NPSNM Grant Report 2016 – I*s species identity or source climate a better predictor of seed germination for native forbs in the Southwest U.S.?: Implications for restoration*

Master's Thesis in the Program of Plant Biology and Conservation

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The funds provided to me by the Native Plant Society of New Mexico annual grant went towards materials needed for germination trials for my thesis research. With the money provided, I was able to purchase thousands of petri plates, opaque containers needed for dark stratification, seed dissection kits, and agar for petri plates. Without these materials I would not have been able to complete the germination trials necessary for my research. Below is the full thesis chapter with the results of my research, which was made possible through the generosity of the Native Plant Society of New Mexico.

Introduction

The next chapter of our environmental history will be defined by ecological restoration (hereafter referred to as 'restoration'). Urban expansion, development, and the influx of invasive species have led to the need for restorations across the United States (Haidet and Olwell 2015). In the last couple of decades there has been an increase in efforts to restore degraded habitats. When thinking about how best to restore plant communities, both short-term and long-term goals must be considered. One short-term goal of a restoration is high establishment success and one long-term goal is obtaining a diverse and resilient community (Johnson et al. 2010). However, before the short-term goal of high establishment success can be achieved, earlier plant life stages must be considered.

As the earliest life stages of a plant, dormancy break and germination have been shown to lead to potential bottlenecks in the restoration process if the seed is not appropriate for the restoration site (Larson et al. 2015). To restore a degraded system to a diverse and resilient community, restoration practitioners must determine the most appropriate seed source that will lead to high germination and subsequent high establishment success. This means using 'the right seed in the right place at the right time' to restore viable communities (Plant Conservation Alliance 2015). In addition to appropriate seed source, diversity of species must be considered. Plant diversity has strong positive effects on the productivity of ecosystem functioning (Tilman et al. 2001). A stronger focus on including forb diversity has been suggested as a way to maintain community functioning on restored grasslands (USDA Forest Service 2012). Determining the appropriate forb species, seed source, and timing of implementation involves understanding the factors that control and drive seed dormancy and germination.

Seed dormancy is the first trait expressed in the life cycle of plants. This important life history trait controls the timing of germination to ensure maximum fitness for the seedling in habitats with unfavorable, extreme, or unpredictable climates (Baskin and Baskin 2001, Donohue et al. 2005, Finch-Savage and Leubner-Metzger 2006, Née et al. 2016). Dormancy is a characteristic of the seed itself and defines what conditions are necessary for the seed to germinate to ensure the greatest probability of survival and growth for the seedling

(Vleeshouwers et al. 1995). Dormancy is regulated by genetics, environmental conditions of the maternal plant, and environmental conditions of the seed. The genetic mechanisms that regulate seed dormancy involve a balance between the hormones abscisic acid and gibberellins (Finch-Savage and Leubner-Metzger 2006). However, when genetic regulators interact with environmental cues, seeds may adapt to variation across different environments (Footitt et al. 2014). Among species, seeds have adapted to the environment through different types of dormancy regulation since the origin of seed plants (Willis et al. 2014).

Species that have a similar phylogenetic history tend to share common traits (Cavender-Bares et al. 2009). Taxonomically-related species and genera often exhibit similar germination patterns independent of geographic distribution and climatic conditions (Thompson et al. 1979), and seed dormancy and germination traits are closely related to phylogeny (Baskin and Baskin 2001, Figueroa and Armesto 2001, Bu et al. 2008, Wang et al. 2009, Burns and Strauss 2011). Previous studies have shown phylogenetic patterns of seed germination in multiple habitats and continents. For example, seed germination requirements of species growing in a wide range of habitat types in England displayed stronger similarities within families than between families (Grime et al. 1981), and in southern Chile, phylogenetic relatedness had a significant effect on interspecific variation in germination timing for 44 species inhabiting a rainforest (Figueroa and Armesto 2001). As evidenced in these studies, seed properties, such as dormancy and germination patterns, are related to phylogenetic distance between species. However, the ecological and geographic distribution of species also influences seed behavior and seed trait adaptation (Nikolaeva 1999). Only a few studies (e.g. (Wang et al. 2009, Xu et al. 2014) have begun to disentangle the relationships between phylogenetic relatedness, life history, and environmental conditions on seed dormancy and germination. Wang et al. and Xu et al. found that both phylogenetic attributes and habitat are major factors contributing to differences in germination percentage and mean germination time among species. However, these two studies primarily examined among-species differences and did not study within-species differences at the population level. Understanding within-species differences in germination is important to understanding how plant populations adapt to the local environment.

Plant populations have shown adaptation to their home environment (Turesson 1922, Linhart and Grant 1996, Kramer and Havens 2009), with local populations outperforming non-local populations when grown under local environmental conditions (Clausen et al. 1941, Leimu and Fischer 2008, Hereford 2009). Patterns in dormancy and germination have shown adaptation to habitat (Ratcliffe 1961, Baskin and Baskin 1971), and have been shown to follow environmental gradients based on latitude (Wagmann et al. 2012), elevation (Fernández-Pascual et al. 2013, Meyer et al. 1995), and temperature (Meyer and Kitchen 1994, Meyer et al. 1995, Pendleton and Meyer 2004, Wagmann et al. 2012, Pendleton and Pendleton 2014). Additionally, dormancy is a highly heritable trait and under strong selective pressure due to the relationship with post-germination trait adaptation and fitness (Baskin and Baskin 2001, Donohue et al. 2010, Walck et al. 2011, Wagmann et al. 2012). Dormancy and germination are essential life history characteristics (Baskin and Baskin 2001, Fenner and Thompson 2005) – once dormancy is broken, and germination has started, the embryo is irreversibly committed to growth. The right environmental conditions can result in germination and survival, and the wrong conditions can result in lack of germination and seedling mortality (Larson et al. 2015). Temperature and precipitation are two major selective forces involved in breaking dormancy and inducing germination (Fenner and Thompson 2005), and they are perhaps the most important

environmental variables for germination cueing and subsequent seedling establishment in suitable climatic conditions (Probert 2000, Walck et al. 2011).

Restoration decisions must identify the correct source material for the environment in which the future project will occur (Hufford and Mazer 2003), as the success and resilience of a restoration is contingent upon sourcing seed from ecologically similar habitats. Seeds used in a restoration project must be adapted to the environmental conditions of the site to ensure the best chances for germination, establishment, and survival. Understanding what drives germination patterns at both the species and population levels is vital to determining seed source and seasonal timing for restoration projects. Some species have particular germination requirements easily circumscribed by the phylogenetic history of the species or family. For example, species in the Fabaceae have physical dormancy and need scarification before they are able to break dormancy (Baskin and Baskin 2001). Furthermore, if the germination patterns of a population are not adapted to particular environmental conditions, then the restoration may fail. Failure to identify the correct seed source and provide the conditions necessary for dormancy break can lead to failed germination, seedling death, or future collapse of the population, resulting from ill-adapted phenotypes (Kramer and Havens 2009).

Time and resources are usually limited for identifying the most suitable source material and developing seed transfer zones that define areas of adaptation within which seeds can be moved with minimal risk of maladaptation (Kramer and Havens 2009, Bower et al. 2014). In many regions seed is often sourced across large geographic and/or environmental distances due to limited availability and economic constraints. This is especially prevalent in the western United States, where there is often less time and funding for the large-scale restoration projects, and local native seed is seldom available (Peppin et al. 2010). It can thus be useful to define the range of germination requirements present among populations of restoration-relevant species to ensure restoration practices support seedling establishment and positive long-term outcomes.

The research presented here builds off of previous phylogenetic and climatic studies, but uses a novel approach of incorporating multiple species and populations to examine both among- and within-species differences in germination. The methods presented in this study allow for the consideration of species relatedness alongside environmental correlates to determine which of these factors is the greater driver in germination differences at the species level and at the population level.

To determine among- and within-species variation in seed germination requirements, I used seed collections from multiple populations of nine forb species native to the southwestern United States to test the following hypotheses: (i) populations from climatically similar habitats will exhibit similar germination patterns regardless of species relatedness; and (ii) intraspecific variation in germination will be present across treatments.

Methods

Study System

This study was undertaken in the Southwest United States, which included areas in Colorado, Utah, Arizona, and New Mexico. The weather patterns and elevation vary significantly across the extent of the southwestern U.S. Climatic conditions of most of the region are classified as semi-arid and fluctuate from hot summers with monsoonal rains in the south to cold winters with substantial snowfall in the north and at higher elevations (Bureau of Land Managment 2014). Total annual precipitation is approximately 250 mm/year, with drier areas receiving as little as 130 mm/year, and high elevations receiving as much as 670 mm/year

(Hereford et al. 2002). Winter and summer precipitation can be highly variable from year to year (Schwinning et al. 2008). Populations of species found across the region experience these extremes in such ecosystems as red rock deserts, high elevation plateaus, woodlands, sagebrush shrublands, salt desert shrublands, and mountain peaks (Bureau of Land Management 2014).

The Southwest is a region of increasing restoration priority, where increased abundance of invasive species, and frequency and intensity of fires challenge the reestablishment of native ecosystems (Bureau of Land Managment 2014, Wood et al. 2015). Regional, genetically appropriate plant material is rarely available for restorations, further constraining restoration efforts. Restoration practices in the western and southwestern United States have largely focused on the use of grass species for revegetation (Beyers 2004, Johnson et al. 2010, Redmond et al. 2013). Forbs are an under-studied, yet vital component of plant communities, and often comprise the majority of plant species richness (Pokorny et al. 2004). Native plant and pollinator initiatives have advocated for the use of herbaceous flowering plants in restoration seed mixes (San Francisco (CA): Pollinator Partnership 2014). Additionally, dormancy and germination in forbs is more complex than in grass species, further adding to the justification to study forbs for use in restorations. Many forb species are highly dependent on ecological conditions of the source site, whereas many grass species can easily germinate without long periods of cold stratification or other ecological treatments (Baskin and Baskin 2001).

