Local *Populus fremontii* genotypes outperformed by transplants: re-examining the home-site hypothesis in restoration

Patrick M. Babbin¹, Zachary A. Miller¹, Jordan A. Erickson¹, Tristan O. Woodsmith¹, Benjamin J. Marcus¹, Dylan G. Fischer¹*, Randy K. Bangert², Gery J. Allan², and Thomas G. Whitham²

¹Environmental Studies, Evergreen State College, Olympia, WA 98505
²Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011

*Correspondence and requests for materials should be addressed to Dylan Fischer, The Evergreen State College, 2700 Evergreen Parkway NW, Olympia, Washington 98505. E-Mail: fischerd@evergreen.edu FAX: (360) 867-5102
Abstract. We examined the hypotheses that individual plant genotype and source population of Fremont cottonwood, *Populus fremontii*, would have a significant effect on the survival of trees in a 6,000 tree restoration site at Cibola National Wildlife Refuge, AZ. Experimental examination of genotypic effects is important because standard restoration practices advocate the use of local genotypes, but tests are rarely conducted to quantify genetic effects and to determine if genotypes derived from local populations actually perform better than genotypes from more distant source populations. Several major patterns emerged. 1. Genetic effects were large; mean mortality rate among genotypes ranged from 9.84% to 88.89%. 2. Environmental and/or maternal effects were not significant (i.e., planting micro-location, sapling diameter, or sapling height). Genotype was a significant factor in determining mortality (*p* < 0.001), explaining 11.26% of the variation among genotypes. 3. Surprisingly, local genotypes performed significantly worse than genotypes from much farther away (up to 900km). Significant single variable relationships were found between mortality and geographic distance from site of origin (*r*² = 0.33), elevation of site of origin (*r*² = 0.33), mean annual precipitation of site of origin (*r*² = 0.28), and mean annual temperature of site of origin (*r*² = 0.29). In combination, these results show that mortality rates generally decrease with increasing distance from the restoration site. These results have significant implications for restoration efforts as they do not support the concept of a ‘home site advantage’ (Montalvo and Ellstrand 2000). They suggest that climate change and other anthropogenic effects on the environment (i.e., altered hydrological and salinity regimes due to flood control and agricultural practices) may have shifted the local environment so much that local genotypes are no longer best adapted to local conditions. By selecting specific genotypes
for specific locations, it may be possible to lower mortality rates, thus increasing
restoration efficiency. We view our findings as a major conundrum for restoration efforts
and have initiated further studies to experimentally examine this initial study that should
be cautiously interpreted, but nevertheless may be an important warning that
anthropogenic-based environmental changes may so alter environments that the strategies
of restoration programs need re-evaluation.

Keywords: common garden, ecological genetics, Fremont cottonwood, home site
advantage, mortality, \textit{Populus fremontii}, restoration

\textbf{Introduction}

Restoration efforts should focus on selecting particular genotypes, rather than
locally adapted stock, in order to more effectively restore habitat. Understanding the
role genetics play in influencing survival of individuals is important, as locally adapted
stock may underperform in the face of climate change and other anthropogenic factors
that are altering ecosystems more quickly than local populations can adapt. Riparian
habitat along the lower Colorado river has declined from 90,000 acres in 1938 to 6,000
acres in 1998 due to anthropogenic factors (Cohn 2001). Recently, multiple public and
private agencies have been involved in the active restoration of the lower Colorado River
Basin. The Bureau of Reclamation in particular has set out a goal of restoring 10,000
acres of riparian forest by 2014. With such extensive restoration efforts, the genetic
composition of the stock may have an important and long-lasting impact on future
riparian forests in the southwestern U.S.
Other studies have examined how distinct genetic populations within a species (ecotypes) influence persistence and genetic composition of populations. Introduced ecotypes within genetically different populations vary in persistence and compositional effects. Some studies have found total dominance by introduced ecotypes (“swamping”) (Hufford and Mazer 2003). For example, a European version of the grass *Phragmites australis* (common reed) was found to rapidly overtake an American epitype of *P. australis* (Saltonstall 2002). Others found greater persistence of locally adapted ecotypes in a home-site when compared to non-native stock. Montalvo and Ellstrand (2000) found that *Lotus scoparius* (deerweed) decreased in persistence as distance from its homesite increased.

