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The effect of plant source location on restoration success: a reciprocal transplant experiment with winterfat (Krascheninnikovia lanata)

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The effect of plant source location on restoration success: a reciprocal transplant experiment with winterfat (*Krascheninnikovia lanata*)

BY

Melanie G. Barnes

B.A., Biology, Reed College, 2001

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy

Biology

The University of New Mexico
Albuquerque, New Mexico

December, 2009

DEDICATION

In memory of my mother, Georgene Grace Barnes.

The completion of this dissertation is also dedicated to my friends and family who have supported me in this endeavor and who taught me many things about life that gave me the perspective I needed to complete this work. I would like to thank Heather Simpson, Jerusha Reynolds, Terri Koontz, Nathan Abrahamson, Jeremy Barlow, Brittany Barker, Laura Calabrese, Jennifer Hollis, Maureen Peters, Helen Barnes, and Tom Barnes. Finally, I want to thank Lisa for her love and emotional support; it means the world to me.

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ABSTRACT OF DISSERTATION

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ABSTRACT

Ecological restoration is becoming more frequent due to the increased pace of land disturbance, more comprehensive government regulations, and the recognition of the valuable ecosystem services that natural areas provide (Rice and Emery, 2003; Dodds et al., 2008). One part of restoration is revegetation, or the introduction of off-site plant materials to the restoration site. As the applications of revegetation have become more diverse, so too have the objectives of these projects. More specifically, it is increasingly important that plant propagules used in revegetation projects are from a location that is geographically or ecologically similar to the planting site.

Local adaptation and population genetic differentiation studies have provided evidence supporting the use of local plant materials for revegetation with several native plant species, primarily grasses. However, along with grass species, shrub species are also frequently used in revegetation seed mixes. To better understand the consequences of using non-local plant materials, I chose to study the population biology of the

widespread shrub winterfat (*Krascheninnikovia lanata*, Chenopodiaceae) among five populations in New Mexico, USA.

The investigation of winterfat population biology included a comparison of winterfat plant morphology *in situ*, quantification of the ecological distance between the five sites, and measurement of the rate of emergence and floral onset in a greenhouse common garden. I also carried out a reciprocal transplant experiment in which individuals from different locations were planted in replicate common gardens. Transplant survival, size, and reproduction were quantified for two years. To complement the morphological studies, the genetic structure of winterfat was quantified using nine isozyme loci. Genetic variation, population differentiation, and correlations among genetic, geographic, and ecological distance were assessed.

The following questions were addressed: (1) Do winterfat populations differ in the vegetative or inflorescence size of individuals in the field? (2) Do winterfat populations differ in emergence phenology in the greenhouse? (3) Do winterfat populations differ in floral phenology in the greenhouse? (4) How do study sites differ in soil characteristics, climate, and plant community composition? (5) Do winterfat plants perform better than plants from other locations at their site of origin? (6) Was the weather at the planting locations during the experiment different from the historical climate at those locations? (7) How much neutral genetic variation do these populations of winterfat possess? (8) How is this neutral genetic variation partitioned among populations? (9) Is genetic distance correlated with geographic or ecological distance? The data from these experiments will assist restoration practitioners in determining appropriate plant material sources for revegetation projects.

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Chapter 1

Winterfat (*Krascheninnikovia lanata*) populations exhibit morphological and phenological variation: implications for arid land restoration

ABSTRACT

Among-population morphological and phenological variation of winterfat (*Krascheninnikovia lanata*) was investigated in order to increase our knowledge of the population biology of shrub species frequently used for revegetation in western North America. For five winterfat populations in north-west and central New Mexico, USA, *in situ* plant vegetative size and inflorescence size were quantified. Seeds from 72 maternal families per site were collected and sown in a greenhouse common garden where emergence and flowering phenology were measured. Environmental differences among sites were quantified by comparing plant community composition, annual climate, and soil properties. Winterfat plants growing at the five sites varied significantly in plant volume and inflorescence size. In the greenhouse common garden, rate of emergence and onset of flowering also varied significantly depending on the population of origin. Though there was no consistent geographic pattern to the rate of emergence, plants originating from more northern or higher elevation sites flowered significantly earlier than plants from more southern or lower elevation sites. Three of the five sites had similar plant communities, but two of the sites had plant communities that differed from the other three sites and from one another. The five sites also differed significantly in

annual climate patterns and soil properties. These results suggest that the environmental heterogeneity among winterfat populations is sufficient to produce potentially adaptive local differentiation.

INTRODUCTION

Ecological restoration, as well as reclamation and rehabilitation, are becoming more frequent due to the increased pace of land disturbance, more comprehensive government regulations, and the recognition of the valuable ecosystem services that natural areas provide (Rice and Emery, 2003; Dodds et al., 2008). Restoration projects, in general, are designed to return a site to its original condition (Society for Ecological Restoration, 2004; Bainbridge, 2007). On the other hand, reclamation projects attempt to stabilize severely disturbed sites. Rehabilitation has an intermediate objective of improving ecosystem function (Society for Ecological Restoration, 2004; Bainbridge, 2007). Nevertheless, all such projects share comparable challenges to implementation, including site preparation methods, species selection, and planting techniques.

Though all restoration projects face similar obstacles, projects in arid regions have the additional limitation of low and unpredictable rainfall. Not surprisingly, practitioners in arid regions frequently have difficulty meeting their restoration goals. In addition, large areas of the arid western U.S. are affected, directly or indirectly, by disturbances ranging from light recreational use to complete alteration via resource extraction. The indirect costs of these disturbances are far-reaching and may slow ecosystem recovery. Thus, the need to reduce the cumulative impact of these disturbances over large areas means that we must develop our knowledge of arid land restoration techniques.

The need for arid land restoration research is highlighted by the many types of disturbances that restoration is used to ameliorate. These include the reclamation of hard rock mining sites (Bjugstad, 1978; Monsen et al., 1979; Reith and Potter, 1986), oil and natural gas drilling pads (Chambers, 1989; Smith and Chambers, 1993), military

installation disturbances (Bainbridge, 2007), over-grazed rangelands (Monsen and Stevens, 2004), wildlife habitat (Monsen and Stevens, 2004), areas burned by wildfires (Loftin, 2004), and road, pipeline, and transmission line corridors (Bainbridge, 2007). Commonly these projects include revegetation, or the planting of seeds or transplants. ('Revegetation' will be used instead of 'restoration,' 'reclamation,' or 'rehabilitation' for the remainder of this paper.) Revegetation assists in soil stabilization and begins the process of building an ecologically diverse site that will eventually become self-sustaining.

Although the importance of revegetation is evident, promoting vegetative growth on disturbed sites is difficult. In the past, non-native grasses were often seeded because they can establish rapidly, but this practice is now discouraged because of the possibility that non-native species may become invasive (Monsen and Stevens, 2004). Another possibility is that non-native species may perform well initially, and then decline in performance relative to native species (e.g., Petersen et al., 2004). Furthermore, we now know that many plantings with native species often failed because the seeds were not adapted to the planting location (Monsen and Stevens, 2004).

Planting seeds collected from a habitat that differs from the destination site may affect revegetation success because performance may vary depending on the site of origin. If this local differentiation allows the individuals of a population to better survive and reproduce in their "home" environment, local adaptation is indicated. Local adaptation can be a major limitation of revegetation success because the source and planting sites may differ in many of the environmental characteristics to which plants become adapted (e.g., soil characteristics, annual precipitation, growing season, or biotic

community interactions; McKay et al., 2005). These abiotic and biotic differences among sites can be thought of as 'ecological distances' rather than geographic distances because geographical proximity does not necessitate ecological similarity. Thus it may be more important to consider the ecological features of a seed source rather than simply its geographic location.

An additional hurdle to using native species for revegetation has been the limited availability of native seeds and plants relative to introduced species (Monsen and Plummer, 1978), although new resources are becoming increasingly common (Sheley et al., 2008; pers. obs.). Direct collection of native seeds from wild populations is an additional source, but this may negatively impact wild populations if not done carefully (Smith et al., 2007). In either case, the purchaser is generally not able to choose the origin of the seeds (Montalvo et al., 1997). For this reason, more information is needed on the importance of seed source to revegetation success for the species commonly used in seed mixes.

Widespread grass and shrub species are the taxa most often used in restoration projects. For example, winterfat (*Krascheninnikovia lanata*) is a widespread Chenopod sub-shrub in western North America that has been used for decades in revegetation projects because of its superior establishment success and tolerance of both drought and salinity (Soil Conservation Service, 1988; McArthur and Monsen, 2004). A better understanding of the importance of winterfat ecotype for plant survival at different locations will improve revegetation success of this species, and inform the planting methods for other woody shrubs as well.

Because the variation leading to local adaptation often occurs in traits that affect survival and reproduction, these are the ideal characters to measure. Emergence phenology is important to seedling survival because mortality is higher when a seedling emerges during unfavorable conditions (e.g., Meyer and Monsen, 1992). If a plant does survive, its vegetative size indicates plant vigor, and larger plants often produce larger inflorescences, that, in turn, produce more seeds (Nagy, 1997; Petersen et al., 2004; Smith et al., 2009; Vergeer et al., 2004; Wright et al., 2006). In addition, plants will have greater reproductive success if they flower when conditions are optimal for pollination, seed development, and seed dispersal (Clausen et al., 1940; Bennington and McGraw, 1995; Olsson and Ågren, 2002; Franke et al., 2006).

A prerequisite for determining if plant populations exhibit local adaptation is to establish that there is intraspecific variation in the characters of interest. I measured winterfat plants from five populations in order to answer the following questions: (1) Do winterfat populations differ in the vegetative or inflorescence sizes of individuals in the field? (2) Do winterfat populations differ in emergence phenology and overall emergence in the greenhouse? (3) Do winterfat populations differ in floral phenology in the greenhouse? I predicted that winterfat populations would differ in all three respects, and I predicted that northern populations would emerge and flower later than southern populations. In addition, to quantify the ecological 'distance' between populations, I asked the following: (4) How do study sites differ in soil characteristics, climate, and plant community composition? I predicted that the five sites would be widely different in these environmental factors and thus may represent very different selective regimes for winterfat.

METHODS

Study species—Winterfat [*Krascheninnikovia lanata* (Pursh) A. Meeuse & A. Smit, Chenopodiaceae] is a widespread, wind-pollinated, perennial sub-shrub common in intermountain western North America. This species was chosen because of its wide distribution, its use in revegetation seed mixes, and its desirable forage quality. Winterfat grows on a variety of soil types and elevations (McArthur and Monsen, 2004) and it is a valuable forage source for wildlife and livestock (Stevens et al., 1977). Winterfat is found from northern Mexico to Saskatchewan and from California to Nebraska (Holmgren, 2004).

Study sites—Study sites were selected over geographic and environmental ranges that would be plausible for seed translocation for revegetation purposes. Sites were 95-115 km apart, located on either Bureau of Land Management- or State of New Mexico-managed land, and had preexisting winterfat populations. All sites were disturbed due to long-term livestock grazing. Two transects, one north-south and one east-west, were established with three sites along each transect (Fig. 1). The towns located nearest to each site were Torreon (Sandoval Co.; 107º11’15.58”W, 35º49’27.42”N), Albuquerque (Bernalillo Co.; 106º34’40.82”W, 34º57’35.49”N), Socorro (Socorro Co.; 106º29’36.92”W, 33º57’34.46”N), Grants (McKinley Co.; 107º52’28.15”W, 35º24’52.59”N), and Gallup (McKinley Co.; 108º57’14.7”W, 35º34’33.47”N), New Mexico, USA. All appropriate permissions for entry and work were acquired from state, federal, and tribal entities.

At each site, three 30 m × 30 m plots were established, except at Albuquerque, where six plots were established because this site was included in both transects. Thus

there were a total of 18 plots. Within each plot, a 20 m × 20 m planting area in the center was designated. The 5 m-wide area surrounding the planting area served as a reference area.

Winterfat measurements—Thirty winterfat plants in the reference area of each plot were randomly selected and marked with a uniquely numbered aluminum tag. For two plots that had fewer than 30 plants, all individuals in the reference area were marked. For each plant, maximum height, maximum width, and the length of the axis running perpendicular to the maximum width were measured to the nearest centimeter and used to calculate plant volume as the volume of an ellipsoid. The number of stems of each plant was also quantified in order to distinguish between spindly and prolific plants. For plants with less than approximately 30 stems, each stem was counted individually. On plants with more than 30 stems, stem number was estimated by counting stems in groups of five. Only green, obviously living portions of plants were measured.

The total inflorescence length of each plant was measured as an estimate of reproduction. Winterfat inflorescences develop at the tip of one or more stems, bearing small (about 3 mm diameter) unisexual flowers. Inflorescence length was used as a measure of reproduction rather than flower number because counting the numerous individual flowers was impractical. Fruit number was not a useful estimate of reproduction either because winterfat plants are heterodichogamous (pers. obs.), meaning plants may have either male or female flowers, or a mix of both, and the male and female phase may or may not be temporally separated. Thus counting fruits would not be an adequate individual-level estimate of reproduction, as it would underestimate male flowering. Inflorescence length included any portion of a stem tip that had evidence of

flowers or seed development from the current growing season. For plants with many stems, the inflorescence length of three stems representing a low, medium, and high length were measured and these were averaged and multiplied by the estimated number of stems for an approximate total inflorescence length. All sites were sampled during the same growing season (Fall 2005).

Emergence—Seeds (diaspores) were collected from each site in October and November, 2005. I collected seeds from 50 individuals within each plot, and kept seeds from each individual separate. When seeds from 50 plants were not available from a plot, plants were targeted for seed collection by increasing distance from the plot until enough seeds were obtained. Due to heavy grazing, there were no seeds available from the Torreon site. Instead, seeds were collected from nearby winterfat populations within 8 km of the site. All collected seeds were stored in paper coin envelopes within paper bags, and chilled in a 4-7˚C refrigerator for at least 9 weeks prior to planting, which has been shown to increase winterfat emergence (Springfield, 1968a; Allen et al., 1987).

Twenty-four maternal plants were randomly selected from the set of seeds collected from each of the 18 plots for observation of emergence. I planted four seeds per pot in eight replicate pots per maternal plant, for a total of 32 seeds per maternal plant. If a randomly selected maternal plant did not have 32 seeds available, a replacement from the same plot, or if necessary, the same site was randomly chosen. A total of 13,824 seeds were planted (18 plots × 24 maternal plants × 8 replicate pots × 4 seeds per pot), with a target of 3,456 plants after thinning. Seeds were planted in 1:1 construction sand:potting soil (MetroMix 360, SunGro Horticulture, Bellevue, WA) in 25.4 cm-deep by 6.4 cm-wide pots (Deepots, Stuewe & Sons, Inc., Albany, OR) and kept in a temperature-

controlled room in the University of New Mexico Research Greenhouse with natural lighting. The greenhouse was set to a day temperature of 15.6-21.1˚C and a night temperature of 10.0-15.6˚C. Winterfat prefers cool emergence temperatures (Springfield, 1968b; Springfield, 1972). Seedlings were watered daily with a misting nozzle.

Within 10 d, seeds began to emerge. Time from sowing to emergence was measured for each seed. Twice a week pots were checked for new seedlings and a colored toothpick corresponding to a particular census day was placed next to each new seedling. After one month, emergence had tapered off and censuses were stopped.

Floral phenology—In March and April 2006, plants were thinned to one seedling per pot. When necessary, thinned seedlings were moved to empty pots of replicates of the same maternal family to achieve the target of eight seedlings per family. If a maternal family had less than six seedlings, 32 seeds (or as many as were available) were re-sown in Petri plates on moist filter paper in a 24ºC growth chamber with 16 h of 220 μmol m^{-2} s^{-1} light (Conviron CMB3244, Controlled Environments, Inc., Pembina, ND), then transplanted to pots in the greenhouse once cotyledons were extended. Maternal families with extremely poor emergence were randomly replaced with a family from the same plot or site. I replaced two-thirds of the maternal families from Grants, and one family from Albuquerque. After thinning, about two months after sowing, I provided each plant with a single application of 5 ml slow-release granular fertilizer (Osmocote Smart Release 14:14:14 N:P:K, Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants were watered daily.

In May 2006, some plants began to flower. At this time, twice-weekly censuses for commencement of flowering were taken in order to compare flowering times among

source populations. Winterfat is heterodichogamous, with unisexual flowers and plants that can be completely male, completely female, or any combination of the two, with or without temporal separation of the sexes. Most plants exhibited a pattern of female flowers opening first, followed by male flowers. For this reason, the sex of the first flower observed was recorded and the date the first male flower was observed was also recorded. Plants that had male flowers initially remained male.

Plant community—In order to quantify a biotic component of ecological distance between sites, I characterized the plant community at each site. Within each plot, 54 1-m^2 quadrats were randomly selected. Within each quadrat, the number of individuals of each plant species was counted. Only species from the current growing season were included, which means that these were conservative approximations of plant community diversity as some annuals were likely not present that year. All sites were sampled during the same growing season (Fall 2005).

Climate—Differences in climate across sites were included as an abiotic measurement of ecological distance. The National Oceanic and Atmospheric Administration's (NOAA) National Climatic Data Center (NCDC) has a network of weather stations that collect data on a regular basis (National Climatic Data Center, 2009). The stations nearest to each site that had the variables and time range of interest were selected. These were: Gallup Municipal Airport (NCDC Co-op no. 293422, McKinley Co., NM; 2054 m elevation, 16.2 km southeast of 'Gallup' site), Grants-Milan Municipal Airport (NCDC Co-op no. 293682, Cibola Co., NM; 1987 m elevation, 23.9 km south of 'Grants' site), Albuquerque International Airport (NCDC Co-op no. 290234; Bernalillo Co., NM; 1618 m elevation, 10.1 km north of 'Albuquerque' site), Torreon

Navajo Mission (NCDC Co-op no. 299031, Sandoval Co., NM; 2106 m elevation, 2.9 km south of 'Torreon' site), and Bingham 2 NE (NCDC Co-op no. 290983, Socorro Co., NM; 1692 m elevation, 14.0 km east of 'Socorro' site). For each of these stations, monthly surface data reports were obtained for the time period between September, 1983 (the earliest month for which all stations had data) and December, 2008 (the latest month available at the time of data analysis). The following variables were included: monthly mean minimum temperature (ºC), monthly mean maximum temperature (ºC), monthly mean temperature (ºC), total monthly precipitation (mm), and total monthly snowfall (cm). Not all variables were available for every month at every station, but this occurred infrequently enough that it is unlikely to have had a large effect on the results.

Soil—The soil characteristics of each site were quantified to represent another abiotic component of ecological distance. Soil cores for analysis of chemical properties were collected from all sites in October 2007. Approximately 16 cores per plot were collected at a depth of 15 cm, and bulked by plot. Samples were allowed to dry, and then sent for analysis to a commercial laboratory (A & L Plains Agricultural Laboratories, Inc., Lubbock, TX). For each sample, the following were quantified: percent organic matter; concentrations of phosphorous (weak bray and strong bray), potassium, magnesium, calcium, sodium, nitrate, soluble salts, pH, and cation exchange capacity.

Soil texture was measured for a second set of soil cores that was collected in the summer of 2006 (with the exception of two Albuquerque plots which were collected in March 2009). These samples were collected in the same manner as the first set. Bulked samples were transferred to paper sacks and dehydrated in a drying oven at 60°C for approximately three days. Soil texture analysis was performed using the hydrometer

method (Gee and Bauder, 1982). Hydrometer measurements (g soil colloids per L at 20°C; model I-8053, Braun Corp., USA) and temperature of soil mixtures were taken 40 sec. and 2 hours after resuspension. Data were reduced according to the methods in Gee and Bauder (1982) to estimate the percentages of sand, silt, and clay in each sample.

Data analysis—Winterfat plant volume was quantified as the volume of an ellipsoid [4/3 π(1/2 maximum width)(1/2 maximum height)(1/2 perpendicular width)]. Total inflorescence length was calculated as the sum of the inflorescence length of each stem, or the mean of three representative inflorescence lengths multiplied by the estimated number of stems. An additional variable, stem density (stems per unit volume), was calculated by dividing the plant volume by the number of stems. Plant volume, total inflorescence length, number of stems, and stem density were natural log-transformed to improve normality, after which the distributions were approximately normal. I performed analyses of variance (ANOVA) with PROC GLM (SAS Institute, 2008) in which site (Gallup, Grants, Albuquerque, Torreon, and Socorro) was an independent fixed effect, plot nested within site was an independent random effect, and plant volume, total inflorescence length, number of stems, and stem density were the dependent variables.

Failure time analysis (PROC LIFETEST; SAS Institute, 2008) was one of two methods used to examine the emergence and floral phenology data, where "failure" was represented by either emergence or flowering. This analysis allows comparison of both the proportion of individuals that "fail" and the rate at which they "fail." The effect of site on rate of emergence or floral initiation was analyzed and multiple comparisons were made with a Tukey-Kramer Test. Within-site variation was determined by examining the effect of maternal family on the failure rate within each site.

Differences in gender expression among plants from different sites were also analyzed using a survival analysis (PROC LIFETEST; SAS Institute, 2008) that compared the rate of female flower initiation and male flower initiation among sites. Post-hoc comparisons were made with a Tukey-Kramer Test. To examine the overlap of male and female flowering, the number of days between male and female flowering was calculated by subtracting the first day that female flowers were observed from the first day that male flowers were observed. Plants produced female flowers before male flowers. An ANOVA (PROC GLM; SAS Institute, 2008) was used to compare the mean number of days between initiation of female and male flowers among sites of origin. Multiple comparisions were done using a Tukey's Studentized Range Test.

