Figure 1. Regional differences for temperature tolerance traits including hot and cold cell membrane stability (HCMS, CCMS), hot and cold chlorophyll fluorescence stability (HCHPL, CCHPL), hot and cold net photosynthetic rate (HPS, CPS). Center line of boxplot is the median value for the region. The letters represent statistically significant differences between regions. Variables with significant differences denoted with asterisks: CCMS (F1,50 = 7.792, p = 0.006), HCHPL (F1,51 = 4.334, p = 0.043), and CCHPL (F1,50 = 64.652, p = 1.6e-10).

**Results**

Table 1. Results from the mixed linear model for the difference in region (north vs south) and the one-way analysis of variance results for the difference between individual genets. Red font color highlights observed outcomes when the result was different from the expected pattern. Asterisk indicates analysis with one outlier removed determined using Grubbs test for one outlier.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Region** | | | **Genet** | |
|  | **Variable** | **Expected** | **Observed** | **p-value** | **Observed** | **p-value** |
| **Sporophyte** | Cell Membrane Stability (Heat) | S > N | - | 0.06102 | **Yes** | **0.013** |
| Cell Membrane Stability (Cold) | N > S | **S > N** | **0.0117** | No | 0.886 |
| Chlorophyll Fluorescence (Heat) | S > N | **N > S** | **0.0405** | No | 0.38 |
| Chlorophyll Fluorescence (Cold) | N > S | N > S | **9.96E-11** | **Yes** | **1.05E-07** |
| Photosynthetic Rate (Heat) | S > N | - | 0.997 | No | 0.127 |
| Photosynthetic Rate (Cold) | N > S | - | 0.77 | No | 0.883 |
| **Gametophyte** | Pollen Germination (Tmax) | S > N | **N > S** | **0.00037** | **Yes** | **0.0251** |
| Pollen Germination (Topt) | S > N | **N > S** | **0.000685** | **Yes** | **0.0351** |
| Pollen Germination (Tmin) | S > N | - | 0.331 | **Yes** | **\*0.0135** |
| Pollen Tube Growth Rate (Tmax) | S > N | - | 0.568 | No | 0.418 |
| Pollen Tube Growth Rate (Topt) | S > N | - | 0.77 | No | 0.608 |
| Pollen Tube Growth Rate (Tmin) | S > N | - | 0.683 | No | 0.496 |

