

## Supporting Information: Figures and Tables

**Article title:** Intraspecific variation in responses to extreme and moderate temperature stress in the wild species, *Solanum carolinense* (Solanaceae)

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**Fig. S2** Cell membrane stability across temporally independent blocks

**Fig. S3** Daily max temperature for spring and summer of 2021

**Fig. S4** Genotype differences for temperature tolerance traits

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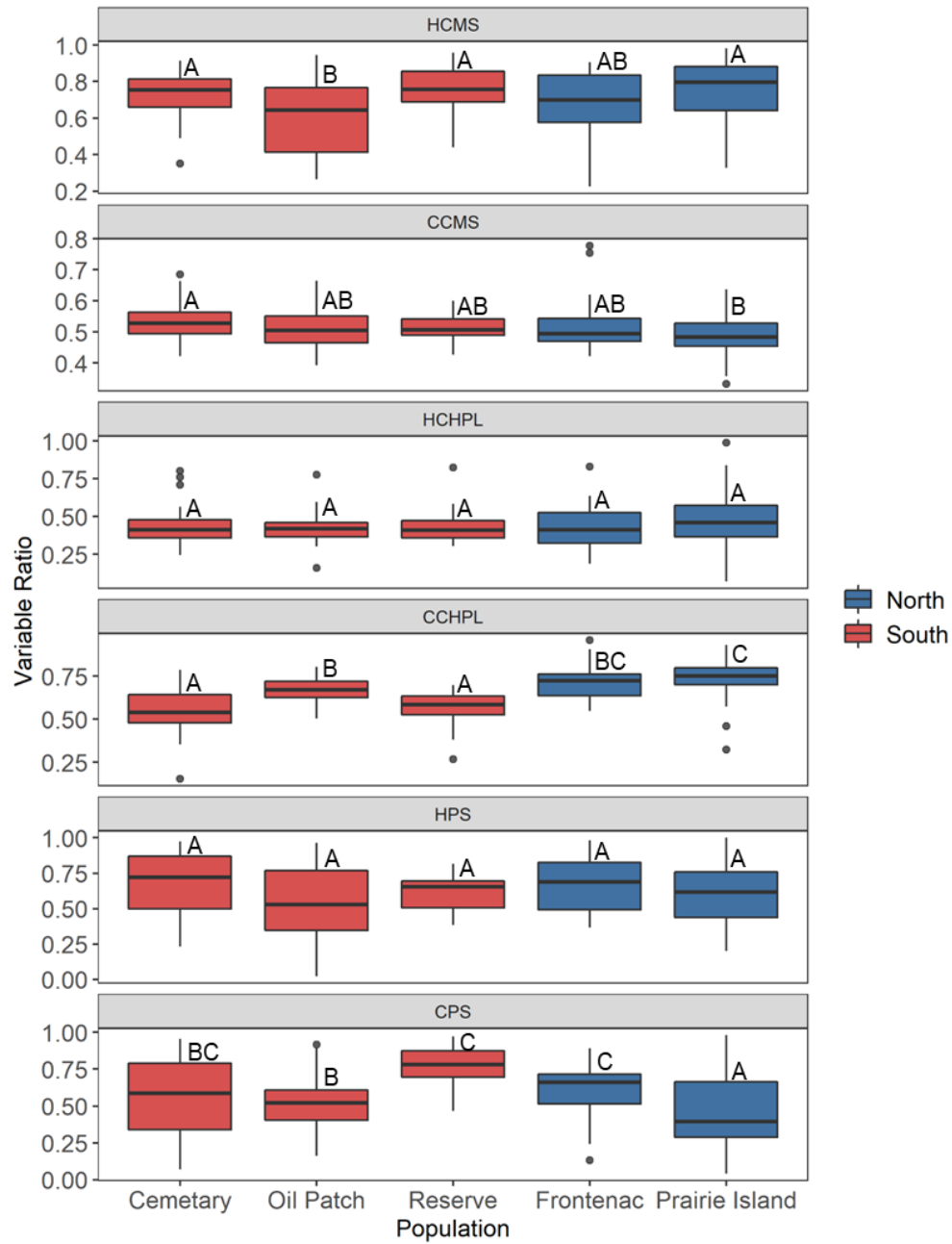
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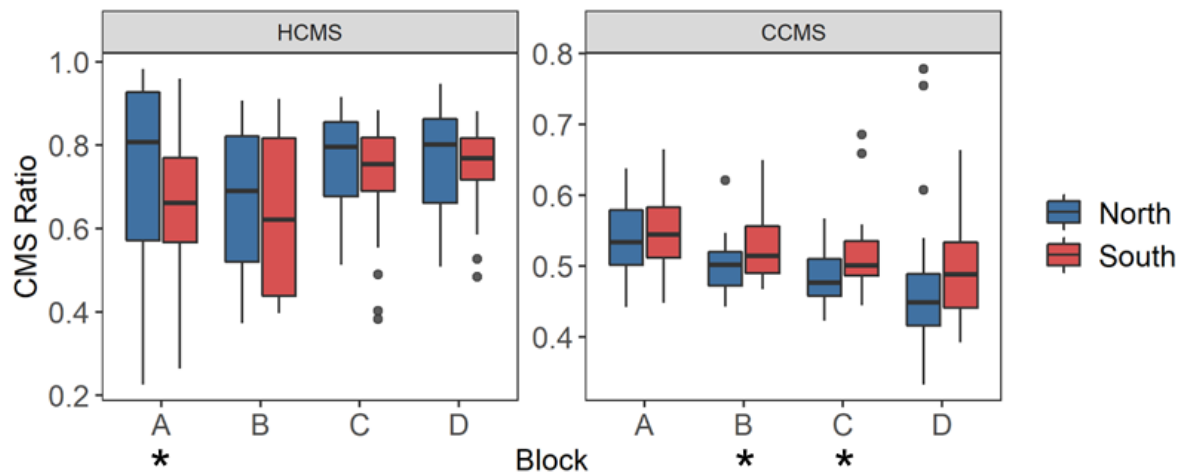
**Table S9** Results from principal component analysis with sporophytic variables

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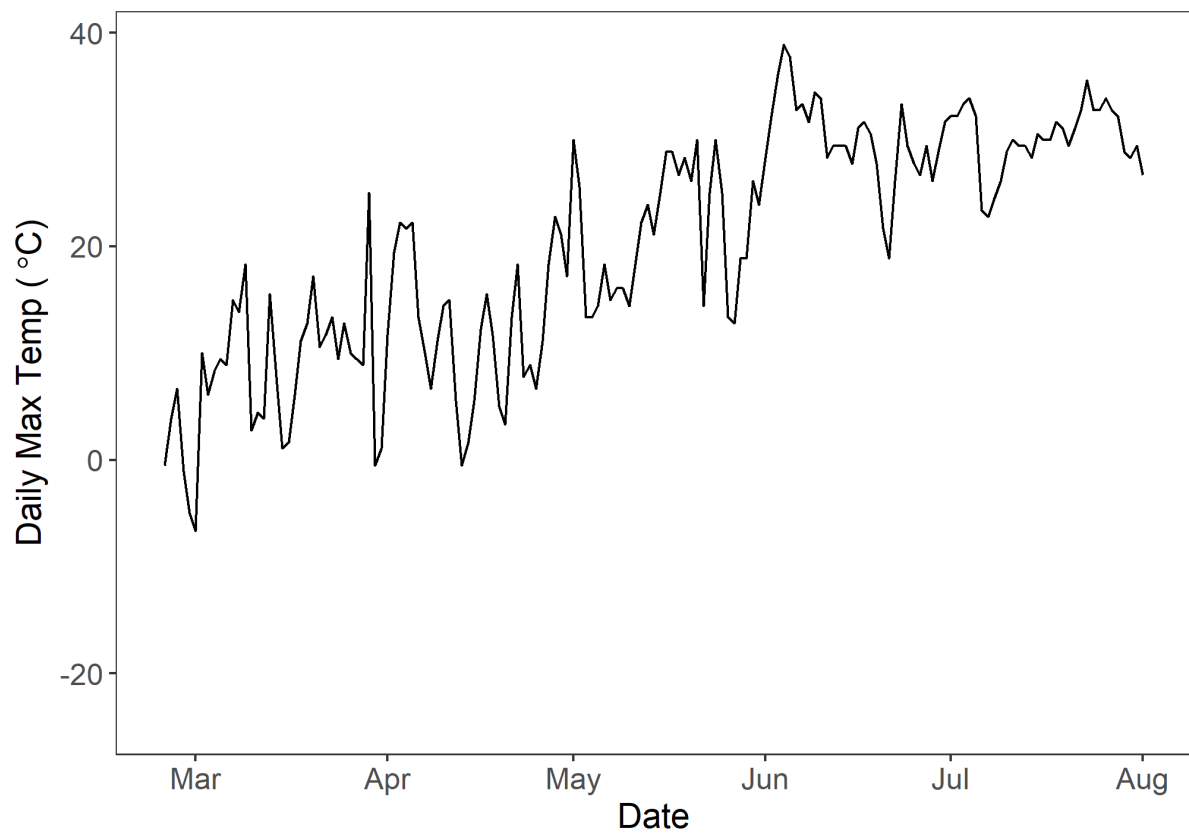
**Table S11** Results from principal component analysis with gametophytic variables



