## *Oecologia* Supporting Information

**Article title**: Evidence of local adaptation in temperature tolerance traits of the gametophytic and sporophytic stages in *Solanum carolinense* (horsenettle)

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Chart, box and whisker chart

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**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.

Chart

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**Fig. S2** Daily max temperature for spring and summer of 2021 from the NOAA station at the Hector International Airport, Fargo, ND.

A picture containing timeline

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**Fig. S3** 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.

Chart

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**Fig. S4** 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).

Chart, diagram

Description automatically generated

**Fig. S5** 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).

Chart

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**Fig. S6** Pollen germination profiles for genets with at least 3 ramets that flowered. Color indicates region of origin.

Chart, box and whisker chart

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**Fig. S7** Comparison of pollen tube growth rate values extracted from a quadratic fit for the maximum, optimal, and minimum temperatures between northern and southern regions.

Timeline

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**Fig. S8** Genotype differences for the maximum (Tmax), optimal (Topt), and minimum (Tmin) pollen tube growth rate temperatures extracted form 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.

Chart

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**Fig. S9** Pollen tube growth rate profiles for genets with at least 3 ramets that flowered. Color indicates region of origin.

Chart

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**Fig. S10** Correlation matrix of all plants. Gametophytic (labels blue font) and sporophytic variables (labels red font) with significant Pearson’s correlations for all study plants.

Chart, bubble chart

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**Fig. S11** 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.

Chart, scatter chart

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**Fig. S12** Scatter plot of the significant correlations between sporophytic variables including plants from the northern and southern regions.

Graphical user interface, chart

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**Fig. S13** 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.

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**Fig. S14** 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.

Chart, radar chart

Description automatically generated

**Fig. S15** 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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | Population | | | Block | |
|  | Variable | F-value | df | p-value | LRT | p-value |
| Sporophyte | Cell Membrane Stability (Heat) | **5.563** | **188.06** | **2.97E-04** | **4.210** | **0.040** |
| Cell Membrane Stability (Cold) | **2.824** | **188.06** | **0.026** | **15.342** | **8.97E-05** |
| Chlorophyll Content (Heat) | 1.732 | 188.25 | 0.145 | 0.188 | 0.665 |
| Chlorophyll Content (Cold) | **32.341** | **188.22** | **2.20E-16** | 1.477 | 0.224 |
| Photosynthetic Rate (Heat) | 1.473 | 126.00 | 0.214 | -2.84E-04 | 0.214 |
| Photosynthetic Rate (Cold) | **3.717** | **138.74** | **6.62E-03** | 0.032 | 0.858 |
| Gametophyte | Pollen Germination (Tmax) | **6.069** | **3** | **1.17E-03** | - | - |
| Pollen Germination (Topt) | **6.861** | **3** | **5.02E-04** | - | - |
| 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.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Region | | | Block | | | Genet | |
|  | Variable | F-value | df | p-value | | LRT | p-value | LRT | p-value |
| Sporophyte | Cell Membrane Stability (Heat) | 3.673 | 1, 50 | 0.061 | | **4.728** | **0.030** | 3.320 | 0.068 |
| Cell Membrane Stability (Cold) | **6.482** | **1, 191** | **0.012** | | **15.731** | **7.30E-05** | - | - |
| Chlorophyll Content (Heat) | **4.418** | **1, 51** | **0.041** | | 0.222 | 0.637 | 0.018 | 0.892 |
| Chlorophyll Content (Cold) | **66.369** | **1, 50** | **9.97E-11** | | 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) | **12.054** | **1, 26** | **0.002** | | - | - | 3.181 | 0.075 |
| Pollen Germination (Topt) | **10.916** | **1, 24** | **0.003** | | - | - | 1.728 | 0.189 |
| Pollen Germination (Tmin)\* | 0.151 | 1, 21 | 0.702 | | - | - | **5.000** | **0.025** |
| 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.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Region | | Genet | |
| Variable | 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.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Block A | | Block B | | Block C | | Block D | |
|  | Variable | Method | t-stat | p-value | t-stat | p-value | t-stat | p-value | t-stat | p-value |
| Sporophyte | Cell Membrane Stability (Heat) | Paired | **-2.910** | **0.015** | -0.853 | 0.403 | -1.640 | 0.113 | -0.539 | 0.595 |
| Cell Membrane Stability (Cold) | Paired | 0.758 | 0.456 | **2.190** | **0.040** | **2.073** | **0.049** | 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 | **-5.889** | **3.82E-06** | **-4.746** | **9.77E-05** | **-5.982** | **3.50E-06** | **-4.106** | **4.33E-04** |
| 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) | **Yes** | **North** | **2.48E-04** |
| 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 | **0.371** | **0.456** | - |  |  |  |  |  |  |
| p-value | 0.576 | **0.003** | **2.18E-04** | - |  |  |  |  |  |  |
| 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 |
| TminGermination | -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 |