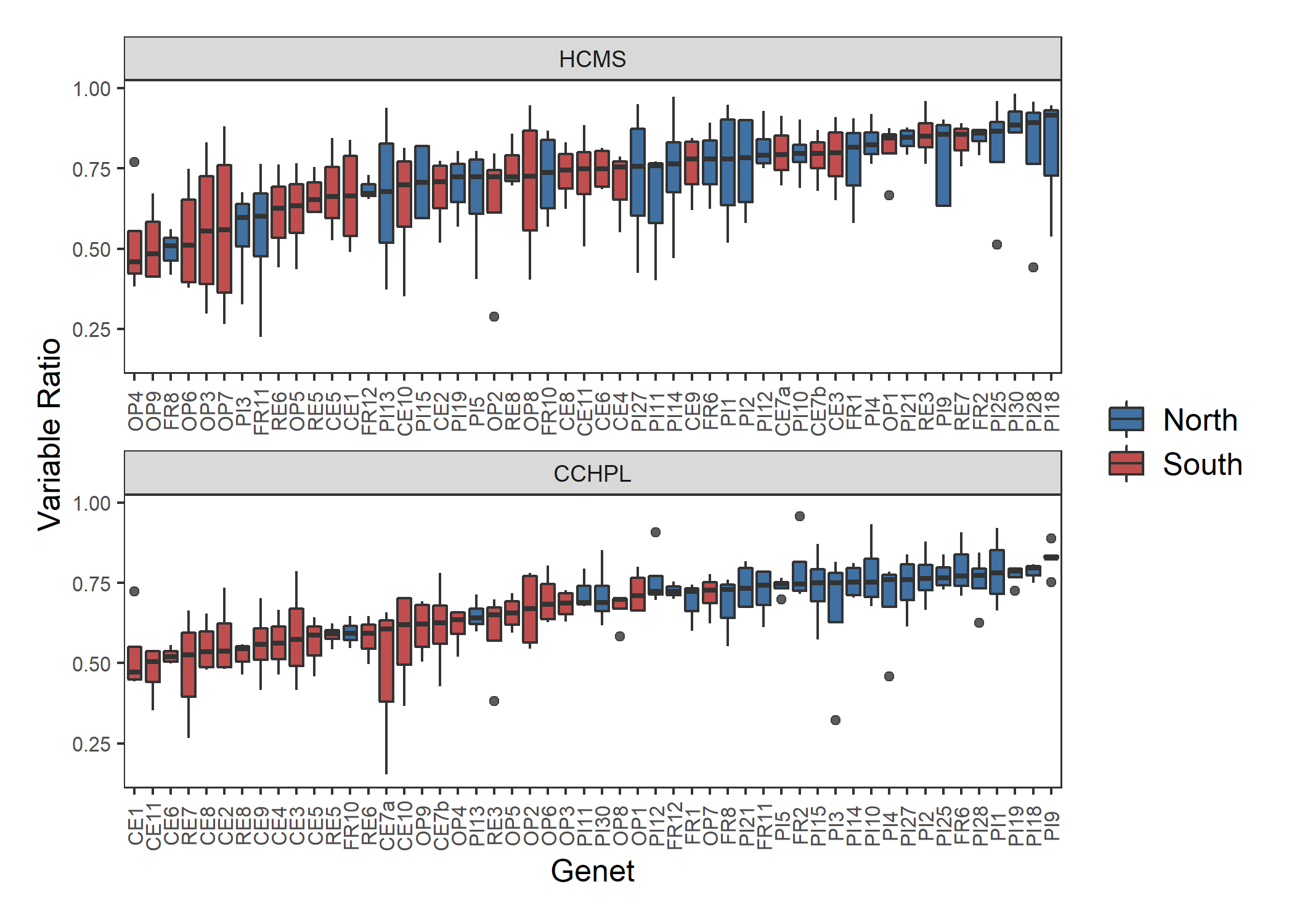


Figure 2. Genotype differences for temperature tolerance traits including hot cell membrane stability (HCMS) and cold chlorophyll fluorescence stability (CCHPL). Genets ordered by increasing ratio for each variable. Center line in boxplot is the median of the measurements taken for the ramets of one genet. There is a significant difference among the genets for HCMS (F = 1.5, p = 0.029) and CCHPL (F = 3.341, p = 6.1e-9). Plots of genet effect for other variables in appendix.

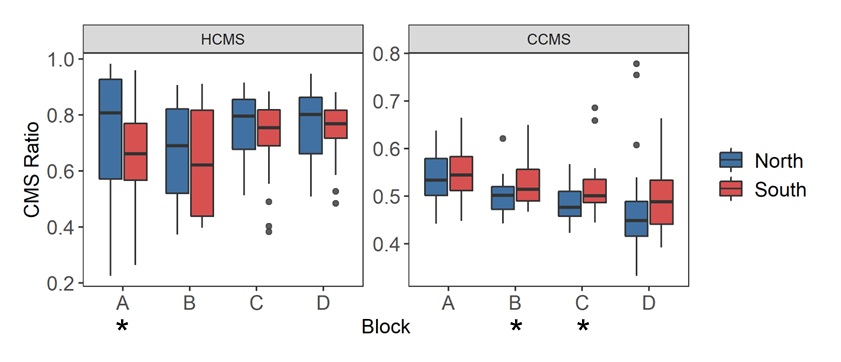


Figure 3. Cell membrane stability across temporally independent blocks and colored by region. The center line of the boxplot is the median of the measurements taken for each region within a ramet. There is a significant difference between blocks for hot cell membrane stability (HCMS, p = 0.0297) and cold cell membrane stability (CCMS, p = 7.30e-05). Astrisks 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). Results from paired t-tests between blocks for each variable located in the appendix.