The second method used to analyze the emergence and floral phenology data was an analysis of variance (ANOVA, PROC GLM; SAS Institute, 2008) where days to emergence or flowering was the dependent variable, while site was an independent fixed effect and family nested within site was an independent random effect. Only plants that emerged or flowered were included in the respective analyses. Days to emergence was natural log-transformed for analysis to improve normality. Multiple comparisons were performed using the Tukey's Studentized Range Test.

To determine differences in community composition among sites, plant community data were analyzed with a canonical discriminant analysis (PROC CANDISC; SAS Institute, 2008) where species abundances were grouped by site. In addition, summary variables were created to examine overall differences among sites. An ANOVA (PROC GLM; SAS Institute, 2008) was used to investigate the fixed effect of site on the total number of individuals, number of shrub individuals, number of

herbaceous individuals, number of grass individuals, and number of species. All plant community summary variables were square-root-transformed for analysis to improve normality. Multiple comparisons were made with Tukey's Studentized Range Test.

For the climate analysis, the three precipitation variables were natural log-transformed to improve normality, after which these variables had approximately normal distributions. To quantify the proportion of precipitation that falls as snow, the variable 'snow:precipitation' was created (snow:precipitation = total monthly precipitation/total monthly snowfall). The overall difference in climate was examined with a principal components analysis (PROC PRINCOMP; SAS Institute, 2008) that found two factors that explained 89% of the variation in the climate variables. These two factors were then included as dependent variables in ANOVAs (PROC GLM; SAS Institute, 2008) where station was an independent fixed effect. Multiple comparisons were made with Tukey's Studentized Range Test.

A principal components analysis (PROC PRINCOMP; SAS Institute, 2008) was also used to examine overall differences in soil characteristics among sites. Four factors were produced that explained 99% of the variation in the soil variables. These four factors were included because they had eigenvalues greater than one, indicating high explanatory power (Quinn and Keough, 2002). The fixed effect of site on these four factors was investigated with ANOVA (PROC GLM; SAS Institute, 2008) followed by Tukey's Studentized Range Test multiple comparisons.

RESULTS

Field populations—The mean volume of winterfat plants growing at the Socorro site was twice as large as the next largest plants in Albuquerque and Gallup, and nearly 20 times as large as the smallest plants in Torreon (Table 1). However, the differences among mean inflorescence lengths at each site were not as variable (Table 1). Plants with large volumes did not necessarily also have a high number of stems (Table 1). Overall differences among sites were statistically significant for all variables (Table 2). And, most of the pair-wise differences among sites were also statistically significant (Table 1, Table 2). There were some differences among plot within site but these were much smaller than the site effects (Table 2).

Emergence—Seeds collected from the five field populations demonstrated relatively high emergence rates (50-75%), except for Grants, which had a 7% emergence rate (Fig. 2). The rate of emergence varied significantly among sites, and among maternal families within sites when examined in a survival analysis (Table 3). In addition, there was also a significant effect of site ($F_{4, 6734} = 24.04$, $P < 0.0001$) and family nested within site ($F_{401, 6734} = 2.53$, $P < 0.0001$) on the number of days to emergence. Gallup and Torreon seeds emerged first, followed by Albuquerque and Socorro seeds, and Grants seeds emerged significantly later (Table 4). The lowest amount of variability in number of days to emergence was found for seeds from Gallup and Torreon, while seeds from Grants had the most variability (Table 4).

Floral phenology—Individuals originating from the Gallup site flowered much earlier than those from other sites, with individuals from the Socorro site beginning to flower when 98% of plants from Gallup had already commenced flowering (Fig. 3). The

higher elevation and more northern sites flowered first, while the two lower elevation southern populations flowered later (Fig. 3). The rate of floral initiation, both overall and for initiation of each gender, varied significantly among sites and among maternal families within each site, except Socorro (Table 5). In addition, there was a significant effect of site ($F_{4, 1280} = 83.7$, $P < 0.0001$) and family nested within site ($F_{364, 1280} = 1.76$, $P < 0.0001$) on the number of days to flowering for those plants that flowered. The plants from colder locations (Gallup, Grants, and Torreon) flowered significantly earlier than plants from warmer locations (Albuquerque and Socorro; Table 4). Plants from colder sites also showed more variability among maternal families for the number of days to flowering (Table 4). In addition, the overlap of male and female flowering also differed among sites ($F_{4,353} = 6.28$, $P < 0.0001$) and among maternal families within sites ($F_{256,353} = 1.43$, $P = 0.0010$). However, there was no geographic or climatic pattern to these differences (Table 6).

Plant community—The Gallup, Grants, and Torreon winterfat study sites had similar plant communities, while the Albuquerque and Socorro sites had communities that were different from the other three sites and from one another (Wilk's $\lambda = 0.0024$, $F_{256,3902} = 53.82$, $P < 0.0001$; Fig. 4). The first canonical variable was positively correlated with species abundant in Socorro and negatively correlated with species abundant in Albuquerque. The second canonical variable was also positively correlated with species abundant in Socorro, but negatively correlated with species abundant in Grants, Gallup, and Torreon.

The Albuquerque and Grants sites had the highest density of winterfat plants, while the Gallup site had the lowest density (Table 7). All five sites had significantly

different mean species richness per square meter, with the Gallup site having the highest species richness and the Grants site having the lowest (Table 7, Table 8). The Albuquerque site had the most dense plant community, while the Grants site had the sparsest community (Table 7). The southern sites, Socorro and Albuquerque, had an abundance of herbaceous individuals, while the Torreon, Gallup, and Grants sites were more abundant in grass and shrub individuals (Table 7). The effect of site, and plot nested within site, on species richness and the plant density variables was significant for all variables except number of grass individuals (Table 8).

Climate—The five sites differed in both temperature and precipitation, with the three northern sites (Gallup, Grants, and Torreon) having colder winters and more winter precipitation than the southern sites (Fig. 5, Fig. 6). Gallup was both cold and dry, while Socorro was warmer and wetter (Fig. 5, Fig. 6).

The six climate variables were collapsed into two factors that accounted for 89% of the variation among climate means. The first factor, which explained 68% of the variation among climate means, was primarily affected by temperature (Table 9). The second factor, which explained 20% of the variation, had a strong relationship with the precipitation measures (Table 9). These factors differed among stations, within years, and across months among stations (Table 10). For factor 1 (highly correlated with temperature), the two southern sites, Socorro and Albuquerque, were not different from one another, but the other three sites were different from each other and from Socorro and Albuquerque (Table 9). For factor 2 (correlated with precipitation), the two driest sites (Albuquerque and Gallup) were indistinguishable, and the three wetter sites (Grants,

Torreon, and Socorro) were indistinguishable (Table 9). These five sites represent a range of annual temperature and precipitation patterns.

Soil—The five sites also differed significantly in soil chemical and texture properties (Table 11, Table 12). Torreon had higher clay content than the other four sites, which ranged from clay loam to sandy clay and sandy loam with Albuquerque being the sandiest site (Table 12). Soil pH was neutral to alkaline and organic matter levels were low for all sites (Table 12). Socorro showed markedly different soil properties from the other sites, with a high cation exchange capacity and calcium level accompanied by a low magnesium level (Table 12).

Four factors that explained 91% of the variation in the 13 soil variables were produced in a factor analysis. The abundance of calcium and the accompanying high cation exchange capacity at the Socorro site were reflected in factor 1 (see factor means and loadings in Table 11), which varied significantly among sites ($F_{4,13} = 40.30$, $P < 0.0001$). Factor 2 represented differences in phosphorous levels and soil texture, with the Albuquerque and Socorro sites (more sandy-silty) having higher factor 2 values than the Torreon site, which had clayey soils (Table 11). Factor 2 also varied significantly across sites ($F_{4,13} = 19.38$, $P < 0.0001$). Factor 3 isolates the Grants site as having a significantly different composition of magnesium levels, nitrate levels, and soil texture from the other sites ($F_{4,13} = 35.64$, $P < 0.0001$). Factor 4 highlighted differences among sites in soil texture and nutrient levels, with the Torreon site having a much higher factor 4 value because its soil has high clay content, and the Gallup site having a low factor 4 value because it is high in sodium and organic matter, but low in magnesium and phosphorous (Table 11; $F_{4,13} = 14.11$, $P = 0.0001$).

DISCUSSION

The presence of local differentiation among populations of a species indicates the potential presence of local adaptation. When the donor plant materials in revegetation projects are locally adapted, this increases the failure rate of recipient plantings to the extent that they are not adapted to local conditions. However, the effect of using non-local donor plant materials on the success of recipient plantings is seldom studied in widespread species used for revegetation. For this reason, I chose to study the potential for local adaptation in the common shrub winterfat.

To explore the potential presence of adaptive variation in winterfat populations, I first asked whether winterfat plants at five locations in New Mexico varied in size, density, or inflorescence length. I found that winterfat growing at each site varied significantly in these characteristics (Table 1, Table 2). Plants growing at the most northern site (Torreon) were markedly smaller and had shorter inflorescence lengths compared to the plants at other sites, which was likely due to overgrazing by horses. Although winterfat is typically able to withstand livestock grazing, overgrazing during the spring and summer growing seasons is detrimental (Stevens et al., 1977; Rasmussen and Brotherson, 1986). While plant size and inflorescence length varied among sites, there was no clear relationship with elevation or latitude. It is important to note that the morphology of plants in the field is a product of both their genetic makeup and their environment (Kawecki and Ebert, 2004). Because the environment of each site is different, it is impossible to say whether genetic or environmental effects have a greater impact on plant morphology. Nevertheless, the significant variation among populations demonstrates the possibility that locally adaptive variation may exist, a hypothesis that

can be tested with a common garden experiment that limits environmental variation (Rowland et al., 2000; Rowland, 2001; Hufford and Mazer, 2003).

Next, I asked whether plants from the five winterfat populations varied in rate of seedling emergence and floral onset. These experiments were carried out in a greenhouse common garden. Seeds from each site varied significantly in rate of emergence (Table 3), with nearly all seeds from Gallup emerging and very few from Grants emerging (Fig. 2). The poor emergence of seeds from Grants was most likely because the growing season was particularly dry at that site, which may have prevented the maternal plants from being able to fill seeds adequately (Stevens et al., 1977). During seed collection and planting, I observed that seeds from Grants were much smaller than the seeds from other sites, and it has been shown that larger seeds of winterfat have better emergence rates (Springfield, 1973). Thus the difference in emergence among seed sources was likely due to variability in seed viability rather than varying responses to environmental factors.

Plants from different sites also varied significantly in the rate of floral onset, with plants from Gallup flowering the most and the earliest, and plants from Socorro flowering much later (Fig. 3, Fig. 4, Table 5). The rate of floral onset was more rapid for higher elevation and higher latitude sites, and slower for lower elevation southern sites (Fig. 3), which was verified by a small, but significant, positive relationship between elevation (R^2 = 0.1928, $P < 0.0001$) and latitude ($R^2 = 0.0799$, $P < 0.0001$) on day of floral initiation. One possible explanation is that the number of frost-free days a winterfat plant experiences triggers floral initiation, with plants from colder areas responding to fewer frost-free days than plants from warmer areas. Because the common garden was in Albuquerque, which has a longer growing season than the other sites, the plants from

colder climates reached the threshold number of frost-free days that cues floral initiation at their home site earlier. In the field, winterfat plants flower in June to July (pers. obs.). In a typical year, plants in Gallup, the coldest site, have experienced about 106 frost-free days (National Climatic Data Center, 2009; not shown) prior to floral onset, while Albuquerque plants have experienced 205 frost-free days. When plants from Gallup began to flower in the greenhouse in Albuquerque in May 2006, they had experienced at least 95 frost-free days (in addition to being sheltered from overnight frosts), which is approximately the number of frost-free days prior to flowering at their location of origin. This argument is supported by the negative relationship between the cumulative number of days at the site of origin with minimum temperatures below 0°C and the day of floral onset (square-root-transformed) in the Albuquerque greenhouse common garden (R^2 = 0.1828, $P < 0.0001$).

In addition, it was observed that the earlier flowering plants were also generally smaller upon floral initiation than plants from southern sites, which was verified with a regression analysis in which I found that plants that flowered at a later date were taller ($R^2 = 0.0439$, $P < 0.0001$). This indicates that warmer locations with long growing seasons enable a strategy of increasing plant size prior to reproduction, which could increase seed production. An analysis of the effect of maternal family nested within site on the number of days to flowering revealed that there is genetic variation in this trait, and that plants from colder sites had more variability than those from warmer sites (Table 4). Earlier flowering for plants from sites with shorter growing seasons has been found to have a genetic basis in other species (Clausen et al., 1940; Olsson and Ågren, 2002; Franke et al., 2006; Bennington and McGraw, 1995).

Winterfat plants were observed to be heterodichogamous, meaning that plants have unisexual flowers and each plant may either have only male flowers, only female flowers, or both. Furthermore, the presence of both male and female flowers on an individual may or may not be temporally separated. The complex breeding system of winterfat has been noted by others (Stevens et al., 1977), and heterodichogamy is also the breeding systems for *Grayia brandegeii*, another chenopod shrub (Pendleton et al., 1988; Pendleton et al., 2000). To explore the patterns of differential gender expression among plants from each site, the rates of onset of female and male flowering were examined. These patterns were similar to those seen for overall flowering, except that the male gender expression generally occurred later than female gender expression. For plants that expressed both sexes, there were more days between sexes for plants from Grants and Socorro (Table 6). This could represent adaptive variation because having temporal separation of male and female function limits the chance of self-fertilization. [Individual flowering winterfat plants isolated in a greenhouse room were able to set seed (pers. obs.).] Self-fertilization may be a reproductive assurance strategy for winterfat individuals in environments with few available mates. Overall, the number of days between male and female flowering of winterfat plants in the greenhouse would allow self-fertilization to occur only rarely.

Because many of my predictions of winterfat morphology and phenology were informed by conjectures about the environment at each site, and because environmental factors are known to promote local adaptation of plant species, (Santamaría et al., 2003; Macel et al., 2007), I also asked how the sites differed in plant community composition, climate, and soil characteristics. Plant community composition was examined because

different species may have altered competitive or facilitative interactions with winterfat, which could provide an additional source of selection. For instance, individuals of a widespread European herb that were transplanted to sites with more similar vegetation communities exhibited higher fitness (Becker et al., 2008). Plants have also been shown to exhibit local adaptation to certain interspecific interactions, including level of competition (Rice and Knapp, 2008), presence and nature of allelopathic chemicals (Grøndahl and Ehlers, 2008; Callaway et al., 2005; Vivanco et al., 2004), and below-ground root interactions (Mahall and Callaway, 1996). The variation in plant community among winterfat field sites represents an additional feature of the surrounding environment to which winterfat plants may be locally adapted.

Patterns in climatic variability among the five sites generally separated the sites into higher elevation and lower elevation groups. The lower elevation sites had higher temperatures and longer growing seasons, while the higher elevation sites were colder, though they did not necessarily receive more precipitation (Fig. 5, Fig. 6). The differences in precipitation were more clearly related to temporal differences in precipitation patterns rather than absolute magnitude of precipitation received. For instance, the southern-most site received the most summer monsoon precipitation, while the higher elevation sites received more winter precipitation in the form of snow (Fig. 6). These distinctly different precipitation patterns could impose selective pressure to time growth and reproduction in a manner that maximizes survival. This could mean that individuals originating from areas with abundant summer precipitation, for example, may not perform as well if moved to a site with little summer precipitation.

Soil properties varied considerably in texture and chemical composition, but there was not an apparent geographic pattern underlying this variation (Table 11, Table 12). High soil variability was expected because the sites were located in different geologic formations (Quaternary to Cretaceous) and soil suborders (Cambids, Argids, and Gypsids suborders of the Aridisols order; Soil Survey Staff, 2009). Though each site had unique soil properties, there were some differences among sites that can be explained by the soil series of each site. For instance, the soil series for the Socorro site originates from the calcium-rich mineral gypsum, which explains the high concentration of calcium in the soils at this site. However, though the soils at the Grants and Albuquerque sites were both of the same soil suborder (Argids), as were the soils at the Gallup and Torreon sites (Cambids), this did not account for the pattern of soil differences among sites. Thus, it may not be assumed that winterfat from similar soil series would perform well when reciprocally transplanted.

The soil characteristics at each site could explain some of the variability in plant stature at each field site. For instance, the sites with sandier soils (Albuquerque, Gallup, and Socorro), had winterfat plants with larger volumes (Table 1), while the plants at the site with the soil highest in clay (Torreon) had plants with smaller volumes, which can be accounted for by the reduced rate of water infiltration in clay soils, which could reduce water availability to deep roots. Other environmental factors may also explain differences in plant volume among sites. Nonetheless, the amount of variability in soil properties among sites is an additional selective pressure that could have produced plants that are locally adapted to site conditions. This has been shown to be the case for winterfat plants in Utah, where reciprocal transplants among four Utah soils that differed in salinity

performed better when seedlings were grown in home-site soil (Workman and West, 1969).

Even though the sites in this study were close enough that translocation would be plausible, the sites were also different enough from one another that abiotic and biotic features among sites were significantly different, and these differences could potentially result in local adaptation. In addition, the relationship between shorter growing season and earlier floral onset suggests the presence of adaptive local differentiation among winterfat populations. To determine whether winterfat plants are locally adapted to the environmental conditions at their site of origin, it is necessary to perform a reciprocal transplant experiment. This would allow comparison of local and non-local winterfat plants at each of their sites of origin. If winterfat plants perform best at their site of origin, this would provide evidence for local adaptation in this species.

Should it be found that winterfat possesses adaptive differentiation, care should be taken in selecting winterfat plant materials for revegetation projects. If seeds that are locally adapted to a particular environment are planted at a site that has different ecological conditions than site of origin, the seeds may exhibit poor emergence rates, reduced seedling vigor, high mortality, reduced growth, or otherwise sluggish performance. The supply of native seeds for revegetation projects (including winterfat), though increasing over time, is still relatively small, and as a consequence native seeds can be costly (Smith et al., 2007). Knowing the extent of local adaptation of the species commonly used for revegetation would conserve limited plant materials and financial resources by choosing seeds that are more likely to perform well at the planting site. Furthermore, increased revegetation success will lead to more rapid achievement of

project objectives, such as creation of wildlife habitat or soil conservation. Such information will augment our knowledge of what factors are important to consider when planning a successful restoration project.

Chapter 2

Effect of plant source on revegetation success: a reciprocal transplant experiment with winterfat (*Krascheninnikovia lanata*)

ABSTRACT

To investigate the importance of the plant material source used for revegetation, I performed a reciprocal transplant experiment with winterfat (*Krascheninnikovia lanata*), a widespread shrub species that is often included in revegetation seed mixes in the western U.S. Five populations located along two transects (approximately 150 km in length) in north-western and central New Mexico, USA, were demarcated. One site, Albuquerque, was included on both transects. Seeds from 72 maternal plants per site were collected and grown in a greenhouse then reciprocally transplanted among the three sites on each transect. Transplants were monitored for survival and flowering, and measurements of vegetative and reproductive size were taken periodically. After two years, there was evidence that winterfat plants are locally adapted to their site of origin, particularly at the coldest site on each transect. However, there was generally no significant effect of the interaction between source site and destination site on the measured characters. These results suggest that plants will perform better if the seed is from a colder or higher-elevation area than the planting location. Care should be taken when selecting plant materials for revegetation projects.

INTRODUCTION

The disturbance of native plant communities through agriculture, urbanization, resource extraction, and energy and transportation development has increased the demand for active restoration of degraded areas. At the same time, restorations have become more complex as practitioners have set more ambitious, ecologically-oriented goals for their projects. Whereas revegetation was commonly applied in the past to range rehabilitation programs (Monsen and Stevens, 2004) and mine reclamation (Bjugstad, 1978; Monsen et al., 1979; Reith and Potter, 1986; Powell, 1988; Munshower, 1993), revegetation is now also applied to restore wildlife habitat (Heady and Bartolome, 1977; Chambers and Germaine, 2003; Monsen and Stevens, 2004a; Bateman et al., 2008), rehabilitate riparian areas (Molles et al., 1998; Rowland et al., 2001; Chambers et al., 2004), increase ecosystem services (Baer et al., 2002; Dodds et al., 2008), and recover plant communities after wildland fires (Buckley et al., 2003). As the applications of revegetation have become more diverse, so too have the objectives of these projects. More specifically, it is increasingly important that plant propagules used in revegetation projects are of a species native to the region and, furthermore, that the propagules are from a location that is geographically or ecologically similar to the planting site.

Decades ago, when revegetation was most often used for rangeland rehabilitation and mine reclamation, native seeds, especially native shrub and forb seeds, were only available in limited quantities (Stoddart and Smith, 1955; Monsen et al., 1979; Monsen and Stevens, 2004) and the use of naturalized introduced species was more prevalent because of their better reliability in establishment (Heady and Bartolome, 1977; Monsen and Plummer, 1978; Brown et al., 1979; Thornburg, 1982; Munshower, 1993). However,

as additional revegetation projects took place and were monitored for longer periods of time, it became evident that native species could perform as well or better than introduced species (Petersen et al., 2004; Gustafson et al., 2004b). In fact, many plantings of native seeds on western rangelands may have failed because the seeds were not adapted to the planting site (Monsen and Stevens, 2004). In addition, concerns about aggressive introduced species becoming invasive added further pressure to find reliable methods for establishing native plant species (Monsen and Stevens, 2004; Sheley et al., 2008).