Species and Population Sampling

The nine native forb species that were selected for this study are widespread throughout the southwestern United States, and have been considered high priority species for use in large-scale restorations in the region by the Colorado Plateau Native Plant Program and collaborators (Wood et al. 2015). The focal species were: *Cleome serrulata* (Capparaceae), *Dieteria canescens* (Asteraceae), *Heliomeris multiflora (Asteraceae), Heterotheca villosa (*Asteraceae*), Machaeranthera tanacetifolia* (Asteraceae), *Packera multilobata (*Asteraceae*), Penstemon comarrhenus* (Plantaginaceae), *Plantago patagonica (*Plantaginaceae*),* and *Sphaeralcea parvifolia* (Malvaceae). All species are common (G4 or G5) taxa, with populations spread across the Southwest. Classification of dormancy type prior to the start of the study was determined based on the taxonomic family of each species. Asteraceae, Capparaceae, and Plantaginaceae are known to have physiological dormancy (PD), while Malvaceae can have physiological and/or physical dormancy (PY). The Malvaceae species used in this study, *S. parvifolia*, is known to have combinational dormancy (PD + PY; (Baskin and Baskin 2001)). The nine species in this study were chosen from a list of 33 priority forb species to include multiple families and multiple genera within families to study a broad range of phylogenetic relatedness.

Populations were sampled from across the range to achieve a representative sampling of the different climatic conditions found throughout the Southwest region. Populations were located using known location information, Southwest Environmental Information Network, and herbarium records. Seeds were collected in the summer and fall of 2015 from two to four populations for each species, for a total of 27 populations across the states of Colorado, Utah, Arizona and New Mexico (Figure 1). I followed Seeds of Success collection protocols (Bureau of Land Management 2012), targeting larger populations and limiting seed collection to 20% of the total population. Seeds were collected from at least 30 maternal lines and then bulked together. Individuals were selected in the field based on maturity of fruits. Some of the collections used in this study were made by colleagues and mailed to the Chicago Botanic Garden. I aimed to collect seed from distant, climatically different populations located along the

range of the monsoonal gradient for each species (Table 1) to identify potential differences in seed ecology resulting from adaptation to climatic conditions. Seeds were cleaned to remove debris, and stored at room temperature for two weeks following collection to allow for after ripening (Pendleton and Pendleton 2014). After two weeks seeds were placed in dryers at 15°C and 15% relative humidity until germination trials began.

Germination Study

Seeds were x-rayed prior to the start of germination trials to determine the proportion of seeds filled with embryos, which has been used as an effective maximum estimate of viability (Riebkes et al. 2015). Seeds that appeared viable were used in the experiment. Immediately before plating, the seeds were sterilized in 5% sodium hypochlorite (bleach) for 30 seconds and rinsed two times in sterile deionized water. The seeds were then placed on 1.5% agar in 15 × 60 mm petri plates, and were treated with 4 stratification treatments as follows: 1) no stratification; 2) 8 weeks at 3°C (simulating a long winter and spring germination); 3) 3 weeks at 3°C (simulating a short winter and spring germination); and 4) 3 weeks at 30°C (simulating a short summer monsoon and fall germination; (Pendleton and Pendleton 2014)). The latter three treatments represent the winter and summer temperatures, and season duration found across the Southwest. *Sphaeralcea parvifolia* has been shown to have combinational dormancy (physiological + physical dormancy), and was therefore scarified (i.e., mechanical scarification by sandpaper; lightly rubbed between sandpaper for 15-30 seconds, or until the seed coat appeared broken) prior to stratification. The seeds were examined for germination every other day during stratification after radicle emergence was noticed for some collections. Following stratification, half the seeds from each treatment were incubated under 20/10°C (simulating spring or fall germination temperatures) and half were incubated under 25/15°C (simulating summer germination temperatures) with an alternating 12 hr. dark/12 hr. light regime for three weeks using climate-controlled incubators (Intellus Environmental Controller, Percival Scientific, Inc. Perry, IA, USA), for a total of eight separate treatments. Each population had four replicates of 25 seeds for each treatment. The petri dishes were randomized inside the incubators, and examined every other day for germinated seeds. Seeds with a radicle extending 1mm were considered germinated and removed from the petri plate. Seeds that did not germinate after 18 days were removed, subjected to a cut test to determine viability, and used in a viability adjusted germination analysis. Seeds that were filled with a white intact embryo were considered viable.

Statistical Analyses

The total proportion of germinated seeds at the end of the study for each treatment was used to construct a data frame of germination traits per species, in which each species had eight traits (mean germination proportion under each of the eight treatments + germination during stratification). In all species, seeds germinated in the dark during the periods of cold or warm stratification. These germinated seeds were added to the final proportion of germinated seeds. All analyses were performed on viability-adjusted germination only and executed using the R environment for statistical computing in the ‘stats’ package, unless otherwise stated (v.3.3.1, R Core Team 2016).

To determine if phylogenetic relatedness or climate was the more significant driver of germination responses, Mantel tests were conducted using distance matrices of germination traits, phylogenetic branch distances, and climatic data. To produce a distance matrix for phylogenetic relationships among species, a phylogenetic tree was created using Phylomatic

version 3 using the stored tree from Zanne 2014 (Figure 2; Webb and Donoghue 2005). The branch lengths of the tree, in millions of years, were used to create a distance matrix with cophenetic distances using the 'cophenetic' function.

Distance matrices were generated for the germination trait data and bioclimatic data using the 'dist' function with Euclidean distances. Climatic data for seven bioclim variables was extracted for each population using raster information from WorldClim and the 'biovars' function in the dismo package (Hijmans et al. 2005). The bioclim variables included, BIO1: Mean Annual Temperature (MAT), BIO2: Mean Diurnal Range (max temp-min temp; MDR), BIO4: Temperature Seasonality 7 (TSeason), BIO8: Mean Temperature Wettest Quarter (MTWetQ), BIO12: Mean Annual Precipitation (MAP), BIO15: Precipitation 8 Seasonality (PSeason), and BIO18: Precipitation Warmest Quarter (PWarmQ). These seven variables represent orthogonal axes of climate variation across the Southwest (Butterfield 2015, Butterfield and Wood 2015). The bioclimatic data was log transformed prior to creating a distance matrix to ensure that all values of the seven variables were evenly scaled.

The trait distance matrix was correlated with both the phylogenetic and the climatic distance matrices and was tested for significance using Mantel tests with 1000 permutations. A third Mantel test was performed with the trait distance matrix and a distance matrix based on the four climatic variables that correspond to temperature (BIO1, BIO2, BIO4, and BIO8), as my germination treatments did not manipulate precipitation in any way. Additionally, each individual bioclimatic variable was tested against the trait distance matrix to determine if any one variable significantly explained germination differences. A Mantel test was also conducted without *Cleome serrulata*, as this species had very little germination across all treatments and likely needs a longer period of cold stratification to show any differences between populations. Because there was high germination during stratification for many populations, Mantel tests were also used to examine germination during stratification. Separate germination trait data were used for this Mantel test, with each species having three traits (mean germination proportion during each of the three stratification treatments).

To visualize the results of the Mantel tests, complete cluster dendrograms were created based on the distance matrices of the climate and germination trait data.

To examine whether the germination response within species differed across treatments and populations, generalized linear models with binomial error and logit link function were fitted to germination results for each species using the three treatment factors (stratification length, stratification temperature, and incubation temperature) and population as predictors. For models that were overdispersed (residual deviance was larger than residual degrees of freedom), a quasibinomial error was used. The best-fit model was determined by AIC using the 'step' function in the stats package with both backwards and forwards elimination. The model was subsequently analyzed using analysis of variance (ANOVA) with a Chi test for significance if the model was evenly dispersed and an F test for significance if the model was overdispersed.

The above methods were also used to generate generalized linear models for germination in stratification. Germination was used as the response with population, stratification length, and stratification temperature as predictors. *Cleome serrulata* was excluded from this analysis, as the germination proportions during stratification were very low.

For each population, tolerance range was calculated using Levins' B (Levins 1968, Feinsinger et al. 1981, Barak et al. 2015):

$$B = 1/R \sum p_i^2 ,$$

$$i$$

where p_i is the percent germination at temperature *i* (mean of 4 petri dishes/replicate) and R is the number of temperature tested (in this case, R = 8). B has a range of 0-1. This measurement is useful in determining the range of requirements that each population needs to germinate. A low Levins' B value indicates a narrow tolerance range, in which a population would need very specific conditions to germinate. A high value indicates a wide tolerance range, whereby a population could germinate over a wide range of conditions.

Results

Drivers of seed germination: species relationships and local climate

The Mantel tests showed that overall phylogenetic relatedness is a more significant driver of germination responses than local climatic conditions. Germination traits were significantly correlated with phylogenetic distance (mantelr = 0.2; p = 0.013), and were not correlated with climatic distance of all bioclimatic variables (mantelr = -0.1; p = 0.89). Additionally, germination response was not correlated with climatic distance of temperature-dependent bioclimatic variables (mantelr = -0.09; p = 0.83), or any individual bioclimatic variable. When *Cleome serrulata* was removed, phylogenetic relatedness was still a more significant driver of climate (mantelr = 0.30; p = 0.008), but climate was also a significant driver of differences in germination proportion (mantelr = 0.19; p = 0.03).

In all nine species, some seeds germinated during cold or warm stratification, *i.e.* in darkness at 3°C or 30°C, respectively (Figure 3). The Mantel tests performed on germination proportion during stratification showed that one climatic variable, precipitation seasonality, is a significant driver of germination variation (mantelr = 0.22; p = 0.01). Germination proportion differences during stratification were not significantly explained by phylogenetic relatedness (mantelr = -0.04; p = 0.61).