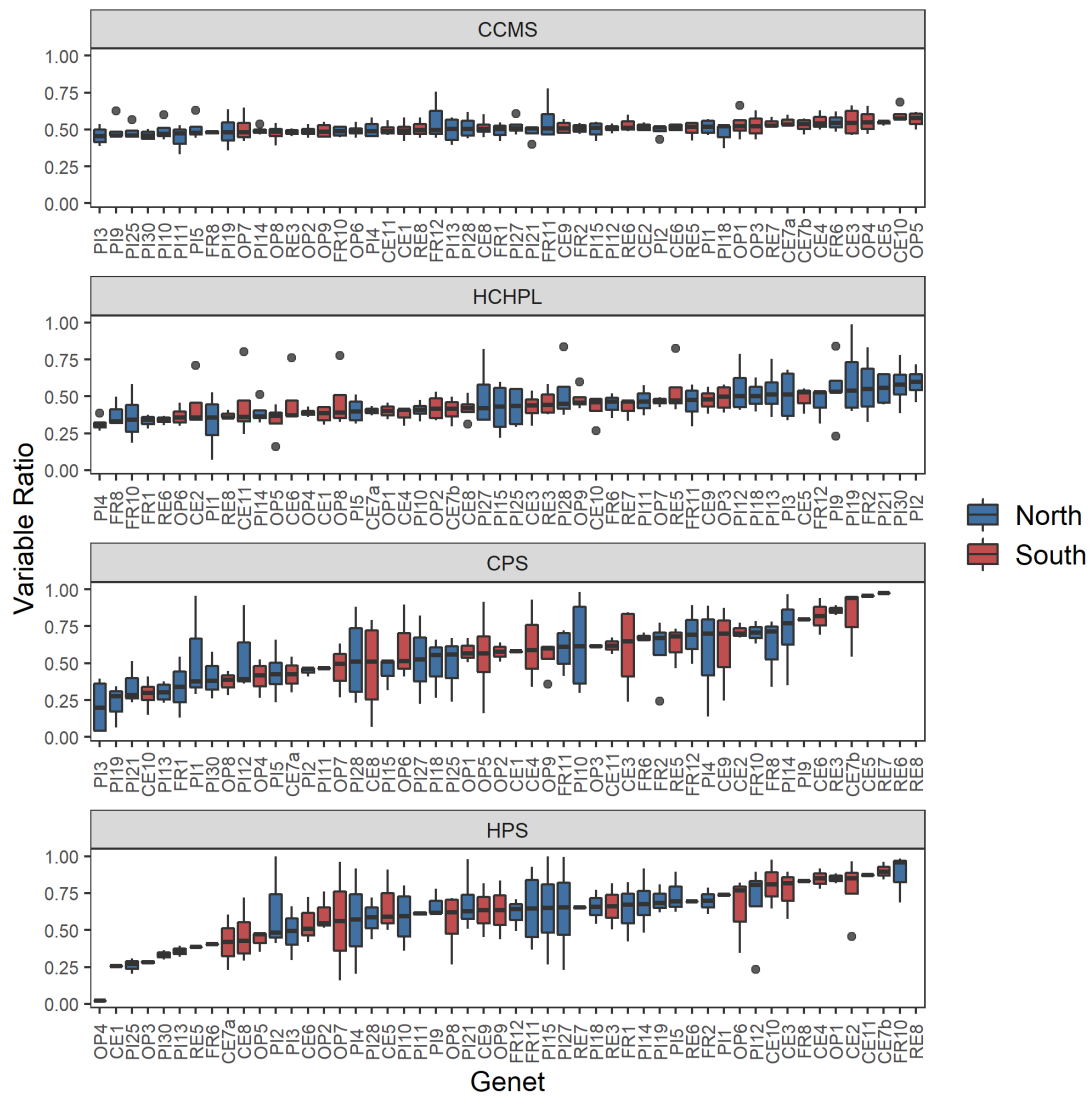
**Fig. S1** Differences between the populations for all sporophytic variables. Letters denote significant differences between populations from a linear mixed effects model with population as the fixed effect and block as the random effect.



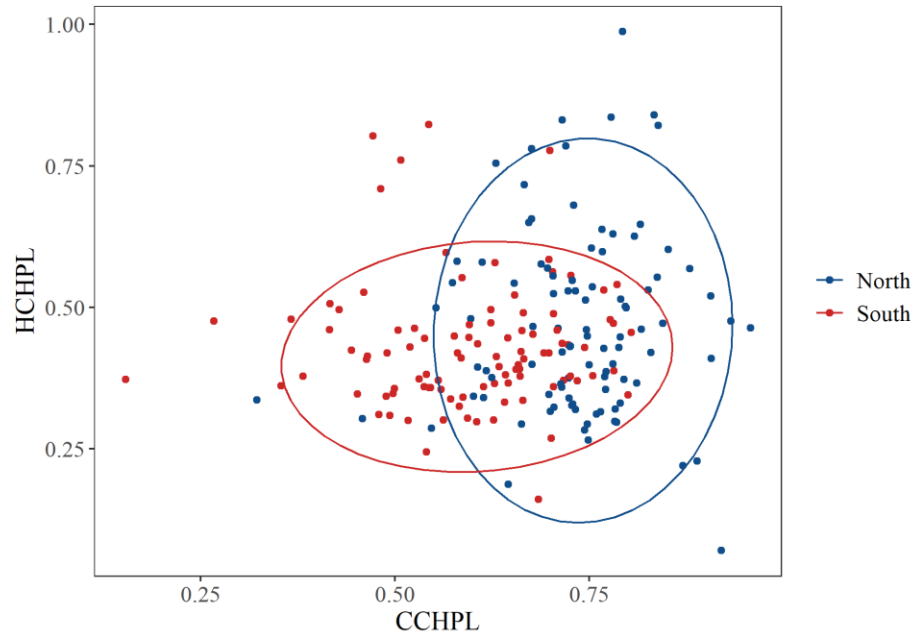
**Fig. S2** Cell membrane stability across temporally independent blocks and colored by region. The center line of the boxplot is the median of the measurements taken for each region within a ramet. There is a significant difference between blocks for hot cell membrane stability (HCMS,  $p = 0.0297$ ) and cold cell membrane stability (CCMS,  $p = 7.30e-05$ ). Asterisks indicate a significant difference between regions from a paired t-test of regions for each block independently. There was a significant difference between regions for HCMS block A ( $t = -2.910$ ,  $p = 0.015$ ), CMS block B ( $t = 2.190$ ,  $p = 0.040$ ), and CMS block C ( $t = 2.073$ ,  $p = 0.049$ ).