The use of local seed sources will be important to revegetation success whenever the species used show local adaptation to their immediate environment. Plants have been shown to be locally adapted to many conditions, including soil properties (Snaydon and Davies, 1982; Wright et al., 2006), salinity (Workman and West, 1969), climate (Keller and Kollmann, 1999; Santamaría et al., 2003; Raabová et al., 2007; Sandquist and Ehleringer, 1997), interspecific and intraspecific interactions (Mahall and Callaway, 1996; Raabová et al., 2007; Leger, 2008), allelopathic chemicals (Callaway et al., 2005; Grøndahl and Ehlers, 2008;), herbivores (Augustine and McNaughton, 1998; Biere and Verhoeven, 2008; Crémieux et al., 2008), and mycorrhizal fungi (Weinbaum et al., 1996; Pánková et al., 2008). If a species included in a revegetation project is locally adapted to different conditions than those that exist at the destination site, the seeds may not germinate, or may germinate at an inappropriate time. If seedlings survive, they may not perform as well as their local counterparts in terms of survival, growth, reproduction, competitive ability, or resistance to herbivory. The reduced performance of a maladapted

genotype could result in failure of a revegetation project (Montalvo et al., 1997; Hufford and Mazer, 2003; Rice and Emery, 2003; McKay et al., 2005).

An additional long-term consequence of planting a large number of non-local genotypes is that the offspring resulting from crosses with local genotypes might also exhibit reduced fitness relative to local parent genotypes, which is known as outbreeding depression (Waser and Price, 1994; Waser et al., 2000; Montalvo and Ellstrand, 2001; Galloway and Etterson, 2005). Outbreeding depression may not manifest itself until at least the second generation of interbred offspring, or later (Fenster and Dudash, 1994; Hufford and Mazer, 2003; Rogers and Montalvo, 2004). The possibility of outbreeding depression is of particular concern when introducing a large number of non-local individuals to a small, fragmented population because most offspring will have at least one non-local parent, which could lead to a genetic swamping of the local genotype (Jones and Johnson, 1998; Rogers and Montalvo, 2004).

Though the importance of using local seed sources for revegetation is often recognized by restoration practitioners, in practice the use of local seeds is hampered by the lack of large, commercially-available amounts of native seed (Rogers and Montalvo, 1994; Sheley, et al., 2008). Though native plant nurseries are more abundant and offer more species of plants than in decades past, selectively bred cultivars are often the only available source for native species. The species offered depends on the consistency of demand for each species, which can vary considerably between years. A further limitation is the duration of seed viability in storage. Winterfat seeds, for instance, only remain viable for a few years in cold storage (Springfield, 1974). An additional complication of the production of native seeds is the need to balance the selection

pressure from propagational increase of seeds with limiting the impacts of seed collection on wild plant populations. Some nursery propagation is necessary because direct collection from wild plant populations for all revegetation needs is impractical, and could damage the ability of wild plant populations to regenerate (Smith et al., 2007). On the other hand, unintentional selection of plants under nursery production may occur and become more pronounced with each additional generation (Campbell and Sorensen, 1984; Young and Evans, 2005), although this can be avoided by crossing individuals from multiple origins during propagation in seed orchards (Booth and Jones, 2001; Burton and Burton, 2002). The high cost and low availability of native plant species has made it imperative for restoration practitioners to limit waste of seed by using practices that increase revegetation success.

The local adaptation of plant populations to their environment has been studied throughout the last century (Turesson, 1930; Clausen et al., 1940; Clausen and Hiesey, 1958). Recently, the concept of local adaptation has been applied to restoration ecology. Because many of the species that are used in revegetation projects are not as amenable to experimentation, there were few local adaptation studies that addressed these species. An exception to this is the abundant research on tree seed zones (e.g., Campbell, 1979; St. Clair et al., 2005; St. Clair, 2006). The methodology for seed zone delineation has been successfully applied to several grass species that are used for revegetation in the western U.S. (Erickson et al., 2004; Johnson et al., 2004; Wilson et al., 2008). Most species used in seed mixes in the intermountain western U.S. are either grasses or shrubs, and are generally wind-pollinated, widespread species. For this reason, I chose to examine the presence of local adaptation in a widespread, wind-pollinated sub-shrub, winterfat

(*Krascheninnikovia lanata*, Chenopodiaceae), that has been used in revegetation projects in the western U.S. for decades (Thornburg, 1982; Soil Conservation Service, 1988; Monsen et al., 2004).

I used a reciprocal transplant experiment to determine whether winterfat plants are locally adapted to their site of origin across two 150-km transects in New Mexico, USA. The plant characteristics I measured are important to revegetation success: survival, growth, and reproduction. All three of these are pre-requisites for a healthy, self-sustaining plant population. The reciprocal transplant experiment was used to answer the following questions: (1) Do winterfat plants perform better than plants from other locations at their site of origin? The presence of local adaptation to climate conditions could be masked if there are unusual weather patterns during the experiment. For this reason, I also asked, (2) was the weather at the planting locations during the experiment different from the historical climate at those locations? I predicted that winterfat plants would exhibit local adaptation to their site of origin because of the strong environmental variation that exists among the field sites (see Chapter 1).

METHODS

Study species—Winterfat (*Krascheninnikovia lanata* (Pursh) A. Meeuse and A. Smit, Chenopodiaceae) is a widespread, wind-pollinated, perennial sub-shrub common in intermountain regions of western North America. This species was chosen because of its wide distribution, its use in revegetation seed mixes, and its desirable forage quality. Winterfat grows on a variety of soil types and is found at elevations from below sea-level to over 3,000 m (Stevens et al., 1977; McArthur and Monsen, 2004). Winterfat is found from northern Mexico to Saskatchewan and from California to Nebraska (Holmgren, 2004).

Study sites—Study sites were selected over a range that would be plausible for seed translocation for revegetation. Sites were 95-115 km apart, were located on either Bureau of Land Management- or State of New Mexico-managed land, and had preexisting winterfat populations. All sites were disturbed due to livestock grazing. Two transects, one north-south and one east-west, were established with three sites along each transect (Fig. 1). The towns located nearest to each site were Torreon (Sandoval Co.; 107º11’15.58”W, 35º49’27.42”N), Albuquerque (Bernalillo Co.; 106º34’40.82”W, 34º57’35.49”N), Socorro (Socorro Co.; 106º29’36.92”W, 33º57’34.46”N), Grants (McKinley Co.; 107º52’28.15”W, 35º24’52.59”N), and Gallup (McKinley Co.; 108º57’14.7”W, 35º34’33.47”N), New Mexico, USA. All appropriate permissions for entry and work were acquired from state, federal, and tribal entities.

At each site, three 30 m × 30 m plots were established, except at Albuquerque, where six plots were established because this site was included in both transects. Thus there were a total of 18 plots. Within each plot, a 20 m × 20 m planting area in the center

was designated. The 5 m-wide area surrounding the planting area served as a reference area.

Plant propagation—To minimize maternal effects, transplants were used rather than direct seeding. Seeds (single-seeded diaspore fruits) were collected from each site in October and November, 2005. I collected seeds from 150 individuals within each site (50 per plot), and kept seeds from each individual separate. When seeds from 50 plants were not available from a plot, plants were targeted for seed collection by increasing distance from the plot until enough seeds were obtained. Due to heavy grazing, there were no seeds available from the Torreon site. Instead, seeds were collected from nearby winterfat populations within 8 km of the site. All collected seeds were stored in paper coin envelopes within paper bags, and chilled in a 4-7˚C refrigerator for at least 9 weeks prior to planting to increase emergence (Springfield, 1968a; Allen et al., 1987).

Twenty-four maternal plants were randomly selected from the set of seeds collected from each plot for emergence observation and eventual reciprocal transplantation. In January 2006, I planted four seeds per pot in eight replicate pots per maternal plant, for a total of 32 seeds per plant. If a randomly selected maternal plant did not have 32 seeds available, a replacement from the same plot, or if necessary, the same site, was randomly chosen. A total of 13,824 seeds were planted (18 plots × 24 maternal plants × 8 replicate pots × 4 seeds per pot), with a target of 3,456 plants (192 per target plot) after thinning. Seeds were planted in 1:1 construction sand:potting soil (MetroMix 360, SunGro Horticulture, Bellevue, WA) in 25.4 cm-deep and 6.4 cm-wide pots (Deepots, Stuewe & Sons, Inc., Albany, OR) and kept in a temperature-controlled room in the University of New Mexico Research Greenhouse with natural lighting. All pots

were randomized, and pots were tagged with a unique plot-family-replicate identifier. The greenhouse was set to a day temperature of 15.6-21.1˚C and a night temperature of 10.0-15.6˚C. No supplemental lighting was provided. Winterfat prefers cooler emergence temperatures (Springfield, 1968b, 1972). Seedlings were watered daily with a misting nozzle.

In March and April 2006, plants were thinned to one seedling per pot. When necessary, thinned seedlings were moved to empty replicate pots of the same maternal family to achieve the target of eight replicate seedlings per family. If a maternal family had less than six seedlings, 32 seeds (or as many as were available) of the same maternal family were re-sown in Petri plates on moist filter paper in a 24ºC growth chamber with 16 h of 220 $\mu molm^{-2}s^{-1}$ light (Conviron CMB3244, Controlled Environments, Inc., Pembina, ND), then transplanted to pots in the greenhouse once cotyledons were extended. Maternal families with extremely poor emergence were randomly replaced with a family from the same plot or site. I replaced two-thirds of the maternal families from Grants, and one family from Albuquerque. In late March 2006, one pesticide application was used to control an aphid infestation. About 5 ml of imidacloprid (1% granular Marathon®; Ohp, Inc., Mainland, PA) was incorporated into the soil of all plants, and cyano-methyl 3-2,2-dimethyl-cylclopropane-caboxylate (11.8%, Tempo®-SC Ultra; Bayer Environmental Science, Research Triangle Park, NC) was applied only to plants that were heavily infested. To assist with uptake of Marathon®, plants were also fertilized on the day of application (2 ml per L of Jack's All-Purpose fertilizer, 20:20:20 N:P:K, J.R. Peters Inc., Allentown, PA). After thinning in April 2006, about two months after sowing, I provided each plant with a single application of 5 ml slow-release granular

fertilizer (Osmocote Smart Release 14:14:14 N:P:K, Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants were watered daily.

Plants originating from each site on a transect were reciprocally transplanted to the other two sites, and replanted to the site of origin. For instance, plants originating from the Gallup site were transplanted to Gallup, Grants, and Albuquerque. To achieve this, plants were allocated to transplant destination plots in such a way that individuals from each maternal family would be located at each of the three sites on a transect. After plant allocation, the plot-family-replicate unique identifiers were replaced with numerical identifiers that did not indicate the plant's site of origin. The plants were placed in random order and affixed with an aluminum tag bearing the numerical identifier. Plants were moved to an outdoor location in August 2006 where they remained until transport to the field sites. Watering was reduced to one or two times a week, depending on amount of precipitation that had been received.

Field planting methods—Field sites were fenced because of the differing grazing levels at each site. The Albuquerque site had no livestock present, so it was not fenced. Standard four-string ranch fence was erected around the plots at the other four sites. The fence was intended to exclude domestic livestock, but allows grazing by other mammalian herbivores (rabbits, prairie dogs, etc.). After sites were fenced, a randomized planting grid was imposed within the 20 m × 20 m planting area in each plot. White plastic pot tags indicating where to transplant plants were placed in a 13 × 13 grid, which allowed 169 possible planting positions within each plot, each 1.5 m apart. When fewer than 169 plants are available, blanks were randomly inserted. The plastic tags bore the numerical identifier of the plant that would be planted in that position.

Planting took place between October 25 and November 24, 2006. On October 24, all plants were provided one application of Root Stimulator (Hi-Yield®, 5:12:13 N:P:K, Voluntary Purchasing Groups, Inc., Bonham, TX) at a rate of 1.3 ml per L to promote rooting. Holes were dug with a motorized one-man augur with a 7.6 cm bit, except the first plot planted, in Torreon, where holes were dug using post-hole diggers and a spade, which I found to be impractical for the number of holes that needed to be dug. Holes were dug to a depth of 30 cm. Plants were transplanted to the hole they were randomly assigned to and soil was filled back in around the plant. Each plant was given about 2 L water. The number of individuals planted at each site from each destination is listed in Table 13. Sites were re-visited once or twice (depending upon planting order) for re-watering until winter snows arrived on Dec. 2, 2006, after which no supplemental water was provided.

Monitoring—In March, May, and September of 2007 and 2008, transplants were monitored for the presence of green leaf tissue. In March, this was interpreted as timing of spring leaf emergence, while in May and September, absence of green leaf tissue indicated plant death. In May and September 2007 and September 2008, the size of each plant was also measured, approximated as the volume of an ellipsoid [4/3 π (1/2 maximum height)(1/2 maximum width)(1/2 width perpendicular to maximum width)]. In addition, I recorded the number of stems on each plant, and if the plant had flowered, I counted the number of inflorescences and measured the lengths of the inflorescences, which were later summed ("total inflorescence length"). Total inflorescence length served as an estimate of reproductive investment. Winterfat inflorescences develop at the tip of one or more stems, bearing small (about 3 mm diameter) unisexual flowers.

Inflorescence length was used as a measure of reproduction rather than flower number because counting the numerous individual flowers was impractical. Fruit number was not a useful estimate of reproduction either because winterfat plants are heterodichogamous (pers. obs.), meaning plants may have either male or female flowers, or a mix of both, and the male and female phase may or may not be temporally separated. Thus counting only fruits would not provide individual-level estimates of reproduction. Inflorescence length included any portion of a stem tip that had evidence of flowers or seed development from the current growing season. For plants with many stems, the inflorescence length of three representative inflorescences was measured and these were averaged and multiplied by the estimated number of stems for an approximate total inflorescence length. For flowering plants in September 2008, I also noted the gender of the plant (male, female, or hermaphrodite).

Climate—The weather during the reciprocal transplant experiment (2006-2008) was compared to the historical (1983-2006) climate at each site to determine if there were significant differences between them. Climate data were obtained from the U.S. National Oceanic and Atmospheric Administration's (NOAA) National Climatic Data Center (NCDC) network of weather stations (National Climatic Data Center, 2009). The stations nearest to each site that had the variables and time range of interest were selected. These were: Gallup Municipal Airport (NCDC Co-op no. 293422, McKinley Co., NM; 2054 m elevation, 16.2 km southeast of 'Gallup' site), Grants-Milan Municipal Airport (NCDC Co-op no. 293682, Cibola Co., NM; 1987 m elevation, 23.9 km south of 'Grants' site), Albuquerque International Airport (NCDC Co-op no. 290234; Bernalillo Co., NM; 1618 m elevation, 10.1 km north of 'Albuquerque' site), Torreon Navajo Mission (NCDC Co-

op no. 299031, Sandoval Co., NM; 2106 m elevation, 2.9 km south of 'Torreon' site), and Bingham 2 NE (NCDC Co-op no. 290983, Socorro Co., NM; 1692 m elevation, 14.0 km east of 'Socorro' site). For each of these stations, monthly surface data reports were obtained for the time period between September, 1983 (the earliest month for which all stations had data) and December, 2008 (the latest month available at the time of data analysis). The following variables were included in the report: monthly minimum temperature (ºC), monthly maximum temperature (ºC), monthly mean temperature (ºC), total monthly precipitation (mm), and total monthly snowfall (cm). Not all variables were available for every month at every station, but this occurred infrequently enough that it is unlikely to have had a large effect on the results.

Statistical analysis—To improve normality, total volume and inflorescence length were natural log-transformed prior to analysis. Mean total plant volume was compared across time by transect with a repeated-measures multivariate analysis of variance (MANOVA, PROC GLM; SAS Institute, 2008). Mean total inflorescence length of surviving individuals was compared among source sites and destination sites using univariate analyses of variance (ANOVA, PROC GLM; SAS Institute, 2008) for each year. For both the repeated measures MANOVA and the univariate ANOVA, source site, destination site, and their interaction were included as independent fixed variables. Multiple comparisons among sites were done using the Tukey's Studentized Range test. A Bonferroni adjustment ($\alpha = 0.05/2$; Rice, 1989) was used to minimize Type I error for the inflorescence length analysis. To compare differences among source sites at each destination site, separate univariate ANOVAs were performed where source site was an independent effect and inflorescence length was a dependent variable. Maternal family

was not included as an independent effect in these analyses because there were not enough surviving individuals from each maternal family to provide sufficient statistical power. Rather, the use of maternal families in the planting design stratified genotypes across sites and ensured that within-population variation was replicated at each site.

Survival at the end of the experiment (September 2008) was compared among source sites and destination sites with logistic regression (PROC LOGISTIC; SAS Institute, 2008). The effects of source site, destination site, and their interaction on the frequency of survivors for each source site × destination site combination were computed separately for each transect. To compare the survival of plants from different source sites at different destination sites, separate logistic regressions were performed that tested the effect of source site on survival.

The frequencies of surviving plants on each transect that flowered in 2008 were compared among source sites, destination sites, and their interaction with a logistic regression analysis. The Gallup destination site was excluded from this analysis because no plants flowered there in 2008. Flowering among sites in 2007 was not analyzed because there were too few plants that flowered on the north-south ('Socorro') transect in 2007.

The mean monthly total precipitation and mean monthly temperature before planting (January 1983 to September 2006) and after planting (October 2006 to October 2008) were compared overall and by weather station using paired *t*-tests (PROC TTEST; SAS Institute, 2008).

RESULTS

Survival—Two years after transplanting, winterfat survival depended upon both the plant's source site and destination site (Fig. 7, Table 14). Differences in survival among plants originating from different source sites was particularly evident at the coldest site on each transect (Gallup and Torreon), where plants originating from those sites were better able to survive (Fig. 7, Table 14). At Gallup in particular, plants from Gallup had double the survival rate of non-local plants (Wald χ^2 for Gallup destination site = 20.00, $P < 0.0001$; Fig. 7, Table 14). At the Grants site on the east-west transect, more plants from Gallup survived than did plants from other sites, though this difference was not significant (Wald χ^2 for Grants destination site = 5.53, $P = 0.0629$; Fig. 7). Significantly more plants from Torreon survived at the home site (Wald χ^2 for Torreon destination site = 14.44, $P = 0.0007$; Fig. 7). The increase in survival rate with increasingly colder climate of origin was only apparent at Grants, Gallup, and Torreon, the three coldest sites. Survival did not depend on source site at the Albuquerque and Socorro sites (Fig. 7)

Plant volume—Changes in plant volume over time exhibited a variety of patterns; monotonically increasing growth only occurred at the Socorro site (Fig. 8). Non-local plants were often significantly larger than local plants. For instance, plants from Socorro were consistently larger than plants from Albuquerque when growing together in Albuquerque (Fig. 8, Table 15). However, local plants did maintain larger volumes than non-local plants in Torreon on the north-south transect (Fig. 8). Plants originating from all source sites grew largest when transplanted to the Albuquerque site, and were smallest when planted in Gallup on the east-west transect or Socorro on the north-south transect

(Fig. 8, Table 15). Plant volumes depended on source site and destination site, but not the interaction between the two (Table 15).

Flowering—Winterfat plants from the colder sites on each transect were more likely to flower in both 2007 and 2008. Although very few plants flowered in 2007, those that did were primarily from Gallup and Grants on the east-west transect and from Torreon on the north-south transect (Table 16). For instance, 13% of surviving plants from Gallup and Grants, on average flowered at all sites on the east-west transect in 2007, as compared to only 4% of plants from Albuquerque (Table 16). The frequency of plants that flowered in 2007 and 2008 on the east-west transect depended on the source site and destination site, but the frequency of flowering on the north-south transect only depended on source site (Table 17). The interaction between source site and destination site did not have a significant effect on the frequency of surviving plants that flowered on either transect (Table 17).

In addition to flowering at higher frequencies, plants originating from the two colder sites on the east-west transect (Gallup and Grants), produced more inflorescence length in 2007 and 2008 than plants from the warmer Albuquerque site, regardless of the destination site (Fig. 9), a difference that varied significantly by source site, destination site, and by source site across destination sites in 2007(Table 18). In 2008, inflorescence lengths on the east-west transect sites only varied significantly by destination site (Table 18). For both the Albuquerque and Grants destination sites on the east-west transect in 2008, plants from Gallup and Grants produced more inflorescence length than plants from Albuquerque (Albuquerque destination site univariate ANOVA $F_{2,354} = 7.19$, $P < 0.0001$; Grants destination site univariate ANOVA $F_{2,446} = 10.12$, $P < 0.0001$; Fig. 9). No

plants flowered at the Gallup site in 2008. On the north-south transect, plants from the most northern site (Torreon), produced significantly more inflorescence length than non-local plants at the Torreon site (Torreon destination site univariate ANOVA $F_{2,456}$ = 11.95, $P < 0.0001$; Fig. 9). However, there was no significant overall effect of source site, destination site, or their interaction on inflorescence lengths of plants on the north-south transect in 2008 (Table 18).

Comparison of climate and planting weather—The overall mean monthly temperature and precipitation during the reciprocal transplant experiment (October 2006 to October 2008) was not significantly different than the historical climate from 1983 to 2008 (t_{59} = -1.54, P = 0.130; paired t-test). Paired comparisons of individual stations also revealed no significant difference in mean monthly temperature or precipitation, except for the Gallup site, which had significantly higher temperatures during the experiment (t_{11} = -2.23, P = 0.048), and the Torreon site, which had marginally significantly less precipitation during the experiment (t_{11} = 1.97, P = 0.054).