Cluster dendrograms to visualize the Mantel tests results agreed with the p-values determined from the Mantel tests (Figure 3). A cluster dendrogram based only on climatic variables identified five main clusters, in which populations located around the same geographic area generally clustered together. A second cluster dendrogram based on germination response of each population additionally identified five main clusters, but did not exhibit a pattern of climate clustering. Instead, populations within species or populations within family generally clustered together, indicating that species relatedness is a driver of germination response. This result agreed with the results of the Mantel tests. Cluster dendrograms based on germination response in stratification and based on the results excluding *Cleome serrulata* showed the same general pattern, in which clustering of climatically similar populations was generally not apparent.

Intraspecific germination variation across and within treatments

The generalized linear models for each species showed that final germination response significantly varied by population within eight of the nine species in this study (Table 2). *Heterotheca villosa*, which had close to 100% germination across all treatments, was the one species in which population was not a significant predictor of germination response. Population differences were more dramatic in some species than in others. For example, the best-fit model for *Packera multilobata* included population as a significant predictor, but germination proportion differences between populations were very small, as germination was close to 100%

across all treatments and populations (Figure 5). *Cleome serrulata* had very low germination in three of the four stratification treatments and only showed some population differences in the long, cold stratification treatment. This suggests that this species likely needs a longer period of cold stratification to reach high levels of germination. Other species, such as *Heliomeris multiflora* and *Plantago patagonica* showed dramatic differences between populations and within and across treatments.

Population significantly interacted with at least one treatment factor in seven of the nine species (Table 2). Populations of *Cleome serrulata* and *Heterotheca villosa* did not interact with any treatment factors to predict germination response. Populations of *Heliomeris multiflora, Machaeranthera tanacetifolia,* and *Plantago patagonica* interacted significantly with all three treatment factors (stratification length, stratification temperature, and incubation temperature) and showed the greatest variation between populations and treatments.

Generalized linear models for germination response in stratification showed that population was significant for seven of the eight species (Table 3). *Sphaeralcea parvifolia* was the one species that did not show significantly different germination responses between populations. This is also the one species with combinational dormancy (physiological + physical), which may contribute to these results. Population significantly interacted with at least one treatment factor (stratification length or stratification temperature) in five of the eight species.

Germination tolerance ranges showed that populations with high germination proportions across all treatments generally had a value close to one, whereas populations that had low germination across many treatments generally had a lower value (Appendix). The Asteraceae species tended to have the highest germination tolerance ranges, as germination was high for most populations across all treatments. *Cleome serrulata* had the lowest tolerance ranges, as this species had very low germination across all treatments and populations.

Discussion

Understanding what drives germination at the species and population level requires the examination of phylogenetics as well as climatic conditions of the population. In knowing the importance of these drivers, restoration practitioners can source the most appropriately adapted seed for the restoration project. The results of this study show that species relatedness is a greater driver of seed germination differences among species overall, but that climate may be a driver of germination response at the population level for some species. These results suggest that germination patterns and requirements are species-specific and there will not be one overarching rule to determine the most appropriate seed source for restorations. However, results from this study do provide some takeaway messages when thinking about defining the best seed source for restorations in the Southwest.

Influence of species relatedness on germination response

Overall, phylogenetic relatedness significantly explained differences in germination response across all treatments. Closely related species tended to exhibit similar germination responses. For example, populations of all of the Asteraceae species, except for *Heliomeris multiflora,* responded similarly under each treatment, with most populations germinating to a high degree regardless of treatment. These results agree with previous studies that found that phylogenetic relatedness is a major factor contributing to differences in germination proportion among species. This pattern was shown in degraded sandy grasslands (Wang et al. 2016),

temperate rain forests (Figueroa 2003), alpine meadows (Bu et al. 2009, Xu et al. 2014), and arid and semiarid zones in northwest China where interspecific variation in germination was explained by phylogeny (Wang et al. 2009). However, these studies did not examine both inter- and intraspecific relatedness to determine if phylogeny was a more significant driver than population climate. Our results show that phylogeny is a greater predictor of differences in seed germination across species and populations, which suggests that germination proportion is primarily driven by long-term phylogenetic constraints (Nikolaeva 1999, Baskin and Baskin 2001, Figueroa and Armesto 2001, Wang et al. 2016). This does not mean that short-term selective pressures, such as climate, do not also drive germination at the population level. For example, many of the populations from New Mexico, among all species, germinated to high degrees in all treatments, suggesting that these populations may be adapted to a climate with periods of high moisture availability. Although phylogenetic relatedness significantly explained differences in germination proportion more so than climate, there were significant differences at the population level, indicating that some species or populations may be more adapted to local climate than others.

Factors contributing to intraspecific variation in germination response

The results of this study show that there is significant intraspecific variation in germination responses to different stratification and incubation treatments. Some species in the study showed dramatic variation between populations (*Heliomeris multiflora, Dieteria canescens, Machaeranthera tanacetifolia, Penstemon comarrhenus,* and *Plantago patagonica*), while others showed little to no variation between populations (*Cleome serrulata, Heterotheca villosa, Packera multilobata,* and *Sphaeralcea parvifolia*). *Cleome serrulata* showed little to no difference in germination response between populations. A study on *C. serrulata* found that date of seedling emergence is a highly heritable trait in this species and is highly correlated with maternal effects (Farris 1988). This could help to explain the germination proportion results that were observed in this study, but it is also likely that this species needs a longer period of cold stratification to truly separate out population-level differences and the influences of genetics and/or maternal effects.

The intraspecific variation identified in this study may be driven by genetic factors (local adaptation or random genetic drift) or by environmental factors (maternal effects or transgenerational plasticity attributed to the environment that the seeds were exposed to prior to harvest). Many previous studies have shown population variation in dormancy and germination that may be driven by adaptation to local conditions. For example, studies on intermountain *Penstemon* species found that germination timing varied based on the source habitat, suggesting that multiple lineages in the genus *Penstemon* have evolved habitat-specific germination strategies to ensure survival in harsh environments (Meyer 1992, Meyer et al. 1995, Kramer et al. 2015). A study on two *Salvia* species in Jordan found that population-level germination requirements are locally adapted to salinity and temperature at the source habitats (Al-Gharaibeh et al. 2016). Dormancy has also been shown to vary within species in relation to climatic conditions. A study on *Centaurium somedanum* in Spain found that degree of dormancy varied by population in relation to altitude and climate. The results suggest that populations have the capacity to adapt to long-term climatic variation and to conditions during seed maturation (Fernández-Pascual et al. 2013).

Environmental conditions can select for local adaptation in dormancy and germination. The yearly and multi-decadal variation in climatic conditions found throughout the southwest

U.S. have likely selected for complex dormancy and germination patterns. The timing of germination-triggering events, such as precipitation seasonality, is the most significant driver of germination response in the Southwest. The timing of these events was found to be a more significant driver than temperature during the growing season (Kimball et al. 2010). The results of this study confirm this hypothesis. The Mantel tests showed that there is a significant relationship between overall germination and climate (when *C. serrulata* is excluded), but more specifically that there is a significant interaction between germination during stratification and precipitation seasonality. The southern populations, which experience a seasonal monsoon during the summer, may be undergoing differential selection from northern populations, which receive most of their precipitation during the winter months (Comstock and Ehleringer 1992, Schwinning et al. 2008, Hintz et al. 2016). Intraspecific populations across the Southwest are thereby likely adapting to variation in seasonal precipitation, which may explain population-level differences in dormancy and germination. However, these differences may not be obvious from final germination results alone. It is therefore important to examine dormancy and germination patterns more deeply, including during stratification.

Changing climatic conditions across the Southwest is leading to plant community composition changes, as well as selection for cold-adapted species. A study based on long-term data in the Sonoran Desert has shown that the warming and drying of the Southwest, as a result of climate change, has led to delayed onset of germination-triggering winter rains and has thereby led to an increase in the abundance of cold-adapted species (Kimball et al. 2010). Germination phenology was found to be the primary life stage determining community shifts in response to climate change, indicating that this early life stage is closely tied to seasonal climatic conditions and the relationship may be quite complex.

The degree of adaptation in seed dormancy and germination strategies is likely the result of a balance between environmental selection and genetic processes, such as drift and gene flow (Linhart and Grant 1996, Galloway and Fenster 2000, Hufford and Mazer 2003, McKay et al. 2005, Debieu et al. 2013). Local adaptation has been shown to be prevalent in large plant populations (i.e., more than 1000 individuals) where there might be restricted gene flow (Leimu and Fischer 2008), whereas trait divergence in small populations is more likely to be affected by genetic drift (McKay et al. 2005). Reciprocal transplant and common garden studies would need to be conducted to disentangle the roles of long-term environmental selection and genetic processes from short-term environmental and maternal effects in the dormancy and germination patterns observed in this study.

Maternal effects have been shown to influence variation seen at early life stages in plants (Bischoff and Müller-Schärer 2010). The effect of yearly ecological or environmental variation on the mother plant during seed maturation, the position of the seed on the mother plant, or the genetics of the mother plant could explain most of the variation in dormancy and germination between years and between populations (Roach and Wulff 1987, Fenner 1991, Gutterman 2000, Baskin and Baskin 2001, Fenner and Thompson 2005).

Among- and within-year ecological variation can greatly influence the level of dormancy of seeds (Fenner 1991, Fenner and Thompson 2005). A study on four weed species, each collected from three populations and in two separate years, found large differences in seed dormancy between individuals, between populations, and between seeds collected in different years (Andersson and Milberg 1998). Among-year variation in dormancy could be an adaptation strategy of some populations to unpredictable environments. A study on *Bromus tectorum* in the southwestern United States found that seeds from an extreme yet predictable environment

showed less year-to-year variation in dormancy than seeds from a more favorable environment (Beckstead et al. 1996). Changes in temperature, day length, and precipitation can influence the seed during maturation within one growing season. A study on *Heterotheca latifolia* found that early-maturing seeds, ray achenes, had a higher level of dormancy than late-maturing seeds, disc achenes (Venable and Levin 1985). This within-year variation could be due to changes in weather or due to position on the mother plant.

