

RESEARCH ARTICLE

Empirical test of increasing genetic variation via inter-population crossing for native plant restoration in variable environments

April M. Goebel^{1,2,3} , Daniel F. Doak⁴, Nolan C. Kane¹

Restoring native plant populations is an essential component of conserving biodiversity, ecological function, and ecosystem services. Restoration using local, ecotypic source materials is largely acknowledged as best practice; however, local populations are not always available or adapted to current or future site conditions. A major challenge in restoration comes from increasingly variable and unpredictable environmental conditions that impose selective pressures and threaten restoration success. Understanding how to conserve and restore populations under changing environments requires attention to within-species genetic diversity, which can exert a number of population-level effects. However, given that these effects can be positive and negative, it remains unclear how increasing genetic diversity via mixing distinct populations may ultimately affect restoration in variable environments. To empirically investigate these effects, we established lineages of a native forb, *Helianthus petiolaris* (prairie sunflower), with higher and lower individual- and population-level genetic diversity by crossing plants using seed from four distinct locations. We planted and tracked a total of 3,200 individual seeds across all lineages in replicated plots in two common gardens representing a range of environmental conditions, and measured fitness components throughout the growing season. We found that populations with increased genetic diversity had intermediate emergence and reproduction, improved survival in the poorest quality plots, and were moderately buffered against environmental variability. Overall, higher diversity led to high or intermediate and stable performance across environments. Our findings support a strategy of increasing genetic diversity when restoring populations, in particular when a lack of information hampers selection of an optimal source population.

Key words: ecological restoration, environmental change, genetic diversity, *Helianthus petiolaris*, inter-population cross, intra-species hybrids

Implications for Practice

- Increasing individual- and population-level genetic diversity in source material can positively impact plant performance and merits consideration in restoration projects, particularly when site conditions are highly variable.
- The decision of if and when to mix seeds from different source populations should be made in light of project size, timeline, species used, source site conditions, and desired outcomes. Mixing seeds of outcrossing species during propagation versus during or after initial restoration planting can result in differences in performance and retention of genetic diversity.

Introduction

Ecological restoration is an essential management tool for protecting plant and wildlife habitat, recovering ecosystem services, and conserving biodiversity. There is an urgent need for restoration across the globe, with multiple ecosystems

devastated and degraded by a range of direct and indirect anthropogenic actions (IPBES 2018). This need is being addressed by current and proposed increases in active restoration worldwide using native plant species (Cross et al. 2020). Successfully restoring diverse, self-sustaining communities however, can be challenging, particularly in heavily degraded habitats, variable environments (e.g. due to natural and anthropogenic disturbance, climate change driven fluctuations in conditions, and invasive species), and locations that differ substantially from available source populations (Kildisheva et al. 2016; Gann

Author contributions: AMG, NCK conceived, designed, and conducted the experiment; AMG performed data collection with logistical support from NCK, DFD; AMG, DFD performed analyses and drafted the manuscript; all authors contributed to interpretation of results and editing of the manuscript.

¹Department of Ecology & Evolutionary Biology, University of Colorado Boulder, Boulder, CO 80302, U.S.A.

²Research & Conservation Department, Denver Botanic Gardens, 909 York Street, Denver, CO 80206, U.S.A.

³Address correspondence to A. M. Goebel, email aprilgoebel@gmail.com

⁴Environmental Studies Program, University of Colorado Boulder, Boulder, CO 80303, U.S.A.

© 2022 Society for Ecological Restoration.

doi: 10.1111/rec.13648

Supporting information at:

<http://onlinelibrary.wiley.com/doi/10.1111/rec.13648/supinfo>

et al. 2019). More field-based research is needed that can inform the success of restored populations in current and changing environments by considering the effects of genetic variation on evolutionary and ecological processes.

Pressures from shifting and variable environmental conditions across both space and time present interconnected aspects of uncertainty when planning restorations. First is uncertainty in selecting optimal source material for a given restoration project. It is widely accepted that locally adapted sources can be optimal (Hufford & Mazer 2003), but these are not always available, large enough, or representative of adaptations to current or future site conditions (Havens et al. 2015). Additionally, because procuring sufficient seed material can take time, the exact locations of all restoration sites may not be known at the time when seed sources must be chosen. Second is uncertainty in site conditions through time. For example, most locations experience high inter-annual climate variability and now habitats are experiencing or expected to show shifts in mean climate conditions due to the effects of climate change (Kilkenny 2015). Both of these aspects of uncertainty are amplified when the site conditions that are most important in driving selection are unclear, or the important drivers of selection are not easy to accurately assess.

An approach to hedge against some of this uncertainty is to increase genetic diversity in restoration materials (Falk et al. 2001; Rice & Emery 2003; Kettenring et al. 2014). This approach, which is analogous to using diverse species in restoration mixes to increase functional diversity (Elmqvist et al. 2003; Hooper III et al. 2005), focuses on increasing genetic diversity within a given species (Munda & Smith 1995). A method for achieving this is to source seed from multiple distinct, yet somewhat geographically and ecologically similar, populations to create an inter-population seed mix (i.e. regional admixture) with increased standing genetic variation and a range of potentially adaptive traits (Lesica & Allendorf 1999; Bucharova et al. 2019).

This approach serves to increase both population-level and individual-level (e.g. heterozygosity) diversity. Note that individual-level diversity applies to outcrossing species and will result as interbreeding between different populations occurs following establishment at a restoration site. The approach of mixing different source populations is commonly recommended in the scientific literature (e.g. Broadhurst et al. 2008; Espeland et al. 2017); however, little research has investigated the nuance of how different levels of diversity (i.e. population versus individual), which may differ depending on mating system and the timing of population mixing, ultimately affects restoration outcomes.

There are several mechanisms by which increased population- and individual-level genetic diversity can exert population level effects. One longer-term effect is heightened adaptive potential, where increased allelic variation at loci for traits that affect fitness provides more targets for natural selection to act on (Gilpin & Soulé 1986; Hoffmann et al. 2017). However, there are also short-term effects of increasing within species diversity on population fitness (Fig. 1), making the net effects of inter-population mixing for restoration less clear. First, outcrossing can increase fitness by reducing inbreeding depression

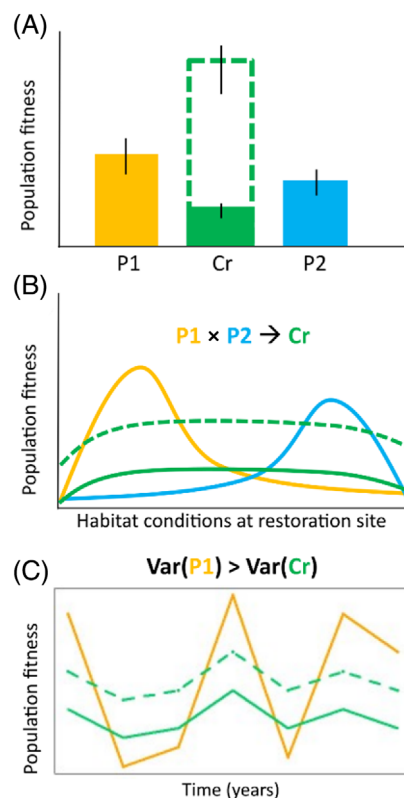


Figure 1. Conceptual diagram showing potential short-term benefits and costs of increased genetic diversity on population fitness. A population (Cr; green) resulting from crossing two distinct populations (P1 and P2; yellow and blue) may; (A) show increased (dashed bar) or decreased (solid bar) fitness ($\pm$ uncertainty) due to heterosis or hybrid breakdown of progeny, respectively; (B) have intermediate (dashed line) or poor (solid line) fitness in a broader range of conditions; (C) be buffered against fluctuations in fitness due to environmental variability (dashed line), which may also result in lower mean fitness (solid line).

