with a Cannon PowerShot® SX130IS placed on a ProMaster® 7050 tripod to ensure a consistent pixel ratio among images. Using these photographs, we calculated PA by outlining photosynthetic tissue using GIMP 2.0 (Peck 2008; Solomon 2009), and calculating the area using ImageJ (Rasband 1997; Schneider et al. 2012). Changes in PA during these two time periods were calculated for each surviving explant by dividing the difference in PA (current PA – initial PA) by the initial PA, where the initial PA was the PA measured at the beginning of the experiment. Changes in PA were calculated for each explant at the midpoint and end of the experiment.

Statistical analyses
Survival. We tested if variation in survival of V. appalachiana experimental explants was explained by population identity, temperature treatment and the interaction between population and temperature using a generalized linear mixed model (PROC GLIMMIX; SAS v. 9.4) with a logit link function to account for the binary response variable (Liang and Zeger 1986; Ziegler et al. 1998). Temperature was treated as a continuous predictor variable, and source population was treated as a fixed categorical predictor variable. The temperature × treatment interaction was also included in the model. We applied a spatial power covariance structure (sppow), specifying ‘census date’ as the spatial factor and the interaction between source population, temperature and growth chamber was identified as the ‘subject’ statement in the model. A separate random statement was also included to specify that growth chamber was a random factor. Census date was included in the ‘random’ statement to account for repeated measurements.

Lifespan. The length of time that explants survived under the different temperature conditions was evaluated in a mixed-model ANCOVA (PROC MIXED; SAS v. 9.4). Lifespan (i.e. the number of days that an explant survived under experimental conditions) was evaluated as a function of population identity (considered a categorical variable) and temperature (considered a continuous variable). The population × temperature interaction was included in the analysis, and growth chamber was included as a random effect.

Changes in PA. The change in PA ((current PA – initial PA)/initial PA) was analysed using a mixed-model repeated-measures ANOVA (PROC MIXED; SAS v. 9.4). We specified an autoregressive covariance structure as a function of population identity, temperature treatment and the interaction between population and temperature using a generalized linear mixed model (PROC GLIMMIX; SAS v. 9.4) with a logit link function to account for the binary response variable (Liang and Zeger 1986; Ziegler et al. 1998). Temperature was treated as a continuous predictor variable. The temperature × treatment interaction was also included in the model. We applied a spatial power covariance structure (sppow), specifying ‘census date’ as the spatial factor and the interaction between source population, temperature and growth chamber was identified as the ‘subject’ statement in the model. A separate random statement was also included to specify that growth chamber was a random factor. Census date was included in the ‘random’ statement to account for repeated measurements.

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conduct pairwise comparisons of statistically significant main effects.

**Pairwise comparisons of thermal performance curves.**

We evaluated population differences in thermal response curves by calculating the offset, slope, curvature, wiggle and total parameters for continuous reaction norms as defined by Murren et al. (2014). Four of these metrics (offset, slope, curvature and wiggle) partition the total variation that exists between a pair of thermal performance curves (TPCs) and the total metric represents the sum of those differences. For all metrics, smaller values indicate greater similarity. The metrics offset and slope are the most similar to traditional linear models and comparisons of performance curves, while curvature and wiggle represent higher-order statistical comparisons between performance curves. Therefore, estimates of slope and wiggle for population pairs may reveal microevolutionary variation in reaction norms that are otherwise obscured using traditional linear analyses (Murren et al. 2014). Each metric was calculated separately for each measurement of explant performance (survival, lifespan, changes in PA), as follows:

The offset represents the average difference in performance between a pair of populations in any treatment, and is calculated as:

$$Offset = \frac{\sum_{i=1}^{n} D_i}{n}$$

where $D$ represents the difference in mean performance in treatment $i$, and $n$ is the total number of treatments (here, $n = 10$).

The slope parameter calculates the average change in $D$ from treatment $i$ to $i + 1$ (e.g. between 6 and 9 °C):

$$Slope = \frac{\sum_{i=1}^{n-1} S_i}{n-1}$$

where $S_i = D_{i+1} - D_i$.

