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Variation in seedling freezing response is associated with climate in *Larrea*

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Abstract

Variation in freezing severity is hypothesized to have influenced the distribution and evolution of the warm desert evergreen genus *Larrea*. If this hypothesis is correct, performance and survival of species and populations should vary predictably along gradients of freezing severity. If freezing environment changes in the future, the ability of *Larrea* to adapt will depend on the structure of variation for freezing resistance within populations. To test whether freezing responses vary among and within *Larrea* populations, we grew maternal families of seedlings from high and low latitude *L. divaricata* and high latitude *L. tridentata* populations in a common garden. We measured survival, projected plant area and dark-adapted chlorophyll fluorescence (F_v/F_m) before and after cold acclimation and for 2 weeks following a single freeze. We detected significant variation in freezing resistance among species and populations. Maternal family lines differed significantly in their responses to cold acclimation and/or freezing for two out of the three populations: among *L. tridentata* maternal families and among low latitude *L. divaricata* maternal families. There were no significant differences across maternal families of high latitude *L. divaricata*. Our results indicate that increased freezing resistance in high latitude populations likely facilitated historical population expansion of both species into colder climates, but this may have occurred to a greater extent for *L. tridentata* than for *L. divaricata*. Differences in the structure of variation for cold acclimation and freezing responses among populations suggest potential differences in their ability to evolve in response to future changes in freezing severity.

Keywords

Freezing resistance; Freezing tolerance; Maternal families; Chihuahuan Desert; Monte Desert

Introduction

Freezing can limit seedling growth (Johnson et al. 2004) and establishment (Coop and Givnish 2008) and ultimately determine plant distributions (Pockman and Sperry 1997; Ewers et al. 2003). Freezing is particularly important for the distribution of warm desert evergreens because they maintain functional leaf area year-round and typically exhibit weak cold acclimation which may be rapidly lost during mid-winter warming periods (Sakai and Larcher 1987). Changes in freezing frequency and intensity have long been hypothesized to influence the encroachment of evergreen deserts into the semiarid grasslands of southwestern North America (Wells and Shields 1964; Hunziker et al. 1977; Van Auken 2000). Over the past 100 years, spring and winter temperatures have increased (Karl et al. 1996), along with a decrease in the number of frost days in North America over the period from 1951 to 2000 at a rate of 3 days per decade (Feng and Hu 2004). Moreover, global climate models project further increases in temperature in the century to come (Ruosteenoja et al. 2003). Therefore, understanding the impacts of freezing on warm desert evergreen populations is necessary to determine the extent to which release from freezing stress has influenced extant distributions, and whether climate change will alter their distributions in the future.

The evergreen shrub *Larrea tridentata* [(Sessé and Moc. ex DC.) Coville] is a dominant species in the Sonoran, Mojave and Chihuahuan Deserts of North America across a broad range of latitudes (Mabry et al. 1977). Its closest relative, *L. divaricata* (Cav.), is similarly distributed across latitude in the Monte Desert of South America (Ezcurra et al. 1991). Across the range of both species, freezing is nearly absent at low latitude and becomes more common at higher latitudes and elevations (Mabry et al. 1977). Though separated by 40° of latitude, *L. tridentata* and *L. divaricata* have only recently been distinguished as separate species (Hunziker et al. 1972), but are very similar physiologically and produce semi-sterile hybrids (Yang et al. 1977). Inability to tolerate the cold climate of the newly elevated Bolivian Andes during the latter part of the Quaternary period has been postulated as the cause for the disjunction of an ancestrally continuous distribution, allowing their eventual divergence (Hunziker et al. 1977).

Increased minimum temperature has also been suggested as a factor in the more recent expansion of *L. tridentata* into short grass prairies of the southwestern US, beginning with the end of the last ice age (Wells and Hunziker 1976). Since that time, *L. tridentata* has become a dominant species in the region, existing as three ploidy races which occur in distinctly different desert habitats. Although the ploidy races are sympatric in some areas, diploids typically occupy the cooler Chihuahuan Desert (Hunter et al. 2001) and are less vulnerable to freezing damage than either Sonoran Desert tetraploid or Mojave Desert hexaploid races (Pockman and Sperry 1997, 2000; Martínez-Vilalta and Pockman 2002; Yang 1967). Seedlings from the three ploidy races also exhibit differential responses to low temperature when grown in a common garden, consistent with genetic differentiation among source populations (Yang 1967). However, the utility of comparisons across ploidy races to understand freezing adaptation is confounded by the positive relationship between polyploidy and cell size (Stebbins 1971; Beaulieu et al. 2008), which increases the vulnerability of both living tissues (Limin and Fowler 2001) and xylem vessels to freezing (Davis et al. 1999; Maherali et al. 2009). In addition, putative ploidy has been shown to be a significant determinant of observed variation in xylem vessel size, which is negatively correlated with freezing tolerance across Sonoran, Mojave and Chihuahuan Desert *L. tridentata* populations (Tyler 2004). Therefore, studies which compare populations of similar ploidy are needed to test whether differential responses to freezing occur across populations of *Larrea* species in the absence of differences in ploidy.

Populations from dissimilar freezing environments may differ not only in their ability to survive freezing but also in reliance on tolerance versus resistance to freezing damage for survival. At low latitude, where freezing is rare and relatively mild, survival may be improved by increasing freezing tolerance, or the ability to recover from freezing damage. Freezing tolerance can be increased through investment in below-ground structures allowing for rapid re-growth or re-sprouting following freezing damage (Ewers et al. 2003). For seedlings, however, their small size likely reduces freezing tolerance. Freezing resistance, or the ability to prevent freezing damage, may evolve when rapid growth out of the seedling stage is not possible and/or at higher latitudes where freezing frequency and intensity are high. In addition, cold acclimation responses, common to freezing resistant species, can dramatically reduce freezing damage, allowing rapid recovery of pre-freezing performance levels (Sakai and Larcher 1987).

Trade-offs between freezing resistance and tolerance may arise as freezing frequency and intensity change (Agrawal et al. 2004). Increased investment in resistance may reduce growth rate and competitive ability in the absence of freezing, while plants which rely on freezing tolerance may incur a high fitness cost as freezing becomes more frequent or intense. In early life stages, when growth is paramount to survival, there may be strong selection against both over- and under-investment in freezing resistance. Furthermore, in the face of altered freezing intensity and frequency, populations may experience a mismatch between freezing intensity and investment in a freezing tolerance or resistance strategy.