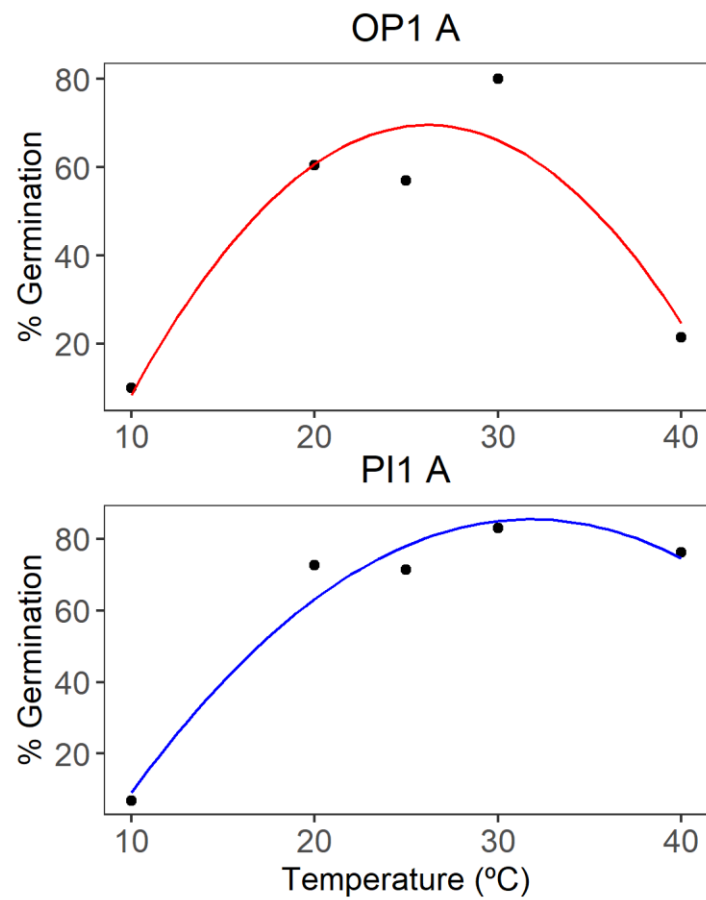
**Fig. S3** Daily max temperature for spring and summer of 2021 from the NOAA station at the Hector International Airport, Fargo, ND.



**Fig. S4** Genotype differences for temperature tolerance traits including cold cell membrane stability (CCMS), hot chlorophyll content (HCHPL), cold photosynthesis (CPS), and hot photosynthesis (HPS). Genets ordered by the sum of median ratios for HCMS and CCHPL. There are no significant differences between the variables shown here.

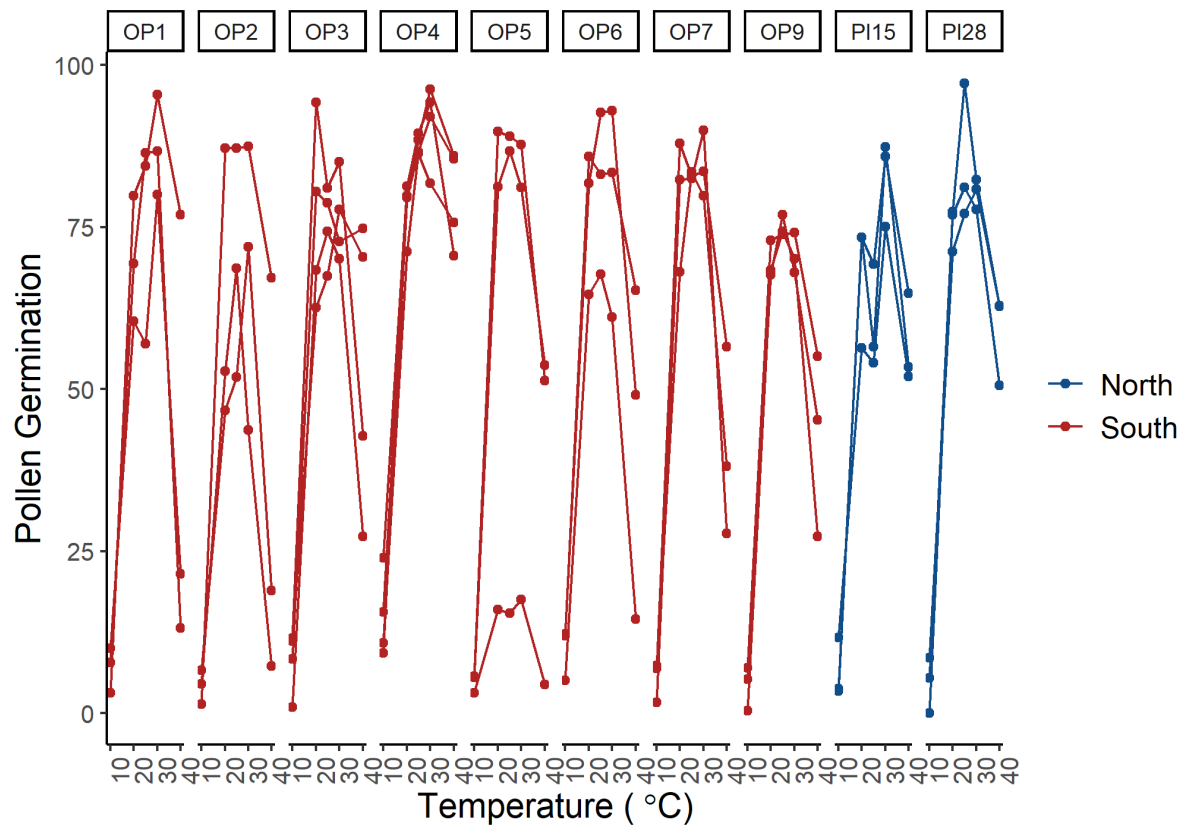


**Fig. S5** Hot chlorophyll content (HCHPL) vs cold chlorophyll content (CCHPL) for plants from the north and south. The ellipse indicates the 95% confidence interval for multivariate T distribution. Results from Bartlett's test for heterogeneity of variance between regions for all variables located in the Supporting Information (Table S5).