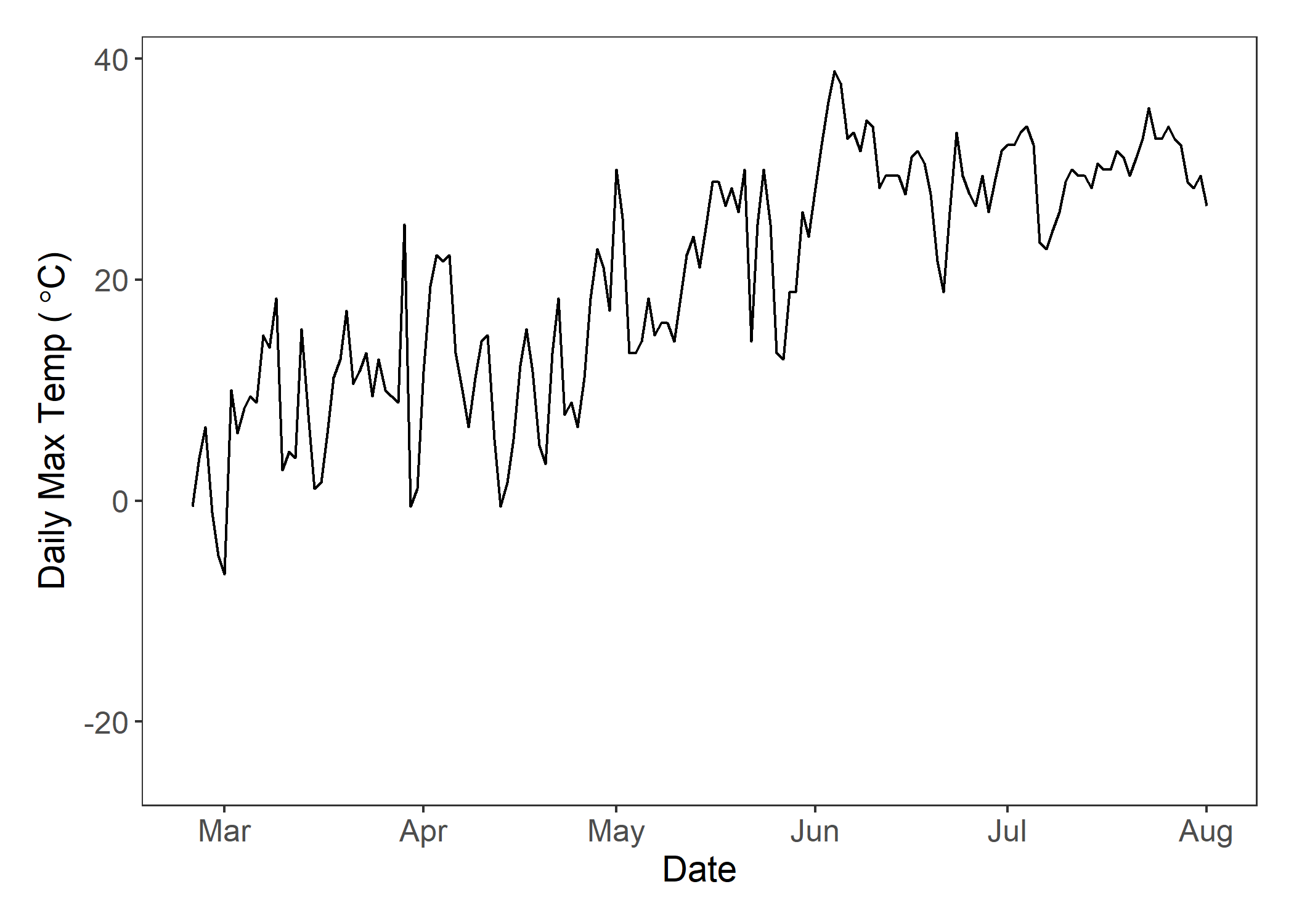


Figure 4. Daily max temperature for spring and summer from the NOAA station at the Hector International Airport, Fargo, ND.

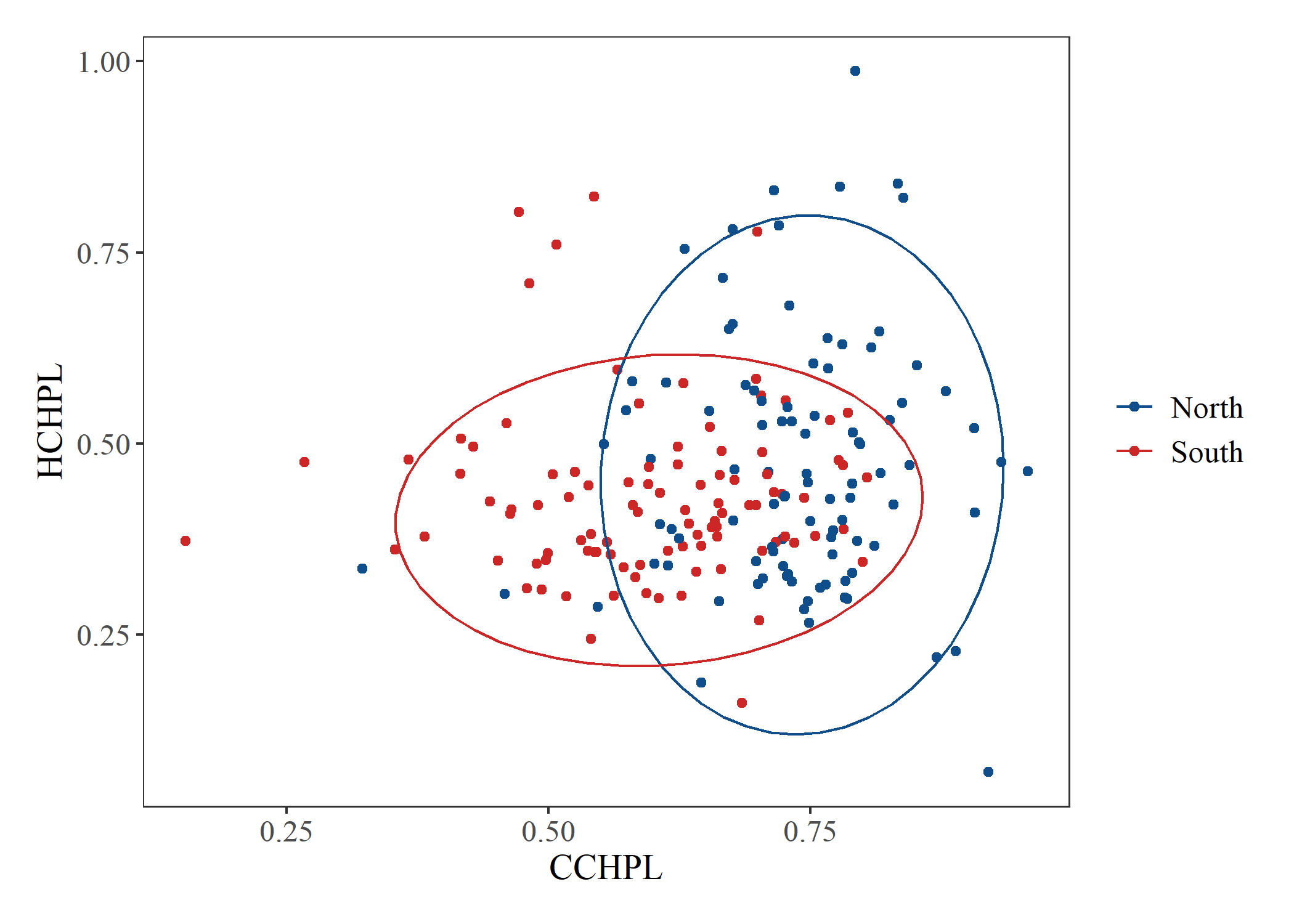


Figure 5. Hot chlorophyll fluorescence stability (HCHPL) vs cold chlorophyll fluorescence stability (CCHPL) for plants from the north and south. Ellipse indicating 95% confidence interval for multivariate T distribution. Results from Bartlett’s test for heterogeneity of variance between regions for all variable located in the appendix.