Although some of the above studies strongly argue for the use of local stock in restoration plantings, climate change represents an unknown ‘wild card’ for conservation and restoration. For example, the most recent IPCC report (IPCC 2007) shows that the American Southwest has undergone and is predicted to show the greatest climatic changes of the 48 contiguous states. In the last 50 years the growing season has increased by about 27 days and an ongoing drought since 1996 has resulted in major vegetation changes. For example, foundation species (ponderosa pine, pinyon pine, aspen, manzanita, cottonwood) from alpine forests to desert and riparian habitats have recently suffered regional mortality rates of 15-41% (Gitlin et al. 2006). As these species are foundation species and some, such as cottonwoods characterize rare habitat types that are hotspots of biodiversity, the community and ecosystem consequences are likely to be great (see also Parmasean 2006). These findings are supported with other studies of high mortality throughout the region (e.g., Allen and Breshears 1998, Breshears et al. 2005,
Mueller et al. 2005) and modeling of climatic envelopes predict grim consequences for
many of these species. For example, modeling by Rehfeldt et al. (2006), suggest that one
of the most common species in Arizona, pinyon pine (*Pinus edulis*) may be extirpated
from the state by 2090. Similarly, modeling by Gitlin and Whitham (in review) argues
that cottonwoods are likely to disappear from the lower Colorado River, which includes
the study site of our current paper.

Genetics have been found to influence leaf size, chlorophyll concentration, and
leaf mass and multiple community and ecosystem traits in *Populus* forests of the
southwest (Rowland 2001, Whitham et al. 2006). Understanding how genetic and
environmental factors (including homesite distance) interact with the planting of native
and non-native ecotypes determines how restoration efforts may be affected.

In restoration projects, as in natural regeneration, early sapling mortality
may define the character of mature forests through the effects of selection. Those saplings
that survive the early stages of restoration have the highest potential to influence the
community and ecosystem in the long-run, while those that fare poorly are less likely to
maintain further ecosystem-level influence. Because genetic differences can affect early
survival of out-planted saplings, restoration efforts may be unintentioned experiments,
where only the genetic stock best fit for early survival will be selected to influence the
future ecosystem.

We addressed two major hypotheses. First, extensive genetic variation in Fremont
cottonwood would be reflected in significantly different mortality rates across genotypes.
Second, local genotypes should perform better than genotypes derived from populations
farther away from our planting site, i.e., the ‘home site advantage’ (Montalvo and
Ellstrand 2000). Answers to these questions are important because they affect our understanding of basic ecological and evolutionary principles, which have important implications for environmental restoration.

Methods

Study Site

Data were collected from three established experimental restoration plots in the Cibola National Wildlife Refuge in southwestern Arizona. The common garden is organized with 16 distinct genotypes of *P. fremontii* randomly transplanted into three plots. The saplings used in the study were propagated cuttings from 16 sites across the southwestern United States (fig. 1). Cuttings were taken before leaf-out, from January to April of 2005 and rooted in a greenhouse for 6 months before transplanting. Each plot was organized in a 16 by 20 grid (320 saplings), with 3 meters between each transplant. Plots were subdivided into twenty 4 by 4 blocks (16 saplings), each containing 1, 2, 4, 8, or 16 varieties, respectively. To account for possible variation in field conditions, the site was laser-leveled and is regularly flood-irrigated.

Sampling Methods

Sapling mortality was examined six months after transplant. There were 17 *P. fremontii* that could not be located (out of 960 total), and were not factored into the final sample size. Genotype, mortality, height, and stem diameter data were collected for each sapling. Height was measured from base to highest apical meristem, while stem diameter was measured at the base of each sapling. Mortality was gauged by assessing whether
chlorophyll was present in the stem by removing a small piece of bark near the base and checking for green pigmentation. To account for any potential effects that environmental factors might have on the fitness of each genotype, a number of variables were obtained from each home-site. Data compiled included geographic distance, latitude, elevation, and annual means of both temperature and precipitation. Geographic distance and latitude were obtained using GIS coordinates. Elevation was recorded at the time when each cutting was taken (Table 1). Long term temperature and precipitation averages were taken from the Western Regional Climate Center (http://www.wrcc.dri.edu/CLIMATEDATA.html). Data from the closest weather station to each home-site was used.