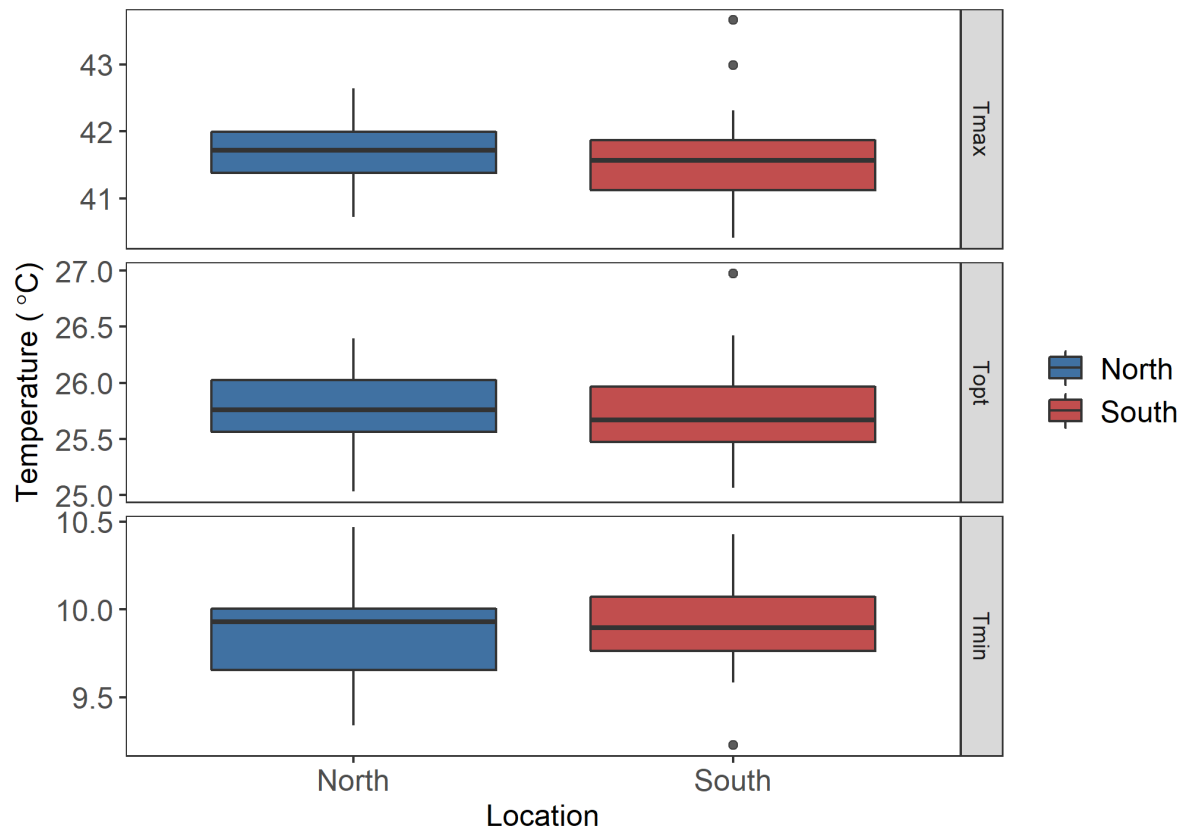


**Fig. S6** Examples of quadratic fit curve for pollen germination of one genet from the southern region (OP1 A, red) and one genet from the northern region (PI1 A, blue).

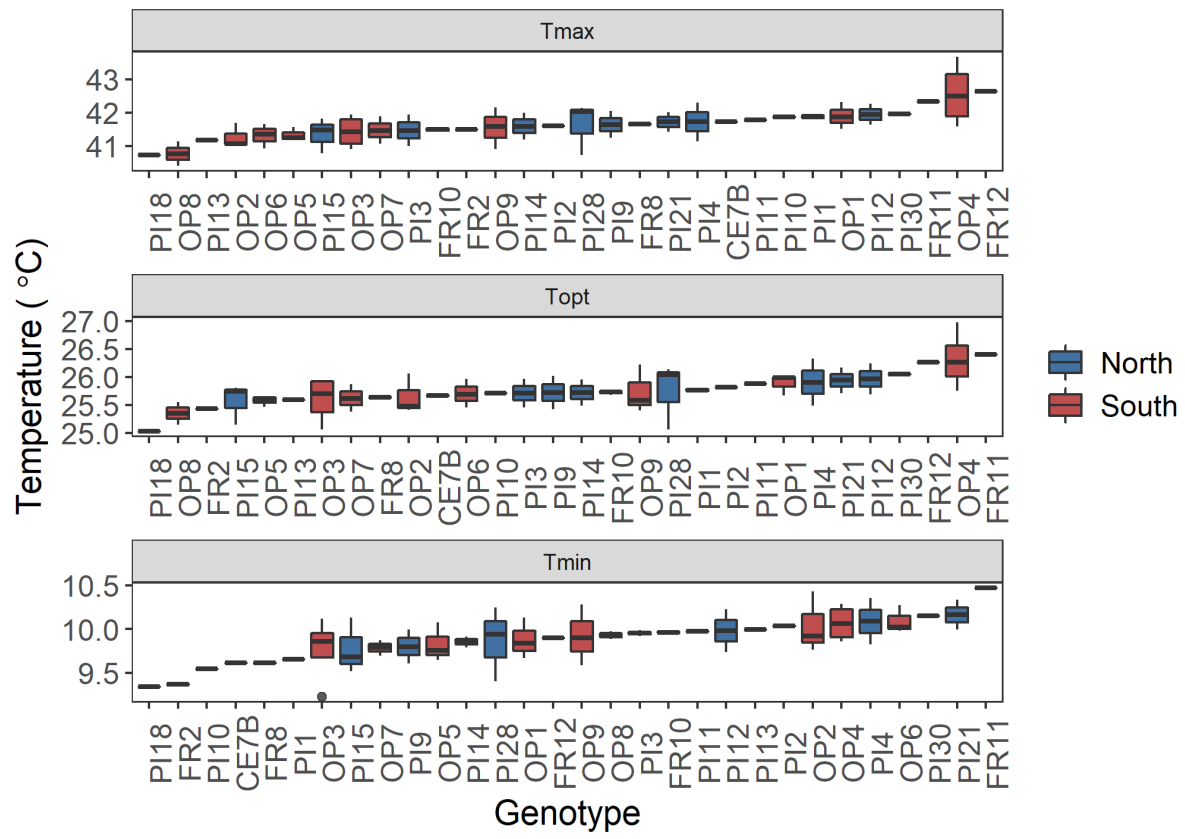




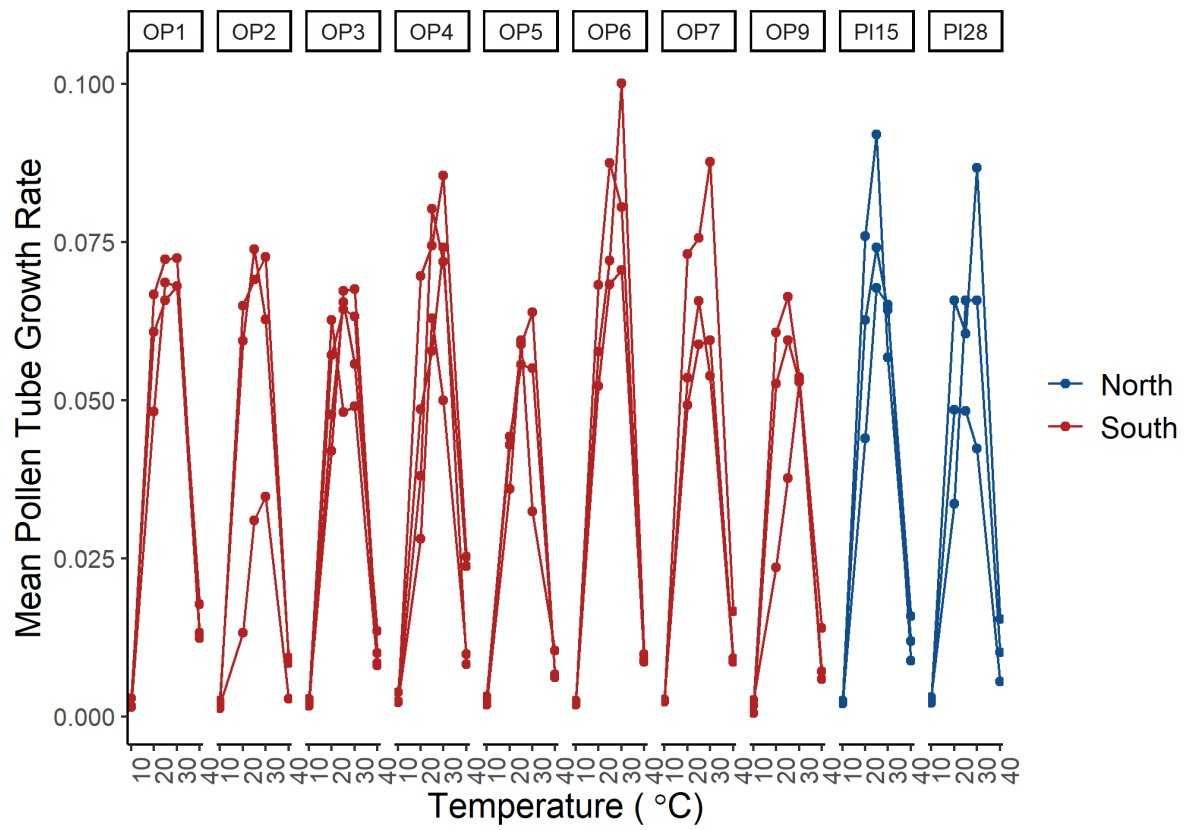
**Fig. S7** Pollen germination profiles for genets with at least 3 ramets that flowered. Color indicates region of origin.