Curvature is the average change in slope across treatments:

$$Curvature = C = \frac{\sum_{i=1}^{n-2} C_i}{n-2}$$

where $C_i = S_{i+1} - S_i$.

Wiggle captures any remaining variation in the comparison of two performance curves, and is calculated as the sum of the absolute value of the change in slope after removing the estimate of curvature:

$$Wiggle = \frac{\sum_{i=1}^{n-2} |C_i|}{n-2} - C$$

A final metric, Total, summarizes the cumulative differences between two tolerance curves, as estimated by the other four metrics:

$$Total = O + S + C + W$$

To facilitate comparisons among population pairs that varied in overall performance, each metric was standardized by dividing the estimated value for offset, slope, curvature, wiggle and total by the grand mean performance measure of both populations in each pairwise comparison. Calculations for these equations were conducted using the base package in R (R Core Team 2014).

**Results**

**Survival**

Survival rates differed significantly across temperature treatments (Temperature, Table 2; Fig. 2A), among populations (Population, Table 2; Fig. 2B), and with respect to populations within each temperature treatment (Temperature × Population, Table 2). Overall survivorship was highest at temperatures below 15 °C and lowest at 15 and 24 °C (Fig. 2A). Explants from Kentucky (KY) had significantly higher survivorship than all other populations followed by New York (NY), which had significantly higher survivorship than Alabama (AL) and North Carolina (NC) (Fig. 2B).

**Lifespan**

The overall mean lifespan of explants during the experiment was ~86 days. Explants exposed to temperatures of 15 °C or higher survived roughly a month less than explants grown at cooler temperatures (Fig. 3A), leading to an overall significant effect of temperature on lifespan (Temperature, Table 2). The length of time that explants remained alive did not vary significantly among populations overall (Population, Table 2; Fig. 2B), and the effects of the temperature treatments were relatively consistent among populations (see non-significant Temperature × Population interaction, Table 2).

**Changes in PA**

We observed a significant increase in the rate of decline in explant PA with increasing temperature (Temperature, Table 2; Fig. 4A), and the total reduction in PA varied significantly among source populations (Population, Table 2), with Kentucky (KY) retaining the greatest amount of PA and Alabama (AL) losing the most, on average, over all temperature treatments and the two time periods (Fig. 4B). The effect of temperature varied significantly between the first and second time periods (Temperature × Time period interaction, Table 2), and explants lost PA faster...
Table 2. Statistical results of all analyses evaluating explant performance from six different populations across 10 temperature treatments. The ‘test statistic value’ column reports F statistics for the main effects in the survival, lifespan and PA analyses. For the latter two analyses, test statistic values for growth chamber are reported as Z statistics because this factor was a random effect. Subscripts identify the numerator and denominator degrees of freedom where appropriate. P-values that were statistically significant are indicated with bold text.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Test statistic value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>49.10, 1,4488</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population</td>
<td>4.52, 1,4488</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature * Population</td>
<td>3.10, 1,4488</td>
<td>0.0086</td>
</tr>
<tr>
<td>Lifespan (days)</td>
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</tr>
<tr>
<td>Temperature</td>
<td>17.801, 1,166</td>
<td>&lt;0.0001</td>
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<tr>
<td>Population</td>
<td>0.77, 1,166</td>
<td>0.5749</td>
</tr>
<tr>
<td>Growth chamber</td>
<td>0.72, 4.2353</td>
<td>0.2353</td>
</tr>
<tr>
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<td>0.70, 1,166</td>
<td>0.6250</td>
</tr>
<tr>
<td>PA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>31.611, 5,330</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population</td>
<td>3.14, 5,330</td>
<td>0.0087</td>
</tr>
<tr>
<td>Time period</td>
<td>94.001, 5,330</td>
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<tr>
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<td>0.1178</td>
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</tr>
<tr>
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<td>0.88, 5,330</td>
<td>0.4978</td>
</tr>
</tbody>
</table>

in the first time period (Time period, Table 2). The change in PA for all remaining factors and interactions in the model were not statistically significant (Table 2).