DISCUSSION

A potential cause of revegetation failure is the use of poorly-adapted plant materials (Monsen and Stevens, 2004; Rogers and Montalvo, 2004). For instance, in a high-elevation experimental Ponderosa pine planting, only trees that originated from a similar elevation survived a hard frost ten years after planting (Squillace and Silen, 1962). However, due to the limited availability of native plant materials, it is often necessary for restoration practitioners to use propagules whose origin site is distant from the revegetation project site (Rogers and Montalvo, 2004; Sheley et al., 2008). For this reason, it would be useful to know the consequences of planting non-local individuals of native species frequently used in restoration projects. To address the effect of plant material source location on revegetation success, I conducted a reciprocal transplant experiment with winterfat, a broadly distributed shrub native to western North America.

I asked whether local winterfat plants perform better than nonlocal winterfat plants when transplanted to their site of origin. Winterfat plants reciprocally transplanted at five sites in New Mexico exhibited population differentiation, but the differentiation was not adaptive in all cases. At the colder sites on each transect (Gallup, Grants, and Torreon), individuals from the planting site (or another cold site) had higher survival, grew larger, flowered at higher frequencies, and produced more inflorescence length (Fig. 7, Fig. 8, Fig. 9, Table 14, Table 15, Table 16, Table 17, Table 18). At the warmer sites on each transect (Albuquerque and Socorro), plants from the three colder sites performed as well as plants originating from the site (Fig. 7, Fig. 8, Fig. 9, Table 14, Table 15, Table 16, Table 17, Table 18). This pattern suggests that the conditions at the three cold sites

(Gallup, Grants, and Torreon) favored gene combinations that promote survival in warmer conditions as well.

Though I refer to Gallup, Grants, and Torreon as the "cold" sites, aspects other than annual temperature varied across the five sites in this study, including latitude, precipitation, and elevation. Due to the mutifactorial nature of climate variation, it is difficult to distinguish which aspects of the climate a plant is locally adapted to (Heslop-Harrison, 1964; Rogers and Montalvo 2004). Typically, the aspect that varies most among the sites in a study is held responsible for any observed ecotypic differences. For instance, climatic differences attributed to latitude were responsible for the smaller stature of plants from more northern locations grown in common gardens for the grass *Festuca roemeri* (Wilson et al., 2008), the aquatic plant *Potamogeton pectinatus* (Santamaría et al., 2003), and 25 European grass and forb species (Turesson, 1930). In other cases, elevation differences among source sites had a stronger effect on plant morphology, as in the smaller size of *Potentilla glandulosa* plants of alpine origin (Clausen et al., 1940), greater survival and growth of Ponderosa pines from a high-elevation source when grown at a high elevation (Conkle, 1973), and higher fitness of *Artemisia tridentata* shrubs when sown at the plant's elevation of origin (Wang et al., 1997). Local adaptation to another aspect of climate, precipitation or moisture availability, was observed in the grass *Sitanion hystrix* (Clary, 1975), the herb *Holcus lanatus* (Macel et al., 2007), and over very small scales (a few meters) in the grass *Avena barbata* in its introduced range (Hamrick and Holden, 1979). Some investigators have used multivariate statistical approaches to quantify climatic or total environmental distance between source sites and planting sites, which can then be correlated with other

distance measures (e.g. geographic, genetic). This method was used to make the determination that *Lotus scoparius* plants are adapted to the environmental conditions of their home site (Montalvo and Ellstrand, 2000). Most of these studies observed decreasing plant performance when planted at sites with increasingly different climates (e.g., Hamrick and Holden, 1979; Montalvo and Ellstrand, 2000), but this was not what was observed for winterfat in this study.

Instead of plants performing less well with increasing planting distance from the home site, plants from some sites performed well at most sites regardless of distance from their home site. Plants originating at the higher elevation/colder sites (Gallup, Grants, Torreon) performed best at home and at sites with similar conditions, but also performed as well as home-site individuals at lower elevation/warmer sites (Albuquerque, Socorro). This is not a unique observation in reciprocal transplant studies. For instance, *Lotus corniculatus* plants from the Czech Republic (cold, moderate precipitation) reciprocally transplanted among sites in the United Kingdom (warmer, drier), Switzerland (cold, most precipitation), and to the site of origin performed well at the home site, but also performed well in the U.K. and in Switzerland (Macel et al., 2007). Asymmetrical reciprocal transplant results from sagebrush (*Artemisia* spp.) provenance trials led to the recommendation that seeds or plants be moved no more than 150 m up in elevation, but can be moved up to 300 m down in elevation (Mahalovich and McArthur, 2004). Similarly, seed transfer guidelines for four-wing saltbush (*Atriplex canescens*) state that moving plants from north to south are more likely to be successful than when moved in the opposite direction (Sanderson and McArthur, 2004). The superior performance of some winterfat seed sources across multiple sites (e.g., Grants

plants) suggests that selection for genotypes that survive and reproduce well under one set of conditions does not eliminate the possibility that the same genotype can do well under other conditions (Rogers and Montalvo, 2004).

The effect of destination site on winterfat plant performance was the most consistent effect in this study. For instance, survival was low in Gallup and Socorro, which was likely due to the abundance of small mammals at each site (Fig. 7). Prairie dog colonies were located within the fenced exclosure at the Gallup site, and hundreds of pocket gopher burrows were observed at the Socorro site. The erratic growth pattern of winterfat plants at most of the sites (except Socorro) suggest that plants were either dying back due to weather conditions (e.g., drought), or were subject to small mammal grazing (Fig. 8). However, sites with high survival, such as Grants, also had many prairie dogs, so other destination site effects may also be important. The effect of planting site was also stronger than the effect of source site in a reciprocal transplant study of a threatened plant, *Hydrasatis canadensis* (Sanders and McGraw, 2005), and a common grass used for restoration, *Arisitida beyrichiana* (Gordon and Rice, 1998). The absence of local adaptation across heterogenous environments is typically due to either a historical event, such as a genetic bottleneck, or a lack of genetic variability, which is often observed in species with high phenotypic plasticity (Linhart and Grant, 1996). Little or no adaptive genetic differentiation was found among populations from differing environmental conditions in several species, including invasive weeds (*Bromus tectorum* and *Alternanthera philoxeroides*) and an alpine forb (*Craspedia lamicola*; Novak et al., 1991; Geng et al., 2007; Byars and Hoffmann, 2009). In all three cases, the observed absence of local adaptation was attributed to phenotypic plasticity. Phenotypic plasticity may have

been a trait produced by natural selection in these species (West-Eberhard, 1989; Scheiner, 1993). Winterfat may possess adaptive phenotypic plasticity that allows individuals from distant locations to survive in environments different from their site of origin.

To determine if the effect of destination site on plant survival and growth was due to abnormal weather patterns during the reciprocal transplant experiment, I also compared the weather during the experiment (2006-2008) to the past climate (1983-2006). Expression of adaptive local differentiation could be distorted if the weather during the experiment differed significantly from the past climate at the site. However, the weather during the experiment was not significantly different from the past climate. Unusual weather cannot explain the observed pattern of plant performance.

The short duration of this experiment (two years) may have prevented observation of local adaptation of winterfat. In provenance testing of tree species, adaptive differentiation often does not appear until an extreme environmental event takes place, such as an extremely cold winter or a prolonged drought (Johnson et al., 2004). For example, Ponderosa pines from various elevations were planted at three elevations in the Sierra Nevada Mountains of California. After twelve years, trees from middle elevations were growing well at all three elevations, but after 29 years, trees from high elevations were significantly taller than trees from lower elevations at the high elevation site (Conkle, 1973). It is reasonable to expect that severe environmental events may reveal local adaptation of winterfat in the future because winterfat plants are long-lived; one Idaho population had a mean age of 72 years, with some plants as old as 136 years

(Yensen and Smith, 1984). Fortunately, because the reciprocal transplants were not destructively sampled, this transplant experiment will continue to be monitored.

The distribution and life history of winterfat is another possible cause of the limited adaptive differentiation observed in this study. Winterfat has a broad distribution and establishes in many different types of habitats, from salt deserts to high elevation mountain meadows (Stevens et al., 1977; Holmgren, 2004). This wide geographic distribution and ability to persist in heterogeneous environments promotes gene flow between populations and limits the ability of adaptive differentiation to arise. Winterfat populations in this study area have a discontinuous distribution, with populations at least 5 km apart (personal observation). However, winterfat had a more continuous distribution prior to the increase in livestock grazing within the last few centuries (Marquiss and Lang, 1959; Stevens et al., 1977). Thus, winterfat individuals in different populations are likely more genetically similar than their distribution suggests. Furthermore, though the five populations in this study were over 70 km apart from one another, gene flow between the populations could take place via intervening populations. There are, nevertheless, many studies that have found local adaptation over similar (or smaller) scales in species with broad distributions, including grass species (*Elymus glaucus*, *Nasella pulchra*, *Festuca roemeri*, and *Bouteloua rigidiseta*), and shrubs (*Lotus scoparius*, *Artemisia* subgenus *Tridentatae*, and *Atriplex canescens*; Millar and Fowler, 1994; Montalvo and Ellstrand, 2000; Erickson et al., 2004; Mahalovich and McArthur, 2004; Sanderson and McArthur, 2004; Rice and Knapp, 2008). However, these species either have breeding systems that promote genetic differentiation (such as self-fertilization or animal pollination) and thus encourage local adaptation (Hamrick and

Godt, 1996), or ecotypic differences that may be driven by varying ploidy levels (Mahalovich and McArthur, 2004; Sanderson and McArthur, 2004).

In contrast, winterfat is wind-pollinated, outcrossing, and there are no ecotypes demonstrated to be the result of variable chromosome numbers. Wind mediates dispersal of winterfat seeds as well. Wind dispersal facilitates gene flow between populations (Hamrick and Godt, 1996). Wind-pollinated species are less likely to exhibit local adaptation, or may be locally adapted at larger scales than species with other mechanisms of pollination (Lesica and Allendorf, 1999; McKay et al., 2005). In comparison, animal-pollinated species exhibit local adaptation at scales as small as 1 m from the home site in hummingbird- and bee-pollinated forbs (Waser & Price, 1985; Waser et al., 2000). Wind-pollination does not preclude local adaptation, however, because many wind-pollinated grass species have been found to be locally adapted to particular habitats (Snaydon and Davies, 1982; Kindell et al., 1996). However, when high levels of population differentiation and local adaptation are found in widespread grass species, the species typically have a mixed mating system (are self-fertile), or are predominantly self-fertilizing. For instance, the self-fertilizing grasses *Elymus glaucus*, *Nasella pulchra*, and *Festuca roemeri* were found to be locally adapted (Knapp and Rice, 1996; Erickson et al., 2004; Wilson et al., 2008).

Therefore, the breeding system of a species is a more important predictor of gene flow among populations than pollination mechanism, with highly outcrossing species exhibiting lower levels of population differentiation (Hamrick and Godt, 1996; Lesica and Allendorf, 1999; Hufford and Mazer, 2003; McKay et al., 2005). Most temperate conifers are highly outcrossing and wind-pollinated, and exhibit lower levels of

population differentiation relative to species that are self-fertile and wind-pollinated (Linhart and Grant, 1996). Wind-pollinated, outcrossing limber pine (*Pinus flexilis*), for example, has an observed population differentiation (F_{ST}) of 0.035, which is relatively low among plant species (Hamrick and Godt, 1996; Schuster and Mitton, 2000). Although the mating system of winterfat has not been conclusively determined, winterfat plants were observed to be heterodichogamous, meaning that plants bore only unisexual female flowers, male flowers, or both, and the genders may or may not be temporally separated. Male and female flowers were usually not observed simultaneously on winterfat plants, but instead the gender that appeared first senesced prior to stigma receptivity or anther dehiscence of the second gender (pers. obs.). For the few winterfat plants that had simultaneous male and female flowers while isolated from other individuals, seed production was observed but the seeds were not tested for viability (pers. obs.). A closely related species, *Grayia brandegei*, also has a heterodichogamous mating system accompanied by wind-mediated outcrossing (Pendleton et al., 1988; 2000). No functional overlap of femaleness and maleness was found in *G. bradegei* (Pendleton et al., 2000). These observations suggest that winterfat is capable of self-fertilization, but selfing occurs only rarely. Thus winterfat is predominantly outcrossing, which could limit population differentiation and the development of local adaptation.

Because the distribution and mating system of winterfat may be limiting genetic differentiation among populations, local adaptation in this species could be found if plants were reciprocally transplanted between populations that are more distant from one another than the five sites in this study. The locations of the sites in this study were chosen because it would be plausible for a restoration practitioner to allow movement of

plant materials over these distances (70 to 150 km). Furthermore, most of the sites are within the same Level III ecoregion (Omernik, 1995), and ecoregions have been proposed as proxy seed transfer zones in the absence of experimentally determined zones (Erickson et al., 2004). The exception is the Socorro site, which is within the Chihuahuan desert ecoregion, but is not far from the ecoregion containing the other sites (desert shrubland). Winterfat occurs at elevations from 0 m in Death Valley, CA, to 3,000 m in the mountains of Utah, and at latitudes from Mexico north to Canada (Stevens et al., 1977; Holmgren, 2004). Thus to determine if winterfat is locally adapted anywhere in its range, it would be necessary to use populations that encompass more of the environmental variation in the range of this species.

Over the relatively short distances among populations included in this study, winterfat plants exhibited differences in plant performance that depended on both plant source location and destination site. By limiting the distances that winterfat plants were reciprocally transplanted to those that would be used in practice for a restoration project, I have demonstrated that care should be taken when transferring plant materials for revegetation purposes. In particular, when local plant materials are not available, practitioners should choose materials from colder or higher elevation sites relative to the planting site. In addition, information on the population biology and breeding system of the species to be used for revegetation can be immensely useful for predicting the likelihood of the species being locally adapted. This information should include the species range, population distribution, life history, mating system (self-fertilizing, outcrossing, mixed mating), pollination mechanism, and seed dispersal mechanism (Linhart, 1995; Rogers and Montalvo, 2004). The difference in environment between the

plant material source site and the planting site should also be considered, particularly for climate and soil conditions (Linhart, 1995; Rogers and Montalvo, 2004). Using as much of this information as is available, a restoration practitioner can make an educated guess as to whether a proposed translocation would be advisable (e.g., Millar et al., 2008). It is not necessary for seeds to come from a narrow distance from the destination site when using species that are widespread and inhabit a variety of environmental conditions.

The population biology of species used in revegetation projects has increasingly been framed within the context of restoration ecology. These studies include the demonstration of population differentiation and local adaptation (or lack thereof) in common grasses (Knapp and Rice, 1996, 1997; Gordon and Rice, 1998; Hufford et al., 2008; Rice and Knapp, 2008), and in shrubs and forbs (Monalvo and Ellstrand, 2000; Bischoff et al., 2006; Smith et al., 2009). Seed transfer guidelines have also been developed for a few grass species (Erickson et al., 2004; Wilson et al., 2008) and shrub species (Mahalovich and McArthur, 2004; Sanderson and McArthur, 2004). However, the population biology of most species used for revegetation remains unstudied. Another approach to defining the appropriateness of seed transfers is the analysis of population genetic structure using molecular markers, which allows assessment of genetic differentiation among the populations of a target species. This methodology has been used to create seed transfer recommendations based on genetic similarity for species to be used in reintroduction or revegetation projects (Iwata et al., 2005; Broadhurst et al., 2006; Iwata et al., 2006; Krauss and He, 2006; Fant et al., 2008). In the meantime, for species that lack experimental studies of population differentiation, restoration practitioners are advised to use as much information as is available on the distributions, life histories, and

mating systems of restoration species in order to choose plant materials that will enhance revegetation success.

Chapter 3

Population genetic differentiation in a common shrub (*Krascheninnikovia lanata*): relationship with geographic and ecological distance and implications for revegetation

ABSTRACT

There is increasing concern that the use of non-local seed sources for revegetation projects will lead to planting failures and the waste of valuable native plant materials. One method that has been used to inform seed source choices for revegetation is the use of molecular markers to quantify genetic diversity and population differentiation in the species of interest for the region that includes both seed sources and revegetation sites. The species examined in this study, winterfat (*Krascheninnikovia lanata*), is a common arid land shrub that has been used in revegetation seed mixes in the western U.S. for decades, but there are no population-level genetic studies of the species. Nine isozyme loci were used to quantify the genetic variation of five winterfat populations in northwestern and central New Mexico, USA. Though the level of genetic variation within winterfat populations was comparable to species with similar distributions and life history characteristics ($P_p = 0.69$, $A_p = 2.39$, $H_o = 0.139$), the genetic differentiation among populations was very low ($F_{ST} = 0.008$, $\Phi_{PT} = 0.015$). The similarity among populations is likely due to the widespread geographic distribution and outcrossing, wind-pollinated breeding system of winterfat. Despite the lack of population genetic

differentiation found among these winterfat populations, seeds should not be moved over large distances to avoid deleterious consequences due to quantitative trait variation that may not have been detected with neutral markers. When population genetic studies are not available for a restoration species, practitioners should use other available information on the species life history, breeding system, and distribution to inform planting decisions.

INTRODUCTION

Differentiation among natural populations has been a continuing interest of evolutionary biologists because of the opportunity that it provides for the study of evolutionary processes, including natural selection, genetic drift, hybridization, and speciation. Population differentiation within plant species has been particularly well studied because of the economic importance of plants and the amenability of plants to experimentation (Heslop-Harrison, 1964; Linhart and Grant, 1996). The observed morphological differences among individuals of different populations often has a genetic basis, as has been demonstrated in common garden and reciprocal transplant studies (Hufford and Mazer, 2003; Kawecki and Ebert, 2004). Genetically-based population differentiation arises from natural selection imposed by contrasting environmental conditions, by genetic drift, or a combination of both processes (Linhart, 1995; Beebee and Rowe, 2004). Local adaptation can result from natural selection for individuals that have higher survival and reproduction at their location of origin. Because genetic drift can create population differentiation, the existence of differentiation does not necessarily mean that the differentiation is adaptive (Falk et al., 2006).

Although there has been theoretical interest in population differentiation for decades, the principle of population differentiation was only recently applied to plant translocation for restoration, conservation, or reintroduction purposes (e.g., Huenneke, 1991; Knapp and Rice, 1996; 1997; Kaye, 2001; Falk et al., 2006; Guerrant and Kaye, 2007; Fant et al., 2008; Menges, 2008). A primary concern is the potential existence of local adaptation and the effects its presence may have on the success of relocating

individuals from one population to another, an aspect of both rare plant reintroductions and large-scale restoration plantings. Movement of locally adapted individuals could be problematic if they are not able to survive and reproduce in the new habitat (Linhart, 1995; Montalvo et al., 1997; Lesica and Allendorf, 1999; McKay et al., 2005). Such an outcome is possible if the selection regimes at the population source and destination sites are dissimilar. Many reciprocal transplant experiments have found this to be the case, with local individuals outperforming non-local individuals in a variety of species (Clausen et al., 1940; Heslop-Harrison, 1964; Bradshaw, 1984; Linhart and Grant, 1996). Conversely, some investigators have not found strong evidence for local adaptation (Gordon and Rice, 1998; Smith et al., 2009). Nevertheless, most investigators have recommended the use of local plant materials in order to minimize the loss of individuals maladapted to the planting site (Linhart, 1995; Keller and Kollman, 1999; Lesica and Allendorf, 1999; McKay et al., 2005).

However, there are multiple obstacles that prevent the use of local plant materials for revegetation. The primary limitation is that local plant materials may not be available commercially, as is the case for many restoration plantings (Jones and Johnson, 1998; Rogers and Montalvo, 2004). When the desired species is available, the seed source may be geographically and ecologically distant from the planting location. Collection of local seeds from wild plant populations is an option, but this can be prohibitively expensive, and uncontrolled harvesting could have negative impacts on the source population (Smith et al., 2007). In addition, the quality of seeds collected from wild populations is unreliable because of variable environmental conditions. The quality of seeds from native plant nurseries is more consistent, and new breeding programs have been developed that

promote the genetic diversity of native plant materials (Booth and Jones, 2001; Burton and Burton, 2002). However, the historical norm is the propagation of a few native cultivars of each species (Rogers and Montalvo, 2004). There is concern that the widespread planting of a few cultivars or seed sources over large geographic areas could cause restoration plantings to fail (Montalvo et al., 1997; Burton and Burton, 2002; Fant et al., 2008). Another concern is that seed increase operations could unintentionally select genotypes that perform well in the nursery environment, which may or may not be genotypes that are able to survive and reproduce in the wild (Campbell and Sorensen, 1984). For these reasons, there is much interest in measuring the degree of population differentiation in native species used for restoration.

Population differentiation can be quantified by comparing both morphological and genetic characteristics among populations. Measurement of quantitative or phenotypic trait variation requires planting seeds or other plant materials from multiple locations in a common garden that minimizes the environmental differences experienced by each individual and randomizes variation within the garden across genotypes. For instance, seed zones for trees and grasses have been delineated using this method (Erickson et al., 2004; Johnson et al., 2004; St. Clair et al., 2005; Wilson et al., 2008). Because creating a common garden experiment can be quite time-consuming and labor-intensive, there has also been interest in using molecular markers to delimit seed zones for revegetation purposes (Iwata et al., 2005; Broadhurst et al., 2006; Iwata et al., 2006; Krauss and He, 2006). This approach relies on the correlation between quantitative trait variation and molecular marker variation, which has not been found consistently (Falk et

al., 2006; Leinonen et al., 2007). Thus it is important to carry out morphological studies in addition to genetic studies.