If a trade-off exists between freezing tolerance and resistance, adaptation to a particular freezing environment may eliminate variation for freezing response among individuals within populations. Therefore, the ability of populations to evolve in response to novel freezing environments could be constrained by prior adaptation (Falconer and Mackay 1996). There is evidence that freezing responses differ among maternal families in cultivated species (for example, see Rapp and Junttila 2000), but information on variation in freezing response among maternal families within natural populations is generally lacking (Geber and Griffen 2003). Variation in damage following freezing has been observed among individual saguaro plants within a Sonoran Desert population (Niering et al. 1963); however, no published studies have examined variation in freezing responses among maternal families within any warm desert evergreen species. Studies which directly investigate trait variation among maternal families within populations are needed to determine the potential for changing selection regimes to result in adaptive shifts in freezing responses. In the case where the reliance on resistance versus tolerance varies within a population, freezing tolerant phenotypes may gain a competitive advantage in the event of climate warming, resulting in evolutionary change.

If *L. tridentata* and *L. divaricata* have adapted to temperature variation in the past, we would expect to find differences in freezing response among species and populations consistent with their climate of origin. If *Larrea* species are capable of further evolutionary response to freezing then we would expect to find variation for freezing response among maternal families within populations. To determine if adaptations to freezing are present within the genus *Larrea*, we asked three questions: (1) has there been differentiation in freezing response among these two closely related diploid species that is consistent with their geographic distribution, (2) has there been differentiation in cold acclimation or freezing response of diploid *L. divaricata* populations across latitude, and (3) is there variation for cold acclimation or freezing response among maternal families within populations? We measured variation in cold acclimation and freezing responses within and among diploid populations of *L. tridentata* and *L. divaricata*. To determine if the two species differ in freezing response, we compared *L. tridentata* and *L. divaricata* seedlings from high latitude populations representing the coldest sites known within their distributions. To determine if

populations of the same species differ in a manner consistent with adaptation, we compared the cold acclimation and freezing responses of *L. divaricata* seedlings from a high latitude population, where freezing is common, with low latitude seedlings from a population which only rarely experiences mild freezing. To quantify variation in freezing tolerance and resistance within populations, we measured freezing responses of 10 maternal families from each population. We also measured cold acclimation of 10 maternal families from both high and low latitude *L. divaricata* populations.

Materials and methods

Seed collection and plant propagation

We grew seedlings under common conditions to minimize the effects of environment-caused variation in cold acclimation and freezing responses, allowing a stronger test of differentiation between species, among populations, and among maternal families. Seeds were collected haphazardly from 30 maternal plants in 2007 at a high latitude *L. tridentata* site, and in 2008 at low and high latitude *L. divaricata* sites. The high latitude *L. divaricata* site is located at the far southern end of the Monte Desert at 42°31'S, 68°15'W near Bajada del Diablo, Chubut, Argentina (Fig. 1). The low latitude *L. divaricata* site is located at the far northern edge of the Monte Desert at 29°25'S, 66°52'W, near Chamental, La Rioja, Argentina (Fig. 1). *L. divaricata* consists of diploid populations throughout its range (Yang et al. 1977). The high latitude *L. tridentata* site is located at the northern edge of the Chihuahuan Desert, near the Five Points area of the Sevilleta LTER at 34°20'N, 106°45'W (Fig. 1). This population is located far from populations where tetraploid and diploid individuals are known to co-mingle and was assumed to consist only of diploid individuals based on studies by Yang et al. (1977) and Hunter et al. (2001).

Because seed storage reduces germination in *Larrea* (McGee and Marshall 1993), experiments were conducted immediately following each round of seed collection. Experiments for *L. tridentata* were conducted in January 2008, while those for *L. divaricata* were conducted in January 2009. Within 3 months of collection, seeds were planted in the University of New Mexico research greenhouse in soil containing a 4:1:1 mixture of sand, peat moss and Perlite. For the period of seedling growth, mean photosynthetic photon flux density in the greenhouse at noon was 1,124 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings were watered twice daily and fertilized with 1/4 strength Peters 20:20:20 (Scotts, Marysville, OH, USA) every 2 weeks.

Seedlings were randomly selected from maternal families using a random number table. Individual seedlings were given a unique number then randomly assigned to racks containing 50 seedlings each; individual racks were treated as blocks. Seedling location within each block was also randomized. Seedlings were grown for 3 months, during which time the racks were randomly assigned a new position in the greenhouse once per week.

Experimental design

Before temperature treatments began, chlorophyll fluorescence and projected plant area measurements were made for all plants; this pre-freezing measurement allows for each individual plant to serve as its own control. Because cold acclimation typically takes place in a field setting prior to freezing, and is known to increase freezing resistance (Sakai and Larcher 1987), *L. tridentata* and high and low latitude *L. divaricata* were cold acclimated before exposure to a single freeze. To characterize cold acclimation response, we measured chlorophyll fluorescence of 100 low and 100 high latitude *L. divaricata* seedlings following cold acclimation. Data on response to cold acclimation were collected only for high and low latitude *L. divaricata* plants. Following cold acclimation, seedlings were randomly assigned

to one of two temperature treatments: -8°C (50 seedlings) and -10°C (100 seedlings) for *L. divaricata*, and -10°C (50 seedlings) and -12°C (50 seedlings) for *L. tridentata*. Immediately following freezing, plants were returned to the greenhouse and thereafter experienced the same conditions as prior to cold acclimation. To characterize the freezing response, we measured chlorophyll fluorescence at 0–24 h and 1 week following freezing. We also measured survival and projected plant area at 1 week following freezing. To test whether populations differ in freezing resistance versus tolerance, we followed seedling recovery by measuring chlorophyll fluorescence again 2 weeks following freezing.