by heterosis, or progeny could perform worse than parents if maladapted gene combinations are introduced (Fig. 1A; Crémieux et al. 2010). Second, a genetically mixed population may have the ability to inhabit a broader range of conditions seen across time or space (Roughgarden 1972; Bolnick et al. 2011). For example, a locally adapted parent population may have high fitness in its home environment, but low fitness elsewhere (Fig. 1B; Rice & Emery 2003; Kawecki & Ebert 2004). The offspring of two locally adapted parents may have lower relative fitness in any one habitat, but intermediate fitness in a wider range of conditions (Fig. 1B; Rius & Darling 2014). However, this mixing could simultaneously negatively impact local populations via genetic swamping (McKay et al. 2005). Lastly, genetically diverse populations may have more consistent population fitness over time in variable conditions due to the presence of some individuals that will perform well in each of many conditions (Kettenring et al. 2014). For example, a homogenous population may have strong fluctuations in fitness due to annual differences in climate or disease pressure, whereas increased genetic variation may lead to a

population being buffered against inter-annual changes (Fig. 1C; Schindler et al. 2015). However, this stability could come at a cost to mean fitness (Fig. 1C). Overall, the outcome associated with combining multiple populations to increase genetic variation for restoration at sites with uncertain or highly variable environmental conditions remains an open question.

Here, we address the practical question of whether increased individual- and population-level genetic diversity actually leads to higher population performance across a range of environments. To test this, we generated higher and lower genetic diversity lineages, by crossing distinct populations, and compared multiple aspects of plant performance in a common garden field experiment. We focused on multiple aspects of performance, which influence population fitness given that fitness encompasses many components that span an individual's life cycle and is often difficult to measure. To quantify the effects of environmental variability, we created a range of conditions by establishing plots across two sites and imposing a precipitation manipulation. We used *Helianthus petiolaris* (prairie sunflower) as our focal species. *Helianthus petiolaris* is an annual native forb that is of interest for restoration in arid and semi-arid prairie and other open habitats that are dominated by low-growing species across much of North America (Heiser et al. 1969; Rogers et al. 1982). We specifically test the following hypotheses: (1) High genetic diversity lineages have higher average performance across a range of environments, and are less sensitive to environmental variability, relative to low diversity lineages. Alternatively, high diversity lineages may have lower mean fitness due to outbreeding depression. (2) Increased individual-level genetic diversity will provide an advantage over population-level only diversity due to heterosis. Alternatively, populations with higher individual diversity may have lower performance due to maladapted gene complexes. (3) Across environments, high genetic diversity lineages will be less likely to experience population failure under varying environmental conditions relative to low diversity lineages. Alternatively, high diversity lineages may be more prone to failure if populations fall to small numbers. This third hypothesis is particularly pertinent in a restoration setting when population size can decline rapidly due to stochastic forces.

Methods

Focal Species

Helianthus petiolaris Nutt. subsp. *fallax* Heiser (Asteraceae) is native to North America, commonly inhabiting sandy soils in prairies of the western United States. Because of its broad distribution, disturbance-adapted nature, importance as a food source for herbivores, pollinators, and seed predators (Charlet & Gavloski 2011), and the advantages of incorporating forb species into restoration mixes (Dickson & Busby 2009), *H. petiolaris* is a potentially important species for a wide range of restoration projects. In the context of this study, *H. petiolaris* is of interest for restoration in and around the Colorado Plateau; an area within its native range that can be challenging to restore due to arid and semi-arid climates and a legacy of destructive land use practices (Winkler et al. 2018).

Additionally, the annual life history and outcrossing mating system make it a good species for experimental manipulations of genetic diversity.

Crossing Design

We selected four source *H. petiolaris* populations (P1, P2, P3, and P4) from sites on and near the Colorado Plateau with varying temperature (mean growing season daily mean temperature ranged from 12.3 to 20.3°C from 1950 to 1980; PRISM Climate Group [Oregon State University, <http://prism.oregonstate.edu>, created 18 Feb 2019]) and precipitation (mean growing season total precipitation ranged from 94.9 to 164.9 mm from 1950 to 1980; PRISM Climate Group) to represent a range of potentially adaptive traits in the experiment (Table S1; Fig. 2). Seeds from three of the source populations were obtained from USDA GRIN (Germplasm Resources Information Network; Table S1); seed from the fourth population was collected by the authors in 2014. Seed collections were made from multiple maternal plants (Seiler & Gulya 2004; N. Kane 2021, University of Colorado, Boulder, CO, personal communication).

We performed crosses with-in and between source populations to generate seed mixes with high and lower levels of both individual and population-level genetic diversity. Inter-population crosses were performed in 2015 and 2016 by first intercrossing plants (via rubbing mature inflorescences together) from the four source populations for one generation and then crossing resulting F1s for a second generation in greenhouse conditions to generate a high genetic diversity seed mix. Specifically, we grew plants from each source population (approximately 40 seeds per source) to sire seeds of plants from other populations. Between 2 and 10 plants per source reached maturity and were used in crosses. Note that the source that only yielded two mature plants (P3) was only crossed with one of the other source populations (P4). Eight different inter-population cross combinations yielded F1 seed (Table S2). We selected four of the resulting F1 types (P1 × P2, P2 × P1, P3 × P4, and P4 × P3), such that nuclear and organellar genomes from each source were represented, to serve as pollen donors and dames for the next generation (Table S2). Next, F1 plants were crossed in a manner that would generate F2s with nuclear genomic representation from all four source populations (Table S3). We selected six resulting F2 types, such that cytoplasm from each parent were represented, to plant in our field experiment. Intra-population crosses of each source population were performed in 2016 for a single generation in greenhouse conditions to decrease maternal effects and generate lower genetic diversity seed (hereafter low diversity lineages). Specifically, we grew plants from each source population (approximately 20 seeds per source) to sire seeds of plants from the same population (Table S4). Seeds from plants of each source that yielded ample seed (>320 per source) were used in our field experiment.

Common Garden Experiment

In the spring of 2017, we sowed seeds originating from inter-population (six F2 high genetic diversity types) and intra-population (four F1 low diversity lineages) crosses of each source