The microenvironment of the seed, or position on the mother plant, can also greatly influence the level of dormancy and germination requirements of the seed (Fenner and Thompson 2005). Asteraceae species have shown two distinct seed morphs in ray florets and disc florets. This could be an adaptation strategy. For example, the achene dimorphism found in *Heterotheca latifolia* could be a form of bet-hedging, whereby the ray achenes offer a cautious germination strategy that delays and spreads germination over time, and the disc achenes offer an advantageous germination strategy that allows germination with the first available rain (Venable and Levin 1985).

The many factors that influence dormancy and germination patterns are quite complex. Understanding which factor is the primary driver of patterns at the population level is valuable when thinking about seed sourcing for restorations.

Restoration implications

Seed germination can lead to major recruitment bottlenecks in restorations if the seed is maladapted to the restoration site (Larson et al. 2015). The results of the study show that among- and within-species variation in germination can be partly explained by differences in climatic conditions between sites, which correspond to other studies that have shown a close relationship with dormancy and germination and environmental conditions, such as temperature and precipitation. Across all species, precipitation seasonality was found to be the most significant climatic driver of germination response, suggesting that this climatic variable should be taken into consideration when defining seed sources. There is not one overarching rule for seed sourcing that can be defined based on the results of this study, however there are some generalizations that can be made that will be important in seed sourcing decisions.

The first is to consider the stratification requirements of each population and the timing of seed sowing for the restoration. Populations from colder climates typically need a period of cold stratification in order to break dormancy and germinate (Meyer 1992, Kramer et al. 2015, Hintz et al. 2016), whereas many of the southern, warmer populations in this study showed little differences between cold and warm stratification treatments. Other species need a long period of cold stratification regardless of population climate. For example, *Cleome serrulata* likely needs at least a 10-week cold stratification period to reach high germination proportions. Many populations had high germination during stratification, which could lead to demographic bottlenecks in the restoration process during later life stages. For example, a population from New Mexico that may be adapted to the monsoonal gradient and time germination to coincide with high levels of precipitation in the summer may germinate at a restoration site with high precipitation in cooler months, in which case the seedling may not survive until the warm growing season.

Phylogenetic relatedness was the most significant driver of differences in germination response overall. Most of the Asteraceae species in this study had high germination across all populations and treatments, which may indicate a lower likelihood of bottlenecks at the germination stage when moving seed from one site to another site that may not be climatically

similar. However, these results do not necessarily translate to later life stages, such as establishment and survival, and could lead to maladaptation later on. Therefore, movement of seeds between dissimilar sites should be done infrequently and with caution. *Heliomeris multiflora* was the one Asteraceae species that showed dramatic variation between populations and a close relationship to source site climate. These results indicate that this species should be sourced from climatically similar sources to ensure the greatest chances of establishment and survival.

Based on the results of this study, certain species may be better suited for use in restoration projects. The species that germinated to high proportions across all treatments may be more likely to germinate and establish at many different sites, regardless of origin. Additionally, these species might be more resilient to climate change if they are able to tolerate wide temperature variation. This was shown through the calculation of tolerance range (Appendix). Populations that germinated to high proportions across all treatments had a high tolerance range, suggesting that these populations have flexible germination conditions, and as such, have a chance of responding well to changing climatic conditions.

In general, the results of this study did not define a clear driver of germination patterns in these species, and there are many other factors not considered in this study that could be contributing to germination variation among- and within-species. Many questions remain unanswered, but it is quite clear that variation in dormancy and germination is largely species-specific. It is therefore very important to know the species with which you are working and the site conditions of the restoration. In other words, use 'the right seed in the right place at the right time' (Plant Conservation Alliance 2015).

All germination results from this study will be uploaded to the Native Plant Propagation Database (http://npn.rngr.net/propagation).

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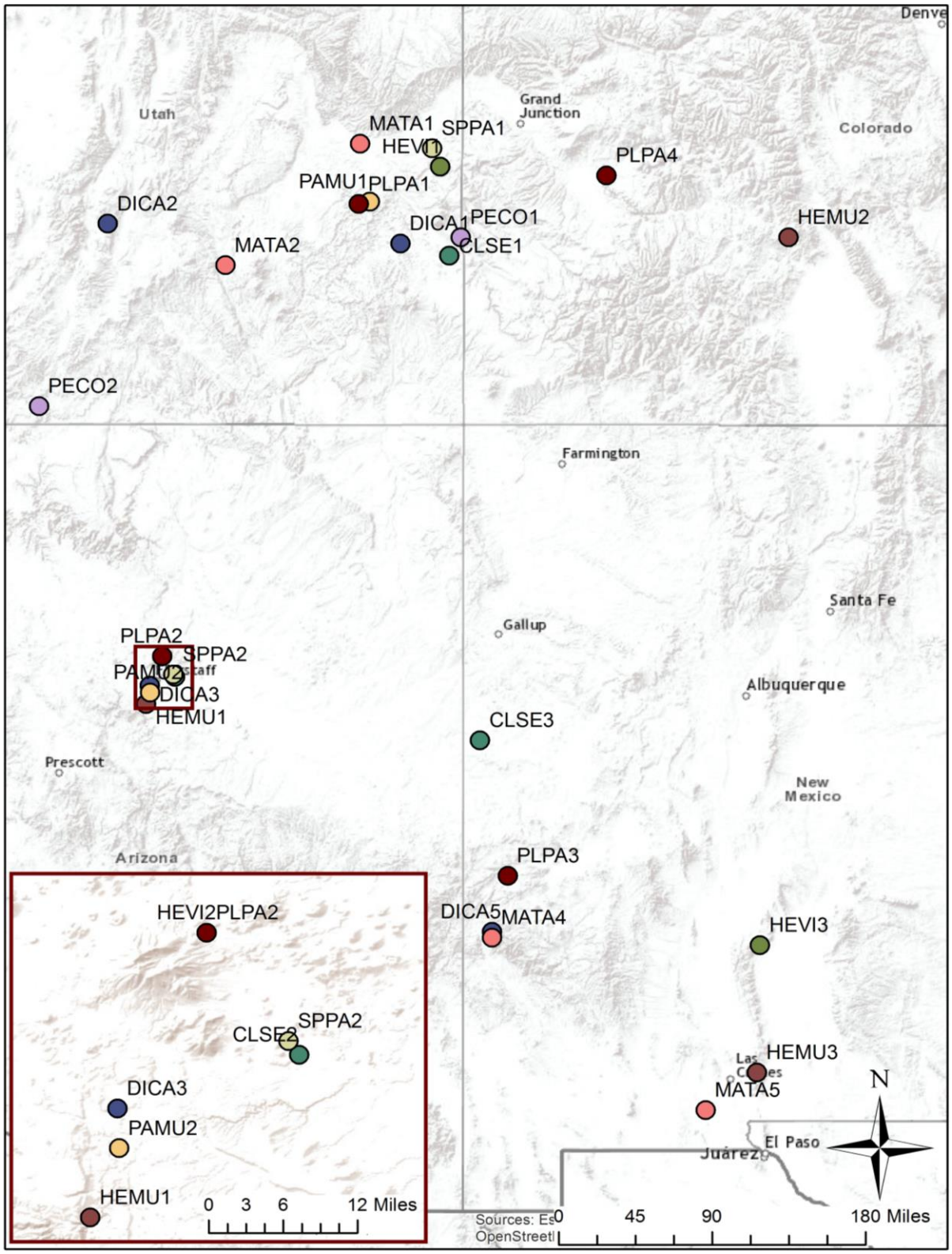


Figure 1. Seed collection locations. Species names are abbreviated with a species code and population number. Each species has a unique color. The insert shows the Flagstaff area at a larger scale.