Increasing geographic distance between two populations of a species is expected to result in a parallel increase in molecular marker variation (Falk et al., 2006; Leinonen et al., 2007). The elucidation of the correlation between genetic distance and geographic distance could be used to infer appropriate seed transfer zones. For instance, if a similar correlation pattern is found for species with similar life histories, then the more easily-measured geographic distance could be used to make seed sourcing decisions without lengthy population differentiation assessment.

An additional use of molecular markers in a restoration context is to determine the level of genetic variation within subpopulations. The level of neutral genetic variation, the type of variation measured by molecular markers, is indicative of differentiation that has occurred to gene flow. Quantitative genetic variation represents the phenotypic expression of genetic variation, which is the variation that is subjected to natural selection. Although neutral and quantitative genetic variation may or may not be correlated for a species or population, knowing the amount of neutral variation can be useful in assessing the genetic characteristics of a population.

When a population lacks quantitative genetic variability, local adaptation is less likely to develop (Linhart and Grant, 1996). This has been observed in several generalist invasive species, which have low genetic variability but are still able to invade new habitats because they possess high phenotypic plasticity (Rice and Mack, 1991; Poulin et al., 2005; Geng et al., 2007). The genetic differentiation of subpopulations can also be compared to infer the likelihood of encountering negative consequences if individuals are

moved from one site to another. That is, populations that are not highly genetically differentiated are presumably connected to other populations via gene flow, which homogenizes the gene pools among sites and limits the development of local adaptation. On the other hand, high levels of genetic differentiation among populations, accompanied by quantitative trait differentiation and low gene flow could result in locally adapted ecotypes (Linhart, 1995; Falk et al., 2006).

There have been several studies that have included marker-based genetic differentiation of restoration species, including trees (Iwata et al., 2006; Liu et al., 2008), grasses (Knapp and Rice, 1996, 1997; Gustafson et al., 2004a; Iwata et al., 2005; Selbo and Snow, 2005; Iwata et al., 2006; Fant et al., 2008), and a few forbs (Schuster et al., 1994; Iwata et al., 2006; Raabová et al., 2007). These studies have found varying levels of differentiation, and have also found that the relationship between quantitative genetic variation and neutral genetic differentiation is not reliable (e.g., Knapp and Rice, 1996, 1997). However, very few shrub species used for restoration have been studied (but see Krauss and He, 2006). The lack of shrub studies is important because life history can impact genetic differentiation, thus studies on native grasses may not be useful for developing management strategies for forbs or shrubs. Shrubs tend to be longer-lived than grasses or forbs, which limits genetic differentiation because the same set of individuals can mate repeatedly over many years, producing genetically similar offspring.

Due to the few genetic studies of long-lived widespread shrubs, I chose to study the population genetics of winterfat (*Krascheninnikovia lanata*), a shrub commonly used in revegetation seed mixes that is broadly distributed throughout the western U.S. Winterfat is a member of the Chenopodiaceae, a family that includes several species of

shrubs that are frequently used in revegetation projects, such as *Atriplex* spp., *Grayia* spp., and others (Monsen et al., 2004). Despite its popularity for restoration and range improvement projects, population-level genetic variation of winterfat is poorly understood. I used isozyme variation of five New Mexico winterfat populations to answer the following questions: (1) How much neutral genetic variation do these populations of winterfat possess? (2) How is this neutral genetic variation partitioned among populations? (3) Is genetic distance correlated with geographic or ecological distance? Because of the wide distribution of winterfat, I predicted that the populations under study would have low genetic variability, and that genetic differentiation among the populations would be low. I expected that genetic distance would be more highly correlated with geographic distance than ecological distance because population differentiation in winterfat, if present, is more likely to be a result of genetic drift than adaptation to local conditions.

METHODS

Study system—*Krascheninnikovia lanata* (Pursh) A. Meeuse & A. Smit (cv. *Eurotia lanata*, *Ceratoides lanata*; Chenopdiaceae) is a long-lived perennial shrub that is broadly distributed throughout western North America (Holmgren, 2004). Winterfat is a valuable forage source for wildlife (Wood et al., 1995). Winterfat is heterodichogamous, potentially self-compatible, and most likely wind-pollinated (personal observation). The populations studied were located on public land in central and northern New Mexico, USA.

Sampling design—Five winterfat populations were selected as study sites for a concurrent reciprocal transplant experiment. The towns located nearest to each site were Torreon (Sandoval Co.; 107º11'15.58"W, 35º49'27.42"N), Albuquerque (Bernalillo Co.; 106º34'40.82"W, 34º57'35.49"N), Socorro (Socorro Co.; 106º29'36.92"W, 33º57'34.46"N), Grants (McKinley Co.; 107º52'28.15"W, 35º24'52.59"N), and Gallup (McKinley Co.; 108º57'14.7"W, 35º34'33.47"N), New Mexico, USA. The populations were arranged along two transects, one north-south and one east-west, with three populations along each transect that were approximately 70 km apart (Fig. 1). Albuquerque was contained in both transects. Each site possessed three 30 m × 30 m replicate plots (six in Albuquerque), for a total of 18 plots.

In spring (late May to early June) 2008 and fall (late August to early September) 2008, leaf tissue was collected from 30 individuals at each site. Samples were collected from ten individuals per plot at Gallup, Grants, Torreon, and Socorro. At Albuquerque, five samples from each of the six plots were collected. Leaf tissue was collected from

randomly selected individuals. This sampling provided 150 individuals from each sampling period (fall and spring), for a total of 300 individuals. However, some individuals were sampled twice, in which case the sample that produced the most readable bands was included in analysis. This gave a total of 222 samples. An additional 49 samples were excluded from analysis because one or more stains were unreadable for the sample. A total of 173 unique individuals were analyzed, and were distributed across the five sites as follows: Albuquerque, 30; Gallup, 38; Grants, 37; Socorro, 36; Torreon, 32.

Protein electrophoresis— Four to six apical meristems were collected from each individual and kept in 1.7 ml microcentrifuge tubes at 4°C for 4 to 48 hours, then crushed. Tissue was crushed on ice in approximately 0.5 ml of crushing buffer (Ellstrand, 1984) and stored at -80°C until starch gel electrophoresis. Extracts were thawed at room temperature, then immediately placed on ice. Chromatography wicks (3MM, Whatman International Ltd., Maidstone, UK) were placed in each sample, allowed to absorb extract, and then loaded on 10% starch gels (Starch Art, Austin, TX). Six individuals from each of the five populations were included on each gel in order to facilitate comparison of alleles across populations. Nine enzyme systems were resolved using four electrophoretic buffer systems, all of which were modifications of the buffer systems listed in Wendel and Weeden (1989). A continuous histidine-citrate buffer (pH = 6.0) was used to resolve aldolase [fructose-bisphosphate aldolase, FBA] (ALD, E.C. 4.1.2.13) and isocitrate dehydrogenase (NADP) (IDH, E.C. 1.1.1.42). A continuous tris-EDTA-borate buffer (pH = 8.6) was used to resolve phosphoglucomutase (PGM, E.C. 5.4.2.2), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), and leucyl amino peptidase

(LAP, E.C. 3.4.11.1). A discontinuous lithium-borate buffer (pH = 8.1) was used to resolve glucose-6-phosphate isomerase (PGI, E.C. 5.3.1.9), aspartate amino transaminase (AAT, E.C. 2.6.1.1), and esterase (EST, E.C. 3.1.1.-). A morphiline-citrate buffer (pH = 8.0) was used to resolve malate dehydrogenase (MDH, E.C. 1.1.1.37). Although multiple loci were apparent for some enzyme systems, only one locus per enzyme system was consistently interpretable. For each enzyme system, alleles were labeled and interpreted according to Weeden and Wendel (1989).

Geographic and ecological data—The location of each population was recorded using a GPS (global positioning system) unit (Juno^{TM} ST; Trimble Navigation Ltd., Sunnyvale, CA). The x and y coordinates for each population in UTMs were derived from ArcMap 9.3 (ESRI, 2008) software, then translated into the linear distance in kilometers between sites.

Ecological data included 23 variables that described the physical and biological environment at each site, composed of elevation, climate, soil, and plant community characteristics. Elevations were derived from GPS data in ArcMap 9.3 (ESRI, 2008). Climate data were obtained from National Oceanic and Atmospheric Administration's (NOAA) National Climatic Data Center (National Climatic Data Center, 2009) for the weather stations closest to each site. The weather stations used were: Gallup Municipal Airport (NCDC Co-op no. 293422, McKinley Co., NM; 2054 m elevation, 16.2 km southeast of 'Gallup' site), Grants-Milan Municipal Airport (NCDC Co-op no. 293682, Cibola Co., NM; 1987 m elevation, 23.9 km south of 'Grants' site), Albuquerque International Airport (NCDC Co-op no. 290234; Bernalillo Co., NM; 1618 m elevation, 10.1 km north of 'Albuquerque' site), Torreon Navajo Mission (NCDC Co-op no.

299031, Sandoval Co., NM; 2106 m elevation, 2.9 km south of 'Torreon' site), and Bingham 2 NE (NCDC Co-op no. 290983, Socorro Co., NM; 1692 m elevation, 14.0 km east of 'Socorro' site). Climate variables included monthly mean temperature (ºC), monthly mean minimum temperature (ºC), monthly mean maximum temperature (ºC), total monthly precipitation (mm), total monthly snow (cm), and the annual number of days with a minimum temperature less than 0ºC. The annual mean value for each variable was computed by site for the years 1983 to 2008.

Three replicate soil samples per site (six samples for Albuquerque) were evaluated for soil texture and soil chemical composition. The soil texture characteristics percent silt, clay, and sand were produced via soil texture analysis using the hydrometer method (Gee and Bauder, 1982). Soil chemical characteristics were obtained by sending samples to an agricultural laboratory (A & L Plains Agricultural Laboratories Inc., Lubbock, TX). The variables included in this analysis were percent organic matter, parts per million (ppm) phosphorous, calcium (ppm), potassium (ppm), magnesium (ppm), sodium (ppm), nitrate (ppm), pH, cation exchange capacity (meq/100g), and soluble salts (measured as conductance in mmhos/cm).

Plant community composition was quantified by measuring the abundance of each plant species in 162 randomly-selected 1-m^2 quadrats at each site. A canonical discriminant analysis (PROC CANDISC; SAS Institute, 2008)was used to generate two factors that explained the most variation in community composition among sites. These two factor variables were included in the computation of ecological distance.

Statistical Analyses—Descriptive statistics were computed by population and by locus using the program Genetic Data Analysis (GDA) 1.1 (Lewis and Zaykin, 2002).

Descriptive statistics calculated included the proportion of polymorphic loci (P_p), mean number of alleles per polymorphic locus (A_p), expected heterozygosity (H_e), observed heterozygosity (H_o), and the fixation index (f). Wright's F statistics (F_{IS}, F_{IT}, F_{ST}) were also computed for each locus using the moment estimators described by Weir and Cockherham (1984), and assuming an n-island model of gene flow.

GenAlEx 6.2 (Peakall and Smouse, 2006) was used to test for conformance with Hardy-Weinberg equilibrium (Hedrick, 2000) and to generate a summary of private alleles (alleles found only at one population) by population. The partitioning of genetic variation among populations was examined with a Φ_{PT}-based AMOVA (analysis of molecular variance; Excoffier et al., 1992; Huff et al., 1993; Peakall et al., 1995; Michalakis and Excoffier, 1996) in GenAlEx 6.2. An estimate of N_m (number of migrants per generation; $N_m = [(1/ \Phi_{PT)} -1)/4]$ and pair-wise F_{ST} values were also generated using GenAlEx 6.2.

Distance matrices for geographic distance, total ecological distance, and the individual components of ecological distance (climate, soil, and plant community) were generated using PROC DISTANCE (SAS, 2008). This procedure computes the pair-wise Euclidean distance between variables. Correlations between genetic distance (pair-wise F_{ST}), geographic distance, and ecological distance were made with Mantel tests in GenAlEx 6.2 (Mantel, 1967; Smouse et al., 1986; Smouse and Long, 1992). A Bonferroni adjustment (Rice, 1989) was used to adjust significance values for multiple comparisons.

RESULTS

Nine isozyme loci were consistently resolved, and eight were polymorphic across populations. Experimental crosses were not performed to confirm the genetic basis for the nine loci, but the observed banding patterns were consistent with the genetic interpretation made by other isozyme studies (Wendel and Weeden, 1989). The only locus that was monomorphic across all populations was aldolase (ALD). Loci that were monomorphic within populations included isocitrate dehydrogenase (IDH) at Albuquerque and Torreon; esterase (EST) at Albuquerque, Socorro, and Torreon; malate dehydrogenase (MDH) at Albuquerque, Grants, and Torreon; and leucine aminopeptidase (LAP) at Gallup. See Appendix 1 for raw isozyme data.

The mean percent of polymorphic loci (P_p) was 69%, with plants from Albuquerque and Torreon having the lowest P_p and the other sites having higher percentages (Table 19). Within populations, the average number of alleles per locus was 2.4, average expected heterozygosity (H_e) was 13.2%, and average observed heterozygosity (H_o) was 13.9% (Table 19). There was no significant difference between the observed level of heterozygosity and the level of heterozygosity that would be expected if the populations were at Hardy-Weinberg equilibrium, assuming random mating, infinitely large population size, no selection, and no migration [$P > 0.05$ for all polymorphic loci within each population, except for the AAT locus for the Albuquerque population ($\chi^2 = 6.44$, $P = 0.011$)].

Despite the low level of genetic variability, several private alleles (alleles unique to a population) were found. Two samples from Gallup had unique alleles at the isocitrate

dehydrogenase (IDH) and esterase (EST) loci, while one sample from Grants also had a unique allele at the IDH locus.

The *F*-statistics indicate that the winterfat populations studied have low genetic variability relative to other plant species. Approximately three-fifths of the inbreeding coefficient values (*f*) for within population variation were not significantly different from zero, indicating a slight excess of heterozygotes (Table 19, Table 20). Most of the F_{IT} values for the amount of variation within populations relative to the total were also negative (Table 20). The F_{ST} values for among population variation were very low, with a mean F_{ST} of 0.008 (Table 20), meaning that only 0.8% of variation was attributable to gene frequency differences among populations. Partitioning of genetic variation was also examined with a Φ_{PT}-based AMOVA, which indicated that 98% of the variation was within populations, while only 2% of the variation was among populations (Table 21). The N_m based on this AMOVA is a high 16 migrants per generation. According to some models, values of N_m less than one are required to produce population differentiation by random genetic drift (Wright, 1951; Slatkin, 1994). These results indicate that there is ample gene flow among populations and that the populations are genetically similar to one another in terms of molecular variation.

Pairwise F_{ST} values ranged from -0.007 to 0.023 (Table 22). The range for F_{ST} is from 0 to 1, thus the negative values produced are likely a product of the calculation method and generally are interpreted not to be significantly different from zero. Winterfat plants from Grants and Socorro were the least differentiated from the other populations, while plants from Gallup were the most divergent (Table 22). However, none of these

values were found to be significantly different from random after a Bonferroni adjustment for multiple comparisons.

Pair-wise ecological similarity comparisons indicated that the Gallup and Torreon sites were most similar ecologically, while Gallup and Albuquerque were the least similar (Table 23). Geographic distances ranged from 77.1 km between Grants and Torreon to 287.5 km between Gallup and Socorro (Table 23). However, there was no relationship between either geographic or ecological distance and genetic distance (Table 24). There was also no relationship between any of the individual components of ecological distance (plant community, soil characteristics, or climate) and genetic distance (Table 24). Furthermore, there was no relationship between geographic and ecological distance (Table 24).

DISCUSSION

The amount of genetic divergence among populations of native species used in restoration plantings has been used to make decisions about appropriate seed transfer distances. However, the majority of species included in seed mixtures in the western U.S. remain unstudied in this respect. In particular, there are few studies of population genetic structure in shrub species used in restoration. To increase our understanding of the relationship between genetic differentiation and revegetation success, I examined the genetic differentiation among five populations of winterfat (*Krascheninnikovia lanata*) in New Mexico, USA.

First, I examined levels of genetic diversity within each of the five populations of winterfat. Nine isozyme loci revealed levels of genetic variation that are comparable to plant species with similar characteristics (Table 19, Table 20). The mean percentage of polymorphic loci within populations was 69%, with a mean of 2.39 alleles per polymorphic locus while the mean level of observed heterozygosity within populations was 0.139 (Table 19). In a review of studies that quantified genetic diversity in woody species based on isozyme markers, Hamrick et al. (1992) classified species based on various characteristics, including the geographic range of the species, breeding system, mode of pollination, and method of seed dispersal. Among woody species that shared the same attributes as winterfat (geographically widespread, outcrossing via wind-pollination and wind-dispersed seeds), the percentage of polymorphic loci was 50.9-74.3%, the number of alleles per polymorphic locus was 1.79 to 2.56, and the level of heterozygosity within populations was 0.149 to 0.228 (Hamrick et al., 1992). Thus, the amount of

genetic variation within winterfat populations is comparable to species with similar characteristics, but low compared to plant species in general.

In contrast, the amount of genetic variation that was distributed among populations (F_{ST} = 0.008; Φ_{PT} = 0.015; Tables 2, 3) was much lower than in similar species, with 0.8 to 2% of variation occurring among populations, depending on the calculation method (Table 20, Table 21). Hamrick et al. (1992) found that woody species that are widespread, outcrossing, wind-pollinated, or wind-dispersed have a higher level of genetic variation distributed among populations (G_{ST} ranged from 0.033 to 0.077) than winterfat, but less genetic variation than the average for woody plant species (G_{ST} = 0.084; Hamrick et al., 1992). That is, the populations of geographically widespread woody species are generally less genetically differentiated than species with smaller distributions; as is the case for species that outcross via wind-pollination relative to species that are self-fertilizing or animal-pollinated, and species whose seeds are dispersed by methods other than wind (Hamrick et al., 1992). The multiple attributes associated with low population differentiation possessed by winterfat may have had a compounding effect on the level of population differentiation in this species, and can explain the lack of population differentiation observed in this species.

The five populations were not genetically differentiated from one another, although there were trends of higher pair-wise F_{ST} values for more distant populations (Table 22). There was also no correlation between genetic distance and either geographic or ecological distance (Table 24). This result was not surprising given the lack of genetic differentiation among populations. Other studies have found a significantly positive correlation between increasing genetic and geographic distance in widespread grass

species (e.g., *Nassella pulchra*, Knapp and Rice, 1997; *Uniola paniculata*, Franks et al., 2004) and shrub species (e.g., *Lotus scoparius*, Montalvo and Ellstrand, 2000; *Larrea tridentata*, Duran et al., 2005). However, no correlation between geographic and genetic distance was found for other widespread grass species (*Elymus glaucus*, Wilson et al., 2001; *Setaria viridis*, Wang et al., 1995) and shrub or tree species (*Quercus gambelii*, Kumar and Rogstad, 1998; *Haloxylon ammondendron*, Sheng et al., 2005). In addition, when the correlation between genetic and geographic distance was evaluated within only the New Mexico portion of the *Larrea tridentata* range, rather than the extent of the range, the correlation was no longer significant (Duran et al., 2005). It is possible that if I had compared samples from a larger extent of the geographic distribution of winterfat, a stronger relationship between genetic and geographic distance may have been revealed.

There was a weak correlation between geographic and ecological distance (R^2 = 0.269, $P < 0.10$; Table 24), which was likely due to the similar climates and vegetation communities shared by populations that were near one another. Ecological distance did not explain the variation in genetic distance (Table 24). Though some investigators have found a significant positive correlation between environmental or ecological distance and geographic distance (e.g., in *Nassella pulchra*, Knapp and Rice, 1997), others did not find evidence for this relationship (e.g., *Lotus corniculatus*, Smith et al., 2009; *Lotus scoaprius*, Montalvo and Ellstrand, 2000). The lack of correlation among geographic, ecological, and genetic distance in winterfat appears to be due to the high level of gene flow in this species, which has prevented population differentiation. The average number of migrants per generation (N_m) was 16, which is more than enough individuals to prevent isolation by distance.

Comparisons of isozyme marker variation with DNA-based molecular marker variation have generally shown that isozyme markers are less variable (e.g., Paupy et al., 1998; Sun et al., 1998; Freville et al., 2004). Isozymes were used for this study because of difficulty obtaining suitably pure DNA extracts for AFLP (amplified fragment length polymorphism) analysis, and because of the relative ease of resolving allozymic loci for winterfat. However, the level of marker variability within populations was comparable to that of similar species (Hamrick et al., 1992), thus the use of isozyme markers is not likely to be responsible for the observed lack of differentiation among winterfat populations.

Another possible explanation for the low level of genetic differentiation among winterfat populations is that its breeding system is primarily outcrossing via wind-pollination (pers. obs.). Winterfat has inconspicuous (3 mm diameter) unisexual flowers (Holmgren, 2004). In a greenhouse common garden study of winterfat floral phenology, plants were observed to be heterodichogamous, meaning that plants had either male flowers, female flowers, or both, and the temporal separation of the sexes varies (see Chapter 1). Of the plants that flowered in the common garden, 44% were hermaphrodites, 46% were female, and 10% were male, and there was an average of 12 days in between onset of female and male flowering on hermaphroditic plants (Table 6). Heterodichogamy has also been found in another closely related Chenopod shrub, *Grayia brandegeii*, and has been suggested as an evolutionary precursor to complete dioecy (Pendleton et al., 1988; 2000), which is the breeding system in other Chenopod shrubs including *Atriplex canescens*. Although there is the potential for self-fertilization to occur in heterodichogamous species, this is a rare occurrence and heterodichogamous species

are primarily outcrossing. In addition, because pollination in winterfat is wind-mediated, gene flow is likely to occur over great distances. Although the distance of pollen dispersal has not been quantified in winterfat, pollen transport in other wind-pollinated species has been shown to occur over tens of kilometers (e.g., 21 km in *Agrostis stolonifera*; Watrud et al., 2004). The wind-pollinated, outcrossing breeding system of winterfat likely promotes high levels of gene flow among populations and reduce population differentiation.