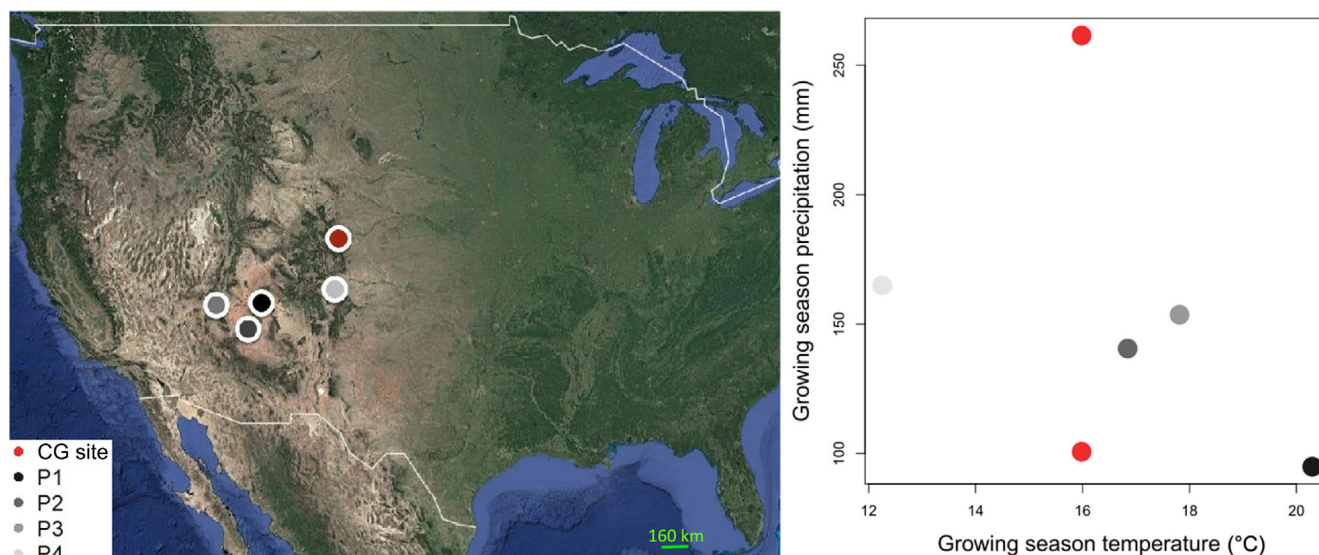


Figure 2. Geographic and climate location of source populations and common garden (CG) sites in the United States (GPS coordinates given in Table S1). Map generated using Google Maps (accessed 12 Dec 2020). Growing season precipitation and temperature for source populations is the total or daily mean, respectively, from April to October averaged from 1950 to 1980. Temperature of the common garden sites is the daily mean from April to October 2017. Climate data obtained from PRISM Climate Group (Oregon State University, <http://prism.oregonstate.edu>, created 18 Feb 2019). Precipitation of the common garden sites was measured using a rain gauge from April to October 2017; red points represent the approximate precipitation experienced by plots with rainout shelters (lower) and supplemental watering (upper).

population in two common gardens located approximately 10 miles NW of Boulder, Colorado, U.S.A. (lat 40.12, long -105.3; Fig. 2). Seeds were cold stratified for 4 weeks at 4°C prior to planting. The location of the gardens was not the home site for any of the source populations (Fig. 2) as our objective was not to compare locally sourced seed, and there were no local populations of *H. petiolaris* in the area so cross pollination from experimental populations was not a concern. The two gardens were located approximately 300 m apart; they differed in soil moisture, soil type, and local plant community (Prevéy & Seastedt 2014; T. Seastedt 2017, University Colorado, Boulder, CO, personal communication). We generated and cleared a total of $32 \times 1\text{-m}$ plots (16 plots per garden) and planted, at approximately 2 cm depth, 10 seeds of each type (source population or inter-population cross type; see Table S5 for example planting design) per plot (100 seeds total per plot, 320 seeds total per type across both gardens). Seeds were planted 10 cm apart and 5 cm from plot edges. Thinning was performed 50 days after planting in plots with high emergence to reduce competitive effects; plots were thinned to a maximum of five randomly selected plants per source or cross type. Half of the plots were pseudo-randomly assigned rain-out shelters, described and previously used by Prévéy and Seastedt (2014), that were designed to exclude approximately 50% of any natural precipitation. Non rain-out plots received supplemental watering, in addition to any natural precipitation, about once per week throughout the growing season.

Data Collection

Using weekly censuses throughout the course of the growing season (April–October), we measured emergence and

survival; reproductive biomass and final plant biomass were measured at the end of the growing season. Emergence was recorded if any seedling tissue was visible above the soil during a census. Survival was assessed for plants that had previously been reported to have emerged. Total above-ground plant tissue was harvested and labeled with unique plant IDs as plants became fully mature and started to senesce. Harvested plants were dried in a drying oven at 60°C until weight stabilization (approximately 24–48 hours). Whole dried plants were weighed to the nearest 10 mg using a Denver Instrument XE-510 balance. All flowering heads on each plant were then removed and weighed to the nearest 1 mg using an Optima OPD-A high precision balance as a measure of relative reproductive output. Although most seeds had dispersed prior to weighing, flower head size is correlated with seed number (Fig. S1).

To guide source population selection and to compare climate between source populations and the common garden sites, we obtained data for total monthly precipitation and mean monthly daily mean temperature from 1950 to 1980 from PRISM Climate Group for each source population and calculated average growing season (April–October) precipitation and temperature. Average growing season temperature at the common garden sites for 2017 was also obtained from PRISM Climate Group. Growing season precipitation for the common garden sites was measured using a rain gauge.

Data Analysis

All analyses were conducted in R version 3.6.2 (R Core Team 2019).

Genetic Variation. To quantify differences in functional genetic variation between lineages, we calculated the coefficient of variation within plot (common environment) for total above-ground mature plant dry biomass and reproductive dry biomass using the function `cv` in the R package `EnvStats` v2.3.1 (Millard 2013). We compared the mean coefficient of variation across plots between lineages and used a one-way analysis of variance (ANOVA) to test for significance.

Performance, Sensitivity to Environment, Individual- Versus Population-Level Diversity, and Likelihood of Failure. We modeled (models described below) performance across the range of plot conditions for each lineage. High genetic diversity types were analyzed individually and when pooled into a single high diversity lineage. We present results from analyses of the pooled high genetic diversity lineage (hereafter high diversity lineage) given that in a restoration context, seeds from different sources or cross types would be pooled into a single mix prior to planting. Additionally, support for models with cross types combined was stronger than for models with each type separately (Table S6).

To quantify the range of conditions experienced by plants, we combined the effects of site, treatment, and plot into a measure of apparent environmental plot quality. To make a single continuous measure of biologically relevant environmental quality, we used total above-ground final dry biomass of all *H. petiolaris* plants in each plot. This bioindicator takes into account environmental characteristics that affect plant performance that differ by site, treatment, and individual plot as all three of these factors were strongly influential (Fig. S2); therefore, this bioindicator is a good surrogate for their combined effects. Additionally, this integrated metric is convenient since, while water was the only manipulated environmental variable, ultimately we are also interested in other, measured and unmeasured aspects of the environment that affect plant performance. To check that lineage-specific differences were not leading to a given lineage(s) dominating our environmental quality index, we tried scaling the plant biomass values for each lineage and recalculating our index. Results were comparable to the raw biomass plot quality values (Fig. S3); we use the simpler (non-scaled) estimates in our analyses.

Performance was evaluated based on four metrics: emergence, survival, reproduction, and an integrated measure that is the product of emergence, survival, and reproduction, hereafter called net performance. We selected the best supported model for each performance metric based on sample-corrected Akaike information criterion (AICc) and a global model containing lineage and environmental plot quality and their interaction as predictor variables. Emergence data (number of seedlings that emerged and survived until census day per plot divided by the number of seeds planted per plot; plants that were later thinned were included in estimates of emergence) were fit using a binomial generalized linear model (GLM) with a logistic link function and family = quasibinomial to account for overdispersion. Survival data (number of plants that survived to flower per plot divided by the number that emerged and were not

thinned per plot) were fit using a binomial GLM with a logistic link function and family = quasibinomial to account for overdispersion. Reproduction for each lineage was estimated using reproductive biomass (summed dry biomass of inflorescences for each individual); these data were fit using a linear model. For each of these three dependent variables, summed or mean estimates per lineage per plot were used as cases. Net performance was calculated as the product of estimated mean emergence, mean survival, and mean adjusted reproduction (inflorescence biomass divided by mean seed mass for each lineage) and fit using linear regression. We ran models for each performance metric using environmental quality as either a continuous or categorical (low, medium, high quality) variable; we present results from the models with the continuous variable in the main text.