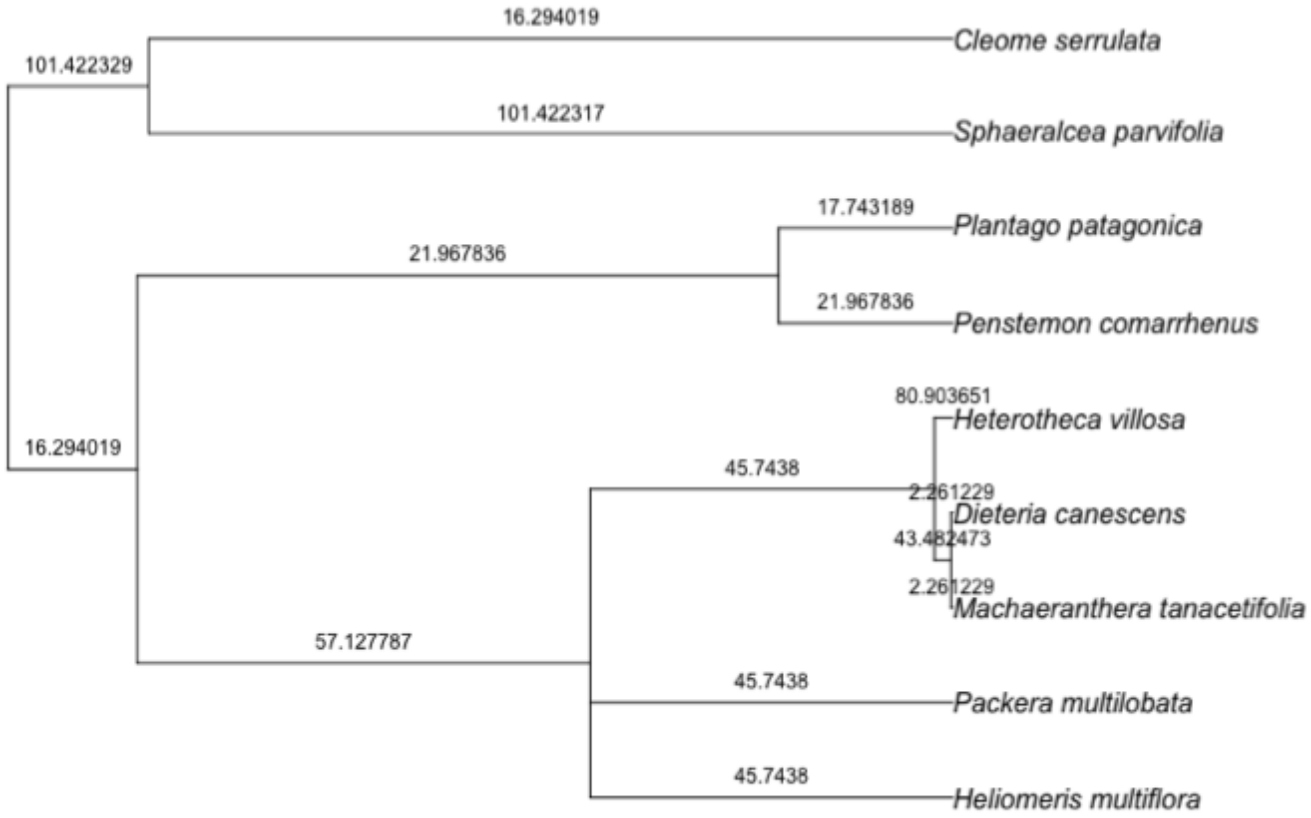


Figure 2. Phylogenetic tree of study species constructed using Phylomatic version 3 (Webb and Donoghue 2005). The tree includes branch lengths measured in millions of years.

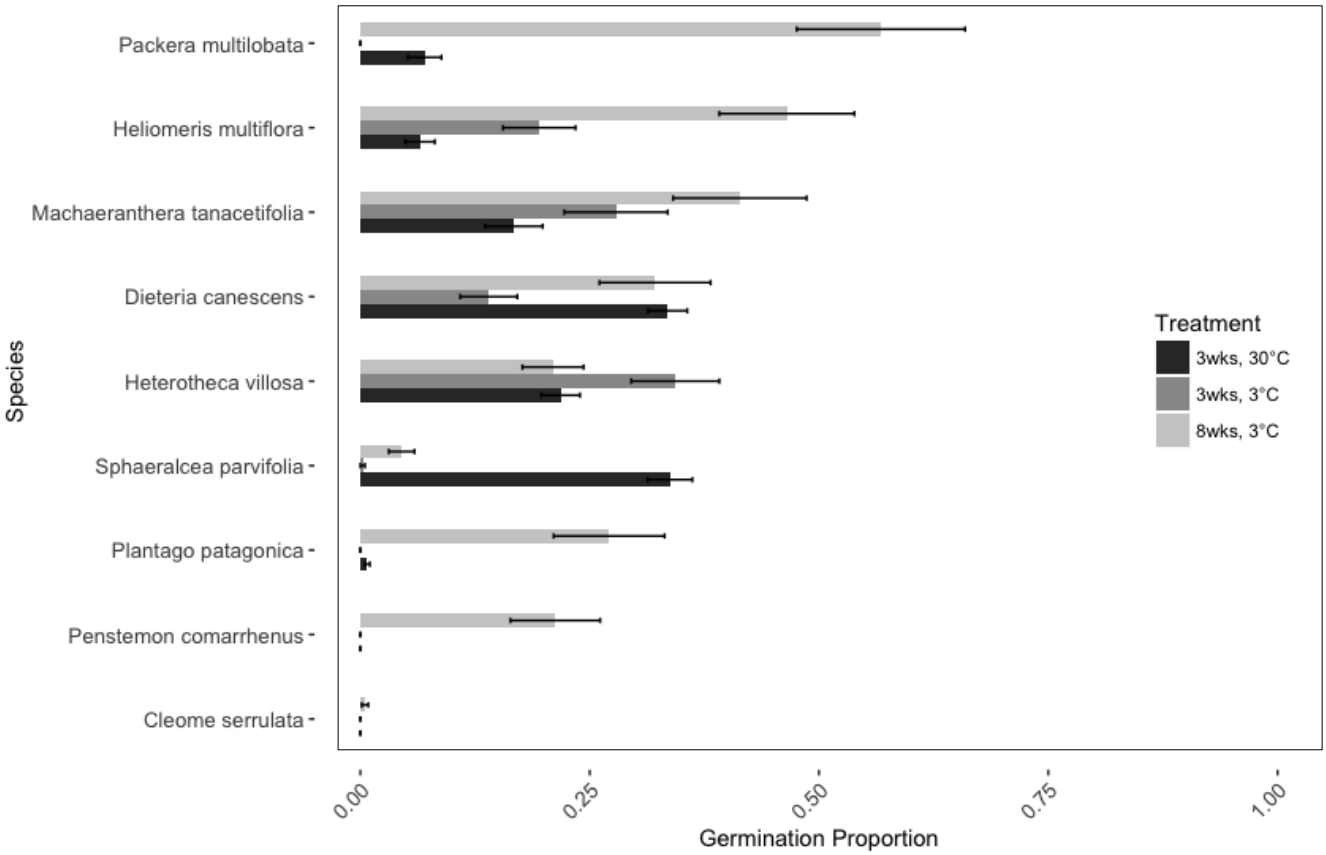


Figure 3. Germination during stratification. Bars represent mean germination proportion for each treatment across all populations for each species. Standard error bars are included.

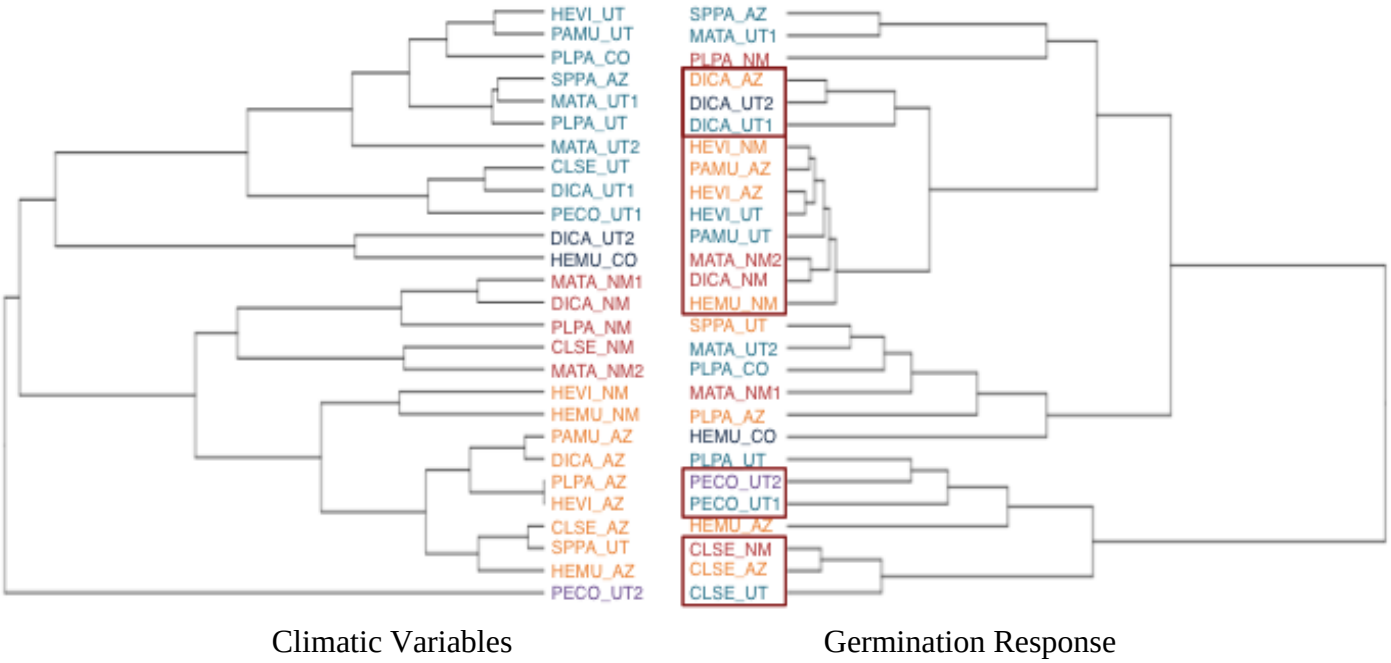
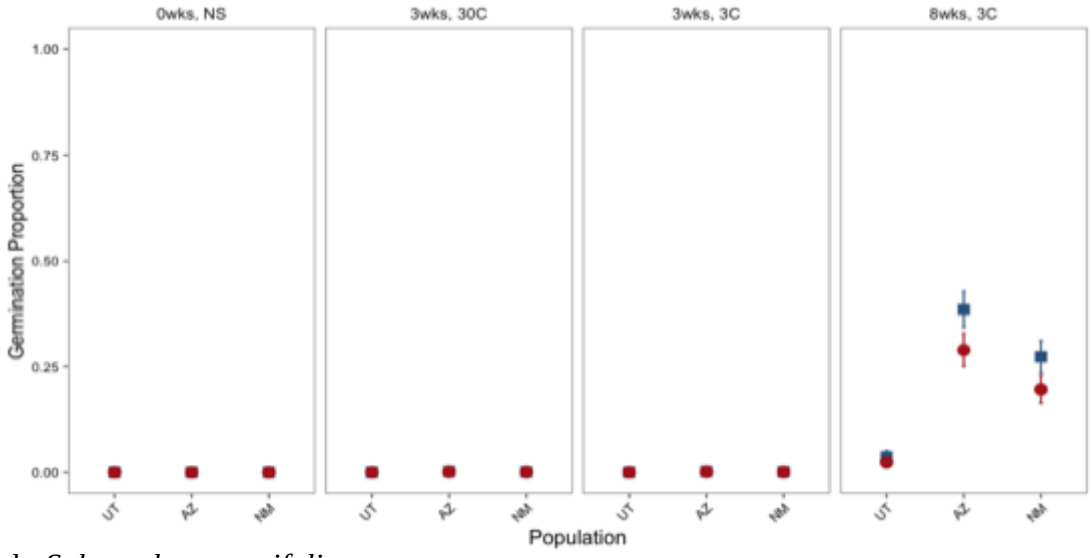
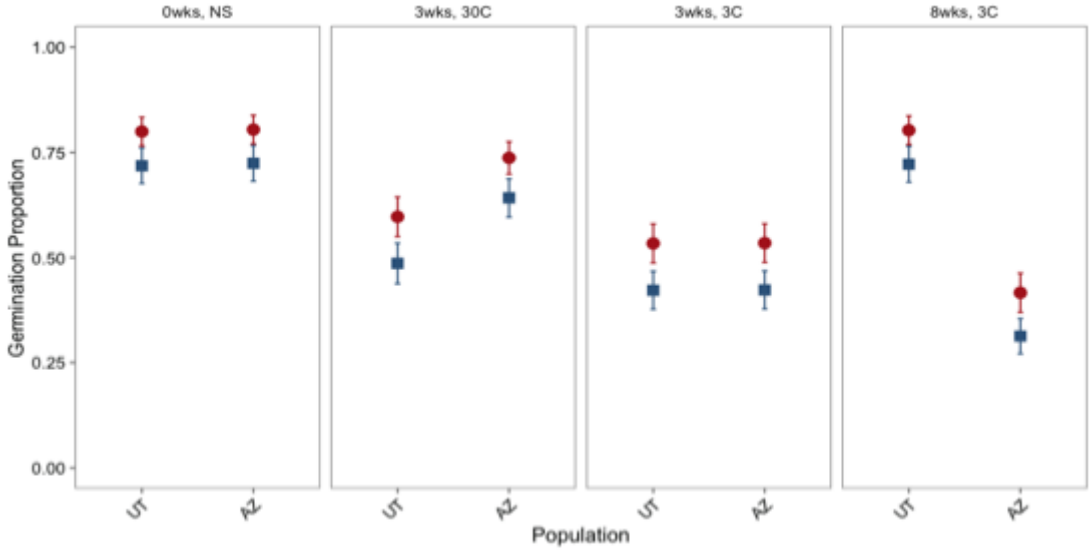