Low temperature treatments

Cold acclimation took place in a Conviron growth chamber (Model #E8; Controlled Environments, Winnipeg, Canada) for 7 days ($12/1^{\circ}\text{C}$ day/night temperature, 12-h day length at a light intensity of $1,160\ \mu\text{mol m}^{-2}\text{ s}^{-1}$). Freezing took place in a chamber designed to expose only the canopy portion of the plant to low temperatures, while maintaining a temperature of 6°C in the root zone (method described in Medeiros and Pockman 2011). Long-term temperature records from the high latitude *L. tridentata* site indicate that minimum temperatures between -9 and -12°C are typically reached 2–3 times per month during January and February (Moore 1989–2009), so high latitude *L. tridentata* seedlings experienced freezing of either -10 or -12°C . In preliminary tests, a single freeze of -12°C resulted in nearly 100% mortality of both high and low latitude *L. divaricata* seedlings, so temperature treatments were shifted to -10 and -8°C for both *L. divaricata* populations.

Chlorophyll fluorescence

Plant responses to cold acclimation and freezing treatments were quantified using dark-adapted chlorophyll fluorescence (F_v/F_m ; PSI Open Fluorcam, Model# FC 1000-H; Photon Systems Instruments, Brno, Czech Republic), a non-invasive measurement of photosynthetic capacity (maximum quantum yield) allowing for repeated measurements over time on the same individuals and the same leaves. F_v/F_m is strongly correlated with leaf dieback (Boorse et al. 1998) and with a more traditional, but destructive, measure of freezing damage, electrolyte leakage (Ehlert and Hinch 2008). Reductions in F_v/F_m may occur as part of cold acclimation, representing a response to cold temperatures rather than damage (Demmig-Adams and Adams 2006). To account for this, we measured pre-acclimation F_v/F_m , quantified the reduction in F_v/F_m following cold acclimation (in the absence of freezing damage; high and low latitude *L. divaricata* seedlings only) and followed the trajectory of F_v/F_m for all seedlings over the 2 weeks following freezing. This approach allowed us to distinguish reduced F_v/F_m due to freezing damage (these plants exhibited relatively slow and incomplete recovery of F_v/F_m) from reductions in F_v/F_m representing an acclimation response (plants exhibited more rapid and complete recovery of pre-freezing F_v/F_m). Plants were dark-adapted for 8 h before measurements. One side of the entire plant was imaged. We calculated F_v (variable fluorescence) from the equation: $(F_m - F_o)/F_m$ where F_o is the initial fluorescence and F_m is the maximum fluorescence (Maxwell and Johnson 2000).

Survival and projected plant area

Survival was monitored for 1 month following freezing. Because plants that died following freezing did so within a few days, we only report survival data for 1 week post-freezing.

Projected plant area was calculated before and 1 week after freezing from fluorescence images generated by the PSI software using Scion Image (Scion, Frederick, MD, USA) and an equation relating pixel area to leaf area.

Data analysis

Data were analyzed using SAS (v.9.2; SAS Institute, Cary, NC, USA). Non-significant block effects and non-significant interactions with block were removed from models to avoid over-specification.

Survival across populations following freezing of -10°C was examined using a logistic model with population as the independent variable. We also tested for differences in survival across maternal families using a logistic model, with maternal family and minimum temperature as the independent variables.

We tested for differences in responses to cold acclimation and freezing across populations using a series of repeated measures analyses (PROC GLM; Potvin 2001). Because F_v/F_m data represent the proportion of $F_m - F_o$ in relation to F_m , we analyzed arcsin-transformed F_v/F_m data. We tested for differences in F_v/F_m following cold acclimation across the two *L. divaricata* populations using two levels of time, pre-treatment and post-acclimation, with population as the independent variable; block was included in this analysis. For analysis of freezing treatments, only plants that survived freezing were included. Comparisons of F_v/F_m 0–24 h post-freeze are not included in analyses since the three populations were measured at different times within the first 24 h, and significant recovery or light damage can take place within this period following freezing (B. Logan, personal communication). Therefore, these data are presented in figures only to provide an indication of the reduction in this factor within the first 24 h following freezing. We tested for differences in F_v/F_m in response to freezing of -10°C across all three populations using population as the independent variable and three levels of time, pre-treatment, 1 week post-freeze and 2 weeks post-freeze. We tested for differences in projected plant area in response to freezing of -10°C using population as the independent variable and two levels of time, pre-treatment and 1 week post-freeze.

We also tested for differences among maternal families using repeated measures analysis. Mortality following freezing resulted in low sample sizes within maternal families of *L. divaricata*, so separate analyses of F_v/F_m and projected plant area were performed on each population. Maternal family was specified as a random effect for all analyses. Though some have recommended the use of PROC MIXED and the Kenward–Roger correction for random effects (Littell et al. 2006), we chose not to use this method since small sample sizes resulted in poor model fits using PROC MIXED, and there were no differences in which dependent variables were determined to be significant using PROC GLM versus PROC MIXED. Since different numbers of seedlings survived in each of the maternal families (see “Results” for details) we used a Satterthwaite approximation to account for the unbalanced design (PROC GLM; Potvin 2001). For each population, we used two levels of time, pre-treatment and 1 week post-treatment, with maternal family, minimum temperature and their interaction as the independent variables. In the high latitude *L. divaricata* population, the effect of block was included in the maternal family level analysis of cold acclimation response.

Results

Survival following freezing

There were significant differences between the three populations in survival following freezing to -10°C . High latitude *L. tridentata* seedlings had the highest survival, followed by high latitude *L. divaricata* (Fig. 2a; Table 1a). A pairwise comparison indicated that survival was significantly higher following the colder freezing treatment only ($P < 0.0001$) for high latitude compared to low latitude *L. divaricata* seedlings.

High latitude *L. tridentata* seedlings had high survival following freezing of both -10 and -12°C , and there was not a significant effect of minimum temperature on survival for this population (Table 1b). For *L. divaricata*, however, survival was significantly higher after freezing to -8 than -10°C for both high and low latitude seedlings (Table 1b).

There were no significant differences in survival across maternal families for the high latitude population of *L. tridentata* or *L. divaricata* (Table 1b). Within the low latitude *L. divaricata* population some maternal families had significantly higher survival than others (Fig. 2b; Table 1b).

Responses to cold acclimation

Both high and low latitude *L. divaricata* seedlings exhibited significantly reduced dark adapted chlorophyll fluorescence (F_v/F_m) following 7 days of cold acclimation (Fig. 3; Table 2a). Low latitude *L. divaricata* seedlings had significantly higher mean post-acclimation F_v/F_m than high latitude seedlings (Table 2a). There was a significant time $\times$ block effect on F_v/F_m , driven by the response of seedlings in the high latitude *L. divaricata* population, which also exhibited a significant effect of block when examined separately (see below).