For each performance measure, we compared the performance of the high diversity lineage (increased individual- and population-level diversity) to increased population-level only genetic diversity. The former was calculated based on model estimates for the high diversity lineage, and the latter was calculated as the mean model estimates of the four low diversity lineages.

Finally, quantile regression (10 and 20 quantiles) using the `rq` function in R package `quantreg` v5.54 (Koenker 2019) was used to assess patterns of the lower end of net performance (calculated as above: the product of estimated mean emergence, mean survival, and mean adjusted reproduction) in each lineage in each plot to inform the relative likelihood of population failure. Models included lineage and environmental quality, as a continuous variable, and their interaction as predictor variables.

Results

Genetic Variation

We calculated the coefficient of variation (CV) within each plot (common environment) as a measure of genetic variation for two continuous traits, above-ground plant dry biomass and reproductive dry biomass. For both traits, as expected, the high diversity lineage showed the greatest mean CV (averaged across plots). Source populations P3 and P4 had slightly lower variation and populations P1 and P2 had significantly lower variation in both traits (Fig. 3).

Plant Performance and Sensitivity to Environmental Variability

Our first hypothesis is that high genetic diversity lineages will have higher average performance across a range of environments and are less sensitive to environmental variability. Model selection using AICc showed support for the full model (lineage, environmental quality, and their interaction) for all performance metrics other than emergence (Table S7). The best supported model for emergence included lineage and environmental quality, but not the interaction term (Table S7). ANOVA analysis indicated that models for all performance metrics showed significant effects of lineage and environmental quality, except for reproduction that did not have a significant effect of lineage

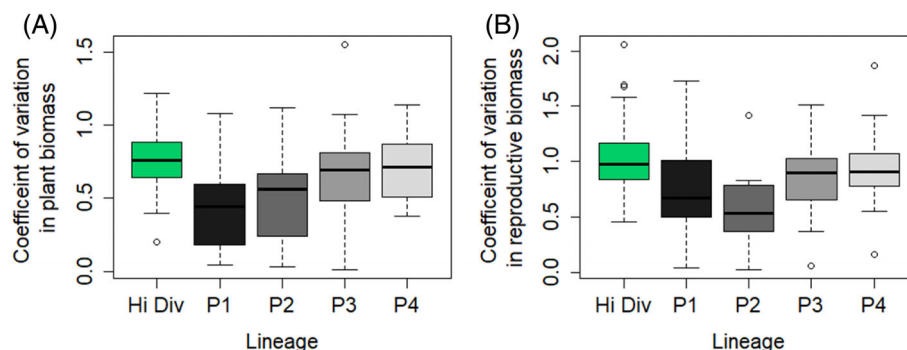


Figure 3. Coefficient of variation across all plots for each lineage in (A) total above-ground mature plant dry biomass ($p = 0.00054$) and (B) reproductive dry biomass ($p = 0.013$). Significant differences between means were assessed using a one-way ANOVA test.

(Tables S9–S12; Fig. 4). Survival, reproduction, and net performance also had significant effects of a lineage-by-environmental quality interaction (Tables S10–S12; Fig. 4). Importantly, the 32 plots used in the experiment all had some plants, which survived to reproduce and therefore represent usable habitat conditions for *H. petiolaris*.

Emergence. Emergence was highest in one of the low diversity lineages (P4) across the entire range of conditions (Fig. 4A); this lineage had notably larger seeds (Table S8) than

the other lineages, which may explain the increased rates of emergence. The high diversity lineage had intermediate emergence across all environment qualities (Figs. 4A & S4A). Sensitivity of emergence to environment was similar across lineages; interestingly all lineages had the lowest emergence in the highest quality plots (Fig. 4A).

Survival. The high diversity lineage had the highest survival in low-quality environments; survival rates were very high and similar across all lineages in high-quality plots (Figs. 4B &

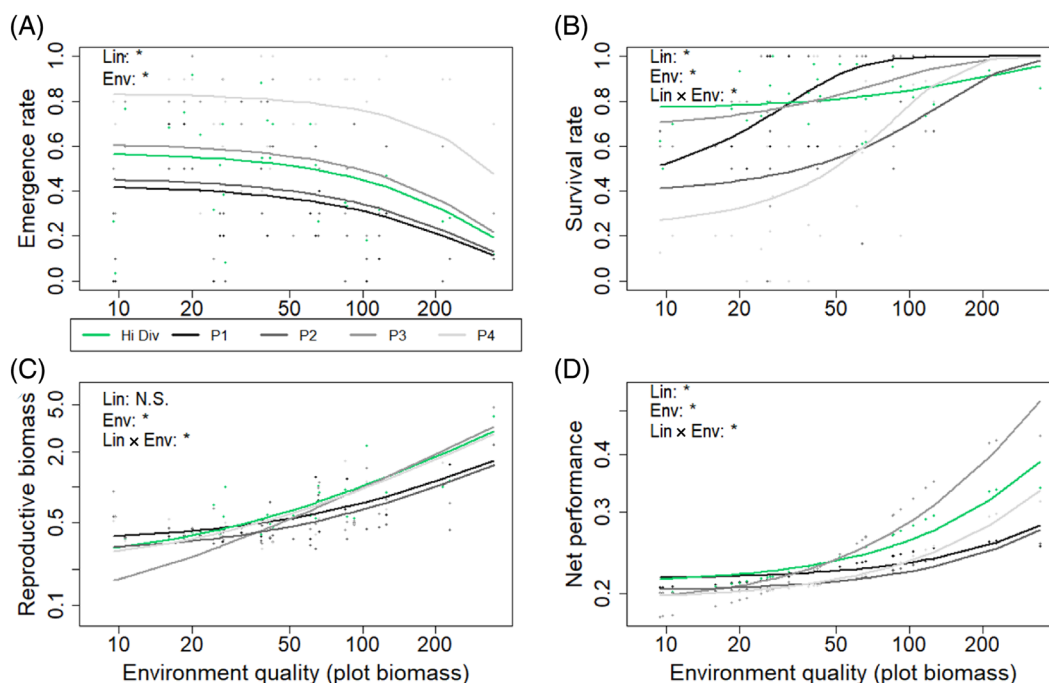


Figure 4. Performance curves using different metrics for each lineage; data are shown as points. The x-axis represents environment quality, estimated as the aboveground dry biomass of all mature experimental plants in each plot; the x-axis is on a log scale. (A) Emergence rates as estimated by a quasibinomial generalized linear model (lines). (B) Seedling-to-adult survival rates as estimated by a quasibinomial generalized linear model (lines). (C) Reproductive biomass as estimated by a linear model (lines); the y-axis is on a log scale. (D) Net performance (product of emergence rate, survival rate, and adjusted reproductive biomass) as estimated by a linear model (lines); the y-axis is on a log scale. N.S. and * indicate p -values $>$ and <0.005 , respectively, for lineage (Lin), environment quality (Env) and lineage-by-environment quality interaction (Lin $\times$ Env).