Another characteristic of winterfat that is associated with low population differentiation is the widespread geographic distribution of this species. Geographic distribution is the strongest predictor of isozyme variation both within and among populations of woody plant species (Hamrick et al., 1992). The distribution of winterfat ranges from Mexico to Saskatchewan and California to Nebraska (Holmgren, 2004). Winterfat is found on a variety of soil types and at elevations ranging from below sea-level elevations in Death Valley, California to over 3,000 m in the mountains of Utah (Stevens et al., 1977). The five populations included in this study represented a small portion of the species distribution, with site elevations ranging between 1566 m to 2159 m, and a maximum linear geographic distance of 288 km between populations. Greater among-population divergence may have been found if more distant populations were considered. A large geographic distribution could lead to reduced population differentiation if there is a high level of migration between populations, which would have a homogenizing effect. If this is the case, populations that are closer to one another will be more genetically similar. Species with large distributions tend to have more populations and thus there is a higher likelihood of gene flow among populations.

In addition, gene flow between populations could take place via intervening populations that were not sampled. Though winterfat populations in the study area had discrete populations, winterfat is known to have had a more continuous distribution prior to the increase in livestock grazing in the last few centuries (Marquiss and Lang, 1959; Stevens et al., 1977). Thus, though winterfat populations may appear disconnected, the pattern of gene flow estimated by the isozyme marker diversity suggests that the species historically had a more continuous distribution.

The origin of winterfat may partially explain the broad distribution of the species. Although winterfat is one of several widely distributed Chenopod shrubs in western North America, its closest relatives are Eurasian. Winterfat is the only representative of the genus *Krascheninnikovia* outside of Eurasia, and its taxonomic treatment varies. I have followed the taxonomic treatment of Meeuse and Smit (1971) in which the species name was changed from *Eurotia lanata* to *Krascheninnikovia lanata* based on palynological evidence, but a more recent taxonomic treatment considering the entire genus recommends a conservative grouping *K. lanata* with its Eurasian congener *K. ceratoides* as the supspecies *K. ceratoides* ssp. *lanata* based on morphological and molecular variation (Heklau and Röser, 2008). The molecular variation, based on an analysis of internal transcribed sequence (ITS) variation, found no support for conclusive taxonomic separation of the species within the genus *Krascheninnikovia*, and supports an expansion of *Krascheninnikovia* into North America during the Pleistocene (Heklau and Röser, 2008). This taxonomic treatment of *K. lanata* is consistent with the low population differentiation found in this study.

Despite the lack of genetic differentiation observed among winterfat populations, evidence for local adaptation of winterfat at the two coldest sites in the experiment (Gallup and Torreon, NM; see Chapter 2) was found in a concurrent reciprocal transplant experiment. This suggests that selection at these sites was strong enough to produce local adaptation despite a high level of gene flow. Restoration practitioners choosing seed sources for revegetation projects should exercise caution when choosing winterfat seed sources. This is due to the evidence for local adaptation among the populations in this study in spite of the homogenizing effects of gene flow. I recommend that seeds from closer sources should be used when possible because, if local adaptation occurred over the relatively small portion of the range included in this study, the differences among more distant ecotypes could be extreme. Future studies of genetic diversity of winterfat should sample a continuum of the species distribution in order to more accurately quantify the amount of genetic variation and population divergence in this species.

Conclusion

The population biology of widespread species has increasingly been framed within the context of restoration ecology. In this study, I investigated the population biology of a widespread shrub (winterfat, *Krascheninnikovia lanata*) that is frequently used for revegetation in the western U.S. at five sites in northwest and central New Mexico. The five sites differed in plant community composition, annual climate, and soil properties. Seeds from the five sites grown in a greenhouse common garden differed in their rates of emergence and floral onset. When individuals were reciprocally transplanted among three sites on two separate transects, evidence for local adaptation was revealed at the two coldest sites on each transect. At these sites, a higher proportion of local individuals survived and reproduced relative to non-local individuals. However, at the low elevation, warmer sites, there was little evidence for local adaptation. This lack of local adaptation despite strong environmental differences among the sites was explained by the low level of genetic differentiation among populations.

The widespread geographic distribution of winterfat, along with its outcrossing, wind-pollinated breeding system, promotes a high rate of gene flow among populations, which has limited population differentiation and local adaptation. Although the consequences of using non-local winterfat plant materials may not be as severe as for species with different life history and breeding system attributes, appropriate considerations should nonetheless be made when choosing seed sources. I recommend that seeds from closer sources should be used when possible because the largest geographic distance between sites in this study was less than 300 km, while winterfat is

distributed throughout western North America (Holmgren, 2004), thus genetic differentiation at the species level was not assessed here. In addition, the more robust performance of winterfat plants from higher-elevation, colder sites suggests that moving plant materials from higher elevations to lower elevations and from higher latitudes to lower latitudes will be more successful than when moved in the opposite direction. When available, information on the population biology and breeding system of target species can be useful for predicting potential consequences of choosing non-local seed sources. This study of winterfat population biology, in addition to augmenting our knowledge of an understudied species, has also provided information that will assist restoration practitioners when choosing plant materials for revegetation projects.

Tables and Figures

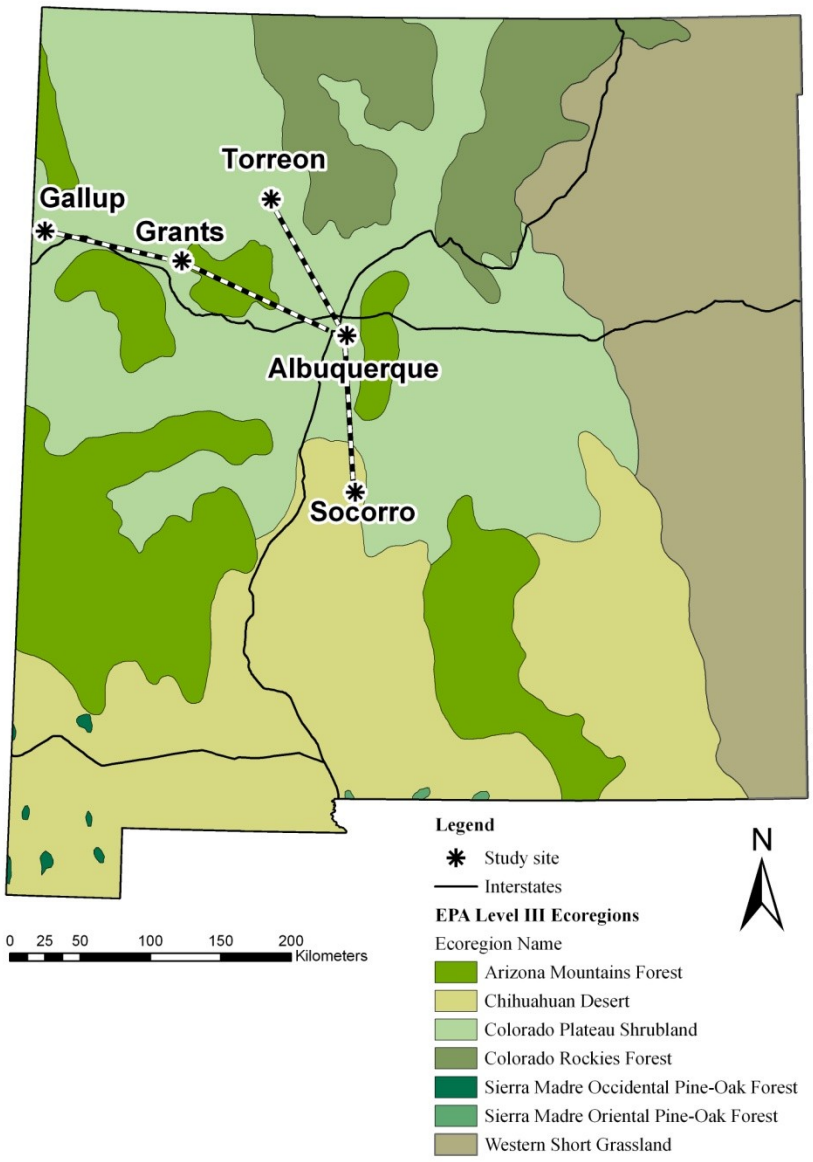


Fig. 1. Location of five winterfat (*Krascheninnikovia lanata*) study sites in New Mexico, USA. Shaded areas indicate Environmental Protection Agency (EPA) Level III Ecoregions (Omernik, 1995).

Table 1. Mean volume, inflorescence length, stem number, and stem density of randomly selected winterfat plants at five study sites in Fall 2005. Standard deviations are below each mean in parentheses. Means within rows with different superscript letters are different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test. Albuq., Albuquerque.

	Site				
	Albuq.	Gallup	Grants	Socorro	Torreon
n	180	70	91	90	91
Plant volume (cm^3)	$27{,}121.5^{b}$ (36,938.7)	$28{,}861.9^{b}$ (34,012.8)	$12{,}285.2^{c}$ (11,561.0)	$59{,}181.3^{a}$ (55,155.6)	$3{,}292.1^{d}$ (3,160.4)
Total inflorescence length (cm)	97.1^{b} (159.2)	260.7^{a} (217.2)	311.4^{a} (213.8)	330.1^{a} (239.1)	2.4^{c} (8.0)
Number of stems	45.1^{b} (33.1)	28.5^{c} (11.4)	63.6^{a} (38.0)	44.0^{b} (24.6)	27.7^{c} (12.6)
Stem density (stems per unit volume)	507.0^{c} (421.3)	$1{,}047.6^{b}$ (1375.9)	178.8^{d} (107.3)	$1{,}287.1^{a}$ (884.9)	130.5^{e} (139.8)

Table 2. *F*-statistics from analyses of variance where site, and plot nested within site, were independent effects and plant volume, inflorescence length, stem number, and stem density were dependent variables. All variables were natural-log transformed for analysis. Df, degrees of freedom.

	Independent variable	
Dependent variable	Site	Plot(site)
df	4, 504	13, 504
Plant volume (cm^3)	71.67****	2.62**
Total inflorescence length (cm)	120.38****	2.42**
Number of stems	17.3****	1.0
Stem density (stems per unit volume)	142.38****	4.98****

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

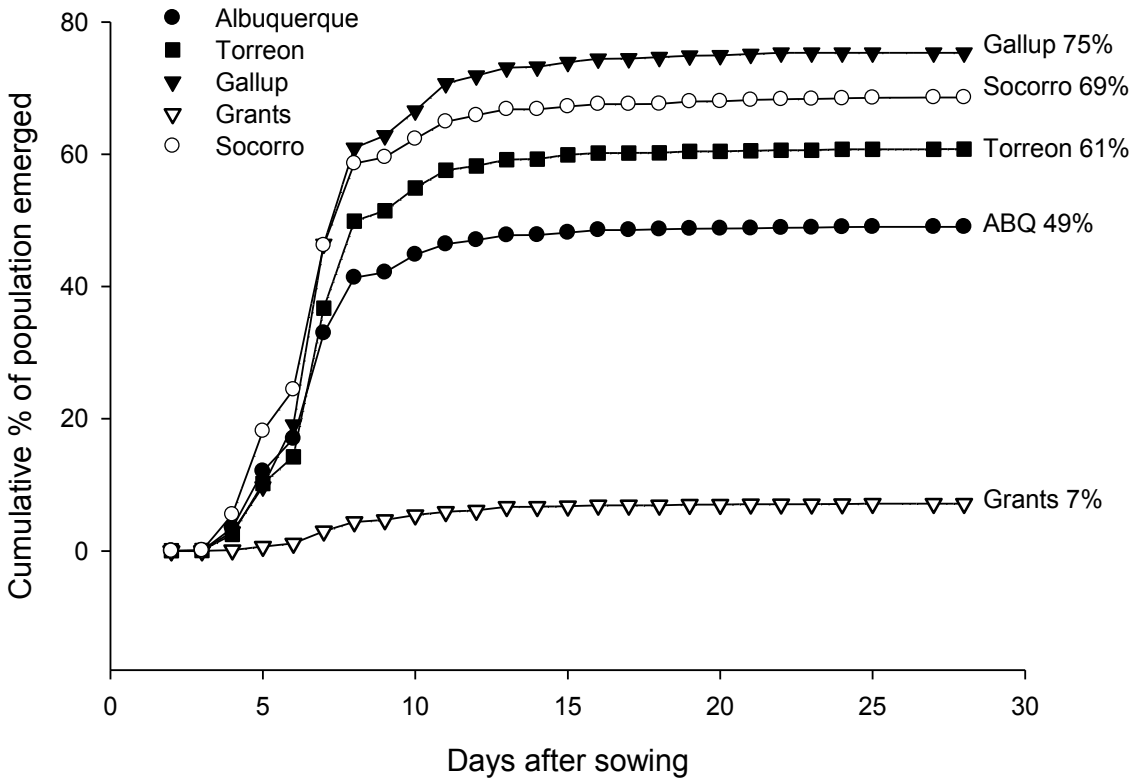


Fig. 2. Cumulative percentage of winterfat plants in a common greenhouse garden that emerged over time by site of origin. Total percentage of emerged seedlings is shown for each site. Abq, Albuquerque.

Table 3. Wilcoxon Chi-square statistics from two failure time analyses of emergence phenology. In the first analysis, emergence rates were analyzed across maternal families for each site. In the second analysis, emergence rates were analyzed across all sites, producing an overall view of the differences among sites. Df, degrees of freedom.

Independent variable	Site	df	Wilcoxon Chi-square
Maternal family	Albuquerque	143	1114.59****
	Gallup	71	336.69****
	Grants	71	468.54****
	Socorro	71	281.54****
	Torreon	71	580.17****
Site	All	4	2143.58****

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Table 4. Mean, standard deviation (1 std. dev.), and coefficient of variation (C.V.) of the number of days to emergence or flowering for winterfat seeds from five sites in northernwestern and central New Mexico. Means within columns with different superscript letters are different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test.

	Days to emergence				Days to flowering			
	n	Mean	1 Std. Dev.	C.V.	n	Mean	1 Std. Dev.	C.V.
Albuquerque	2255	7.25[b]	2.55	35.2	497	27.38[a]	12.35	45.1
Socorro	1400	7.59[b]	2.49	37.6	134	27.13[a]	11.08	40.9
Gallup	1740	7.67[a]	2.49	32.5	531	15.38[c]	11.09	72.1
Grants	165	8.98[c]	3.51	39.1	89	19.98[b]	11.44	57.3
Torreon	1580	7.23[a]	2.72	32.8	315	22.17[b]	13.45	60.7

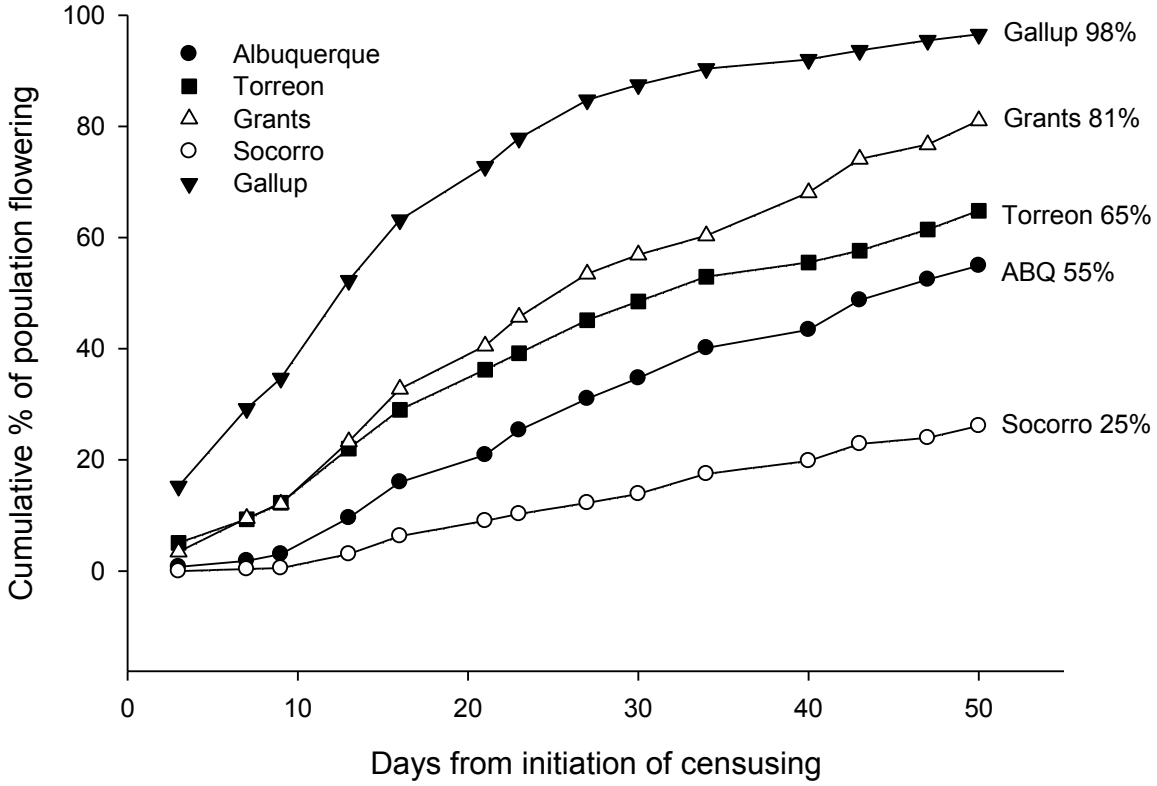


Fig. 3. Cumulative percentage of winterfat plants in a common greenhouse garden that flowered over time by site of origin. Final percentage of plants from each site that flowered is also shown. Abq, Albuquerque.

Table 5. Wilcoxon Chi-square statistics from two failure time analyses of flowering phenology. In the first analysis, flowering rates were analyzed across maternal families for each site. In the second analysis, flowering rates were analyzed across sites, producing an overall view of the differences among sites. Df, degrees of freedom.

			Dependent variable		
Independent variable	Site	df	Flowering	Female flowering	Male flowering
Maternal family	Albuquerque	138	290.46****	280.57****	279.12****
	Gallup	70	181.45****	169.65****	154.59****
	Grants	46	127.19****	128.42****	103.97****
	Socorro	71	80.93	80.63	79.87
	Torreon	69	240.77****	196.79****	158.55****
Site	All	4	1071.14****	1013.21****	641.55****

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Table 6. Mean number of days between initiation of female and male unisexual flowers on heterodichogamous winterfat individuals in a greenhouse common garden. Means with different superscript letters are significantly different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test. Df, degrees of freedom; std. dev. = 1 standard deviation of the mean.

Site	df	Mean (std. dev.)
Albuquerque	201	8.91^{b} (6.18)
Gallup	277	9.83^{b} (7.83)
Grants	38	15.61^{a}(11.23)
Socorro	52	14.27^{a} (10.78)
Torreon	129	10.39^{b} (8.57)
Mean	139	11.80 (8.92)

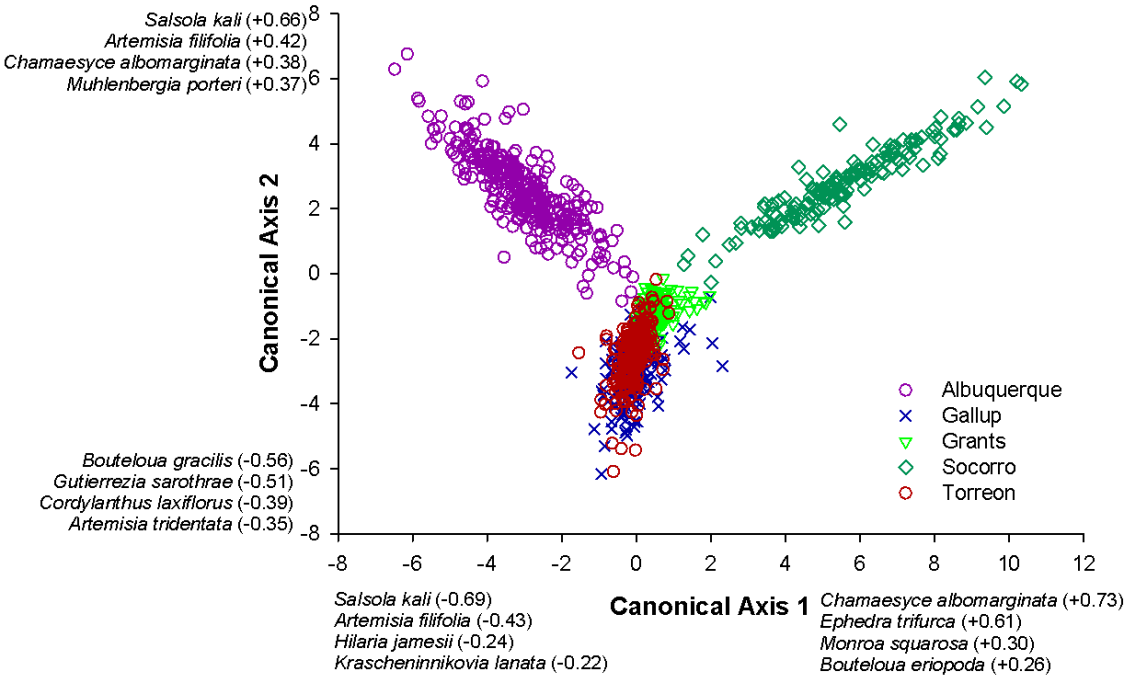


Fig. 4. Scatter plot of the first two canonical axes from a canonical discriminant analysis of the species abundances at five winterfat study sites. The species with the four highest and lowest loadings (total-sample correlations between the canonical variable and the original variables) for each axis are shown.