F_v/F_m of high latitude *L. divaricata* seedlings was significantly reduced by cold acclimation (Table 2b), but there were no significant differences between maternal families. Among low latitude *L. divaricata* seedlings, F_v/F_m was also significantly reduced by cold acclimation (Table 2c). Though the overall effect of maternal family was not a significant factor in determining F_v/F_m following cold acclimation, there was a significant difference in the response of maternal families pre- to post-acclimation (Table 2c, time $\times$ maternal family effect).

Responses to freezing

All three populations exhibited a significant response of F_v/F_m (Table 3) and there were significant differences across the three populations in the response of F_v/F_m to a single freeze of -10°C . Seedlings from both *L. divaricata* populations had lower F_v/F_m than *L. tridentata* seedlings at every measurement period (Fig. 3), while high latitude *L. divaricata* seedlings exhibited smaller reductions in F_v/F_m following freezing compared to low latitude *L. divaricata*.

Prior to freezing treatments, *L. tridentata* seedlings had larger projected plant area ($7.83 \pm 5.12 \text{ cm}^2$) than either low latitude ($3.98 \pm 2.00 \text{ cm}^2$) or high latitude *L. divaricata* seedlings ($2.72 \pm 1.34 \text{ cm}^2$). Freezing of -10°C had a significant effect on projected plant area for all three populations (Table 3) and resulted in significant differences in projected plant area across the three populations (Table 3). The relative sizes of seedlings across the three populations, however, were similar pre- and post-freeze. High latitude *L. divaricata* seedlings were still the smallest, but had retained the greatest proportion of their pre-freeze plant area, with an average reduction in plant size of 52% (mean 1 week post-freeze projected plant area $1.41 \pm 0.93 \text{ cm}^2$). Greater reductions in projected plant area in response to freezing (61% reduction) occurred among high latitude *L. tridentata* (mean 1 week post-freezing projected plant area $4.75 \pm 4.75 \text{ cm}^2$) and low latitude *L. divaricata* (mean 1 week post-freezing projected plant area $2.43 \pm 2.00 \text{ cm}^2$).

Both high and low latitude *L. divaricata* responded more negatively to the -10°C freezing treatment than to the -8°C treatment (Fig. 2a), and there was a significant effect of temperature treatment on F_v/F_m for both high (Table 4a) and low latitude populations (Table 4b). There was also a significant effect of the interaction between maternal family and temperature treatment on projected plant area for low latitude *L. divaricata* (Table 4b). The

response of high latitude *L. tridentata* seedlings also differed significantly based on the intensity of freezing (Table 4c).

There were no significant differences in F_v/F_m across maternal families following freezing for any of the three populations (Table 4a–c). There was a significant effect of freezing on projected plant area for high latitude *L. divaricata* seedlings (Table 4a), but not a significant effect of maternal family. Among low latitude *L. divaricata*, there were significant differences between maternal families in the response of projected plant area to freezing (Table 4b, time $\times$ maternal family effect). This population exhibited a significant negative correlation between pre-freeze projected plant area and the change in projected plant area in response to freezing (Pearson product moment correlation = -0.41 , $P = 0.0361$). The relationship between pre-freeze plant size and freezing response was maintained when comparing maternal family lines. Pairwise comparisons (Tukey test, $\alpha = 0.05$) indicated that reduction in projected plant area pre- to post-freeze (following freezing of -10°C) was significantly greater for maternal family 3, which had the largest mean pre-freeze projected plant area (Fig. 4a), than for maternal family 6, which had an intermediate mean pre-freeze projected plant area.

In the high latitude *L. tridentata* population, there was also a significant negative correlation between pre-freeze plant size and the change in plant size following freezing (Pearson product moment correlation = -0.78 , $P < 0.0001$) in conjunction with a significant effect of maternal family on projected plant area following freezing (Table 4c, time $\times$ maternal family effect and maternal family effect). Pairwise comparisons (Tukey test, $\alpha = 0.05$) indicated that maternal families with significantly larger mean pre-freezing seedling size (4, 8 and 10) had significantly greater reductions in projected plant area following freezing to -12°C compared to family 9, which had a smaller mean pre-freeze seedling size. We observed re-sprouting in seedlings of five maternal families from the high latitude *L. tridentata* population, though post-hoc logistic regression revealed no significant differences across the maternal families in proportion of plants re-sprouting (data not shown).

Discussion

Variation in freezing response among species and populations

Growth in a common environment revealed differentiation in freezing responses between the two diploid *Larrea* species, with Chihuahuan Desert *L. tridentata* having higher freezing survival (Fig. 2a) and dark-adapted chlorophyll fluorescence (F_v/F_m ; Fig. 3) following freezing of -10°C than either population of *L. divaricata*. Though isozyme data indicate that the two species are closely related (co-efficient of genetic similarity above 0.93; Cortes and Hunziker 1997), our data suggest that functional trait divergence has taken place since their disjunction 0.6–1.2 million years ago (Cortes and Hunziker 1997). Although we did not directly measure genetic differences, the variation we observed among the three populations grown in a common garden are consistent with genetic differentiation in freezing resistance across *Larrea* populations experiencing dissimilar freezing intensity and frequency. The differences we observed between *L. divaricata* populations were consistent with higher freezing resistance in the high compared to the low latitude population. High latitude plants exhibited a stronger response to cold acclimation (greater reduction in F_v/F_m), accompanied by more rapid recovery of pre-freezing F_v/F_m (Fig. 3), smaller reductions in leaf area (see “Responses to freezing”) and higher survival following freezing to -10°C (Fig. 2a).

Though we did not compare high and low latitude *L. tridentata* in our study, other published accounts indicate that diploid adults and juveniles from lower latitudes have lower freezing resistance than seedlings of the population currently under study. Adult *L. tridentata* growing in the Chihuahuan Desert 180 miles to the south of our field site, at the Jornada

LTER, where a minimum temperature of -10°C is rare, experienced leaf loss following a freezing of -5°C (Gutschick and BassiriRad 2003). Furthermore, greenhouse-grown *L. tridentata* from Querétaro, México (20°N , mean minimum January temperature 5.4°C ; Hijmans et al. 2005), exhibit 40% mortality following exposure to single freezing of -8°C (Medeiros, unpublished data). In contrast, 98% of seedlings in our study survived freezing to -12°C . This provides further evidence that differentiation in freezing responses has occurred among diploids of the genus *Larrea*, and that increased freezing resistance of seedlings may have facilitated past establishment at higher latitudes and elevations.