***Sporophytic Variables***

*Cell Membrane Stability*

Cell membrane stability (CMS) was quantified using the ratio of a conductivity measurement after a temperature treatment to a conductivity measurement after a maximum damage treatment. An increased CMS ratio indicates higher tolerance of the temperature treatment. When *Solanum carolinense* plants from the north were compared to the south, we found no significant difference in the hot treatment, but there was a significant difference in the cold treatment (figure 1). We found a significant difference among genotypes in the hot treatment, but not in the cold treatment (figure 2).

Because we could not grow all the experimental plants at the same time due to lack of space, we made the above comparisons among regions and genotypes in five different temporal blocks over the course of the spring and summer. To avoid confounding treatments with temporal effects plants from different regions were paired with each other within blocks. When we tested for the presence of block effects, we found significant effects for both hot and cold CMS (figure 3). Plants grown at different times in the greenhouse had different CMS ratios. We started growing the plants in the winter and early spring and outside temperatures gradually rose during that time (figure 4). Acclimation to higher temperatures later in the year could account for the block differences observed. The most even temperatures were in the winter, when the earliest ramet was planted. When plants from the north and south were compared for hot CMS (HCMS) in just ramet A in a paired t-test, there was a significant difference between the regions (figure 3). Northern plants had a higher CMS in extreme heat than those from the south. For cold CMS (CCMS), there was a significant difference between regions for blocks B and C (figure 3). In both cases, southern plants were more tolerant of the cold temperatures than northern plants.

*Chlorophyll fluorescence stability*

Chlorophyll fluorescence was measured before and after either a heat stress or cold stress and the inverse ratio of the measurements was used as a proxy for temperature tolerance. As the chlorophyll ratio increases, the individual sporophyte is more tolerant of the temperature treatment. There was a significant difference between plants originating in the north and south for both the hot and cold treatments (figure 1). Northern plants were more tolerant than southern plants in both treatments regardless of block. We found a significant difference between individual genotypes for the cold treatment, but not for the hot treatment (figure 2). The two regions also differed in variation for hot CHPL. Northern plants had significantly more variation in the hot treatment than southern plants (Bartlett’s test p-value = 1.68E-4) (figure 5). No other variable showed a difference in variance between the two regions.

*Net Photosynthetic Rate*

We used net photosynthetic rate after thermal stress as a physiological indicator of temperature tolerance. Both previous variables mentioned could directly influence temperature tolerance through their involvement in photosynthesis. Net photosynthetic rate (PS) was the proportion of the net photosynthetic rate after the treatment (heat and cold) to the net photosynthetic rate before the treatment. Increased PS indicates higher temperature tolerance of the thermal stress. For both the cold and hot treatments, there was no significant difference between north and south (figure 1). There were no significant differences between blocks and genotypes for both the hot and cold treatments.