Table 7. Means and standard deviations (below means, in parentheses) for variables that summarize the overall plant community characteristics at five winterfat study sites. Means within rows with different superscript letters are significantly different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test. Albuq., Albuquerque.

	Site				
	Albuq.	Gallup	Grants	Socorro	Torreon
n	324	198	162	162	198
Winterfat individuals per m^2	3.09^{c} (6.31)	0.10^{a} (0.46)	3.07^{d} (4.34)	0.30^{ab} (0.82)	0.49^{b} (0.94)
Total individuals per m^2	38.59^{d} (13.35)	20.76^{b} (10.31)	11.27^{a} (6.49)	23.73^{c} (11.19)	20.52^{b} (9.18)
Shrub individuals per m^2	1.60^{b} (1.64)	3.79^{c} (3.24)	3.37^{c} (2.99)	0.99^{a} (0.99)	7.37^{d} (5.55)
Herb individuals per m^2	31.24^{e} (12.72)	9.30^{c} (9.28)	0.96^{a} (1.85)	19.23^{d} (11.95)	7.33^{b} (7.57)
Grass individuals per m^2	2.66^{a} (1.87)	7.57^{d} (3.33)	3.87^{b} (1.77)	3.22^{a} (3.50)	5.32^{c} (3.66)
Number of species per m^2	4.20^{c} (1.43)	5.90^{e} (1.73)	3.25^{a} (1.24)	3.69^{b} (1.40)	5.28^{d} (1.65)

Table 8. *F*-statistics from analyses of variance with site, and plot nested within site, as independent effects and total number of winterfat plants, total plant abundance, shrub abundance, herb abundance, grass abundance, and total species per m^2 as dependent effects. All variables were square-root transformed for analysis. Df, degrees of freedom.

	Independent variable			
	Site		Plot(site)	
Dependent variable	df	*F*	df	*F*
Winterfat abundance	4, 1026	71.97****	13, 1026	4.69****
Plant abundance	4, 1026	251.26****	13, 1026	14.77****
Shrub abundance	4, 1026	129.99****	13, 1026	8.23****
Herb abundance	4, 1026	526.46****	13, 1026	22.12****
Grass abundance	4, 1026	136.43	13, 1026	19.52
Species richness	4, 1026	101.04****	13, 1026	12.16****

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

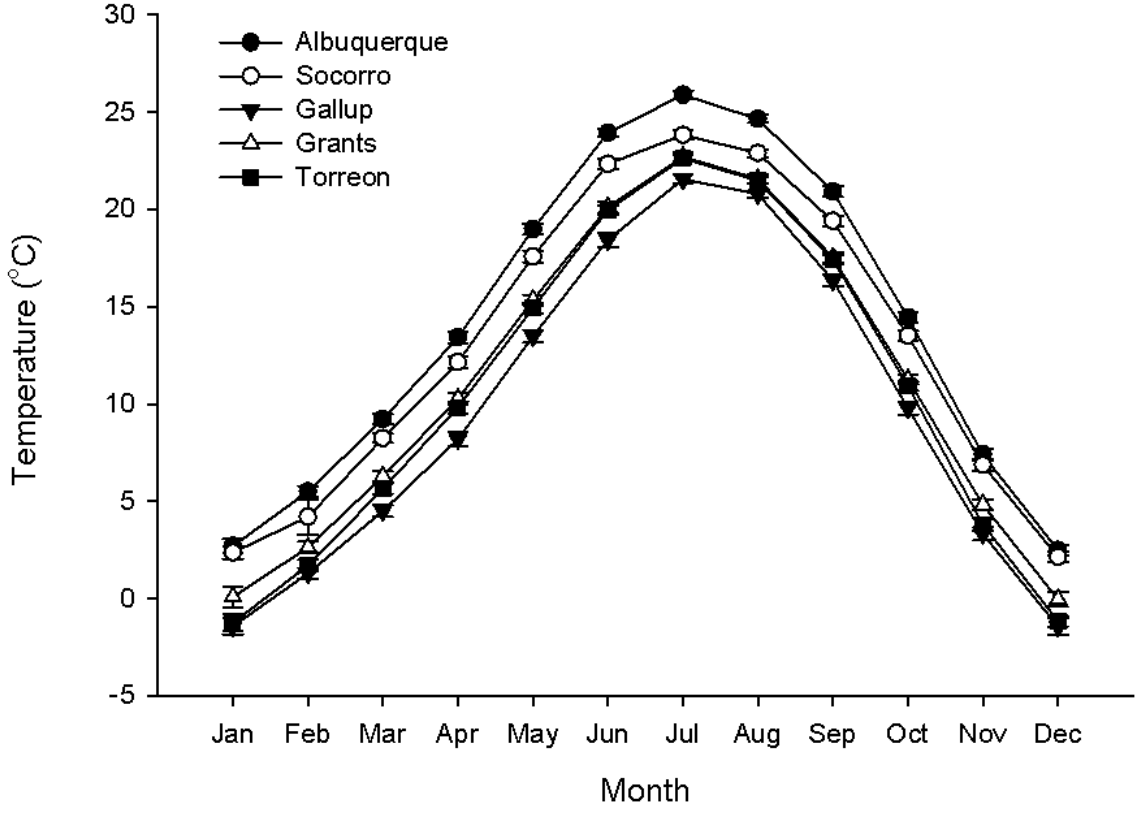


Fig. 5. Monthly mean temperature (ºC) from 1983 to 2008 for five weather stations closest to the winterfat study sites. Data were obtained from NOAA (monthly surface data; National Climatic Data Center, 2009). Bars are ± 1 standard error.

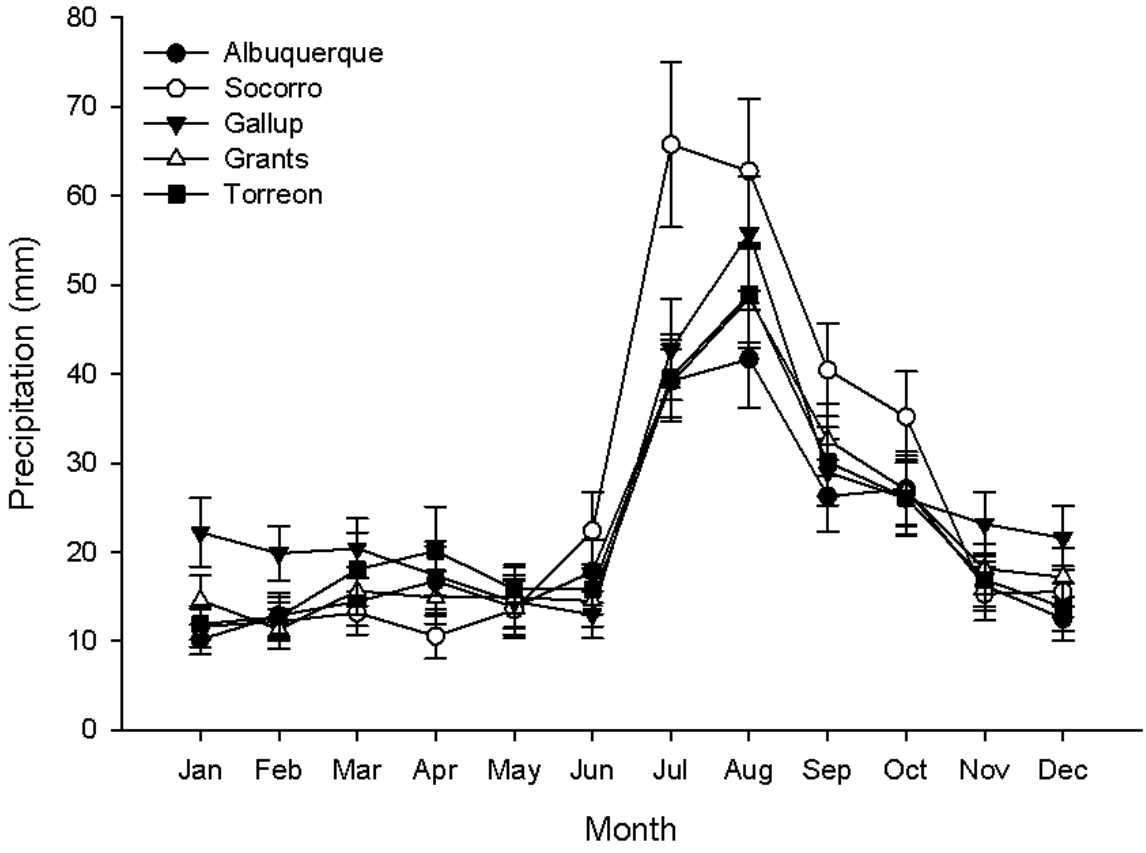


Fig. 6. Total monthly precipitation (mm) from 1983 to 2008 for five weather stations closest to the five winterfat study sites. Data were obtained from NOAA (monthly surface data; National Climatic Data Center, 2009). Bars are ± 1 standard error.

Table 9. Means and loadings for two factors created from a principal components analysis of six climate observations taken from 1983-2008 NOAA monthly surface data (National Climatic Data Center, 2009) for five weather stations closest to the winterfat study sites. Means within rows with different superscript letters are different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test. Loadings (total-sample correlations between the canonical variable and the original variables) are shown for the variables with the largest and smallest correlations with each factor. Total monthly precipitation, total monthly snow, and the proportion of precipitation that fell as snow were natural-log transformed for analysis. Albuq., Albuquerque; Soc., Socorro; Tor., Torreon; min. temp., monthly mean minimum temperature; mean temp., monthly mean temperature; max. temp., monthly mean maximum temperature; precip., total monthly precipitation; snow, total monthly snow; snow:precip., proportion of precipitation that fell as snow.

	Site					Loadings	
Variable	Albuq.	Gallup	Grants	Soc.	Tor.	High	Low
Factor 1	0.15^{a}	-0.32^{d}	0.07^{b}	0.20^{a}	-0.12^{c}	Min. temp. (0.92)	Snow (-0.85)
	(1.03)	(1.08)	(0.89)	(0.88)	(1.03)	Mean temp. (0.95)	Snow:precip. (-0.85)
						Max. temp. (0.94)	Precip. (0.07)
Factor 2	0.20^{a}	0.23^{a}	-0.16^{b}	-0.19^{b}	-0.05^{b}	Precip. (0.88)	Max. temp. (0.15)
	(0.95)	(0.76)	(0.90)	(1.22)	(1.02)	Snow (0.40)	Mean temp. (0.21)
						Snow:precip. (0.39)	Min. temp. (0.27)

Table 10. *F*-statistics for analyses of variance for the effect of station, month, and their interaction on two factors created from six climate variables in a principal components analysis. The *F*-statistics for analyses of variance of the effect of station, month, and their interaction on monthly mean temperature and total monthly precipitation (natural-log transformed) are also shown to accompany Figs. 6 and 7. Monthly mean precipitation was natural-log transformed for analysis.

		Dependent variable			
Independent variable	df	Factor 1	Factor 2	Monthly mean temperature (ºC)	Monthly mean precipitation (mm)
Station	4, 1421	125.09****	13.60****	416.51****	4.66***
Month	11, 1421	1029.04****	20.08****	3607.28****	16.69****
Station×month	44, 1421	3.29****	1.96****	1.28	1.00

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Table 11. Means and factor pattern of four factors produced in a factor analysis of 13 soil characteristics. These four factors explained 91% of the variation in the soil variables. Means within rows with different superscript letters are significantly different at $\alpha = 0.05$ according to a Tukey's Studentized Range Test. The three highest and lowest factor coefficients are shown. CEC, cation exchange capacity; org. matter, organic matter; Albuq., Albuquerque; Soc., Socorro; Tor., Torreon.

	Site means					Factor pattern	
Variable	Albuq.	Gallup	Grants	Soc.	Tor.	High	Low
Factor 1	-1.14^{c} (0.08)	0.25^{b} (0.17)	0.11^{b} (0.48)	1.56^{a} (0.42)	0.35^{b} (0.42)	CEC (0.82) Calcium (0.82) Soluble salts (0.80)	pH (-0.87) Potassium (-0.83) Sand (-0.42)
Factor 2	0.55^{a} (0.36)	-0.71^{bc} (0.46)	-0.17^{ab} (0.15)	1.24^{a} (0.50)	-1.47^{c} (0.63)	Sand (0.82) Phosphorous (0.72) CEC (0.49)	Clay (-0.72) Silt (-0.57) Sodium (-0.48)
Factor 3	-0.32^{bc} (0.41)	0.05^{b} (0.30)	1.99^{a} (0.24)	-0.52^{bc} (0.22)	-0.87^{c} (0.32)	Org. matter (0.62) Magnesium (0.61) Phosphorous (0.47)	Nitrate (-0.93) Clay (-0.55) pH (-0.27)
Factor 4	0.14^{a} (0.58)	-1.84^{b} (0.49)	0.60^{a} (0.55)	0.04^{a} (0.24)	0.91^{a} (0.38)	Magnesium (0.60) Phosphorous (0.37) Clay (0.35)	Sodium (-0.53) Sand (-0.36) Org. matter (-0.34)

Table 12. Means for 13 soil characteristics of five winterfat study sites. In parentheses, below means, are one standard deviation of the mean. Means within rows with different superscripts are significantly different in a Tukey's Studentized Range test at $\alpha = 0.05$. Albuq., Albuquerque.

	Site				
Variable	Albuq.	Gallup	Grants	Socorro	Torreon
Silt (%)	10.74^{b} (0.90)	20.48^{a} (2.20)	26.52^{a} (2.03)	19.93^{a} (4.13)	24.79^{a} (1.13)
Clay (%)	33.40^{b} (1.57)	32.32^{b} (1.61)	29.07^{b} (0.67)	30.24^{b} (0.99)	44.81^{a} (2.08)
Sand (%)	55.86^{c} (2.11)	47.19^{c} (3.71)	44.41^{bc} (1.70)	49.83^{c} (5.12)	30.40^{ab} (2.62)
Organic matter (%)	0.73^{a} (0.08)	1.07^{a} (0.17)	1.10^{a} (0.00)	0.80^{a} (0.10)	0.87^{a} (0.03)
pH	7.80^{c} (0.03)	7.60^{bc} (0.06)	7.40^{ab} (0.15)	7.27^{ab} (0.03)	7.57^{bc} (0.13)
Cation exchange capacity (meq/100g)	16.27^{b} (0.59)	21.67^{b} (1.61)	22.27^{b} (0.80)	67.57^{a} (0.92)	22.47^{b} (3.67)
Soluble salts (mmhos/cm)	1.15^{a} (0.04)	1.20^{a} (0.10)	0.97^{ab} (0.03)	0.30^{b} (0.00)	1.03^{a} (0.18)
Phosphorous (ppm)	151.17^{ab} (8.51)	41.67^{c} (14.67)	101.33^{b} (6.23)	99.33^{a} (38.02)	30.67^{c} (5.70)
Potassium (ppm)	406.00^{a} (31.27)	259.33^{bc} (15.07)	340.00^{ab} (18.82)	188.67^{c} (17.84)	214.67^{bc} (4.33)
Magnesium (ppm)	180.50^{c} (8.89)	79.00^{d} (11.72)	370.67^{a} (15.17)	52.33^{d} (7.54)	239.00^{b} (17.04)
Calcium (ppm)	2718.00^{c} (92.53)	4034.33^{b} (320.29)	3630.00^{bc} (145.60)	13318.00^{a} (203.45)	3952.00^{bc} (730.63)
Sodium (ppm)	42.17^{c} (0.65)	58.33^{a} (1.45)	51.00^{b} (1.53)	49.67^{b} (1.76)	49.67^{b} (1.45)
Nitrate (ppm)	18.50^{ab} (1.98)	14.33^{b} (0.33)	5.33^{c} (0.88)	17.33^{ab} (0.88)	23.00^{a} (1.53)

Table 13. Number of winterfat plants from each source site that were planted at each destination site in Fall 2006: (a) east-west ('Gallup') transect; (b) north-south ('Socorro') transect. The low number of seeds from Grants that emerged resulted in small sample sizes for the Grants source site. Total $N = 2{,}633$.

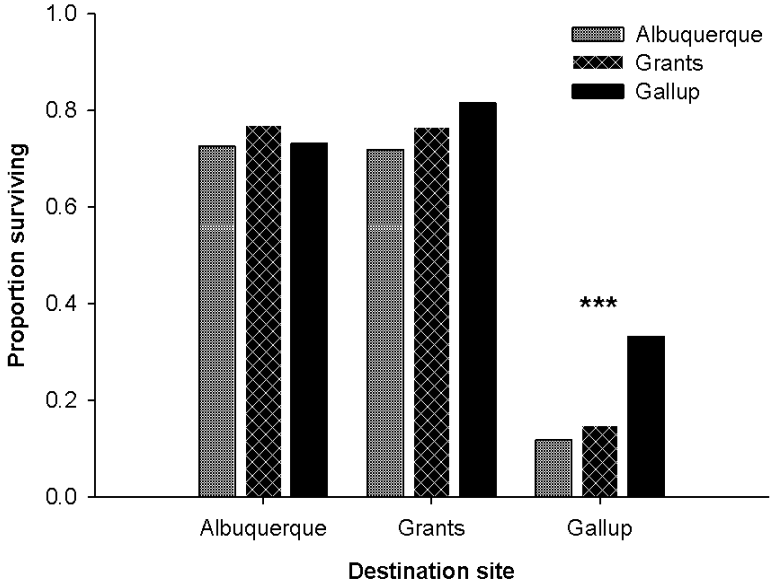
(a) East-west transect

	Source site			
Destination site	Albuquerque	Grants	Gallup	Total
Albuquerque	159	38	184	381
Grants	155	41	186	382
Gallup	154	40	188	382
Total	468	119	558	1145

(b) North-south transect

	Source site			
Destination site	Socorro	Albuquerque	Torreon	Total
Socorro	184	151	157	492
Albuquerque	184	152	158	494
Torreon	187	151	159	497
Total	555	454	474	1483

a) East-west transect survival



b) North-south transect survival

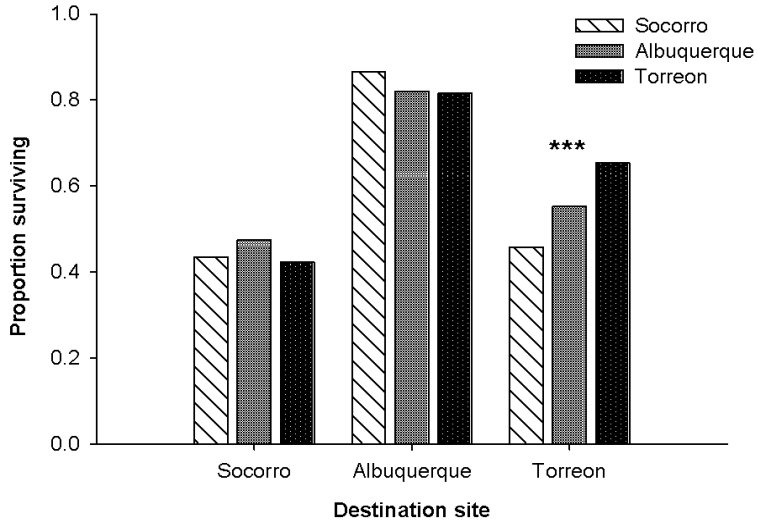


Fig. 7. Cumulative percent of surviving individuals from each source location (individual bars) at each destination (group of bars, x-axis). (a) East-west ('Gallup') transect sites; (b) North-south ('Socorro') transect sites. *** Indicates destination-level differences that were significant at $\alpha = 0.05$ (see text for details).

Table 14. Wald chi-square statistics from a logistic regression analysis of the effect of source site, destination site, and their interaction on survival of winterfat plants from three source locations reciprocally transplanted to three destination locations along two transects, one east-west ('Gallup') and one north-south ('Socorro'). Df, degrees of freedom.