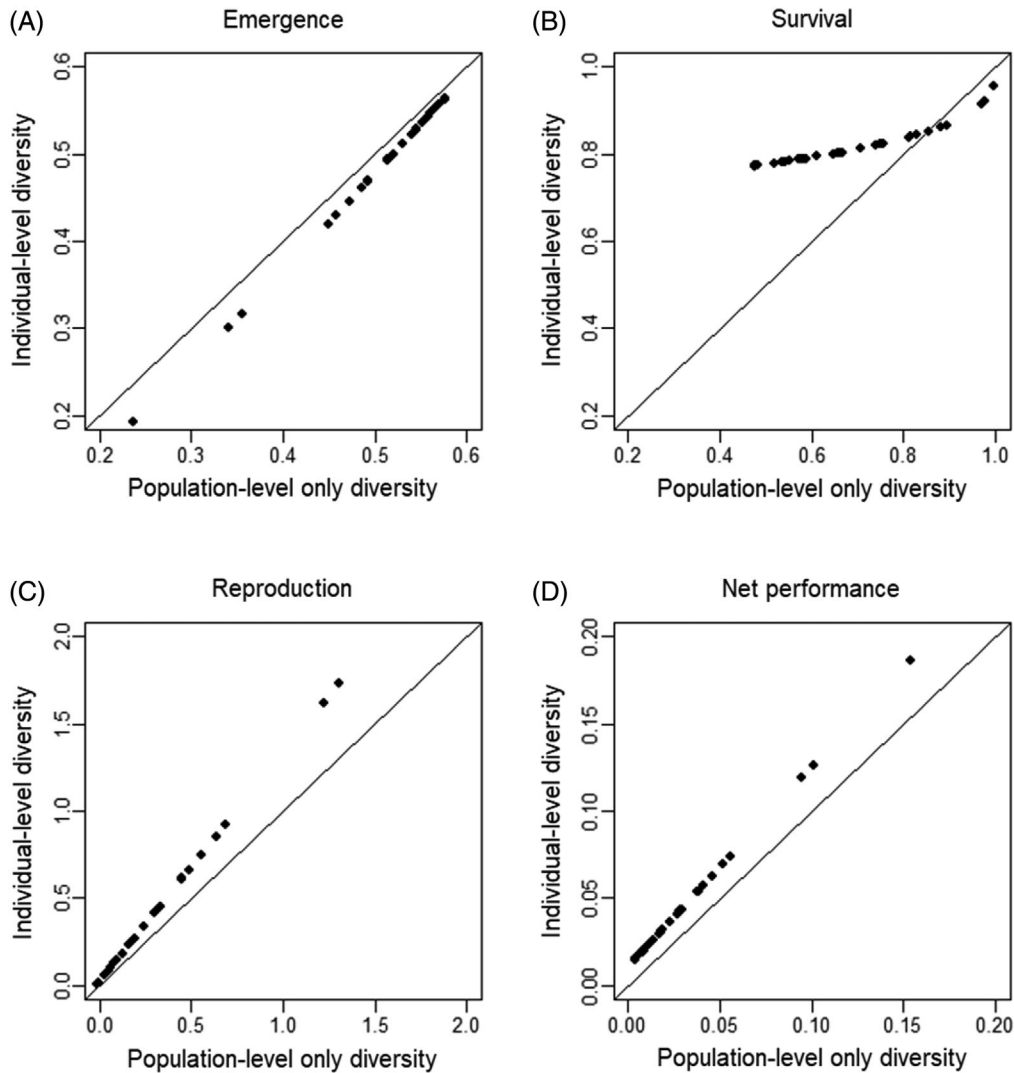


Figure 5. Performance of populations with increased individual- and population-level genetic diversity compared to increased population-level only genetic diversity. Points represent model estimated values of the high diversity lineage versus mean of the model estimated values of all low diversity lineages for (A) emergence, (B) survival, (C) reproduction, and (D) net performance in each plot. The 1:1 line represents performance values where individual-level is equal to population-level only diversity.

S4B). The high diversity lineage showed the most gradual change over the environmental range, indicating low sensitivity to environmental differences compared to most of the low diversity lineages (namely P1, P2, P4; Table S10; Fig. 4B).

Reproduction. The high diversity lineage had intermediate reproductive biomass in poor conditions and was among the highest in high quality plots (Figs. 4C & S3C). Sensitivity of reproductive output to environmental change of the high diversity lineage was intermediate (Table S11; Fig. 4C).

Net Performance. When considering net performance, which combines contributions from all three preceding performance metrics after adjusting reproductive output by seed mass, all

lineages performed similarly in low quality plots, with the high diversity lineage performing among the top two. In high quality plots, the high diversity lineage ranked higher than three, and lower than one (P3), of the low diversity lineages (Figs. 4D & S4D). The sensitivity of net performance to environmental change of the high diversity lineage was intermediate relative to the low diversity lineages (Table S12; Fig. 4D).

To investigate if adaptation (or maladaptation) to local climate might in part explain the above results for the low diversity lineages, we looked at the relationship between home climate of the four source populations and climate at the site of the experiment. We did not see a strong correspondence when considering precipitation or temperature (Figs. S6–S8).

Finally, we considered correlations between performance metrics to assess possible tradeoffs in different lineages. The inverse relationship between emergence and plot quality for all

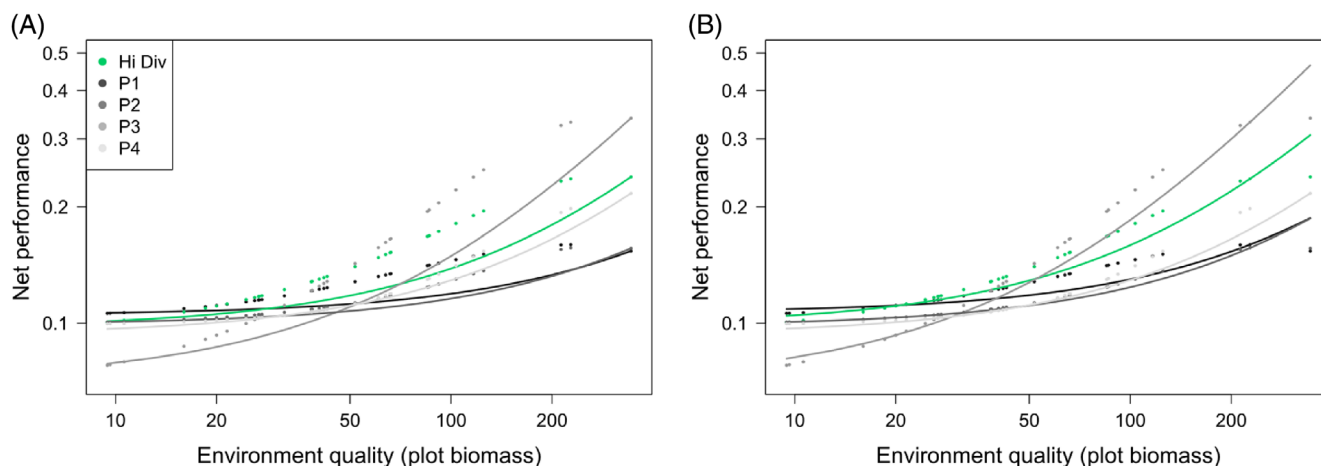


Figure 6. Quantile regression of net performance (lines) for the lower (A) 10th and (B) 20th quantiles of plants in each lineage. Data are shown as points. The x-axis represents environment quality, calculated as the aboveground dry biomass of all mature experimental plants in each plot. The x- and y-axes are on a log scale.

lineages resulted in negative correlations between emergence and the other performance metrics (Fig. S5). Correlations between survival and reproduction were positive for all lineages, with the high diversity lineage having one of the strongest positive correlations (Fig. S5).

Individual- Versus Population-Level Genetic Diversity

To test whether increased individual-level genetic diversity will provide an advantage over increased population-only diversity, we compared performance of the high diversity lineage (individual- and population-level) to the mean performance of all low diversity lineages (population-level only) in each plot. Performance of the two groups was positively correlated for all performance metrics (Fig. 5). With respect to emergence, population-only diversity was superior but similar to individual-level diversity (Fig. 5A). For survival, individual-level diversity resulted in improved performance relative to population-only diversity in lower-quality plots, whereas population-level only showed improved performance in the highest quality plots (Fig. 5B). Both reproduction and net performance showed individual-level diversity being advantageous or comparable to population-level only diversity (Fig. 5C & 5D).