Pairwise comparisons of thermal performance curves

Overall patterns in the cumulative differences between performance curves (total) indicated that Indiana (IN) and North Carolina (NC) had the most similar TPCs while New York (NY) and Indiana (IN) exhibited the greatest differences in all three measures of explant performance [see Supporting Information—Table S1a–c]. Additional differences among populations emerged when the four Murren metrics—offset, slope, curvature and wiggle—were individually examined. The mean difference in explant performance (offset) for all three explant performance metrics was greatest between explants from New York (NY) and Indiana (IN) [see Supporting Information—Table S1a–c]. On the other hand, the average change in mean performance between temperature steps (slope) was greatest between Ohio (OH) and Alabama (AL) for survival, Ohio (OH) and Indiana (IN) for lifespan, and New York (NY) and Indiana (IN) for change in PA [see Supporting Information—Table S1a–c]. We detected a relatively large degree of dissimilarity between Alabama (AL) and Indiana (IN) in curvature and wiggle when lifespan was used as the measure of performance [see Supporting Information—Table S1b], but no clear patterns emerged for these two metrics when examining survival and change in PA [see Supporting Information—Table S1a and c].

Discussion

Our results indicate that patterns of population differentiation in V. appalachiana that had been previously documented in the field and physiological studies (Chambers and Emery 2016; Chambers et al. 2017) are not driven by population variation in temperature tolerances. These previous studies detected a pattern of countergradient variation (Conover and Schultz 1995) in V. appalachiana in which explants from New York outperformed all other populations over much of the species’ geographic range. However, the results from this experiment suggest that in general populations have very similar temperature tolerance curves, with explants from Kentucky (KY) having higher survivorship and PA retention than several other populations (Figs 2B and 4B). The Murren metrics identified more subtle differences between specific population pairs that were not evident in the linear analyses, though the nature of these differences depended on the performance metrics that is analysed. For example, estimates of curvature and wiggle detected the greatest difference between explants from Alabama and Indiana with respect to lifespan, suggesting that there may be slight differences in the effects of temperature variation on explant longevity between these two populations [see Supporting Information—Table S1b]. Nonetheless, only faint patterns of population differentiation in thermal performance emerged in V. appalachiana using both traditional linear analyses and higher order comparisons, suggesting that TPCs are relatively conserved within this species even though its populations span a relatively broad latitudinal range in North America.

The significant differences we detected among populations in the TPCs for explant survival and change in PA appeared to be driven by two populations, Kentucky (KY) and New York (NY). All other populations exhibited similar TPCs across the temperature gradient tested here, suggesting that temperature variation has not driven the patterns of population differentiation previously detected.
in field transplant experiments (Stevens and Emery 2015; Chambers and Emery 2016). It is possible that the previously detected patterns of population differentiation have been driven by factors other than mean temperature that are known to vary across the species’ range, such as relative humidity levels (Chambers et al. 2017). Responses to dry conditions share a metabolic pathway with responses to cold temperatures, such that organisms that can tolerate colder temperatures can also tolerate lower levels of relative humidity (Knight and Knight 2001; Sinclair et al. 2013). Therefore, if we had examined population responses to low temperatures, perhaps we would have identified the same pattern of differentiation that had been documented in field studies. While the high overall levels of senescence that we observed could generate concern that the experimental environment was unusually stressful for the explants, these levels of senescence are actually relatively typical for this species. A previously conducted reciprocal transplant experiment indicated similar patterns of senescence among gametophytes transplanted back in to their home environment (Chambers and Emery 2016). Additional work conducted some 40 years ago also comments on the overall slow growth rate (Farrar 1978). Thus, the senescence rates observed here are not far from the norm with respect to experiments in this species.