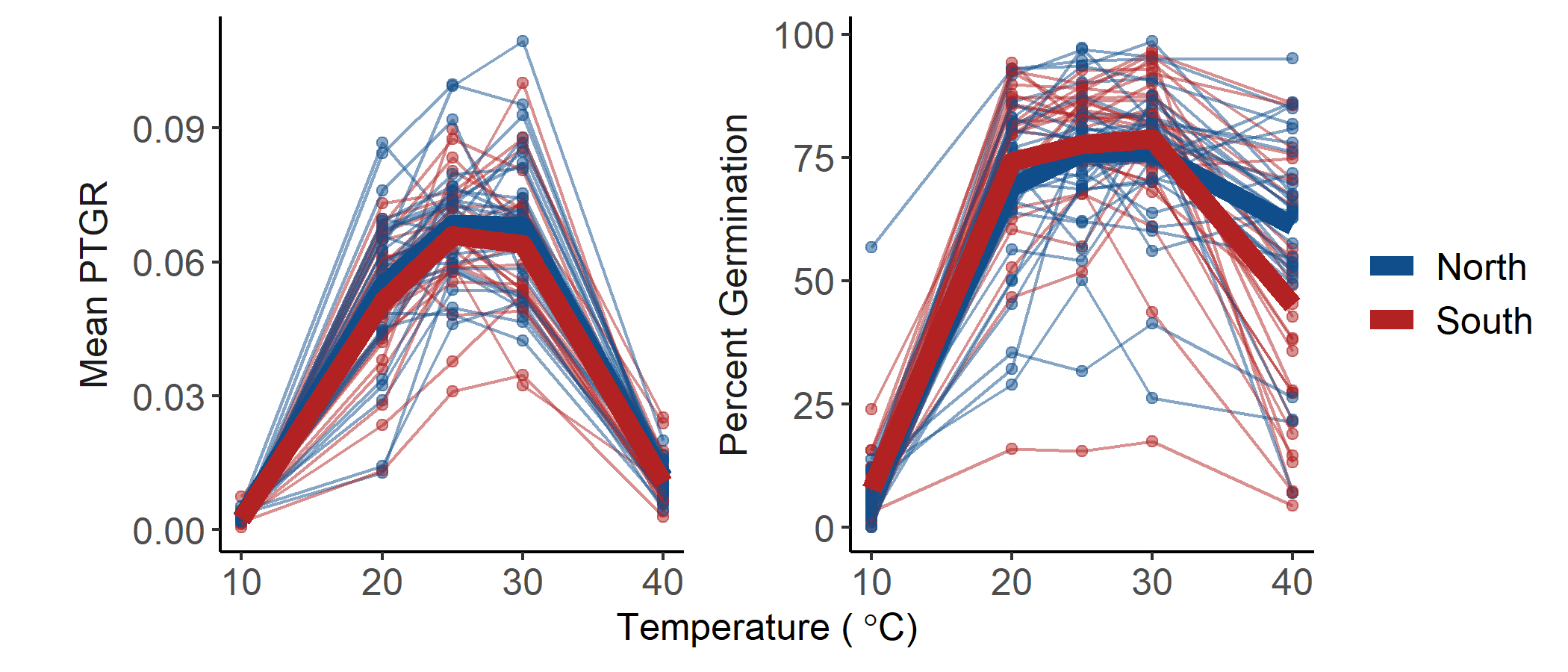


Figure 6. Percent germination and mean pollen tube growth rate (PTGR) for *Solanum carolinense* pollen grains from the north (blue) and south (red) across a temperature gradient (10°C, 20°C, 25°C, 