**Statistical Methods**

Average mortality was calculated for each genotype. An analysis of variance was run using SPSS 13.1 for Windows (SPSS Inc, Chicago, IL USA) to determine significance \( p \leq 0.05 \) of mortality rates between genotypes. We used linear regressions to examine relationships between each continuous variable and mortality. Broad sense heritability, the ratio of variance between genotypes, was also calculated using standard methods (Bailey et al. 2006). We were unable to directly compare genotype and environmental variables of the homesite since a single genotype was propagated from each homesite independently.

To determine the relative influence of co-varying environmental factors on sapling mortality we used a model selection approach that allows ranking of multiple predictive models based on information-theoretic criteria (Burnham and Anderson 2002).
This approach avoids the pitfalls of other model selection techniques such as stepwise regression which may have unreasonable assumptions, or preferentially pick models based on order of inspection (Burnham and Anderson 2002). Briefly, the information-theoretic approach uses maximum likelihood theory and the principle of parsimony to assess the strength of evidence for each model in a candidate set of a priori defined models. We used Akaike’s Information Criterion, adjusted for small sample size (AICc), an estimate of model likelihood, Akaike weights ($w_i$), and an evidence ratio computed from these variables, to simultaneously compare and rank multiple models from a set of a priori candidate models (see Burnham & Anderson, 2002). Each measure provides an index of the best model given the data, and the evidence ratio gives a comparative “odds” of the top-ranked model being the best model given the data. All models were single variable linear models due to low sample sizes. The models included “altitude of homesite,” “temperature of homesite,” “precipitation of homesite,” “basal diameter of sapling,” and an “intercept-only” model. Models whose $\Delta$AIC (AICc relativized to the lowest value) differed by less than 2.0 were not considered statistically distinguishable, as is common practice (Burnham & Anderson, 2002). The AICc values were calculated from AIC values given in JMP stepwise regression analyses (JMP Discovery statistical package 4.0, SAS Institute Inc., Cary, NC, USA).

Results
As predicted, we found a significant difference in mean mortality rates between genotypes ($p < 0.001$). Average mortality ranged from 9.84% to 88.89% (Fig. 2). The average mortality rate for all transplants studied was 31.55%.

Although elevation and environmental factors were significant predictors of mortality, highest mortality occurred among genotypes from areas proximal to the restoration site, and lowest mortality occurred in genotypes from distant, higher elevations, with lower average temperatures and higher annual precipitation (Figure 3). There were significant negative linear relationships between mortality and geographic distance from homesite (where cuttings originated; $p < 0.001$), elevation of homesite ($p < 0.001$), and mean annual precipitation of homesite ($p < 0.001$). A significant positive relationship was found between mortality and mean annual temperature of homesite ($p < 0.001$).

Although we found elevation to be the best ranked model for predicting mortality according to our model selection criteria, it was indistinguishable from mean annual temperature and precipitation. The elevation model best predicted mortality according to all model selection criteria, including: the $\Delta$AICc values (lowest), the likelihood value for the best model (LIKELIHOOD; 1.0), the Akaike weight of evidence ($w_i$; closest to 1), and the evidence ratio (EVID. RATIO; next best model has a 1:1.5 chance of being better, given the data). This is reflected by the delta AICc which suggests this model is statistically indistinguishable from the next three. The size of the cutting (diameter) is a far worse predictor of mortality than the cutting’s origin.

Some variation in planting conditions existed in the common garden, but neither location nor sapling size were significant predictors of mortality. We found no significant
correlation between mortality and plant height ($p = 0.184$), basal diameter of sapling ($p = 0.957$), or orientation in the garden (east-west: $p = 0.056$; north-south: $p = 0.153$).