Figure 4. Cluster dendrograms based on distance matrices of climatic variables and germination response. The dendrogram on the left is clustered based on climatic relatedness of populations and the dendrogram on the right is clustered based on germination response of each population to the eight treatments. Red boxes show clustering of closely related species.

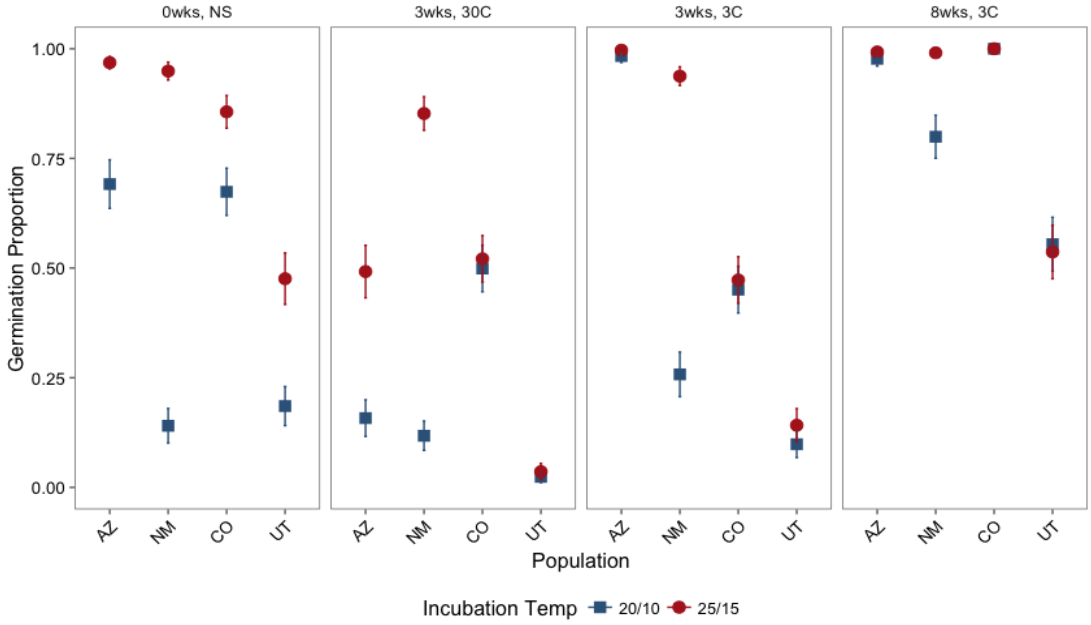
a. *Cleome serrulata*



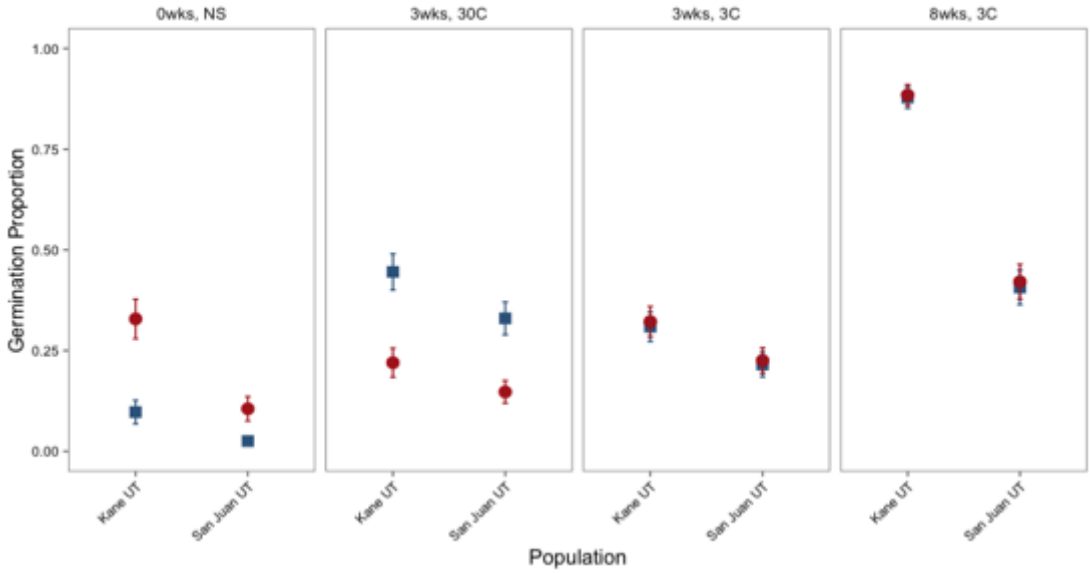
b. *Sphaeralcea parvifolia*



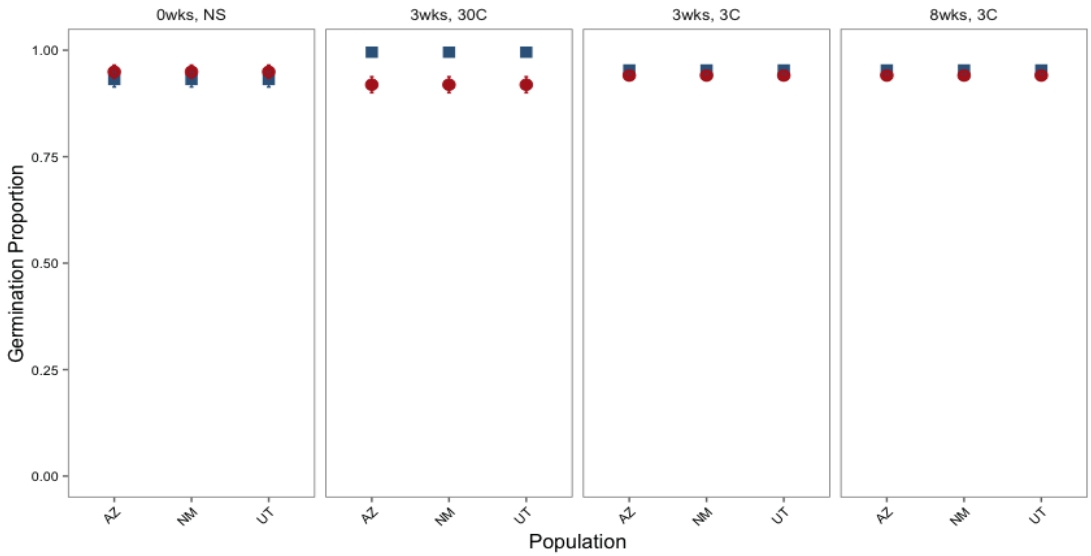
c. *Plantago patagonica*



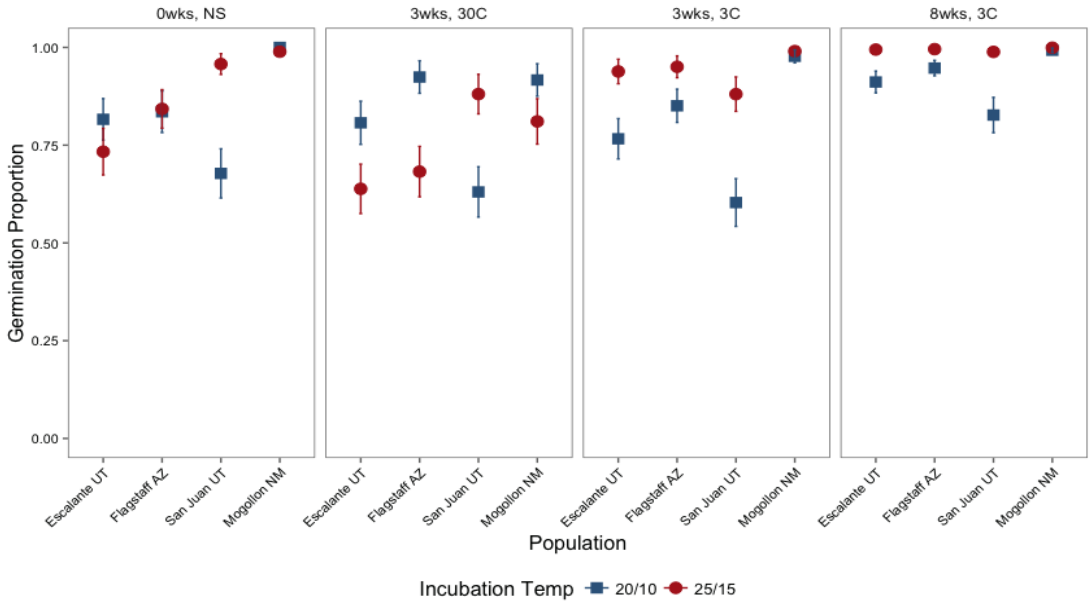
d. *Penstemon comarrhenus*



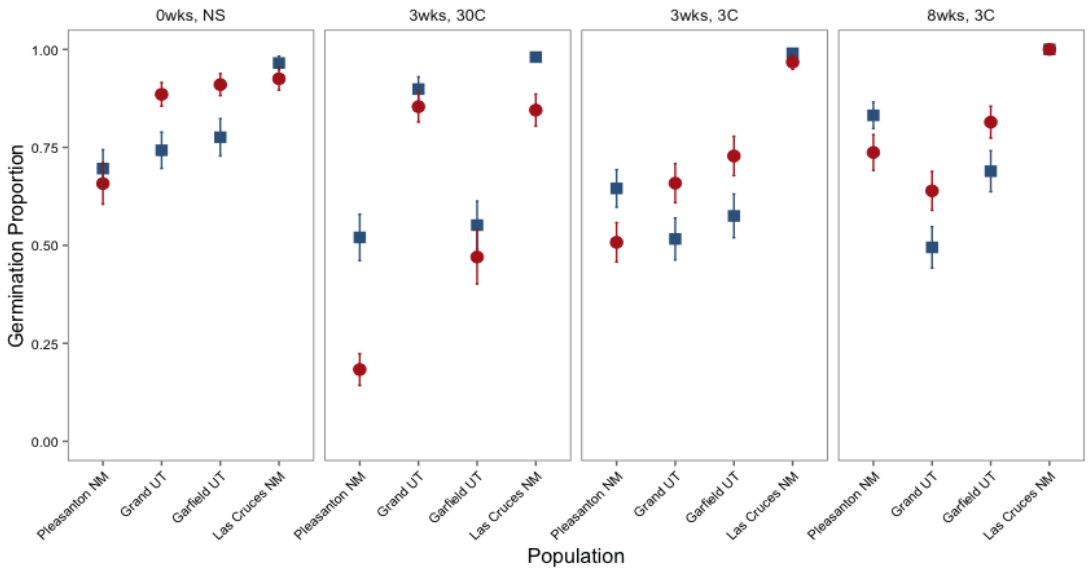
e. *Heterotheca villosa*



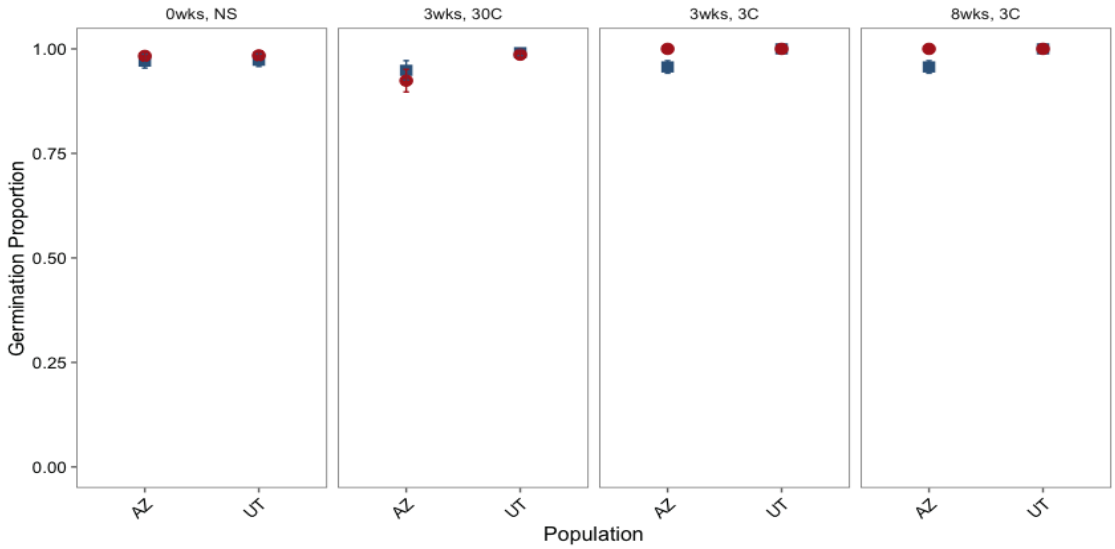
f. *Dieteria canescens*



g. *Machaeranthera tanacetifolia*