30°C, 40°C). Thin lines and points represent each individual plant that flowered. Thick lines indicate the mean value for the region at each temperature.

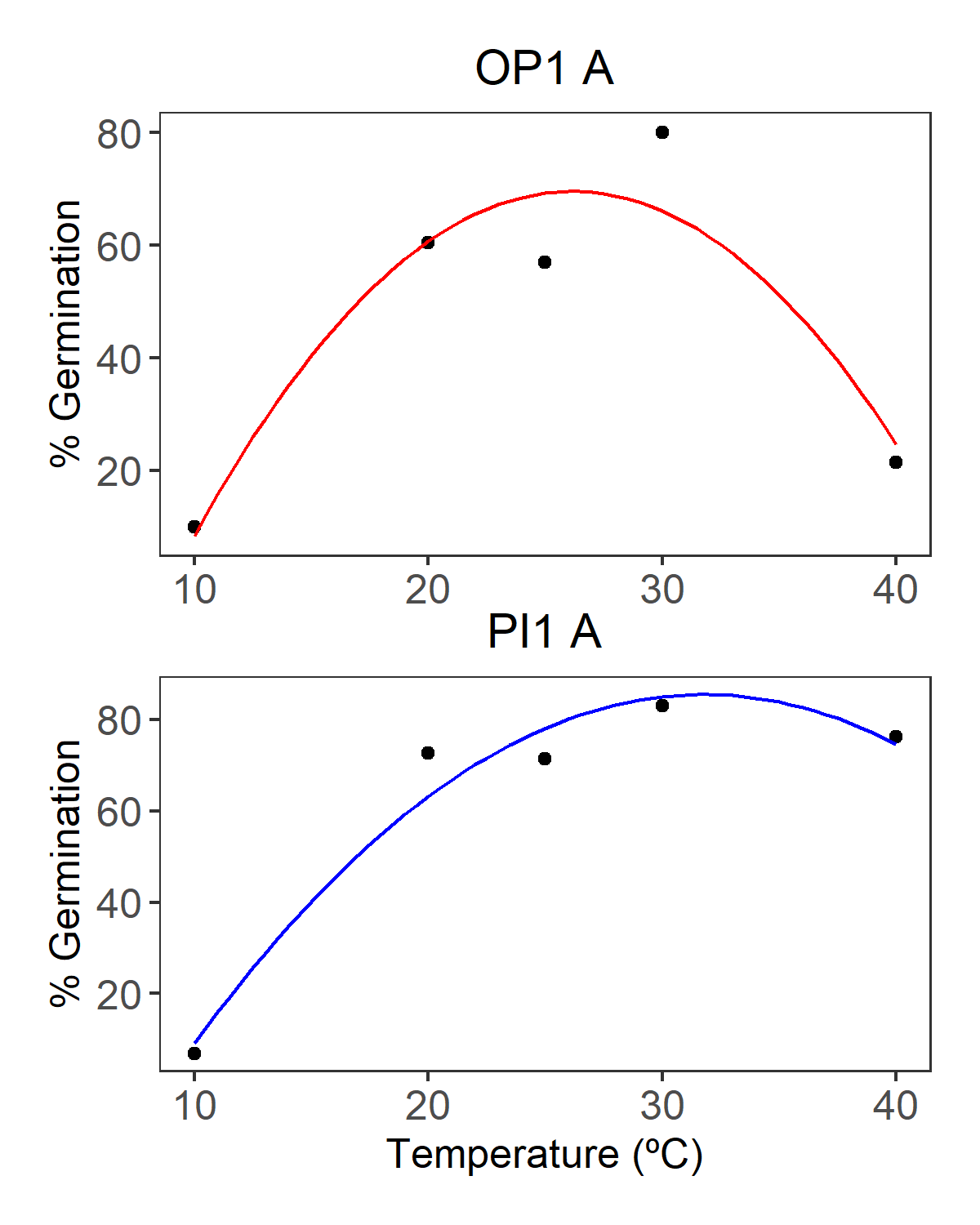
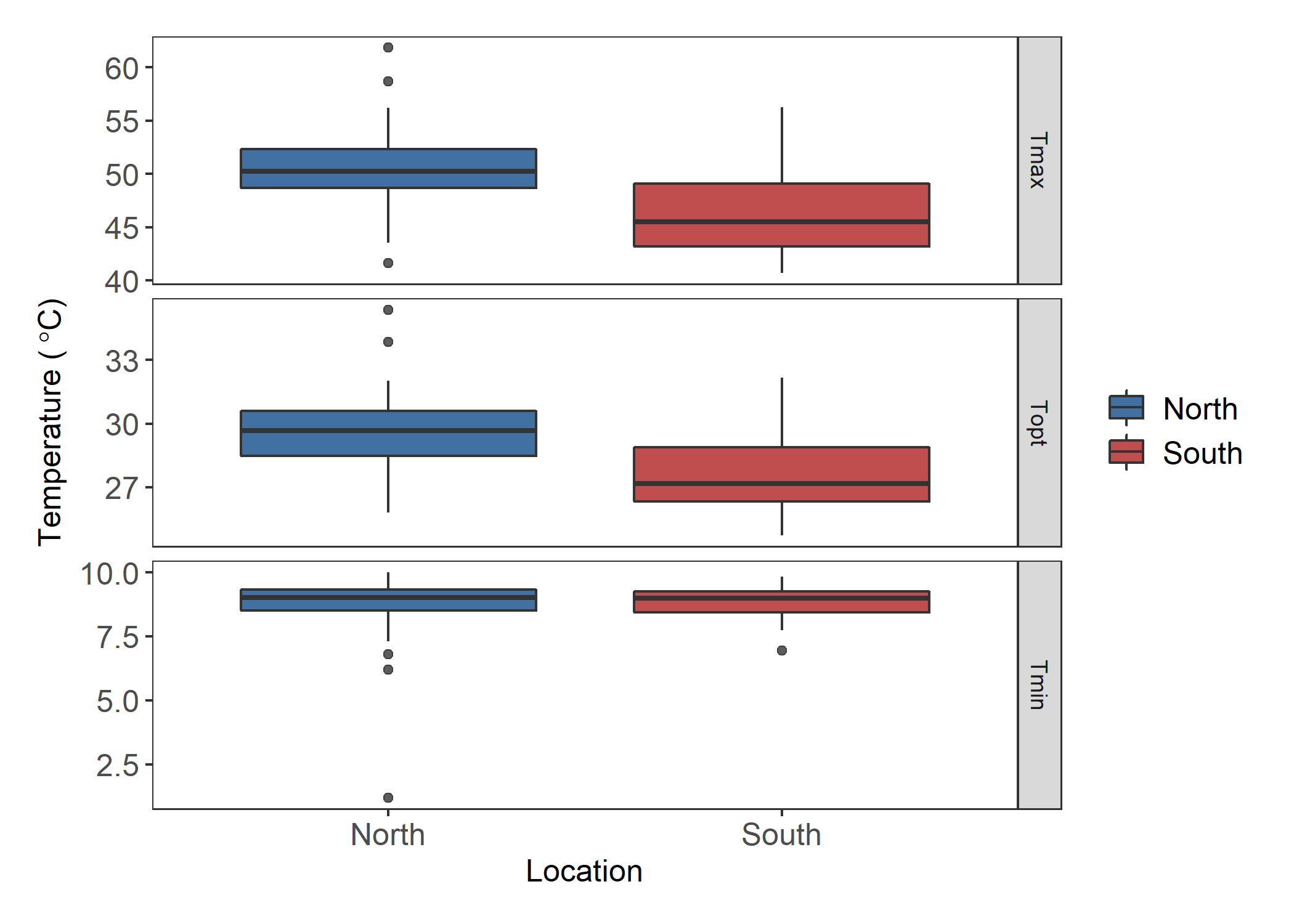