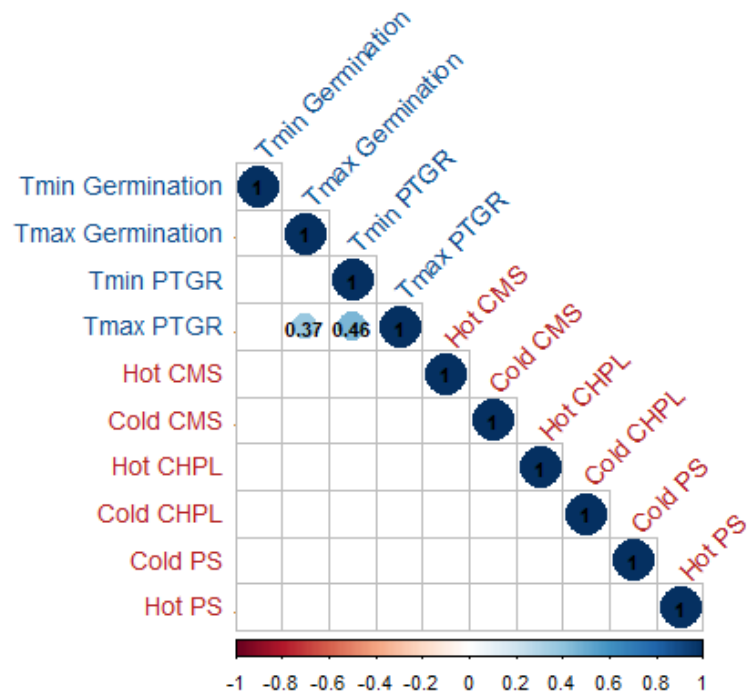
**Fig. S8** Comparison of pollen tube growth rate values extracted from a quadratic fit for the maximum, optimal, and minimum temperatures between northern and southern regions.



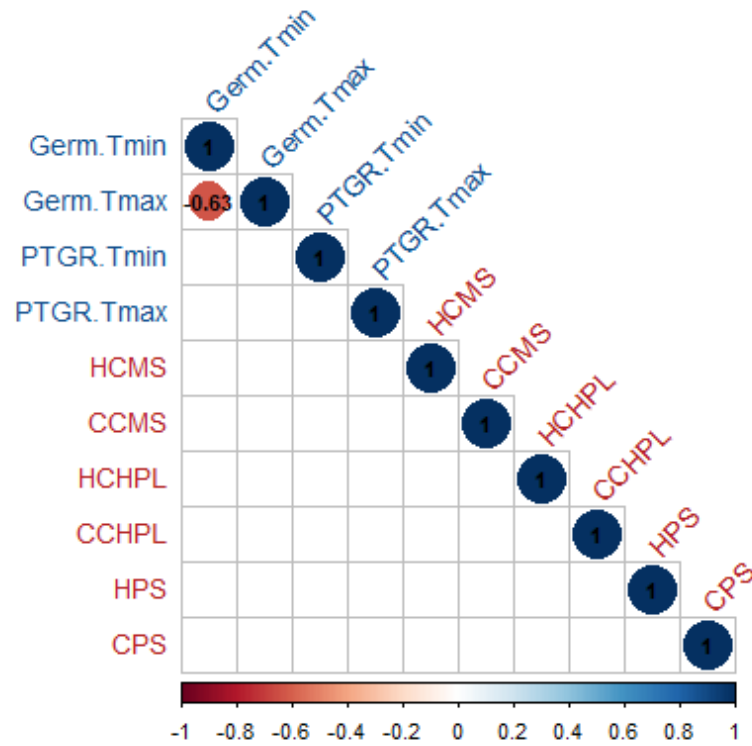
**Fig. S9** Genotype differences for the maximum ( $T_{max}$ ), optimal ( $T_{opt}$ ), and minimum ( $T_{min}$ ) pollen tube growth rate temperatures extracted from the quadratic fits of the pollen tube growth rate data for each individual. There were no significant differences among the genets for pollen tube growth rate. Each plot is in order of increasing PTGR.



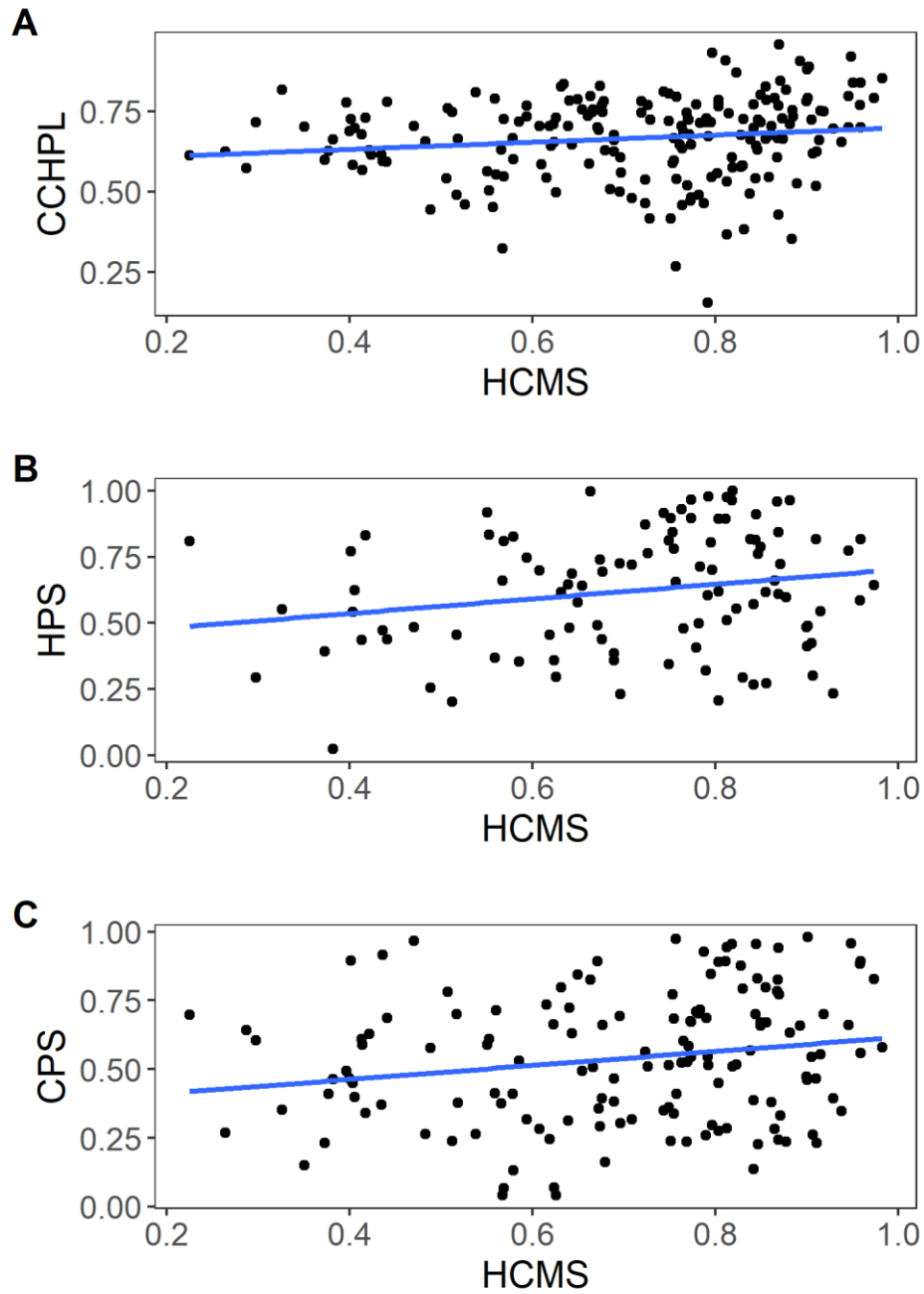
**Fig. S10** Pollen tube growth rate profiles for genets with at least 3 ramets that flowered. Color indicates region of origin.