h. *Packera multilobata*



i. *Heliomeris multiflora*

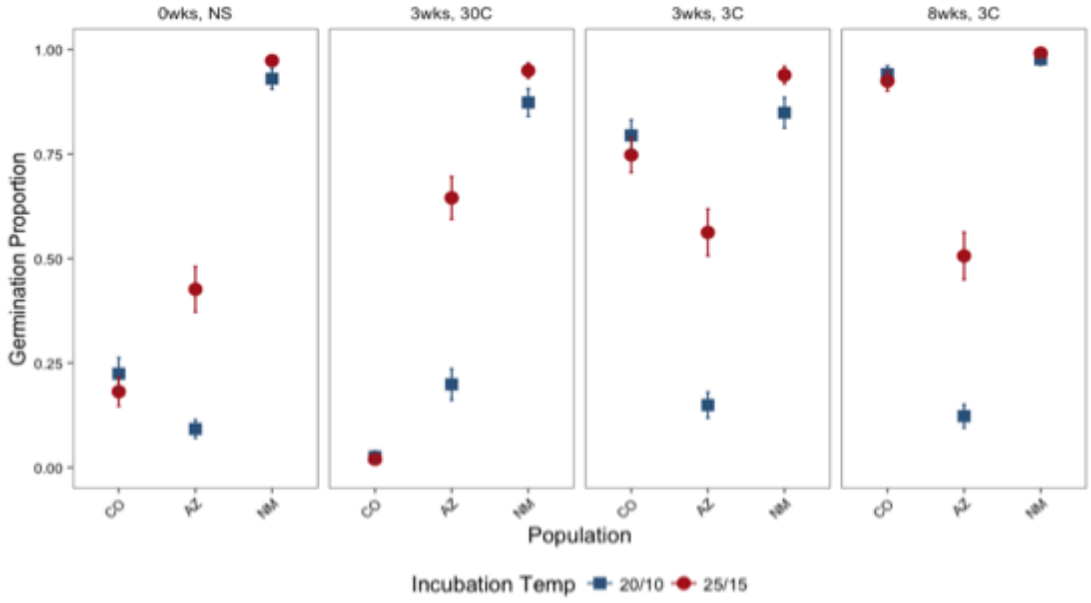


Figure 5. Final germination proportion for each species across all treatments. The results are based on best-fit GLMs with treatment factors and population as predictors (binomial or quasibinomial error with logit link function). Standard error bars are displayed. (a) *Cleome serrulata*; (b) *Sphaeralcea parvifolia*; (c) *Plantago patagonica*; (d) *Penstemon comarrhenus*; (e) *Heterotheca villosa*; (f) *Dieteria canescens*; (g) *Machaeranthera tanacetifolia*; (h) *Packera multilobata*; (i) *Heliomeris multiflora*.

Table 1. Collection information for the study species. Numbers in parentheses correspond to species ID codes found in figures and Appendix. MAT = mean annual temperature (°C); AP = annual precipitation (mm).