Likelihood of Failure in Restored Populations

To ask whether high genetic diversity lineages will be less likely to experience population failure relative to low diversity lineages, we compared net performance of the lowest performing plants from each lineage. When considering the lower 10 and 20 quantiles, the high diversity lineage performed similar to most low diversity lineages (P1, P2, P4) in low-quality plots, ranked second in performance (P3 was first) in high-quality environments, and had intermediate sensitivity to environmental change (Fig. 6).

Discussion

We investigated the effects of increasing genetic diversity on plant performance across a range of plot conditions in the context of ecological restoration. An outstanding challenge in native plant restoration is how to achieve successful establishment and long-term population persistence given that the environmental conditions of restored habitats are variable, changing, and introducing new selective pressures (Kilkenny 2015; Wilsey 2020). This challenge requires plants to be suited to current site conditions while maintaining enough genetic variation to buffer populations from environmental variability and adapt as conditions change (Kettenring et al. 2014; Wood et al. 2015). By experimentally manipulating levels of genetic variation in *H. petiolaris* and growing plants across a range of conditions, we show that higher genetic variation results in intermediate or improved performance and sensitivity to plot quality, and possibly a lower likelihood of population failure compared to most of the low diversity lineages tested.

Genetic Variation

By analyzing the genetic variation in two quantitative traits, we showed that the high diversity lineage did indeed show higher levels of variation compared to the four low diversity lineages. This comparison of genetic variation is strictly relative and does not say anything about the absolute levels of variation in the source populations. However, we expect that the source populations did not have low genetic variation in an absolute sense, as *H. petiolaris* is a widely distributed species with a very large effective population size (Strasburg et al. 2011) and substantial within population genetic variation (Kane et al. 2009).

Plant Performance and Sensitivity to Environmental Variability

The performance and sensitivity of each lineage differed by performance metric. All lineages showed increasing performance as environmental quality improved for all performance metrics

except emergence. This may in part be explained by differences in plot quality being less pronounced at the emergence stage. Environmental quality was in part driven by our water treatment, which was initiated at planting, not long before emergence; therefore, the effects of soil moisture differences may not have been fully felt by the plants at the emergence stage. Further research on germination and emergence patterns of common restoration species are important given that these early life history stages commonly account for the low success rates of restoration projects in arid regions (James et al. 2011).

When considering net performance, no single low diversity lineage performed better than the high diversity lineage across the range of environments tested. Low diversity lineages were either comparable in low-quality environments (P1, P2, P3, P4), performed worse when plot quality improved (P1, P2, P4), or were more sensitive (P3) compared to the high diversity lineage. The site of the experiment was not the home site for any of the source populations and therefore we did not expect a home site advantage. However, if the source populations were adapted to their local climate, we might expect to see patterns of performance explained in part by differences in climate between the source and experimental sites. We did not see a strong correspondence between precipitation or temperature in the plots and the home locations of the four source populations.

Our measure of net performance combines three components of fitness that span the entire vegetative portion of the annual life cycle; however, it does not consider contributions to fitness occurring during the seed stage. To obtain a more complete picture of performance and estimates of population growth rate, future studies should include estimates of seed survival (James et al. 2011), which captures the probability that a seed disperses to suitable habitat, survives fungal attack, predation, and any other sources of mortality occurring between seed maturity in the fall and emergence the following spring. This is particularly pertinent in the context of restoration in arid and semiarid environments where many restoration projects fail due to unsuccessful initial establishment (Kildisheva et al. 2016). Including fitness components that span the entire life cycle would allow for estimates of absolute population fitness (i.e. whether a population is expected to persist or not) (Metcalf & Pavard 2006; Takada & Shefferson 2018), in addition to relative performance as assessed here.

Individual- Versus Population-Level Genetic Diversity

Our experiment involved deliberate crossing of different populations to generate seeds containing genetic material from all source populations. While this careful crossing approach is not feasible in most restoration contexts, it allowed us to tease apart potential genetic effects of using crosses that contain higher levels of both individual and population diversity versus directly planting seed from multiple source populations to augment population-level diversity (Munda & Smith 1995; Burton & Burton 2002; Broadhurst et al. 2008). When using outcrossing species, the latter approach assumes that crossing between different sources will occur with subsequent generations; however, the importance of that assumption for restoration success is not

clear. Our results show that individual- and population-level diversity differs from, but is positively correlated with, population-only diversity. We found that higher individual-level diversity was advantageous for reproduction and net performance, disadvantageous for emergence, and had both positive and negative effects on survival. Regardless of the mechanism, any advantage that leads to increased short-term success will in turn lead to maintenance of genetic diversity, which would otherwise be lost if populations are small. We expect that a fair amount of initial diversity at the onset of restoration will be lost due to selection and drift; therefore, any advantage that leads to increased population establishment and growth will result in more genetic diversity overall.

Crossing distinct populations requires attention to the possibility of outbreeding depression, which can result from maladapted gene combinations, cytonuclear incompatibility, or ploidy differences when populations that are too divergent are mixed (Etterson et al. 2007; Kramer et al. 2018). On the other hand, increased individual diversity at functional loci can be beneficial when populations with segregating alleles have similar genetic backgrounds. For example, increased heterozygosity can lead to increased fitness when inbreeding depression, caused by recessive deleterious mutations, is alleviated (Frankham 2015). Heterozygosity at adaptive loci can be beneficial in cases where different populations contain contrasting adaptations at the same locus; for example, different pathogens can select for alternate alleles in different populations each associated with resistance to its respective pathogen. Additionally, different populations may contain adaptations to different selective pressures (e.g. herbivores and climate). In both cases, mixing the different populations would result in the restored population having alleles that improve fitness in the face of a broader range of selective pressures (Engelhardt et al. 2014). Having this adaptive diversity at the individual level at the time of population founding, in addition to having potential inherent benefits, serves to maintain variation in the population by helping to ensure less total genetic diversity is lost if strong selection is experienced in the first few years that could lead to loss of a subset of the population with particular alleles.

On a practical note, if using multiple source populations is deemed appropriate for a given restoration project, practitioners will need to decide on the timing of mixing. This decision will likely depend on the size and timeline of the project, species used, and whether seed propagation is needed. For example, if no propagation is needed, the focal species is self-pollinating, and/ or there are timeline constraints, then direct seeding may be the most efficient (Erickson & Halford 2020). However, if seed propagation is required, the focal species is outcrossing, and the resulting seed will be used for specified local projects, then allowing source populations to cross pollinate during propagation may have inherent advantages and will improve retention of genetic diversity post-restoration (Munda & Smith 1995). In a third scenario, if seed propagation is done commercially and the resulting seed is to be used widely for diverse projects in a range of locations, propagating seed from different sources separately will allow for more flexibility. Mixing of diverse populations can then be tailored for specific

projects in specific locations, and knowledge about how much of each source is planted in a given project can be recorded (Espeland et al. 2017). The benefits of individual-level variation may still be realized after several generations of random mating. In cases where mixing different populations is not deemed suitable, or multiple appropriate source populations are not available, then using a single source with high genetic diversity is recommended (McKay et al. 2005). In the absence of data, approximate population size could be used as a proxy for levels of within population genetic variation (Hoffmann et al. 2017).