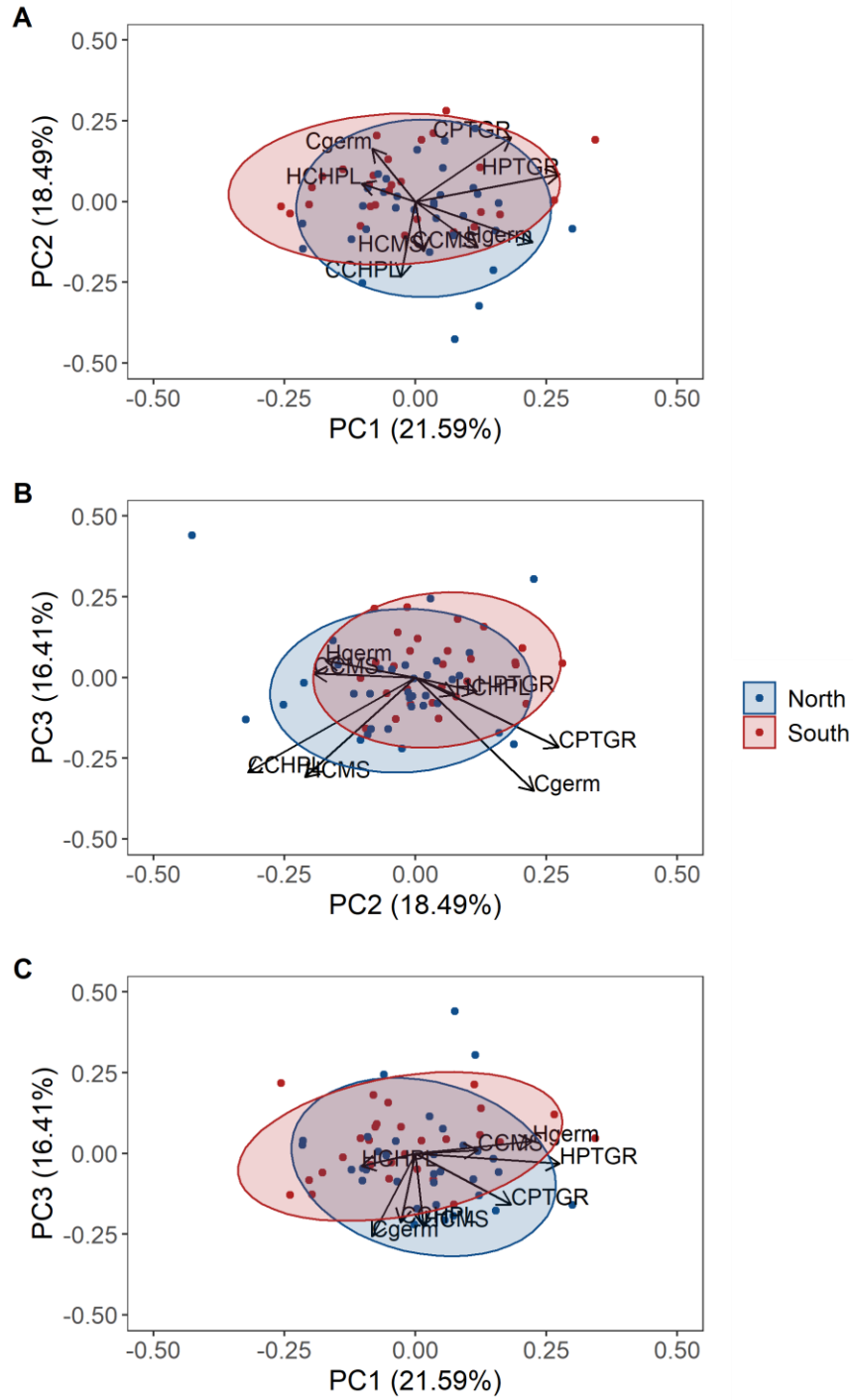
**Fig. S11** Correlation matrix of all plants. Gametophytic (labels blue font) and sporophytic variables (labels red font) with significant Pearson's correlations for all study plants.



**Fig. S12** Correlation matrix of southern plants with significant Pearson's correlations. Sporophytic (red font) and gametophytic variables (blue font) included. Blue colors indicate positive correlations and red colors indicate negative correlations.

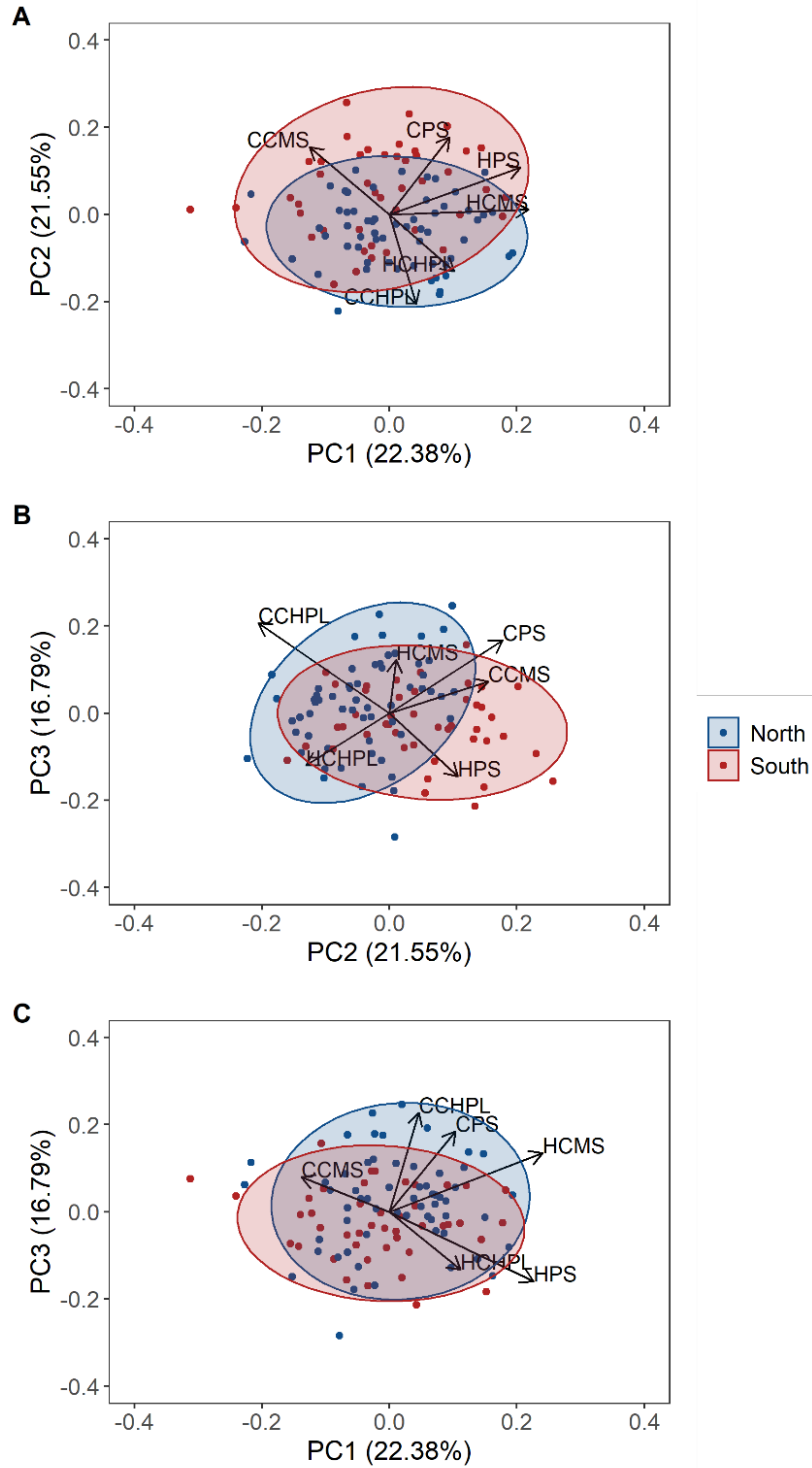


**Fig. S13** Scatter plot of the significant correlations between sporophytic variables including plants from the northern and southern regions.