Figure 7. Examples of quadratic fit curve for pollen germination of one genet from the southern region (OP1 A, red) and one genet from the northern region (PI1 A, blue).



A

B

\*

A

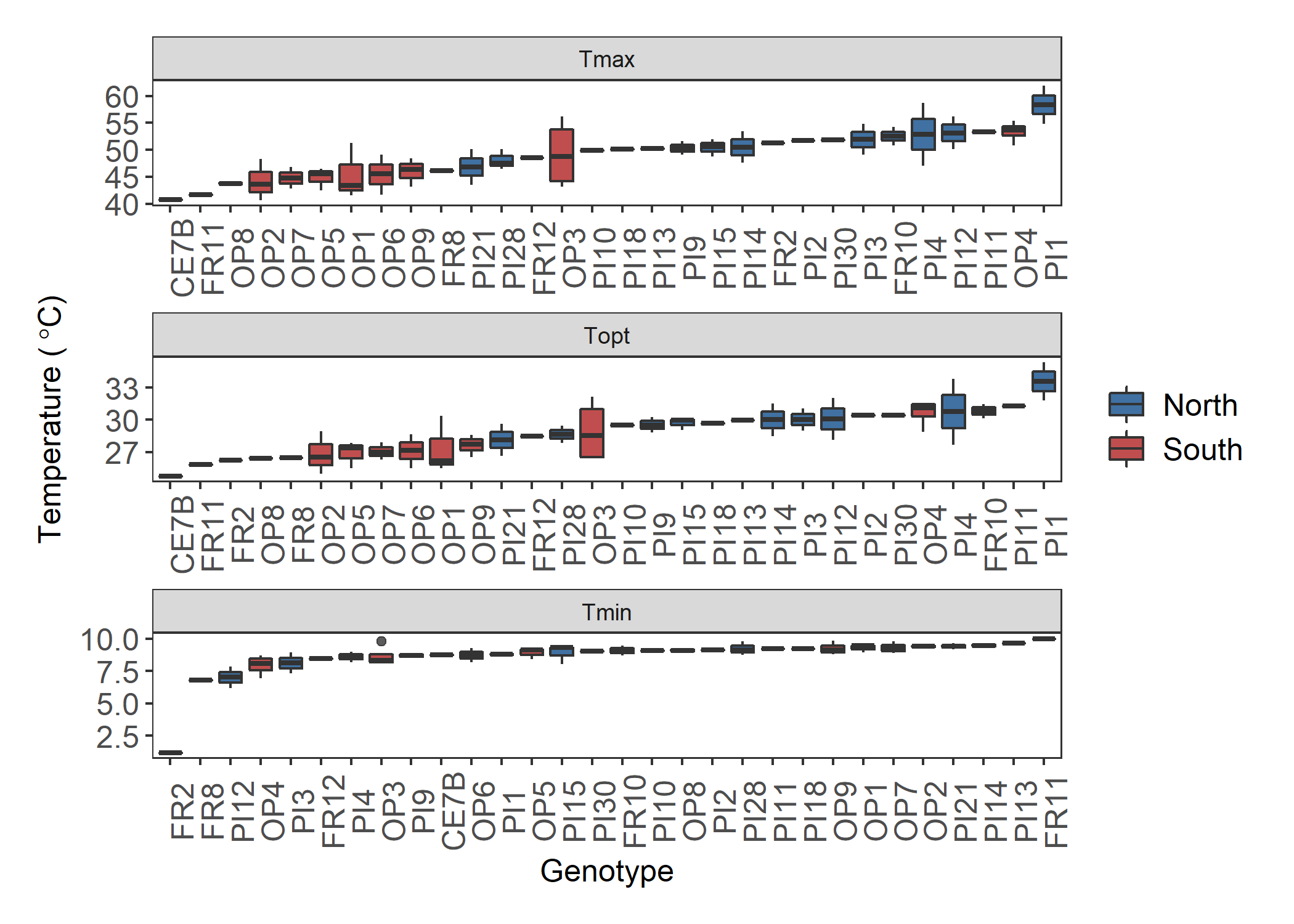
B

\*

A

A

Figure 8. Estimates for the maximum (Tmax), optimal (Topt), and minimum (Tmin) germination temperature extracted from quadratic fits of the germination data for each individual. Asterisks and different letters indicate significant differences. There is a significant difference between regions for Tmax (F = 14.28, p = 3.7E-4) and Topt (F = 12.85, p = 6.85E-4).



\*

Figure 9. Boxplots of the maximum (Tmax), optimal (Topt), and minimum (Tmin) pollen germination temperatures by genet. There is a significant difference between the genets for Tmax (F = 2.064, p = 0.025), Topt (F = 1.952, p = 0.035), and Tmin (F = 2.284, p = 0.0135). Asterisk indicates the outlier removed for analysis. Plots of genet effect for other variables in appendix.

***Gametophytic Variables***

*Pollen Germination*

We fit quadratic curves to temperature performance profiles of each genet for pollen germination at five temperatures (figure 7). From the quadratic fit, we calculated the minimum (Tmin), maximum (Tmax), and optimal (Topt) temperature of pollen germination for each individual. There was a significant difference between regions for Tmax and Topt (figure 8). Genets from the north germinated more readily at high temperatures than genets from the south. There was no significant difference between the two regions for Tmin. The genets were significantly different from one another for Tmin, Tmax, and Topt (figure 9). One outlier was identified using the Grubbs test for one outlier (outliers; function grubbs.test) and subsequently dropped from the analysis.

*Pollen Tube Growth Rate*

The pollen tube growth rates for each individual were also fit with a quadratic curve to estimate the Tmin, Tmax, and Topt. There was no significant difference between genets from the north and south for any of the calculated variables (Appendix). There were also no significant differences between genets for the calculated variables (Appendix).

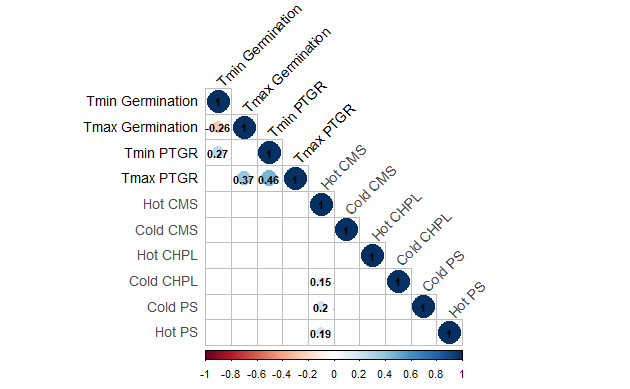


Figure 10. Correlation matrix of sporophytic (dark gray) and gametophytic variables (black) with significant Pearson’s correlations. Blue colors indicate positive correlations and red colors indicate negative correlations.