Likelihood of Failure in Restored Populations

Restored populations are particularly vulnerable if initial population sizes are small. Small populations are prone to decreases in genetic diversity leading to bottlenecks and inbreeding depression as well as being susceptible to extirpation from demographic and environmental stochasticity (Hoffmann et al. 2017). Small population size may occur because initial establishment is low, or strong biotic or abiotic forces act to negatively impact population numbers. Low establishment or population declines are common following restoration as projects are often located on degraded land with poor habitat quality (James & Svejcar 2010; Knutson et al. 2014). Restoration efforts on the Colorado Plateau, for example, often contend with sites degraded from legacies of resource extraction and grazing, as well as increasing aridity and invasive species (Winkler et al. 2018).

To assess how increasing genetic diversity may affect the likelihood of successful restoration if population size is small, we compared the net performance of the bottom fraction of plants in each lineage. Based on the net performance of the 10th and 20th quantiles, we found that the high diversity lineage had improved or intermediate performance and sensitivity to environmental change. This suggests that if population numbers are low, such that population persistence is dependent on the performance of a random sample of surviving individuals (in the worst-case scenario, this would be the worst performing plants), higher genetic diversity may be beneficial for maintaining population viability, particularly if environmental conditions are unpredictable.

In conclusion, this study focuses on population level effects of genetic diversity within a single species. Other research has reported how intra-species genetic diversity can also impact community and ecosystem level functions (Vellend 2006; Hughes et al. 2008; Kettenring et al. 2014). This is an important area of research warranting expansion in the context of ecological restoration where goals often include improvements to ecosystem function in addition to simple revegetation (Gann et al. 2019). Additionally, future studies should examine individual- and population-level genetic diversity in restoration species with a range of life histories, beyond a single annual species as studied here.

Acknowledgments

Research was conducted upon lands of the Arapaho, Cheyenne, and Ute peoples; we thank current guardians S. and L. Sanabria. The authors thank T. Seastedt and T. Wood for research

contributions. T. Meyers, H. Monahan, Z. Litoff, E. Bach, G. Kendzierski, T. Goebel, and J. Stanley helped with fieldwork and data collection. The authors thank N. Emery, R. Hufft, and two anonymous reviewers for feedback on an earlier manuscript draft. This work was supported by a NSERC Postgraduate Scholarship (AMG) and NSF IGERT grant number 1144807 (AMG).

LITERATURE CITED

- Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* 26:183–192. <https://doi.org/10.1016/j.tree.2011.01.009>
- Broadhurst LM, Lowe A, Coates DJ, Cunningham SA, McDonald M, Vesk PA, Yates C (2008) Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1:587–597. <https://doi.org/10.1111/j.1752-4571.2008.00045.x>
- Bucharova A, Bosdorf O, Hölzel N, Kollmann J, Prasse R, Durka W (2019) Mix and match: regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. *Conservation Genetics* 20:7–17. <https://doi.org/10.1007/s10592-018-1067-6>
- Burton PJ, Burton CM (2002) Promoting genetic diversity in the production of large quantities of native plant seed. *Ecological Restoration* 20:117–123. <https://doi.org/10.3368/er.20.2.117>
- Charlet L, Gavloski J (2011) Insects of sunflower in the Northern Great Plains of North America. Pages 159–178. In: *Arthropods of Canadian grasslands: inhabitants of a changing landscape*. Ottawa, Ontario: Biological Survey of Canada
- Crémieux L, Bischoff A, Müller-Schärer H, Steinger T (2010) Gene flow from foreign provenances into local plant populations: fitness consequences and implications for biodiversity restoration. *American Journal of Botany* 97:94–100. <https://doi.org/10.3732/ajb.0900103>
- Cross AT, Pedrini S, Dixon KW (2020) Foreword: international standards for native seeds in ecological restoration. *Restoration Ecology* 28:S225–S227. <https://doi.org/10.1111/rec.13173>
- Dickson TL, Busby WH (2009) Forb species establishment increases with decreased grass seeding density and with increased forb seeding density in a Northeast Kansas, U.S.A., experimental prairie restoration. *Restoration Ecology* 17:597–605. <https://doi.org/10.1111/j.1526-100X.2008.00427.x>
- Elmqvist T, Folke C, Nyström M, Peterson G, Bengtsson J, Walker B, Norberg J (2003) Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment* 1:488–494. [https://doi.org/10.1890/1540-9295\(2003\)001\[0488:RDECAR\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0488:RDECAR]2.0.CO;2)
- Engelhardt KAM, Lloyd MW, Neel MC (2014) Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. *Biological Conservation* 179:6–16. <https://doi.org/10.1016/j.biocon.2014.08.011>
- Erickson VJ, Halford A (2020) Seed planning, sourcing, and procurement. *Restoration Ecology* 28:S216–S224. <https://doi.org/10.1111/rec.13199>
- Espeland EK, Emery NC, Mercer KL, Woolbright SA, Kettenring KM, Gepts P, Etterson JR (2017) Evolution of plant materials for ecological restoration: insights from the applied and basic literature. *Journal of Applied Ecology* 54:102–115. <https://doi.org/10.1111/1365-2664.12739>
- Etterson JR, Keller SR, Galloway LF (2007) Epistatic and cytonuclear interactions govern outbreeding depression in the autotetraploid *Campanulastrum americanum*. *Evolution* 61:2671–2683. <https://doi.org/10.1111/j.1558-5646.2007.00234.x>
- Falk DA, Knapp EE, Guerrant EO (2001) An introduction to restoration genetics. Society for Ecological Restoration, Washington, D.C.
- Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology* 24:2610–2618. <https://doi.org/10.1111/mec.13139>