It is quite possible that the biogeography and history of selection in V. appalachiana can largely explain...
the surprisingly similar thermal responses we observed among widely distributed populations. *Vittaria appalachiana* was likely once widespread in eastern North America, fully capable of producing a sporophyte when the climate resembled that of the contemporary neotropics prior to the Pleistocene glaciations (Farrar 1998). It is thought that *V. appalachiana* retreated into the rockshelters at that time to escape the cold climates of the Pleistocene glacial period. Selection during this time period may also have favoured the obligate gametophyte life history strategy because the sporophyte may have been less tolerant of cold temperatures (Farrar 1998). These pressures would have been imposed on all populations, and the loss of the sporophyte generation and highly fragmented distribution left these populations with no potential for sexual reproduction and little potential for gene flow. As a result, there is little opportunity for adaptive genetic variation to arise in these populations, highly restricting their potential for adaptive differentiation. Furthermore, the buffered climate within rockshelters may limit the extent to which populations experience temperature variation occurs across their geographic range. Under this biogeographic hypothesis, differences observed in the previous field studies are more likely due to genetic drift among populations that established in the Pleistocene rather than patterns of local adaptation to contemporary environmental conditions.

While our results did not find strong evidence for adaptive differentiation among populations in their TPCs, we certainly observed that the species as a whole is highly sensitive to the temperature gradient that we experimentally imposed. All populations exhibited a decline in performance in temperatures above 12 °C for all populations. **Figure 3.** (A) The lifespan, or number of days that explants remained alive in each temperature treatment, averaged across all populations (raw means ± 1 SE). (B) Mean lifespan for explants from each source population, averaged across all temperature treatments. Data shown are raw means ± 1 SE.
performance metrics (Figs 2A, 3A and 4A). Previous studies have found that 15 and 18 °C are the highest average and maximum temperatures known to occur in natural populations (Table 1; Chambers and Emery 2016); thus, 12–15 °C may represent a critical species-wide temperature threshold for *V. appalachiana*. The experimental temperatures that represented climate change scenarios (i.e. 21, 24, 26, 27 and 30 °C) resulted in rapid explant deterioration and senescence. The clear negative effects of temperatures above the 15 °C threshold for all populations indicate that many, if not all, populations of *V. appalachiana* will be pushed beyond their physiological tolerance limits as temperature rapidly increases due to global climate change (IPCC 2013). Transplant experiments beyond *V. appalachiana*’s northern range boundary have shown that suitable habitat exists at higher latitudes but has not been colonized due to the highly limited dispersal potential of the species (Stevens and Emery 2015). Just as the lack of sexual reproduction and inability to disperse may have restricted the potential for populations to adapt to their local temperature regimes, these characteristics will also likely restrict the potential for rapid adaptive evolution in response to changing thermal environments. Management practices that aim to conserve this species, and others that are dispersal limited, asexual and physiologically sensitive to climate, may be required to use assisted migration techniques, which would facilitate the colonization of suitable habitat that becomes available as climate change unfolds.

**Conclusions**

The results of this study indicate that the thermal performance curves of *V. appalachiana* populations are highly
conserved despite a relatively broad latitudinal range occupied by the species. This suggests that adaptive variation may not arise and spread in taxa with limited potential for gene flow within and among populations. Our results also highlight the importance of examining different aspects of reaction norm shapes to uncover differences not captured by traditional reaction norm comparisons.

Sources of Funding
This work was supported by a Purdue Andrews Environmental Travel Grant and the Botany and Plant Pathology Department at Purdue University.

Contributions by the Authors
S.M.C. and N.C.E. conceived and designed the experiment. S.M.C. conducted the experiment, and analysed the data. S.M.C. and N.C.E. wrote the manuscript.

Conflict of Interest
None declared.

Acknowledgements
The authors thank G. Tryon, S. DeSimini, A. Chen, C. Chandler, S. Marshall, D. Moore and C. Sieradzki for their assistance with this experiment. M. Zanis contributed useful discussions during the design and implementation of this experiment. J. Banks, J. Dukes and E. Watkins provided constructive feedback throughout this project and helpful comments on an earlier draft of this manuscript. We also thank numerous landowners for supporting this study by providing access to populations. Publication of this article was funded by the University of Colorado Boulder Libraries Open Access Fund.

Supporting Information
The following additional information is available in the online version of this article—
Table S1a–c. Pairwise values for all five Murren metrics, for all three measurements of explant performance.

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