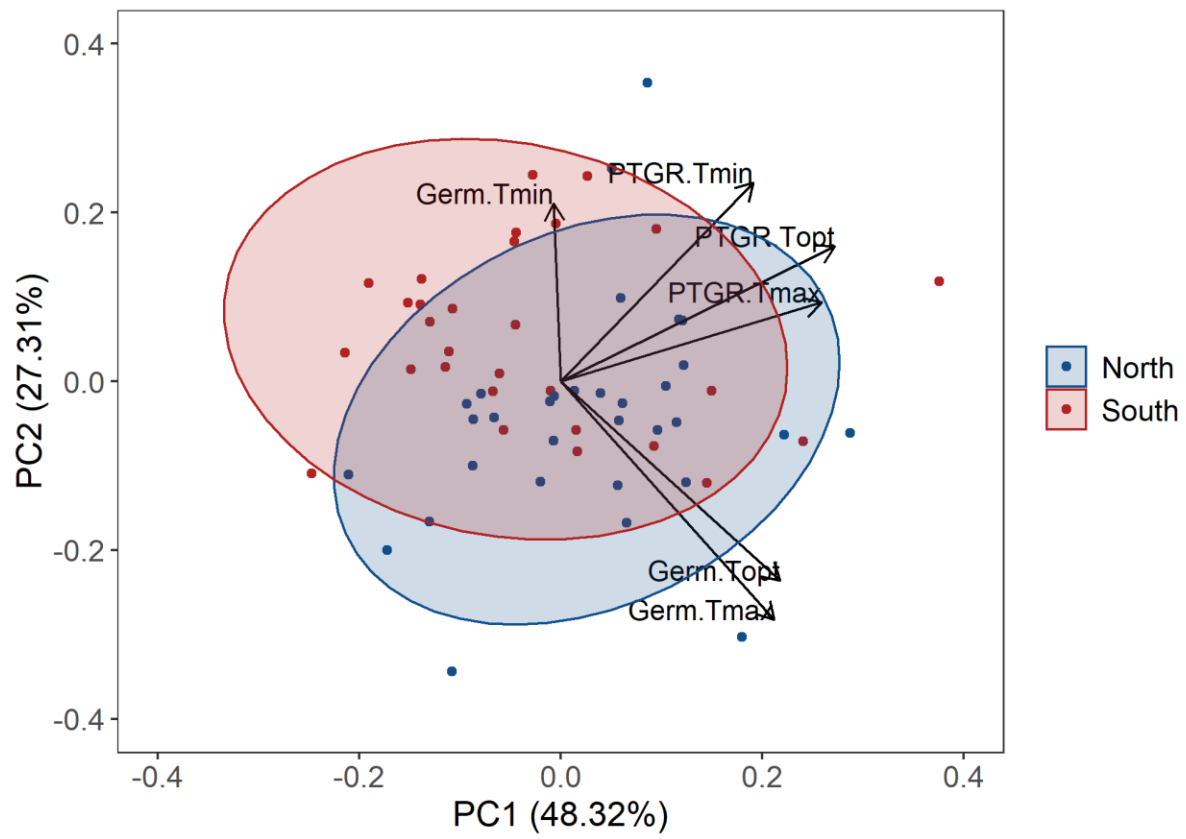


**Fig. S14** Principal component analysis with sporophytic and gametophytic variables, excluding photosynthesis. A) PC1 and PC2, B) PC2 and PC3, C) PC1 and PC3. Ellipsoid indicating 95% confidence interval.





**Fig. S15** Plots of the results of principal component analysis for the sporophytic variables. A) PC1 and PC2, B) PC2 and PC3, C) PC1 and PC3. Ellipsoid indicating 95% confidence interval. PC1 explains 22.38% of the variance, PC2 explains 21.55% of the variance, and PC3 explains 16.79% of the variance.



**Fig. S16** Plot of the results of principal component analysis of the gametophytic variables. PC1 describes 48% of the variation and PC2 explains 27%.

**Table S1.** Mixed effects model results for the difference between population for all sporophytic variables. One-way ANOVA results for the gametophytic variables for the difference between population. Block overfit the model when included for the gamtophyte and was therefore excluded from the gametophytic variable models. Bolded values indicate significant relationships.

	Variable	Population			Block	
		F-value	df	p-value	LRT	p-value
Sporophyte	Cell Membrane Stability (Heat)	<b>5.563</b>	<b>188.06</b>	<b>2.97E-04</b>	<b>4.210</b>	<b>0.040</b>
	Cell Membrane Stability (Cold)	<b>2.824</b>	<b>188.06</b>	<b>0.026</b>	<b>15.342</b>	<b>8.97E-05</b>
	Chlorophyll Content (Heat)	1.732	188.25	0.145	0.188	0.665
	Chlorophyll Content (Cold)	<b>32.341</b>	<b>188.22</b>	<b>2.20E-16</b>	1.477	0.224
	Photosynthetic Rate (Heat)	1.473	126.00	0.214	-2.84E-04	0.214
	Photosynthetic Rate (Cold)	<b>3.717</b>	<b>138.74</b>	<b>6.62E-03</b>	0.032	0.858
Gametophyte	Pollen Germination (Tmax)	<b>6.069</b>	<b>3</b>	<b>1.17E-03</b>	-	-
	Pollen Germination (Topt)	<b>6.861</b>	<b>3</b>	<b>5.02E-04</b>	-	-
	Pollen Germination (Tmin)	2.656	3	0.057	-	-
	Pollen Tube Growth Rate (Tmax)	0.400	3	0.753	-	-
	Pollen Tube Growth Rate (Topt)	0.197	3	0.898	-	-
	Pollen Tube Growth Rate (Tmin)	0.459	3	0.712	-	-

**Table S2.** Mixed effects model results for each variable. Full model included region as a fixed effect with block and genet nested in population as random effects. Random effect terms were dropped when the model overfit the data. Bolded values indicate significant relationships.

	Variable	Region			Block		Genet	
		F-value	df	p-value	LRT	p-value	LRT	p-value
Sporophyte	Cell Membrane Stability (Heat)	3.673	1, 50	0.061	<b>4.728</b>	<b>0.030</b>	3.320	0.068
	Cell Membrane Stability (Cold)	<b>6.482</b>	<b>1, 191</b>	<b>0.012</b>	<b>15.731</b>	<b>7.30E-05</b>	-	-
	Chlorophyll Content (Heat)	<b>4.418</b>	<b>1, 51</b>	<b>0.041</b>	0.222	0.637	0.018	0.892
	Chlorophyll Content (Cold)	<b>66.369</b>	<b>1, 50</b>	<b>9.97E-11</b>	1.018	0.313	2.082	0.149
	Photosynthetic Rate (Heat)	1.24E-05	1, 129	0.997	-	-	-	-
	Photosynthetic Rate (Cold)	3.269	1, 47	0.07702	0.148	0.701	1.674	0.196
Gametophyte	Pollen Germination (Tmax)	<b>12.054</b>	<b>1, 26</b>	<b>0.002</b>	-	-	3.181	0.075
	Pollen Germination (Topt)	<b>10.916</b>	<b>1, 24</b>	<b>0.003</b>	-	-	1.728	0.189
	Pollen Germination (Tmin)*	0.151	1, 21	0.702	-	-	<b>5.000</b>	<b>0.025</b>
	Pollen Tube Growth Rate (Tmax)	0.446	1, 29	0.509	-	-	1.455	0.228
	Pollen Tube Growth Rate (Topt)	0.121	1, 29	0.731	-	-	0.309	0.578
	Pollen Tube Growth Rate (Tmin)	0.168	1, 59	0.683	-	-	-	-

**Table S3.** Mixed effects model of control values used in calculation for variable proportions to determine baseline differences between regions without the temperature treatments.

