**Discussion**

***Sporophytic Variables***

If *Solanum carolinense* has locally adapted to the respective temperature regimes in TX and MN, we would expect that plants from the north would be more tolerant of cold temperatures and plants from the south would be more tolerant of hot temperatures. Rather than a clear-cut difference between north and south for hot and cold treatments, there were mixed results for the sporophytic variables. Plants from the south had a more stable cell membrane when exposed to an extreme cold treatment. Cold stress reduces the fluidity of the cell membrane and produces ROS that have the potential to oxidize lipids and damage the membrane (Valitova et al., 2019). Valitova et al. (2019) found that wheat seedlings upregulate the production of sterols and their incorporation in the cell membrane. Sterols, specifically 24-ethyl and 24-methyl sterols specific to plants, function by maintaining the membrane fluidity required for normal function (Dufourc, 2008a). In plants, the incorporation of sterols in the cell membranes are an adaptation that increases the temperature range that tissues can withstand and reduce the shock experienced at extreme temperatures (Dufourc, 2008b). There was a significant difference between genets for cell membrane stability hot treatment. The variation between genets could again be due to different levels of sterols incorporated in the cell membranes or variation in the proportion of saturated fatty acids in the cell membrane. When exposed to heat, the membrane becomes more fluid. Saturated fatty acids reduce the fluidity of the cell membrane at high temperatures and have been shown to be an adaptation for species growing in environments with high heat (Knight & Ackerly, 2001, 2002; Zhu et al., 2018). We expected that southern plants would have more stable membranes at the higher temperatures, since plants in TX experience much higher temperatures more regularly. This was not the case, suggesting that the populations haven’t been in the location long enough to evolve or do not possess the variation required for selection to act on the populations. Though there was a significant difference between all the genets, there was no significant difference between gents when the regions were separated from one another (Appendix) supporting the idea that there is little variation in traits related to cell membrane stability within populations of the respective regions.

Plants from the north had more stable chlorophyll fluorescence in both the hot and cold treatment. Less degradation of chloroplasts in heat and cold for northern plants may be explained by northern plants experiencing a larger range of temperatures allowing them to outperform the southern plants at both extreme high and extreme low temperatures. Since the temperate conditions of Minnesota are more variable, populations in the north may have evolved to have higher levels of temporary phenotypic plasticity, at least when it comes to chloroplast and chlorophyll stability than those in the south. The two regions also differed in variation within the regions. For the cold treatment, there was more variation in the south. Conversely, there was more variation in the north for the hot treatment. This may suggest that there is stabilizing selection occurring in the respective regions. There was a significant difference between the individual genets for chlorophyll fluorescence cold treatment. This difference might be driven by the difference observed between the two regions. However, when north and south were analyzed separately, genets from the south remained different from one another (Appendix).

There was no significant difference between northern and southern plants for net photosynthetic rate in both the hot and cold treatments. Net photosynthesis was the only sporophytic variable where the whole plant was placed in a temperature treatment and leaves were measured on the plant. The plant may compensate for temperature stress through physiological mechanisms, such as increasing transpiration. Therefore, the temperature treatments may not have stressed the plants to the extent that temperature tolerance for the northern and southern plants was distinguishable from one another.

***Gametophytic Variables***

To test gametophytic temperature tolerance in *Solanum carolinense*, we measured pollen performance variables over a temperature gradient. The variables of interest were pollen germination and pollen tube growth rate, both of which directly impact the capacity of a pollen grain to compete with other pollen grains and fertilize an ovule within a flower. We fit a quadratic curve to the measurements of each individual at five temperatures to extract information about the individual’s level of temperature tolerance, including maximum temperature (Tmax), optimal temperature (Topt), and minimum temperature (Tmin) of germination. Pollen germination was higher in pollen grains from the north than the south for both Tmax and Topt. This means that pollen from the north have a higher propensity to produce pollen tubes at high temperatures than their southern counterparts. The distinct difference between north and south suggests that there is sensitivity to high temperatures and likely an adaptive response occurring in the populations of the south. Rutley et al. (2022) proposed the two-baskets model categorizing pollen, which states that there are high-ROS (reactive oxygen species) and low-ROS subpopulations of pollen within anthers of flowering species. The low-ROS pollen have a lower metabolic rate than high-ROS pollen due to partial dehydration during development. The two subpopulations of pollen are adaptive as they allow for asynchrony in pollen germination, permitting some pollen to remain dormant in a stressful environment and grow pollen tubes later in more favorable conditions. Keller et al. (2018) found that *Solanum lycopersicum* (tomato) pollen had two responses during heat stress – direct and delayed translation. Luria et al. (2019) later showed that *Solanum lycopersicum* has pollen that fall in the low-ROS and high-ROS groups, supporting the two-basket model in a species closely related to *Solanum carolinense*. We hypothesis that *Solanum carolinense* populations in the south have higher proportions of low-ROS to high-ROS pollen grains than those in the north due to increased selective pressures of high temperatures in the south. Low-ROS pollen that remains dormant would not be adaptive in northern populations, with little exposure to high temperature stress.

There was no significant difference between northern and southern populations for Tmin. Pollen of *Solanum carolinense* may be constrained by a lower temperature limit for the physiological processes necessary for pollen tube growth. Though there was no difference between the plants form the two regions, there was a significant difference between individual genets. The presence of variation in pollen germination at low temperatures suggests that if the selective pressure were there, populations have the potential to evolve.

Pollen tube growth rate remained constant across regions and genets for the five temperatures. Since pollen tube growth rate is constrained by the physiological processes involved in cell division, there is likely little variation upon which selection can act.

***Correlation between the Gametophyte and Sporophyte***

Tanksley et al. (1981) first described the correlation between selection in the gametophyte and sporophyte when they found a correlation between allozymes expressed in both stages. Based on their findings and several studies that followed (Pedersen et al., 1987; Willing & Mascarenhas, 1984), including studies on temperature tolerance (Hedhly et al., 2005; Poudyal et al., 2019), we hypothesized that there would be a correlation between temperature tolerance in the sporophyte and the gametophyte. There were no significant correlations between any of the gametophytic and sporophytic variables, suggesting that there are different mechanisms mitigating temperature stress in the two stages. This is not the first study to find inconsistencies in the selection for cold tolerance in the sporophyte and gametophyte. Dominguez et al. (2005) conducted a study to determine if pollen selection can be used to improve cold tolerance in the gametophyte by selecting pollen from cold tolerant plants. They found that pollen selection did not improve pollen viability and formation in cold and explained their results by describing how the genes mediating cold stress may be expressed in the sporophyte tissue surrounding the site of pollen formation, rather than the pollen grains themselves. To resolve the discrepancies, we will need to understand the molecular underpinnings of temperature stress mitigation for both life stages.

**Conclusion**

We had mixed results for the sporophytic variables that did not clearly follow our predictions, suggesting that the response to temperature tolerance is less coordinated than we originally anticipated. Since all populations included in this study were located toward the edge of the range for this species, time for selective pressures to act on the populations may also be insufficient for local adaptation to occur in the sporophyte. Our results did show evidence of local adaptation in the gametophyte, indicating that the temperature difference between the northern and southern regions has led to differential selection in pollen. We found no evidence of a relationship between temperature tolerance in the sporophyte and the gametophyte. However, to fully understand how plants respond to temperature, we need to examine the underlying mechanisms involved in the temperature stress response in both the gametophyte and sporophyte.

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