Population	State	Latitude	Longitude	MAT	AP
Cleome serrulata (CLSE)					
San Juan	UT	38.169356	-109.164725	8.5	354
Flagstaff	AZ	35.227639	-111.491444	9.4	452
Fence Lake	NM	34.77413	-108.90502	9.7	305
Dieteria canescens (DICA)					
San Juan	UT (1)	38.252142	-109.579425	9.3	322
Escalante	UT (2)	38.388056	-112.059444	4.1	381
Flagstaff	AZ	35.163417	-111.702944	7.6	564
Mogollon	NM	33.39235	-108.80225	9.3	483
Heliomeris multiflora (HEMU)					
Flagstaff	AZ	35.03292	-111.73454	10.4	556
Ouray County	CO	38.2949	-106.2919	1.9	422
Organ Mtns	NM	32.3625	-106.55866	12.4	363
Heterotheca villosa (HEVI)					
Rio Mesa	UT	38.77689	-109.24421	11.3	267
Flagstaff	AZ	35.373861	-111.598944	6.2	540
Salinas Peak	NM	33.29552	-106.53376	8.8	506
Machaeranthera tanacetifolia (MATA)					
Grand	UT (1)	38.93292	-109.921313	11.2	210
Garfield	UT (2)	38.103889	-111.063333	11.6	177
Pleasanton	NM (1)	33.3496	-108.80441	7.8	529
Las Cruces	NM (2)	32.08456	-106.99344	16.0	240
Packera multilobata (PAMU)					
Arch Rock	UT	38.538581	-109.839934	10.1	266
Griffith's Spring	AZ	35.115833	-111.701111	8.2	564
Penstemon comarrhenus (PECO)					
Kane	UT (1)	38.295722	-109.067611	6.8	427
San Juan	UT (2)	37.131111	-112.6425	9.3	390
Plantago patagonica (PLPA)					
Grand	UT	38.52194	-109.932206	11.1	228
Flagstaff	AZ	35.373861	-111.598944	6.2	540
Apache Creek	NM	33.79833	-108.67032	9.1	387
Delta County	CO	38.716883	-107.83345	9.3	294
Sphaeralcea parvifolia (SPPA)					
Winona	AZ	38.901841	-109.311857	12.0	229
Grand	UT	35.244111	-111.503806	8.9	465

Table 2. Results from Mantel tests.

	Phylogenetics	Climate	Temperature	Precip Seasonality
Final Germination	r = 0.2 p = 0.013	r = -0.1 p = 0.89	r = -0.09 p = 0.83	
Final Germ minus *Cleome serrulata*	r = 0.3 p = 0.008	r = 0.19 p = 0.03		
Germination in stratification	r = -0.04 p = 0.61			r = 0.22 p = 0.01

Table 3. Results from ANOVA models evaluating the effects of treatment factors and population on germination response for each species.

	D.f.	Deviance	Resid. D.f.	Resid. Dev	F value	P value
Cleome serrulata						
Population	2	65.21	93	388.65		< 0.001 ***
Strat Length	2	351.70	91	36.95		< 0.001 ***
Inc. Temp	1	4.05	90	32.89		< 0.05 *
Dieteria canescens						
Population	3	115.50	124	457.58	23.62	< 0.001 ***
Strat Length	2	90.96	122	366.63	27.90	< 0.001 ***
Strat Temp	1	21.61	121	345.02	13.26	< 0.001 ***
Inc. Temp	1	16.36	120	328.65	10.04	< 0.01 **
Population x Strat Temp	6	36.55	114	292.10	3.74	< 0.01 **
Population x Inc. Temp	3	53.95	111	238.15	11.03	< 0.001 ***
Strat Length x Inc. Temp	2	14.87	109	223.28	4.56	< 0.05 *
Strat Temp x Inc. Temp	1	25.39	108	197.89	15.57	< 0.001 ***
Heliomeris multiflora						
Population	2	672.07	93	747.39	294.63	< 0.001 ***
Strat Length	1	136.52	92	610.88	119.70	< 0.001 ***
Strat Temp	2	86.92	90	523.96	38.11	< 0.001 ***
Inc. Temp	1	54.16	89	364.55	47.48	< 0.001 ***
Population x Strat Length	2	105.25	87	364.55	46.14	< 0.001 ***
Population x Strat Temp	4	204.20	83	160.35	44.76	< 0.001 ***
Population x Inc. Temp	2	56.70	81	103.66	24.86	< 0.001 ***
Heterotheca villosa						
Inc. Temp	1	3.98	94	107.99		< 0.05 *
Inc. Temp x Strat Temp	4	16.90	90	91.10		< 0.01 **
Machaeranthera tanacetifolia						
Population	3	326.03	124	412.66	85.14	< 0.001 ***
Strat Temp	2	43.56	122	369.10	17.06	< 0.001 ***
Population x Strat Length	7	91.75	115	277.35	10.27	< 0.001 ***
Population x Strat Temp	3	61.62	112	215.74	10.09	< 0.001 ***
Population x Inc. Temp	4	44.17	108	171.56	8.65	< 0.001 ***
Strat Temp x Inc. Temp	2	22.27	106	149.29	8.72	< 0.001 ***
Packera multilobata						
Population	1	11.42	62	83.67		< 0.001 ***
Strat Temp	2	8.22	60	75.45		< 0.05 *
Population x Strat Temp	2	7.31	58	68.14		< 0.05 *
Strat Temp x Inc. Temp	3	12.07	55	56.06		< 0.01 **

Penstemon comarrhenus						
Population	1	71.33	62	409.56	63.03	< 0.001 ***
Strat Length	2	263.31	60	146.25	116.33	< 0.001 ***
Population x Strat Length	2	41.22	58	105.03	18.21	< 0.001 ***
Strat Temp x Inc. Temp	4	42.20	54	62.83	9.32	< 0.001 ***
Plantago patagonica						
Population	3	531.09	124	1517.35	112.78	< 0.001 ***
Strat Length	2	513.58	122	1003.77	163.59	< 0.001 ***
Strat Temp	1	84.63	121	919.14	53.92	< 0.001 ***
Inc. Temp	1	251.90	120	667.24	160.48	< 0.001 ***
Population x Strat Length	6	83.98	114	583.26	8.92	< 0.001 ***
Population x Strat Temp	3	186.61	111	396.66	39.63	< 0.001 ***
Population x Inc. Temp	3	203.41	108	193.25	43.20	< 0.001 ***
Strat Length x Inc. Temp	2	22.78	106	170.47	7.26	< 0.01 **
Sphaeralcea parvifolia						
Population	1	5.08	62	202.01	4.16	< 0.05 *
Strat Length	2	47.63	60	154.39	19.50	< 0.001 ***
Strat Temp	1	13.74	59	140.65	11.25	< 0.01 **
Inc. Temp	1	14.83	58	125.82	12.14	< 0.001 ***
Population x Strat Length	2	52.55	56	73.27	21.52	< 0.001 ***
Population x Strat Temp	1	3.87	55	69.40	3.17	< 0.1 .

Table 4. Results from ANOVA models evaluating the effects of treatment factors and population on germination response for each species during stratification. *Cleome serrulata* was not included in this analysis because of low germination proportions in stratification.

	D.f.	Deviance	Resid. D.f.	Resid. Dev	F value	P value
Dieteria canescens						
Population	3	226.89	92	565.84	48.46	< 0.001 ***
Strat Length	1	10.79	91	555.05	6.91	< 0.05 *
Strat Temp	1	154.17	90	400.89	98.79	< 0.001 ***
Population x Strat Length	3	149.33	87	251.56	31.90	< 0.001 ***
Population x Strat Temp	3	109.38	84	142.18	23.36	< 0.001 ***
Heliomeris multiflora						
Population	2	349.70	69	509.20	179.43	< 0.001 ***
Strat Length	1	322.45	68	186.75	330.90	< 0.001 ***
Strat Temp	1	54.36	67	132.39	55.79	< 0.001 ***
Population x Strat Temp	2	66.17	65	66.22	33.95	< 0.001 ***
Heterotheca villosa						
Population	2	30.48	69	201.63	12.33	< 0.001 ***
Strat Length	1	9.37	68	192.26	7.58	< 0.01 **
Strat Temp	1	23.42	67	168.85	18.95	< 0.001 ***
Population x Strat Length	2	8.69	65	160.15	3.52	< 0.05 *
Population x Strat Temp	2	77.20	63	82.95	31.24	< 0.001 ***
Machaeranthera tanacetifolia						
Population	3	880.44	92	444.66		< 0.001 ***
Strat Length	1	161.75	91	282.91		< 0.001 ***
Strat Temp	1	37.40	90	245.51		< 0.001 ***
Population x Strat Length	3	72.21	87	173.30		< 0.001 ***
Population x Strat Temp	3	92.36	84	80.94		< 0.001 ***
Packera multilobata						
Population	1	121.14	46	643.51	65.44	< 0.001 ***
Strat Length	1	528.37	45	115.15	285.42	< 0.001 ***
Strat Temp	1	41.89	44	73.26	22.63	< 0.001 ***
Penstemon comarrhenus						
Population	1	70.66	46	227.22		< 0.001 ***
Strat Length	1	209.45	45	17.77		< 0.001 ***
Plantago patagonica						
Population	3	306.43	92	685.97		< 0.001 ***
Strat Length	1	572.37	91	113.60		< 0.001 ***
Strat Temp	1	8.31	90	105.29		< 0.01 **
Population x Strat Temp	3	51.17	87	54.12		< 0.001 ***
Sphaeralcea parvifolia						
Strat Length	1	45.31	46	274.07	34.11	< 0.001 ***
Strat Temp	1	221.38	45	52.68	166.63	< 0.001 ***

Appendix


Population ID
Non dormant + cool
Non dormant + warm
Monsoon + cool
Monsoon + warm
Short winter + cool
Short winter + warm
Long winter + cool
Long winter + warm
Tolerance range
CLSE_UT 0.23
CLSE_AZ 0.25
CLSE_NM 0.25
DICA_UT1 0.97
DICA_UT2 0.98
DICA_AZ 0.99
DICA_NM 1
HEMU_CO 0.62
HEMU_AZ 0.72
HEMU_NM 1
HEVI_UT 1
HEVI_AZ 1
HEVI_NM 1
MATA_UT1 0.96
MATA_UT2 0.96
MATA_NM4 0.9
MATA_NM5 1
PAMU_UT 1
PAMU_AZ 1
PECO_UT1 0.72
PECO_UT2 0.75
PLPA_CO 0.9
PLPA_UT 0.59
PLPA_AZ 0.88
PLPA_NM 0.75
SPPA_UT 0.95
SPPA_AZ 0.92
Germination percentage
0-20%
20-40%
40-60%
60-80%
80-100%