Variable	Region		Genet	
	Difference	p-value	Difference	p-value
Conductivity of cell membrane max damage	No	0.445	No	0.097
Chlorophyll content initial value	No	0.795	No	0.869
Net photosynthetic rate initial value	No	0.303	No	0.380

**Table S4.** T-test results for differences between region within block. Paired t-tests were used as a northern and southern plant were paired with one another and experienced the same green house conditions. An unpaired t-test was used for photosynthesis because there were missing data points for some genets. Bolded values indicate significant relationships.

	Variable	Method	Block A		Block B		Block C		Block D	
			t-stat	p-value	t-stat	p-value	t-stat	p-value	t-stat	p-value
Sporophyte	Cell Membrane Stability (Heat)	Paired	<b>-2.910</b>	<b>0.015</b>	-0.853	0.403	-1.640	0.113	-0.539	0.595
	Cell Membrane Stability (Cold)	Paired	0.758	0.456	<b>2.190</b>	<b>0.040</b>	<b>2.073</b>	<b>0.049</b>	0.939	0.358
	Chlorophyll Content (Heat)	Paired	-0.374	0.712	-1.650	0.113	-1.933	0.065	-0.728	0.474
	Chlorophyll Content (Cold)	Paired	<b>-5.889</b>	<b>3.82E-06</b>	<b>-4.746</b>	<b>9.77E-05</b>	<b>-5.982</b>	<b>3.50E-06</b>	<b>-4.106</b>	<b>4.33E-04</b>
	Photosynthetic Rate (Heat)	Unpaired	0.541	0.594	1.144	0.261	-1.367	0.187	0.021	0.984
	Photosynthetic Rate (Cold)	Unpaired	-0.664	0.511	1.542	0.137	1.219	0.231	1.782	0.083

**Table S5.** Difference in variation between the two regions for all variables using Bartlett's test of homogeneity of variances. Bolded text indicates differences that are statistically significant. Asterisk denotes variable with one data point removed after an outlier test.

	Variable	Difference	More Variation	p-value
Sporophyte	Cell Membrane Stability (Heat)	No	-	0.896
	Cell Membrane Stability (Cold)	No	-	0.131
	Chlorophyll Content (Heat)	<b>Yes</b>	<b>North</b>	<b>2.48E-04</b>
	Chlorophyll Content (Cold)	No	-	0.057
	Photosynthetic Rate (Heat)	No	-	0.444
	Photosynthetic Rate (Cold)	No	-	0.602
Gametophyte	Pollen Germination (Tmax)	No	-	0.515
	Pollen Germination (Topt)	No	-	0.972
	Pollen Germination (Tmin)	No	-	0.1557*
	Pollen Tube Growth Rate (Tmax)	No	-	0.107
	Pollen Tube Growth Rate (Topt)	No	-	0.532
	Pollen Tube Growth Rate (Tmin)	No	-	0.487

**Table S6.** Correlation matrix with correlation coefficient and p-value for each combination of variables. Bolded text indicates correlations that are statistically significant with p-values adjusted using the Holm's-Bonferroni method for multiple correlations.

		Tmin Germ	Tmax Germ	Tmin PTGR	Tmax PTGR	Hot CMS	Cold CMS	Hot CHPL	Cold CHPL	Cold PS	Hot PS
Tmin Germination	Corr	-									
	p-value	-									
Tmax Germination	Corr	-0.264	-								
	p-value	0.040	-								
Tmin PTGR	Corr	0.266	0.061	-							
	p-value	0.038	0.639	-							
Tmax PTGR	Corr	-0.073	<b>0.371</b>	<b>0.456</b>	-						
	p-value	0.576	<b>0.003</b>	<b>2.18E-04</b>	-						
Hot CMS	Corr	0.015	0.112	-0.004	0.030	-					
	p-value	0.911	0.392	0.977	0.818	-					
Cold CMS	Corr	-0.130	0.167	0.042	0.106	-0.131	-				
	p-value	0.317	0.198	0.745	0.416	0.067	-				
Hot CHPL	Corr	0.112	-0.078	-0.069	-0.103	0.060	-0.131	-			
	p-value	0.392	0.552	0.596	0.428	0.401	0.066	-			
Cold CHPL	Corr	0.101	0.065	-0.127	-0.144	0.145	-0.093	0.102	-		
	p-value	0.440	0.621	0.331	0.267	0.043	0.191	0.154	-		
Cold PS	Corr	0.240	0.062	0.164	0.133	0.205	-0.084	0.092	-0.151	-	
	p-value	0.228	0.758	0.412	0.508	0.038	0.402	0.358	0.129	-	
Hot PS	Corr	-0.052	0.054	0.066	0.137	0.194	0.076	-0.069	-0.030	0.131	-
	p-value	0.730	0.719	0.662	0.358	0.019	0.362	0.408	0.718	0.186	-



**Table S7.** Results from principal component analysis with gametophytic and sporophytic variables. Loadings for each variable on all principal components.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Tmin Germination	-0.189	0.377	-0.586	0.224	-0.069	0.422	0.385	0.313
Tmax Germination	0.511	-0.286	0.088	-0.299	-0.103	0.625	0.324	-0.232
Tmin PTGR	0.417	0.456	-0.360	0.123	-0.009	-0.206	-0.112	-0.646
Tmax PTGR	0.628	0.195	-0.072	-0.150	-0.025	-0.069	-0.354	0.639
HCMS	0.035	-0.352	-0.516	-0.468	0.215	-0.466	0.351	0.058
CCMS	0.271	-0.325	0.021	0.575	-0.522	-0.319	0.326	0.095
HCHPL	-0.235	0.126	-0.090	-0.476	-0.815	-0.037	-0.160	-0.053
CCHPL	-0.064	-0.534	-0.489	0.215	-0.025	0.253	-0.595	-0.082

**Table S8.** Loadings from principal component analysis of only sporophytic variables.

	PC1	PC2	PC3	PC4	PC5	PC6
HCMS	0.614	0.030	0.345	0.147	0.574	0.390
CCMS	-0.352	0.435	0.204	-0.575	0.521	-0.208
HCHPL	0.285	-0.368	-0.338	-0.758	-0.087	0.295
CCHPL	0.117	-0.578	0.580	-0.141	-0.038	-0.543
HPS	0.578	0.303	-0.409	0.000	0.044	-0.637
CPS	0.265	0.499	0.471	-0.230	-0.624	0.132

**Table S9.** Results from principal component analysis with sporophytic variables. The proportion of variance explained by each of the components.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.159	1.1372	1.0038	0.968	0.8775	0.8058
Proportion of Variance	0.224	0.2155	0.1679	0.1562	0.1283	0.1082
Cumulative Proportion	0.224	0.4394	0.6073	0.7634	0.8918	1

**Table 10.** Loadings from principal component analysis of only gametophytic variables.

	PC1	PC2	PC3	PC4	PC5	PC6
Germ.Tmin	-0.013	0.406	0.812	0.376	-0.001	-0.184
Germ.Topt	0.419	-0.454	0.390	-0.003	0.002	0.683
Germ.Tmax	0.408	-0.545	0.165	-0.101	-0.002	-0.707
PTGR.Tmin	0.368	0.453	0.128	-0.751	-0.281	0.001
PTGR.Topt	0.524	0.308	-0.203	0.124	0.758	-0.002
PTGR.Tmax	0.499	0.180	-0.322	0.518	-0.589	0.001

**Table S11.** Results from principal component analysis with gametophytic variables. The proportion of variance explained by each of the components.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.703	1.28	1.0046	0.673	0.0008	0.0001
Proportion of Variance	0.483	0.2731	0.1682	0.0755	0	0
Cumulative Proportion	0.483	0.7563	0.9245	1	1	1