We detected a trade-off between plant growth and freezing resistance across the two populations of *L. divaricata*, as has been demonstrated previously for other plant species (Clausen et al. 1941; Benowicz et al. 2000). Differential investment in construction of freezing-resistant xylem and/or leaves could have diverted resources away from the production of leaf area in high latitude plants, reducing their pre-freeze size compared to low latitude plants. Previous work has demonstrated differences in xylem vessel diameter (a trait related to xylem freeze-thaw embolism vulnerability) among *L. tridentata* populations experiencing different freezing regimes (Tyler 2004). A trade-off between xylem freeze-thaw embolism vulnerability and plant growth could arise because a relative reduction in vessel diameter also reduces water transport capacity (Hacke and Sperry 2001), which in turn has been shown to reduce relative growth rate of seedlings across a variety of woody species (Castro-Diez et al. 1998).

Trade-offs between growth rate and investment in leaf freezing resistance could also play a role in the patterns we observed. Though low latitude *L. divaricata* seedlings were larger than high latitude *L. divaricata* seedlings before freezing, they exhibited greater percent reductions in leaf area following freezing (see “Responses to freezing”) and never recovered pre-freeze values of F_v/F_m during the course of the study (Fig. 3). In contrast, high latitude *L. divaricata* seedlings were smaller but quickly recovered to pre-freeze values of F_v/F_m , within 2 weeks. The more rapid recovery of F_v/F_m following freezing among high compared to low latitude plants indicates that high latitude plants were better able to either resist or rapidly reverse the negative effects of freezing. The activation of photo-protective mechanisms could cause a reduction, followed by rapid recovery in F_v/F_m , such as we observed among high latitude plants (Adams and Demmig-Adams 2004). Differences between populations in the timing of F_v/F_m recovery might also indicate differential reliance on sustained versus rapidly reversing photo-protective mechanisms (Osmond 1994). Future work will be required, however, to determine the ultimate causes of the observed patterns in F_v/F_m and the potential effect of energetic requirements of slow and rapidly reversible photo-protective mechanisms on plant growth rate, as these data are lacking in the current literature.

Accumulation of cations, amino acids, soluble sugars and proline (known as osmolytes) during winter are also known to increase leaf freezing resistance (Walker et al. 2010). Investment in osmolytes could reduce seedling growth rate. Although a direct connection between the accumulation of such stress-related compounds and reduced growth rate is difficult to make, since stresses themselves can cause growth cessation, increased accumulation of proline results in reduced growth of yeast even in the absence of stress (Maggio et al. 2002).

Variation in freezing response within populations

Our data indicate that high latitude *L. divaricata* seedlings are freezing resistant enough to establish during most winters under current freezing regimes at their home field site (Fig. 1). Seedlings from this population were uniformly small, there were no differences across maternal families, and re-sprouting was not observed. Cold mean annual temperatures in

conjunction with very low precipitation at this site (Fig. 1) may make re-growth untenable following freezing, placing a higher premium on the development of freezing resistance. Low variability among maternal family lines within this population could indicate strong selection in the past, as has been suggested for other uniform-sized populations of perennials (Hiesey et al. 1942). Though we observed a freezing response consistent with adaptation, founder effects could also have reduced variation among maternal families in this population, which lies at the far southern edge of the *L. divaricata* distribution. Reduced variation among maternal families could result from reproductive isolation if populations became established at the limits of the range of pollinators, or if a different suite of pollinators is present at colder sites. *L. divaricata* is capable of self-fertilization, though outcrossing is strongly favored (Rossi et al. 1999), suggesting that even rare pollination events could counter genetic drift. Regardless of the mechanism of reduced variation, future evolution of freezing resistance and/or tolerance would be expected to occur slowly in this population. Similarity of freezing response across maternal families may also make the population as a whole more vulnerable to rare extreme events. The amount of variation within populations has been suggested as an important factor determining their ultimate fate in the face of changing climate (Jump and Penuelas 2005). In addition, given the relationship we observed between seedling size and freezing resistance, continued investment in freezing resistance may reduce the ability of seedlings to compete with faster growing species in the event that freezing becomes less frequent.

Among low latitude *L. divaricata* seedlings, freezing survival was greatly reduced just below the long-term minimum for their home field site of -8°C (Fig. 2a), suggesting that even small increases in minimum temperature could result in increased establishment at this site. Freezing resistance was lowest in this population; however, we observed a significant negative correlation between pre-freeze plant size and the change in plant size in response to freezing (Fig. 4a). Freezing resistance was higher among maternal families with smaller seedlings; maternal families with the smallest mean pre-freeze projected plant area exhibited the smallest reductions in plant area in response to freezing. This suggests a trade-off between investment in high relative growth rate and investment in freezing resistance. Freezing tolerance, on the other hand, may be favored by a high relative growth rate, since larger plants are likely to have larger root systems and larger reserves, allowing them to better accommodate re-growth following freezing damage. The relative rarity of low temperature events at this site may drastically reduce the fitness benefits of freezing resistance if investment in resistance reduces competitive ability. In contrast, weak freezing resistance could place fewer constraints on plant growth if freezing frequency and intensity decrease, such that freezing tolerance may evolve at low latitude where freezing is mild and rare. Significant variation for cold acclimation response, freezing survival, and freezing response among maternal families in this population (Figs. 2b, 4a) leaves open the possibility of the evolution of increased freezing tolerance and expansion into higher elevation sites. Our data on freezing tolerance should be interpreted with some caution, however, given that recovery from freezing was measured over only a short time period.