- Gann GD, McDonald T, Walder B, Aronson J, Nelson CR & Jonson J et al. (2019) International principles and standards for the practice of ecological restoration. Second edition
- Gilpin ME, Soulé ME (1986) Minimum viable populations: the processes of species extinctions. In: Soulé ME (ed) Conservation biology: the science of scarcity and diversity. Sunderland, MA: Sinauer Associates
- Havens K, Vitt P, Still S, Kramer AT, Fant JB, Schatz K (2015) Seed sourcing for restoration in an era of climate change. *Natural Areas Journal* 35:122–133. <https://doi.org/10.3375/043.035.0116>
- Heiser C, Smith D, Clevenger S, Martin W (1969) The North American sunflowers (*Helianthus*). *Memoirs of the Torrey Botanical Club*. 22:Durham, North Carolina: Club by the Seeman Printery, 1–218
- Hoffmann AA, Sgrò CM, Kristensen TN (2017) Revisiting adaptive potential, population size, and conservation. *Trends in Ecology and Evolution* 32: 506–517. <https://doi.org/10.1016/j.tree.2017.03.012>
- Hooper DU III, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35. <https://doi.org/10.1890/04-0922>
- Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* 18:147–155. [https://doi.org/10.1016/S0169-5347\(03\)00002-8](https://doi.org/10.1016/S0169-5347(03)00002-8)
- Hughes AR, Brian D, Johnson MTJ, Underwood N (2008) Ecological consequences of genetic diversity. *Ecology Letters* 11:609–623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>
- IPBES (2018) The IPBES assessment report on land degradation and restoration. In: L Montanarella, R Scholes, A Brainich (eds) Secretariat of the intergovernmental science-policy platform on biodiversity and ecosystem services, Bonn, Germany. 744 pages. <https://doi.org/10.5281/zenodo.3237>
- James JJ, Svejcar TJ (2010) Limitations to postfire seedling establishment: the role of seeding technology, water availability, and invasive plant abundance. *Rangeland Ecology and Management* 63:491–495. <https://doi.org/10.2111/REM-D-09-00124.1>
- James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology* 48: 961–969. <https://doi.org/10.1111/j.1365-2664.2011.02009.x>
- Kane NC, King MG, Barker MS, Raduski A, Yatabe Y, Knapp SJ, Rieseberg LH, True J (2009) Comparative genomic and population genetic analyses indicate highly porous genomes and high. *Evolution* 63:2061–2075. <https://doi.org/10.1111/j.1558-5646.2009.00703.x>
- Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters* 7:1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Kettenring KM, Mercer KL, Reinhardt Adams C, Hines J (2014) Application of genetic diversity-ecosystem function research to ecological restoration. *Journal of Applied Ecology* 51:339–348. <https://doi.org/10.1111/1365-2664.12202>
- Kildisheva OA, Erickson TE, Merritt DJ, Dixon KW (2016) Setting the scene for dryland recovery: an overview and key findings from a workshop targeting seed-based restoration. *Restoration Ecology* 24:S36–S42. <https://doi.org/10.1111/rec.12392>
- Kilkenny FF (2015) Genecological approaches to predicting the effects of climate change on plant populations. *Natural Areas Journal* 35:152–164. <https://doi.org/10.3375/043.035.0110>
- Knutson KC, Pyke DA, Wirth TA, Arkle RS, Pilliod DS, Brooks ML, Chambers JC, Grace JB (2014) Long-term effects of seeding after wildfire on vegetation in Great Basin shrubland ecosystems. *Journal of Applied Ecology* 51:1414–1424. <https://doi.org/10.1111/1365-2664.12309>
- Koenker R (2019) quantreg: Quantile Regression. R package version 5.54. <https://CRAN.R-project.org/package=quantreg>
- Kramer AT, Wood TE, Frischie S, Havens K (2018) Considering ploidy when producing and using mixed-source native plant materials for restoration. *Restoration Ecology* 26:13–19. <https://doi.org/10.1111/rec.12636>
- Lesica P, Allendorf FW (1999) Ecological genetics and the restoration of plant communities: mix or match? *Restoration Ecology* 7:42–50. <https://doi.org/10.1046/j.1526-100X.1999.07105.x>
- McKay JK, Christian CE, Harrison S, Rice KJ (2005) “How local is local?” – a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13:432–440. <https://doi.org/10.1111/j.1526-100X.2005.00058.x>
- Metcalfe CJE, Pavard S (2006) Why evolutionary biologists should be demographers. *Trends in Ecology and Evolution* 22:205–212. <https://doi.org/10.1016/j.tree.2006.12.001>
- Millard SP (2013) EnvStats: An R package for environmental statistics. New York City, NY: Springer
- Munda B & Smith S (1995) Proceedings: wildland shrub and arid land restoration symposium. In USDA general technical report INT-GTR-315. http://www.fs.fed.us/rm/pubs_int/int_gtr315/int_gtr315_335_338.pdf. Accessed 11 February 2020.
- Prevéy JS, Seastedt TR (2014) Seasonality of precipitation interacts with exotic species to alter composition and phenology of a semi-arid grassland. *Journal of Ecology* 102:1549–1561. <https://doi.org/10.1111/1365-2745.12320>
- R Core Team (2019) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. <https://www.r-project.org>
- Rice KJ, Emery NC (2003) Managing microevolution: restoration in the face of global change. *Frontiers in Ecology and the Environment* 1:469–478. [https://doi.org/10.1890/1540-9295\(2003\)001\[0469:MMRITF\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0469:MMRITF]2.0.CO;2)
- Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology and Evolution* 29:233–242. <https://doi.org/10.1016/j.tree.2014.02.003>
- Rogers C, Thompson T, Seiler G (1982) Sunflower species of the United States. National Sunflower Association
- Roughgarden J (1972) Evolution of niche width. *The American Naturalist* 106: 683–718. <https://doi.org/10.1086/282807>
- Schindler DE, Armstrong JB, Reed TE (2015) The portfolio concept in ecology and evolution. *Frontiers in Ecology and the Environment* 13:257–263. <https://doi.org/10.1890/140275>
- Seiler GJ, Gulya T (2004) Exploration for wild *Helianthus* species in North America: challenges and opportunities in the search for global treasures. Proceedings of the 16th international sunflower conference
- Strasburg JL, Kane NC, Raduski AR, Bonin A, Michelmore R, Rieseberg LH (2011) Effective population size is positively correlated with levels of adaptive divergence among annual sunflowers. *Molecular Biology and Evolution* 28:1569–1580. <https://doi.org/10.1093/molbev/msq270>
- Takada T, Shefferson R (2018) The long and winding road of evolutionary demography: preface. *Population Ecology* 60:3–7. <https://doi.org/10.1007/s10144-018-0622-9>
- Vellend M (2006) The consequences of genetic diversity in competitive communities. *Ecology* 87:304–311. <https://doi.org/10.1890/05-0173>
- Wilsey B (2020) Restoration in the face of changing climate: importance of persistence, priority effects, and species diversity. *Restoration Ecology* 29:1–10. <https://doi.org/10.1111/rec.13132>
- Winkler DE, Backer DM, Belnap J, Bradford JB, Butterfield BJ, Copeland SM, et al. (2018) Beyond traditional ecological restoration on the Colorado Plateau. *Restoration Ecology* 26:1055–1060. <https://doi.org/10.1111/rec.12876>
- Wood TE, Doherty K, Padgett W (2015) Development of native plant materials for restoration and rehabilitation of Colorado plateau ecosystems. *Natural Areas Journal* 35:134–150. <https://doi.org/10.3375/043.035.0117>

Supporting Information

The following information may be found in the online version of this article:

Figure S1. Relationship between flower head size and seed number from nine different *H. petiolaris* populations.

Figure S2. Total above-ground dry biomass of all mature *H. petiolaris* in each plot.

Figure S3. Relationship between total above-ground dry biomass of all mature *H. petiolaris* in each plot (raw biomass values) and scaled (by lineage) total above-ground biomass in each plot.

Figure S4. Means and standard errors of performance metrics for each lineage in low, medium, and high environmental quality.

Figure S5. Correlations between different model estimated performance metrics in each lineage.

Figure S6. Relationship between four different performance metrics and temperature distance (absolute value of source mean growing season daily mean temperature minus common garden site mean growing season daily mean temperature).

Figure S7. Relationship between four different performance metrics and precipitation distance (absolute value of source total growing season precipitation minus common garden site total growing season precipitation in plots with rainout shelters).

Figure S8. Relationship between four different performance metrics and precipitation distance (absolute value of source total growing season precipitation minus common garden site total growing season precipitation in plots that received supplemental water).

Table S1. Location, climate, and accession numbers of source populations.

Table S2. Design for the first round (F1 generation) of inter-population crosses.

Table S3. Design for the second round (F2 generation) of inter-population crosses.

Table S4. Design for the one round (F1 generation) of intra-population crosses.

Table S5. Example planting design for a single 1×1 -m plot.

Table S6. Corrected Akaike information criterion (AICc) from models of each performance metric showing support for using lineage (lineage; combines different cross types) instead of cross types separately (Type).

Table S7. Corrected Akaike and quasi-Akaike information criterion (AICc and QAICc) from nested models of each performance metric using a global model containing lineage, environment quality, and their interaction as predictor variables.

Table S8. Mean seed mass $\pm$ standard error (SE) for each lineage.

Table S9. Parameter estimates from a generalized linear model of seedling emergence rates.

Table S10. Parameter estimates from a generalized linear model of seedling-to-adult survival rates.

Table S11. Parameter estimates from a linear model of reproductive biomass.

Table S12. Parameter estimates from a linear model of net performance.

Coordinating Editor: Paul G Nevill

Received: 17 February, 2021; First decision: 20 April, 2021; Revised: 3 February, 2022; Accepted: 9 February, 2022