***Correlations and PCA***

We used correlation analysis and principal component analysis to identify relationships between hot and cold tolerance for the sporophytic and gametophytic variables. Pearson’s correlations were determined for all pairings of variables. There were no significant correlations between the gametophytic and sporophytic variables (figure 10). Of the sporophytic variables, only three correlations were significant (correlation plots located in appendix). Both hot and cold photosynthesis had a slight positive correlation with HCMS (HPS Pearson’s correlation 0.19, CPS Pearson’s correlation 0.2). CCHPL was also weakly correlated with HCMS (Pearson’s correlation 0.15). There were three significant correlation coefficients between the gametophytic variables. Maximum and minimum pollen tube growth rate were positively correlated (Pearson’s correlation 0.45). Maximum and minimum pollen germination were negatively correlated (Pearson’s correlation -0.33). Maximum pollen tube growth rate and maximum pollen germination were positively correlated (Pearson’s correlation 0.3).

We conducted principal component analysis to further explore relationships among all variables and the sporophytic and gametophytic variables separately. For the full PCA, we included all gametophytic and sporophytic variables, except HPS and CPS. There were missing values for both photosynthesis and the gametophytic variables, due to plants dying or not flowering. To increase the sample size for the full PCA and incorporate both the sporophyte and gametophyte, we excluded the photosynthesis variables. The first three principal components accounted for 57% of the variation (full PCA plots and loadings located in the appendix). There was little divergence between regions. When the eigenvalues of the principal components were compared between region, PC2 was the only principal component with a significant difference between the regions (t58 = -2.69, p = 0.0092). CHPL loads primarily on PC2 and is likely driving the divergence between northern and southern plants. This pattern also occurs in the PCA for only sporophytic variables.

*Sporophytic PCA*

In the sporophytic PCA, the first three principal components explained 60% of the variation. HCMS and HPS primarily loaded on PC1 (Table 2, figure 11). PC2 was mostly influenced by CCHPL and PC3 by HCHPL. CCMS and CPS loaded evenly on two or more of the three axes. There was a significant difference between the regions for the eigenvalues extracted from both PC2 (t78 = -5.09, p = 2.39e-06) and PC3 (t101 = 2.38, p = 0.019). The divergence in PC2 can be explained by the opposite responses we observed for CCMS and both chlorophyll fluorescence treatments. Northern plants have a higher chlorophyll fluorescence ratio for both treatments, while southern plants had less cell membrane damage in the cold treatment. PC1 did divide HCMS and CCMS, suggesting an antagonistic relationship between the two variables, though there was no correlation between the two that was statistically significant. Hot and cold treatment variables were also divided on PC3. HPS and HCHPL were opposite in direction to CPS and CCHPL.

*Gametophytic PCA*

In the gametophytic PCA, the first three components explained 92.5% of the variance. Pollen germination variables divided the northern and southern plants. Tmax and Topt loaded evenly in the opposite direction of Tmin for both PC1 and PC2 (Table 3, figure 12). There was a significant difference between north and south for the eigenvalues extracted from PC2 (t46 = -3.17, p = 0.0025). PTGR variables loaded evenly on the first two principal components and in the same direction.

Chart

Description automatically generated

Figure 11. 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. Tables with principal component importance for PC1 through PC6 in the Appendix.

Table. Results from principal component analysis with sporophytic variables. Loadings for each of the variables on the principal components

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** | **PC6** |
| **HCMS** | 0.613999 | 0.02998 | 0.344975 | 0.147492 | 0.573797 | 0.390002 |
| **CCMS** | -0.35207 | 0.435008 | 0.204072 | -0.57534 | 0.520509 | -0.20789 |
| **HCHPL** | 0.284794 | -0.36797 | -0.33836 | -0.75803 | -0.08744 | 0.294536 |
| **CCHPL** | 0.117118 | -0.57752 | 0.579996 | -0.14114 | -0.03843 | -0.5431 |
| **HPS** | 0.577968 | 0.302596 | -0.40867 | 0.000171 | 0.044159 | -0.63673 |
| **CPS** | 0.264909 | 0.499375 | 0.470594 | -0.22955 | -0.6235 | 0.13244 |

Chart, radar chart

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Figure 12. Plot of the results of principal component analysis of the gametophytic variables. PC1 describes 48.32% of the variation and PC2 explains 27.31%. Tables with principal component importance for PC1 through PC6 in the Appendix.

Table 3. Results from principal component analysis with gametophytic variables. Loadings for each of the variables on the principal components

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** | **PC6** |
| **Germ.Tmin** | -0.01334 | 0.405643 | 0.812217 | 0.376238 | -0.00058 | -0.18446 |
| **Germ.Topt** | 0.418665 | -0.45436 | 0.390064 | -0.00262 | 0.001723 | 0.682727 |
| **Germ.Tmax** | 0.407763 | -0.5446 | 0.164764 | -0.10069 | -0.00179 | -0.707 |
| **PTGR.Tmin** | 0.367661 | 0.452838 | 0.127813 | -0.75119 | -0.28131 | 0.00071 |
| **PTGR.Topt** | 0.523981 | 0.308129 | -0.20261 | 0.123985 | 0.757676 | -0.00193 |
| **PTGR.Tmax** | 0.498538 | 0.180049 | -0.3219 | 0.518304 | -0.58888 | 0.001496 |