Our data indicate that nearly 100% of *L. tridentata* seedlings can survive winter temperatures close to the mean yearly minimum temperature at their home field site ($-14.4 \pm 1.1^{\circ}\text{C}$; Moore 1999–2009). Germination of *L. tridentata* typically occurs in the fall (Beatley 1974), and establishment at the current field site under study occurs rarely (Moore 1999–2009), however, during warmer than average winters (Moore 1989–2009). Our study suggests that establishment may increase with increasing mean minimum temperatures. It is important to note that we also observed a strong negative relationship between plant size and the relative strength of freezing resistance versus freezing tolerance within this population (Fig. 4b), again indicating a trade-off between investment in relative growth rate and investment in freezing resistance. Furthermore, we detected significant variation in

resistance versus tolerance among maternal families (Fig. 4b), suggesting that this population could evolve in response to natural selection by freezing. Again, interpretation of our data on freezing tolerance must proceed with caution, given the short period that time plant recovery was measured following freezing. Our data do show, however, that differences in response to climate warming may exist between maternal families with smaller, more freezing-resistant seedlings and those with larger, potentially more freezing-tolerant, seedlings. As freezing becomes rarer and less intense, the selective advantage of freezing resistance may decrease, resulting in reduced representation of freezing-resistant lineages, while establishment of freezing-tolerant lineages may increase.

Our experiment provides evidence for variation in freezing response among diploid *Larrea* species and populations consistent with adaptation to minimum temperature. Given the current study design, however, we are unable to determine the relative contributions of genetic and maternal environmental effects to the differences in freezing response we observed among populations and maternal families. Though variation in the responses of first-generation seedlings grown in a common garden is consistent with genetic differentiation among populations, future studies are needed to reveal what, if any, genetic differences underlie the variation we observed. Few studies have investigated maternal environmental effects on freezing resistance; however, in *Picea abies*, changes in the timing of cold acclimation, de-hardening and spring bud set were associated with changes in gene transcription in seedlings experiencing cold versus warm temperatures during embryogenesis (Johnsen et al. 2005). Future experiments investigating the effects of cold acclimation and freezing on seedlings grown from seeds produced in a greenhouse common garden would be required to tease apart maternal effects from the effects of climate per se.

In conclusion, we provide support for the hypothesis that minimum temperature has played a role in determining the distribution of the genus *Larrea*. Our results also indicate that the evolution of higher freezing tolerance and/or resistance have also likely facilitated establishment of populations at higher latitudes or elevations. Finally, the structure of within-population variation in freezing resistance and tolerance reported here warrants further study because it suggests that some populations may be better poised than others to evolve in response to changing climate conditions in the future.

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References

- Adams, WW., III; Demmig-Adams, B. Chlorophyll fluorescence as a tool for monitoring plant response to the environment. Massachusetts: Springer; 2004.
- Agrawal AA, Conner JK, Stinchcombe JR. Evolution of plant resistance and tolerance to frost damage. *Ecol Lett.* 2004; 7:1199–1208.
- Beatley JC. Phenological events and their environmental triggers in Mojave Desert ecosystems. *Ecology.* 1974; 55:856–863.
- Beaulieu JM, Leitch IJ, Patel S, Pendharkar A, Knight CA. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytol.* 2008; 179:975–986. [PubMed: 18564303]

- Benowicz A, Guy RD, El-Kassaby YA. Geographic pattern of genetic variation in photosynthetic capacity and growth in two hardwood species from British Columbia. *Oecologia*. 2000; 123:168–174.
- Boorse GC, Bosma TL, Meyer A-C, Ewers FW, Davis SD. Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. *Int J Plant Sci*. 1998; 159:513–521.
- Castro-Diez P, Puyravaud JP, Cornelissen JHC, Villar-Salvador P. Stem anatomy and relative growth rate in seedlings of a wide range of woody plant species and types. *Oecologia*. 1998; 116:57–66.
- Clausen J, Keck DD, Hiesey WM. Regional differentiation in plant species. *Am Nat*. 1941; 75:231–250.
- Coop JD, Givnish TJ. Constraints on tree seedling establishment in montane grasslands of the Valles Caldera, New Mexico. *Ecology*. 2008; 89:1101–1111. [PubMed: 18481534]
- Cortes MC, Hunziker JH. Isozymes in *Larrea divaricata* and *Larrea tridentata* (Zygophyllaceae): a study of two amphitropical vicariants and autopolyploidy. *Genetica*. 1997; 101:115–124. [PubMed: 16220366]
- Davis SD, Sperry JS, Hacke UG. The relationship between xylem conduit diameter and cavitation caused by freezing. *Am J Bot*. 1999; 86:1367–1372. [PubMed: 10523278]
- Demmig-Adams B, Adams WW III. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytol*. 2006; 172:11–21. [PubMed: 16945085]
- Ehlert B, Hinch DK. Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in *Arabidopsis* leaves. *Plant Methods*. 2008; 4:12. [PubMed: 18505561]
- Ewers FW, Lawson MC, Bowen TJ, Davis SD. Freeze/thaw stress in *Ceanothus* of southern California chaparral. *Oecologia*. 2003; 136:213–219. [PubMed: 12740694]
- Ezcurra E, Montaña C, Arizaga S. Architecture, light interception and distribution of *Larrea* species in the Monte Desert, Argentina. *Ecology*. 1991; 72:23–34.
- Falconer, DS.; Mackay, TFC. Introduction to quantitative genetics. 4th edn. Essex: Pearson; 1996.
- Feng S, Hu Q. Changes in agro-meteorological indicators in the contiguous United States: 1951–2000. *Theor Appl Climatol*. 2004; 78:247–264.
- Geber MA, Griffen LR. Inheritance and natural selection on functional traits. *Int J Plant Sci*. 2003; 164:S21.
- Gutschick VP, BassiriRad H. Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytol*. 2003; 160:21–412.
- Hacke UG, Sperry JS. Functional and ecological xylem anatomy. *Perspect Plant Ecol Evol Syst*. 2001; 4:97.
- Hiesey WM, Clausen J, Keck DD. Relations between climate and intra-specific variation in plants. *Am Nat*. 1942; 76:5–22.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol*. 2005; 25:1965–1978.
- Hunter KL, Betancourt JL, Riddle BR, Van Devender TR, Cole KL, Spaulding WG. Ploidy race distributions since the Last Glacial Maximum in the North American desert shrub, *Larrea tridentata*. *Glob Ecol Biogeogr*. 2001; 10:521–533.
- Hunziker JH, Palacios RA, de Valesi AG, Poggio L. Species disjunctions in *Larrea*: evidence from morphology, cytogenetics, phenolic compounds, and seed albumins. *Ann Mo Bot Gard*. 1972; 59:224–233.
- Hunziker, JH.; Palacios, RA.; Poggio, L.; Naranjo, CA.; Yang, TW. Geographic distribution, morphology, hybridization, cytogenetics, and evolution. In: Mabry, TJ.; Hunziker, JH.; Difeo, D., editors. Creosote bush: biology and chemistry of *Larrea* in New World deserts. Dowden: Hutchinson and Ross, Stroudsburg; 1977. p. 10-46.
- Johnsen O, Fosdal CG, Nagy N, Møllmann J, Dæhlen OG, Skråppa T. Climatic adaptation in *Picea abies* progenies is affected by the temperature during zygotic embryogenesis and seed maturation. *Plant Cell Environ*. 2005; 28:1090–1102.