	East-west transect		North-south transect	
	df	Survival	df	Survival
Source site	2, 1074	18.32***	2, 1342	0.93
Destination site	2, 1074	145.01****	2, 1342	132.43****
Source site × destination site	4, 1074	10.50*	4, 1342	14.51**

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

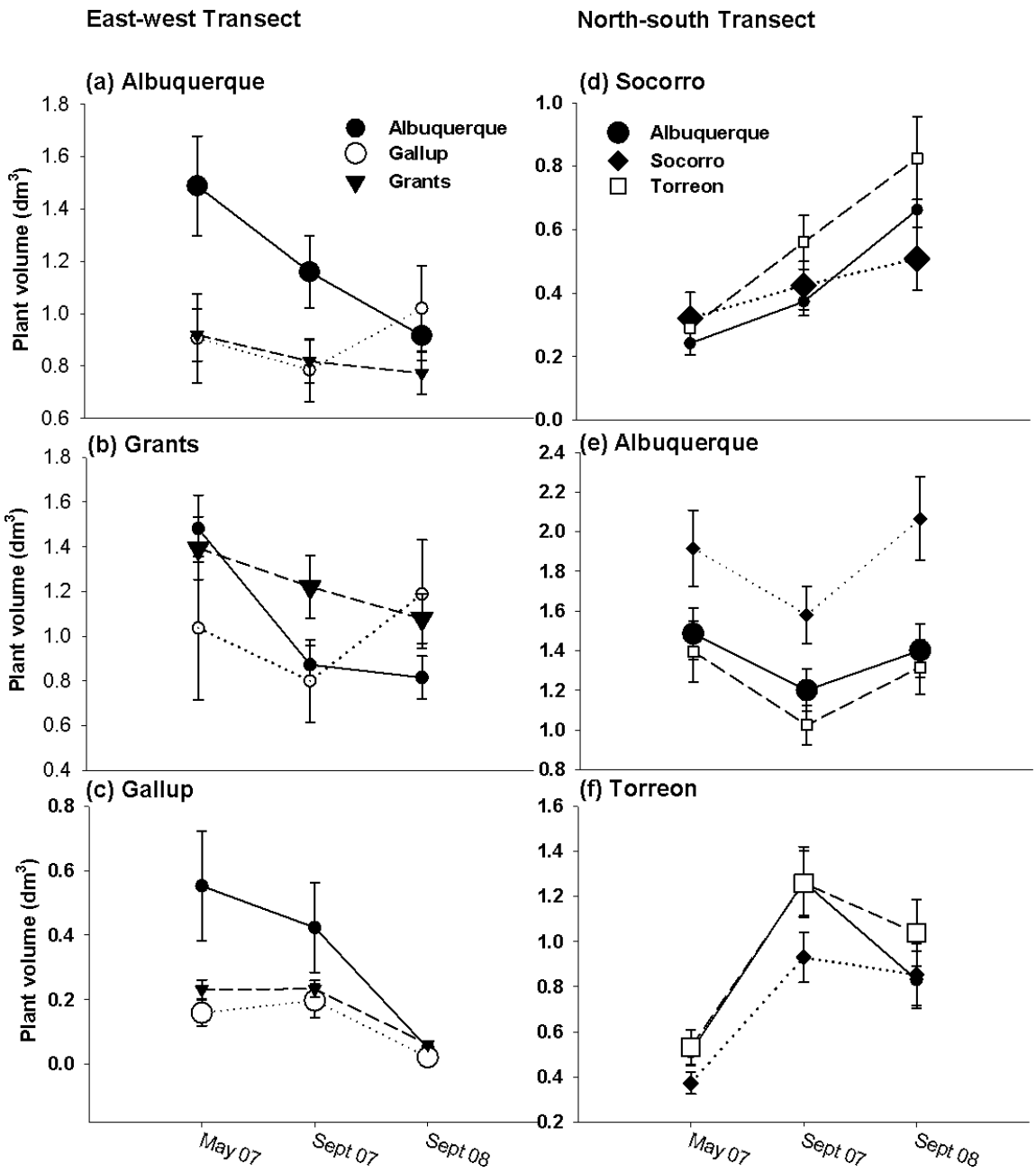


Fig. 8. Average plant volume (cubic decimeters) of winterfat plants from three source locations (lines) reciprocally transplanted to three destination sites (panels) along two transects, one east-west ('Gallup'; a-c) and one north-south ('Socorro'; d-f). Bars represent ± 1 standard error. Large symbols correspond to values for transplants at their home site. Note that vertical axes have different scales.

Table 15. *F*-statistics from a repeated-measures MANOVA testing for the effect of source site, destination site, and their interaction (fixed effects) on mean plant volume among winterfat plants from three source sites reciprocally transplanted to three destination sites along two transects, one east-west ('Gallup') and one north-south ('Socorro'). Only plants that were alive were included in the analysis. Df, degrees of freedom.

	East-west transect		North-south transect	
Independent effect	df	*F*	df	*F*
Source site	2, 545	3.90*	2, 795	6.51**
Destination site	2, 545	12.41****	2, 795	48.56****
Source site × destination site	4, 545	1.79	4, 795	1.00

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Table 16. Frequencies of winterfat plants from three source sites reciprocally transplanted to three destination sites on two transects [Gallup (a, c) and Socorro (b, d)] that flowered in 2007 (a, b) and 2008 (c, d). The Gallup destination site was excluded from the flowering analysis (c) because no plants flowered there in 2008. Albuq., Albuquerque.

(a) East-west transect, 2007

		Destination site			
		Albuq.	Grants	Gallup	Total
Source site	Albuq.	0.01	0.03	0.09	0.04
	Grants	0.03	0.11	0.25	0.13
	Gallup	0.08	0.16	0.17	0.13
	Total	0.03	0.09	0.18	0.10

(b) North-south transect, 2007

		Destination site			
		Soc.	Albuq.	Tor.	Total
Source site	Soc.	0.00	0.06	0.06	0.03
	Albuq.	0.00	0.07	0.03	0.03
	Tor.	0.02	0.07	0.20	0.09
		0.01	0.07	0.10	0.05

(c) East-west transect, 2008

		Destination site		
		Albuq.	Grants	Total
Source site	Albuq.	0.14	0.06	0.10
	Grants	0.32	0.25	0.28
	Gallup	0.37	0.26	0.32
	Total	0.25	0.18	0.21

(d) North-south transect, 2008

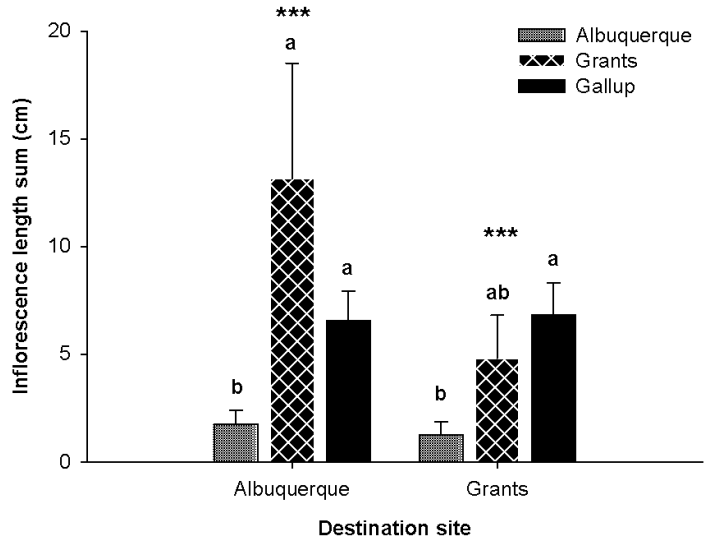
		Destination site			
		Soc.	Albuq.	Tor.	Total
Source site	Soc.	0.11	0.16	0.16	0.14
	Albuq.	0.20	0.18	0.15	0.18
	Tor.	0.23	0.25	0.35	0.28
	Total	0.18	0.20	0.24	0.20

Table 17. Wald chi-square statistics from a logistic regression analysis of the effect of source site, destination site, and their interaction on the presence of flowers on winterfat plants from three source locations reciprocally transplanted to three destination locations along two transects, one east-west ('Gallup') and one north-south ('Socorro'). Only living plants were included in this analysis. 2007 flowering on the north-south transect was excluded from the table because too few plants flowered. Df, degrees of freedom.

	East-west transect				North-south transect	
	df	2007	df	2008	df	2008
Source site	2	9.80**	2	25.39****	2	13.64**
Destination site	2	17.49***	1	4.79*	2	1.12
Source site × destination site	4	3.40	2	1.23	4	3.56

*$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

(a) East-west transect



(b) North-south transect

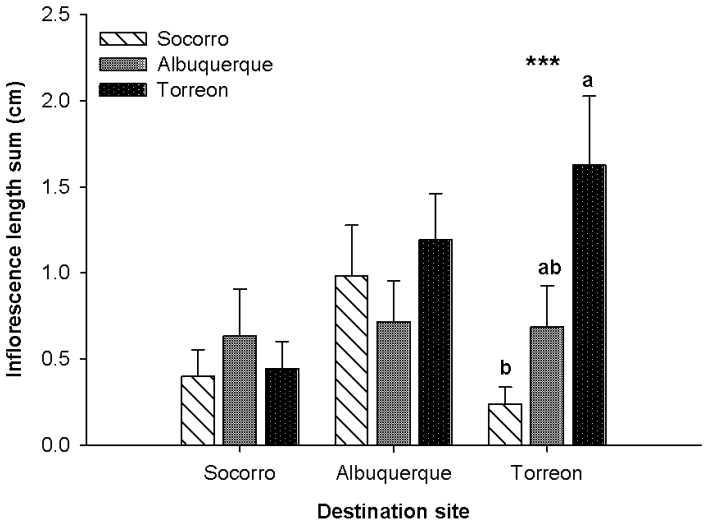


Fig. 9. Mean inflorescence length (cm) of winterfat plants from different source locations (individual bars) planted at different destinations (x-axis bar groupings) for two transects: (a) east-west ('Gallup') and (b) north-south ('Socorro'). 'Inflorescence length' is the sum of the lengths of all inflorescences on an individual. The Gallup destination site is not included because no plants flowered at that site in 2008. Bars represent ± 1 standard error. Note that vertical axes have different scales.

Table 18. *F*-statistics from two univariate analyses of variance where source site, destination site, and their interaction were independent fixed effects and mean inflorescence length was the dependent variable. *P*-values were adjusted with a Bonferroni multiple comparison correction ($\alpha = 0.025$) across the two years for each transect. Df, degrees of freedom.

(a) 2007 Inflorescence length

	East-west transect		North-south transect	
Independent effect	df	*F*	df	*F*
Source site	2, 785	8.93***	2, 967	2.60
Destination site	2, 785	7.53***	2, 967	6.67**
Source site × destination site	4, 785	3.93**	4, 967	2.25

*$P \leq 0.025$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

(b) 2008 Inflorescence length

	East-west transect		North-south transect	
Independent effect	df	*F*	df	*F*
Source site	2, 610	3.21	2, 810	2.46
Destination site	2, 610	5.40**	2, 810	0.96
Source site × destination site	4, 610	1.59	4, 810	1.84

*$P \leq 0.025$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Table 19. Descriptive statistics for five populations of winterfat in New Mexico: mean samples size (*N*), proportion of polymorphic loci (P_p), mean number of alleles per polymorphic locus (A_p), expected heterozygosity (H_e), observed heterozygosity (H_o), and fixation index (*f*).

Population	*N*	P_p	A_p	H_e	H_o	*f*
Albuquerque	29.0	0.56	2.20	0.108	0.126	-0.176
Gallup	38.0	0.78	2.43	0.152	0.167	-0.096
Grants	37.0	0.78	2.71	0.145	0.150	-0.033
Socorro	37.0	0.78	2.43	0.130	0.126	0.029
Torreon	32.0	0.56	2.20	0.127	0.125	0.016
Mean	34.6	0.69	2.39	0.132	0.139	-0.049

Table 20. *F*-statistics and gene diversity statistics for nine isozyme loci assayed in five populations of winterfat in New Mexico. 95% confidence intervals are included for the fixation indices. H_e, expected heterozygosity; H_o, observed heterozygosity. *F*-statistics follow computation method of Weir and Cockherham (1984).

Locus	H_e	H_o	f (F_{IS})	F (F_{IT})	θ (F_{ST})
Ald	0.000	0.000	--	--	--
Aat	0.478	0.462	0.038	0.032	-0.007
Est	0.017	0.017	-0.003	-0.004	-0.0007
Idh	0.121	0.127	-0.110	-0.041	0.062
Lap	0.068	0.058	-0.040	-0.021	0.018
Mdh	0.012	0.012	0.001	-0.004	-0.005
Pgi	0.095	0.098	-0.040	-0.034	0.005
6Pgd	0.067	0.058	0.143	0.144	0.001
Pgm	0.354	0.416	-0.185	-0.174	0.009
Mean	0.135	0.140	-0.047	-0.038	0.008
Lower 95%	--	--	-0.148	-0.133	-0.004
Upper 95%	--	--	0.039	0.039	0.035

Table 21. Results of an AMOVA that computed the partitioning of genetic variation within and among five winterfat (*Krascheninnikovia lanata*) populations in New Mexico. Df, degrees of freedom.

Source	df	Sum of squares	Mean square	Estimated variation	Percent of variation
Among populations	4	6.93	1.733	0.018	2%
Within populations	168	189.32	1.127	1.127	98%
Total	173	196.25		1.144	100%

Table 22. Pair-wise F_{ST} values for five populations of winterfat (*Krascheninnikovia lanata*) in New Mexico. Albuq., Albuquerque.

	Albuq.	Gallup	Grants	Socorro	Torreon
Albuq.	--				
Gallup	0.018	--			
Grants	0.008	0.007	--		
Socorro	0.011	0.017	-0.007	--	
Torreon	-0.005	0.023	-0.003	-0.001	--

Table 23. Pair-wise geographic distance (km; below diagonal) and pair-wise ecological distance (above the diagonal). Geographic distances were the linear distance in kilometers between populations. Ecological distance consisted of 23 soil, climate, and plant community variables. Albuq., Albuquerque.

	Albuq.	Gallup	Grants	Socorro	Torreon
Albuq.	--	7.80	6.78	7.18	7.02
Gallup	227.3	--	5.36	7.47	4.47
Grants	128.5	99.9	--	6.85	5.47
Socorro	111.2	287.5	205.6	--	7.14
Torreon	110.3	161.8	77.1	216.2	--

Table 24. R^2 values and *P*-values from Mantel tests for the correlations between pair-wise comparison matrices. Genetic distances were pair-wise F_{ST} values (Table 4). Geographic distances were the linear distance in kilometers between populations (Table 5). Ecological distance consisted of 23 soil, climate, and plant community variables (Table 5). The soil, climate, and plant community distance matrices were also compared individually to genetic distance. *P*-values represent the probability of randomly generated values being greater than the observed values.

Distance matrix comparison	R^2	*P*
Geographic vs. genetic	0.110	0.219
Ecological vs. genetic	0.012	0.428
Soil vs. genetic	0.236	0.154
Climate vs. genetic	0.097	0.252
Vegetation vs. genetic	0.017	0.424
Geographic vs. ecological	0.269	0.093

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Appendix 1

Raw haploid data for nine isozyme loci for five populations of winterfat in New Mexico, USA. ALD, aldolase; IDH, isocitrate dehydrogenase; PGI, phosphoglucose isomerase; EST, esterase; PGD, phosphoglucose dehydrogenase; PGM, phosphoglucose mutase; LAP, leucine amino-peptidase; MDH, malate dehydrogenase; AAT, amino-aspartate transferase; pop., population; ABQ, Albuquerque; GAL, Gallup; GRT, Grants; SOC, Socorro; TOR, Torreon.

Pop.	Sample	ALD	IDH	PGI	EST	PGD	PGM	LAP	MDH	AAT
ABQ	AB111	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB114	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB117	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB119	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB146	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB331	1 1	2 2	2 2	2 2	2 2	2 2	1 2	1 1	1 1
ABQ	AB333	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB341	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB345	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
ABQ	AB352	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
ABQ	AB359	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
ABQ	AB364	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
ABQ	AB393	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB395	1 1	2 2	2 2	2 2	2 2	1 1	2 2	1 1	1 1
ABQ	AB413	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB422	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
ABQ	AB435	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
ABQ	AB437	1 1	2 2	2 2	2 2	1 2	1 2	2 2	1 1	1 2
ABQ	AB444	1 1	2 2	1 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB444	1 1	2 2	2 2	2 2	2 2	1 1	2 2	1 1	1 2
ABQ	AB458	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB463	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB466	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB473	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
ABQ	AB483	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB489	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB496	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
ABQ	AB497	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
ABQ	AB544	1 1	2 2	2 2	2 2	2 2	4 1	2 2	1 1	1 1
ABQ	AB545	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1

Appendix 1. (continued)

Pop.	Sample	ALD	IDH	PGI	EST	PGD	PGM	LAP	MDH	AAT
GAL	GL051	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL053	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL054	1 1	2 2	2 2	1 2	2 2	2 2	2 2	1 1	1 2
GAL	GL055	1 1	2 2	2 2	2 3	2 2	1 2	2 2	1 1	1 2
GAL	GL271	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
GAL	GL273	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL275	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GAL	GL276	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GAL	GL277	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL278	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL283	1 1	2 3	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL284	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL284	1 1	2 2	1 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL286	1 1	2 4	1 2	2 2	2 2	2 2	2 2	1 2	1 1
GAL	GL288	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL289	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	2 2
GAL	GL291	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GAL	GL293	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GAL	GL297	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GAL	GL299	1 1	2 3	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL311	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL312	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GAL	GL313	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL314	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL318	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL321	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GL322	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GAL	GL324	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	2 2
GAL	GL326	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GAL	GL328	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL341	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GAL	GL343	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
GAL	GL344	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GL346	1 1	2 2	2 2	2 2	1 2	1 2	2 2	1 1	1 2
GAL	GLUM1	1 1	2 2	2 2	2 2	2 2	4 2	2 2	1 1	2 2
GAL	GLUM2	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GAL	GLUM4	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GAL	GLUM5	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1

Appendix 1. (continued)

Pop.	Sample	ALD	IDH	PGI	EST	PGD	PGM	LAP	MDH	AAT
GRT	GT062	1 1	2 2	1 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT063	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT064	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT064	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT065	1 1	2 2	2 2	2 2	1 2	2 2	1 2	1 1	1 2
GRT	GT069	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GRT	GT072	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT074	1 1	2 2	2 3	2 2	2 2	1 2	2 2	1 1	1 2
GRT	GT075	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GRT	GT076	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT081	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GRT	GT084	1 1	2 2	2 2	2 2	2 2	2 2	2 3	1 1	1 1
GRT	GT211	1 1	2 2	2 2	1 2	2 2	2 2	2 2	1 1	4 1
GRT	GT215	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GRT	GT218	1 1	1 2	2 2	2 2	1 2	1 2	2 2	1 1	2 2
GRT	GT219	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	1 1
GRT	GT224	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT225	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT226	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT228	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT231	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GRT	GT233	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT236	1 1	1 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT239	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GRT	GT242	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
GRT	GT244	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GRT	GT245	1 1	2 4	2 2	2 2	2 2	2 2	1 2	1 1	2 2
GRT	GT246	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
GRT	GT248	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
GRT	GT251	1 1	2 2	2 2	2 2	1 2	2 2	2 2	1 1	1 2
GRT	GT254	1 1	2 4	2 2	2 2	1 2	1 2	2 2	1 1	1 1
GRT	GT257	1 1	2 2	2 2	2 2	1 2	1 2	2 2	1 1	1 2
GRT	GT258	1 1	2 2	2 2	2 2	2 2	4 1	2 2	1 1	1 1
GRT	GT263	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT264	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
GRT	GT265	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
GRT	GT269	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1

Appendix 1. (continued)

Pop.	Sample	ALD	IDH	PGI	EST	PGD	PGM	LAP	MDH	AAT
SOC	SO121	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO122	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO124	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
SOC	SO125	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 2	1 1
SOC	SO126	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO133	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO134	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO136	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
SOC	SO145	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO146	1 1	2 2	1 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO148	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
SOC	SO369	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
SOC	SO371	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO372	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
SOC	SO373	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
SOC	SO376	1 1	2 2	1 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO377	1 1	2 2	2 2	2 2	1 2	2 2	2 2	1 1	1 1
SOC	SO378	1 1	2 2	1 2	2 2	2 2	1 2	2 2	1 1	2 2
SOC	SO384	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO385	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
SOC	SO387	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO394	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
SOC	SO516	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO517	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO521	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
SOC	SO522	1 1	2 2	1 2	2 2	2 2	2 2	2 3	1 1	1 2
SOC	SO523	1 1	2 2	2 2	2 2	2 2	2 2	2 3	1 1	4 1
SOC	SO525	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
SOC	SO526	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO527	1 1	2 4	2 2	2 2	2 2	2 2	2 2	1 1	1 1
SOC	SO529	1 1	2 2	2 2	2 2	2 2	2 2	2 3	1 1	2 2
SOC	SO531	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
SOC	SO533	1 1	2 4	2 2	2 2	2 2	1 2	2 2	1 1	1 1
SOC	SO534	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
SOC	SO534	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
SOC	SO538	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2

Appendix 1. (continued)

Pop.	Sample	ALD	IDH	PGI	EST	PGD	PGM	LAP	MDH	AAT
TOR	TO021	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO024	1 1	2 2	2 2	2 2	2 2	1 2	1 2	1 1	2 2
TOR	TO032	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
TOR	TO034	1 1	2 2	2 2	2 2	2 2	2 2	1 2	1 1	4 2
TOR	TO037	1 1	2 2	2 2	2 2	2 2	2 2	1 2	1 1	1 1
TOR	TO041	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO044	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
TOR	TO044	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
TOR	TO048	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO154	1 1	2 2	2 2	2 2	1 2	1 2	2 2	1 1	1 2
TOR	TO156	1 1	2 2	2 2	2 2	2 2	2 2	1 2	1 1	1 2
TOR	TO158	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO159	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	2 2
TOR	TO162	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 2
TOR	TO165	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
TOR	TO167	1 1	2 2	2 2	2 2	1 2	1 2	2 2	1 1	1 2
TOR	TO169	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO172	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
TOR	TO174	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 2
TOR	TO175	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO183	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	2 2
TOR	TO189	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO191	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO192	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO194	1 1	2 2	2 2	2 2	2 2	2 2	2 2	1 1	1 2
TOR	TO197	1 1	2 2	2 3	2 2	2 2	2 2	2 2	1 1	1 1
TOR	TO199	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO241	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO244	1 1	2 2	2 3	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO244	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1
TOR	TO245	1 1	2 2	2 2	2 2	2 2	1 2	1 2	1 1	1 2
TOR	TO249	1 1	2 2	2 2	2 2	2 2	1 2	2 2	1 1	1 1