- Johnson DM, Germino MJ, Smith WK. Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings below and above the alpine timberline. *Tree Physiol.* 2004; 24:377–386. [PubMed: 14757577]
- Jump AS, Peñuelas J. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecol Lett.* 2005; 8:1010–1020.
- Karl TR, Knight RW, Easterling DR, Quayle RG. Indices of climate change for the United States. *Bull Am Meteorol Soc.* 1996; 77:279–292.
- Limin AE, Fowler DB. Inheritance of cell size in wheat (*Triticum aestivum* L.) and its relationship to the vernalization loci. *Theor Appl Genet.* 2001; 103:277–281.
- Littell, RC.; Milliken, GA.; Stroup, WW.; Wolfinger, RD.; Schabenberger, O. SAS for mixed models. 2nd edn. Cary: SAS Institute; 2006.
- Mabry, TJ.; Hunziker, JH.; DiFeo, DR., editors. Creosote bush: biology and chemistry of *Larrea* in New World deserts. Stroudsburg: Dowden, Hutchinson, & Ross; 1977.
- Maggio A, et al. Does proline accumulation play an active role in stress-induced growth reduction? *Plant J.* 2002; 31:699–712. [PubMed: 12220262]
- Maherali H, Walden AE, Husband BC. Genome duplication and the evolution of physiological responses to water stress. *New Phytol.* 2009; 184:721–731. [PubMed: 19703115]
- Martínez-Vilalta J, Pockman WT. The vulnerability to freezing-induced xylem cavitation of *Larrea tridentata* (Zygo-phylaceae) in the Chihuahuan desert. *Am J Bot.* 2002; 89:1916–1924. [PubMed: 21665620]
- Maxwell K, Johnson GN. Chlorophyll fluorescence—a practical guide. *J Exp Bot.* 2000; 51:659–668. [PubMed: 10938857]
- McGee KP, Marshall DL. Effects of variable moisture availability on seed germination in three populations of *Larrea tridentata*. *Am Midl Nat.* 1993; 130:75–82.
- Medeiros JS, Pockman WT. Drought increases freezing tolerance of both leaves and xylem of *Larrea tridentata*. *Plant Cell Environ.* 2011; 34:43–51. [PubMed: 20825578]
- Moore, D. Sevilleta LTER meteorological data. 1989–2009. <http://sev.lternet.edu>
- Moore, D. Sevilleta *Larrea* seedling monitoring study. 1999–2009. <http://sev.lternet.edu>
- Niering WA, Whittaker RH, Lowe CH. The Saguaro: a population in relation to the environment. *Science.* 1963; 142:15–23. [PubMed: 17812501]
- Osmond, CB. What is photoinhibition? Some insights from comparisons of sun and shade plants. In: Baker, NR.; Bowyer, JR., editors. Photoinhibition of photosynthesis: from molecular mechanisms to the field. Oxford: BIOS; 1994. p. 1–24.
- Pockman WT, Sperry JS. Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia.* 1997; 109:19–27.
- Pockman WT, Sperry JS. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Am J Bot.* 2000; 87:1287–1299. [PubMed: 10991900]
- Potvin, C. ANOVA: experimental layout and analysis. In: Scheiner, SM.; Gurevitch, J., editors. Design and analysis of ecological experiments. 2nd edn. New York: Oxford University Press; 2001. p. 63–76.
- Rapp K, Junttila O. Heritability estimates of winter hardiness in white clover based on field and laboratory experiments. *Acta Agric Scand B Soil Plant Sci.* 2000; 50:143–148.
- Rossi BE, Debandi GO, Peralta IE, Palle EM. Comparative phenology and floral patterns in *Larrea* species (Zygophylla-ceae) in the Monte desert (Mendoza, Argentina). *J Arid Environ.* 1999; 43:213.
- Ruosteenoja, K.; Carter, TR.; Jylha, K.; Tuomenvirta, H. Future climate in world regions: an intercomparison of model-based projections for the new IPCC emissions scenarios. Vol. vol 2010. Finnish Environment Institute; Helsinki: 2003. <http://www.environment.fi/download.asp?contentid=25835>
- Sakai, A.; Larcher, W. Frost survival of plants. Berlin: Springer; 1987.
- Stebbins, GL. Chromosomal evolution in higher plants. London: Edward Arnold Ltd; 1971.

- Tyler, AP. Regional variation in xylem anatomy of a wide-ranging desert shrub, *Larrea tridentata*. University of New Mexico: Master's Thesis; 2004.
- Van Auken OW. Shrub invasions of North America semiarid grasslands. *Annu Rev Ecol Syst.* 2000; 31:197–215.
- Walker DJ, Romero P, Correal E. Cold tolerance, water relations and accumulation of osmolytes in *Bituminaria bituminosa*. *Biol Plant.* 2010; 54:293–298.
- Wells PV, Hunziker JH. Origin of Creosote bush (*Larrea*) deserts of Southwestern North America. *Ann Mo Bot Gard.* 1976; 63:843–861.
- Wells PV, Shields LM. Distribution of *Larrea divaricata* in relation to a temperature inversion at Yucca Flat, southern Nevada. *Southwest Nat.* 1964; 9:51–55.
- Yang TW. Ecotypic variation in *Larrea divaricata*. *Am J Bot.* 1967; 54:1041–1044.
- Yang TW, Hunziker JH, Poggio L, Naranjo CA. Hybridization between South American Jarilla and North American diploid Creosotebush (*Larrea*, *Zygophyllaceae*). *Plant Syst Evol.* 1977; 126:331–346.

Fig. 1.

Climate at the three sites where seeds were collected. High latitude *L. divaricata*, Bajada del Diablo Argentina; low latitude, Chamental, Argentina; high latitude *L. tridentata*, Sevilleta LTER, USA. Bars indicate mean monthly precipitation; lines indicate mean maximum and minimum monthly temperature. Agreement between the WORLDCLIM data (Hijmans et al. 2005) and those from nearby Five Points meteorological station (~2 km away Moore 1999–2009) was generally very good

Fig. 2. Survival of *L. divaricata* and *L. tridentata* following freezing **a** across populations and **b** across maternal families of low latitude *L. divaricata*. *Open bars* represent seedling survival following freezing to -8°C , *black bars* represent survival following freezing to -10°C and *gray bars* represent survival following freezing to -12°C

Fig. 3. Time course of dark-adapted chlorophyll fluorescence (F_v/F_m) in high and low latitude *L. divaricata* and high latitude *L. tridentata* seedlings. Error bars standard error

Fig. 4. Projected plant area before and 1 week post-freeze across maternal families of a low latitude *L. divaricata* -10°C, and b high latitude *L. tridentata* following freezing to -12°C. Note the difference in scale for the two panels. Maternal families are ranked from *left to right* in order of mean pre-freeze projected plant area. *Error bars* standard error

Table 1

χ^2 statistics from logistic regression testing for differences in survival

Independent variables	Dependent variable	
	<i>df</i>	Survival (χ^2)
(a) Across populations		
Population	2	24.46 ***
<i>n</i>	250	
(b) Across maternal families		
<i>L. tridentata</i> : high latitude		
$T_{\min}$	1	0.01
Maternal family	9	0.01
<i>n</i>	100	
<i>L. divaricata</i> : high latitude		
$T_{\min}$	1	17.07 ***
Maternal family	9	3.70
<i>n</i>	150	
<i>L. divaricata</i> : low latitude		
$T_{\min}$	1	19.26 ***
Maternal family	9	17.70 *
<i>n</i>	150	

(a) Across the three populations, high latitude *L. tridentata* and high and low latitude *L. divaricata*, following a single freeze of -10°C . (b) Across maternal families of all three populations

* $P < 0.05$;

*** $P < 0.0001$

Table 2

F statistics from repeated measures ANOVA testing for differences in dark-adapted chlorophyll fluorescence (F_v/F_m) following 7 days cold acclimation

Independent variables	Dependent variable	
	<i>df</i>	F_v/F_m (<i>F</i>)
(a) Across populations		
MANOVA		
Time	1	552.02 ***
Time × population	1	7.70 *
Time × block	3	4.25 **
Repeated measures		
Population	1	8.73 **
Block	3	0.35
<i>n</i>	200	
(b) Across maternal families: high latitude <i>L. divaricata</i>		
MANOVA		
Time	1	647.09 ***
Time × maternal family	9	1.71
Time × block	3	5.22 **
Repeated measures		
Maternal family	9	0.92
Block	3	2.88 *
<i>n</i>	100	
(c) Across maternal families: low latitude <i>L. divaricata</i>		
MANOVA		
Time	1	1098.96 ***
Time × maternal family	9	2.28 *
Repeated measures		
Maternal family	9	0.64
<i>n</i>	100	

(a) Across populations of high and low latitude *L. divaricata*. (b) Across maternal families of high latitude *L. divaricata*. (c) Across maternal families of low latitude *L. divaricata*

* $P < 0.05$;

** $P < 0.01$;

*** $P < 0.0001$

Table 3

F statistics from repeated measures ANOVA testing for differences in dark-adapted chlorophyll fluorescence (F_v/F_m) and projected plant area across the three populations, high latitude *L. tridentata* and high and low latitude *L. divaricata*, following a single freeze of -10°C

Independent variables	Dependent variable		
	<i>df</i>	F_v/F_m (<i>F</i>)	Projected plant area (<i>F</i>)
MANOVA			
Time	2	54.78 ***	53.34 ***
Time $\times$ population	4	12.63 ***	0.84
Repeated measures			
Population	2	36.23 ***	21.46 ***
<i>n</i>	112		

 $P < 0.0001$

Table 4

F-statistics from repeated measures ANOVA testing for differences in dark-adapted chlorophyll fluorescence (F_v/F_m) and projected plant area following freezing across maternal families

Independent variables	Dependent variables		
	<i>df</i>	F_v/F_m (<i>F</i>)	Projected plant area (<i>F</i>)
(a) High latitude <i>L. divaricata</i> Bajada del Diablo			
MANOVA			
Time	1	23.56 ***	69.46 ***
Time × maternal family	9	0.67	0.86
Time × T_{min}	1	1.20	1.10
Time × maternal family × T_{min}	9	1.42	1.04
Repeated measures			
Maternal family	9	0.50	1.05
T_{min}	1	4.17 *	1.78
Maternal family × T_{min}	9	0.21	0.80
<i>n</i>	85		
(b) Low latitude <i>L. divaricata</i> Chamical			
MANOVA			
Time	1	58.50 ***	82.98 ***
Time × maternal family	9	1.46	2.29 *
Time × T_{min}	1	9.24 **	7.67 **
Time × maternal family × T_{min}	7	0.38	2.80 *
Repeated measures			
Family	9	0.97	1.44
T_{min}	1	1.31	2.52
Maternal family × T_{min}	7	1.07	0.85
<i>n</i>	71		
(c) High latitude <i>L. tridentata</i> Sevilleta LTER			
MANOVA			
Time	1	16.67 **	55.86 ***
Time × maternal family	9	0.44	3.32 **
Time × T_{min}	1	0.32	7.53 **
Time × maternal family × T_{min}	9	1.10	2.22 *
Repeated measures			
Maternal family	9	1.17	12.89 ***
T_{min}	1	1.61	8.55 **
Maternal family × T_{min}	9	0.92	1.38
<i>n</i>	98		

(a) A high latitude *L. divaricata*. (b) Low latitude *L. divaricata*. (c) High latitude *L. tridentata*. Both within subject (MANOVA) and between subject (repeated measures) results are shown

*
 $P < 0.05$;

**
 $P < 0.01$;

 $P < 0.0001$