Discussion

In a restoration context, genotypic variation in early sapling mortality can affect project efficacy dramatically. In phreatophytic and facultatively phreatophitic (see Cox et al. 2005) species such as *P. fremontii*, early sapling survival may help determine whether individuals live long enough to make contact with the water table, ensuring later-year survival (Amlin and Rood 2002). This study shows high variation in mortality among genotypes in a common garden. Mortality rates ranged from 88.89% in a genotype from the Hassayampa Preserve, AZ (HP), to 9.84% in a genotype from the Weber River, UT (B161). Calculation of broad sense heritability suggests that 11.26% of mortality rates may be attributed to differences among genotype. While this difference is small, it is significant enough to be useful in silvicultural and restoration applications. For example, in agricultural applications, a $H^2_b$ of greater than XXX is frequently exploited for breeding purposes.

The results of this study suggest that temperature, elevation, and precipitation of home-site are all effective predictors of *P. fremontii* sapling persistence in restoration efforts. However, the results of these relationships run counter to our expectations. We hypothesized that mortality would increase with increasing home-site distance from the location of the restoration site. Home-site climatic variables that were more similar to the restoration site’s environmental variables would predict survival of out-planted saplings.
We found the reverse pattern, where as geographic distance of home-site, and precipitation increased, mortality rates generally decreased. In contrast to our own expectations and the previous data of other researchers (Montalvo and Ellstrand 2000), these results suggest that locally planted stock may not always be the best adapted to local conditions. In an evolutionary context, this may be due to inbreeding or changing environmental conditions. Locally planted stock that does not receive new genetic material may be at risk when environmental and climate factors change, and locally adapted stock populations are greatly reduced, rather than molded by selection pressures. Because correlation does not necessitate causation, we suggest multiple explanations for this pattern: 1) phenological differences (e.g., length of winter dormancy) associated with climatic differences in environments provides for a higher regeneration success in the field. Differences in collection techniques and timing may have contributed similarly to these differences; 2); flood irrigation of out-planted saplings creates an environment advantageous to saplings from northern, moister climates where trees are in constant contact with a water-table 3) the correlation arises from a correlation with another variable that we did not measure that co-varies with elevation and precipitation; 4) by sampling from a larger and potentially more diverse genetic pool (in more northern or higher elevation climes), survival rates may increase relative to smaller gene pools due to sampling effect; and finally 5) susceptibility to local pathogens by local stock increases their mortality relative to distant genotypes which may not have as many locally adapted pests and pathogens due to high genetic specificity of fungal and arthropod communities(Whitham et al. 2006). This last explanation is identical to the argument often used at the species level to explain success of exotic species.
All cuttings were collected using the same methodology and grown to leaf-out and then induced into dormancy in a greenhouse before planting. However some lower latitude trees have only brief dormancy periods, and so dormancy of cuttings at time of collection could not be ensured in all cases. Because some trees may have not been completely dormant at time of collection, those trees may have stored fewer resources, and may have been less prepared for out-planting. However, this may be a perpetual pattern with cuttings taken from lower elevation warmer climates. More northern locations may have more complete dormancy, making cutting propagation more successful in restoration attempts. This could have important implications for large restoration plantings where low mortality is important, re-planting of dead trees is unrealistic, and managers must balance tree survival with a desire to plant local stock.

Previous studies have shown that genetic variation is associated with mortality rates in many phenotypes of Cottonwood. Mortality rates may vary with factors such as productivity and water use, which have been shown to be controlled by genetics. For example, Lojewski et al. (personal communication 2006) and Oleksyn (2000) found that differences in tree genotypes may lead to differences in productivity. Other studies have also shown that water use may vary between Populus genotypes (Rowland et al. 2000). Genetic variation also affects mortality in species other than cottonwood. A study examining the effect of temperature selection pressure on mortality rates in Solidago vigaurea (goldenrod) found significant differences in survival rates between genotypesBjorkman 1966), and classic ecotype studies by Clausen, Keck, and Hiessey (1940), found higher performance of ecotypes in environments more similar to the environments of their origin. However, provenance studies in forestry frequently show
higher performance of higher latitude species in lower latitude climes. In the case of
dramatic restoration efforts along the lower Colorado river where more than 90% of an
environment requires direct replanting for restoration of dominant foundational species
(Ellison et al. 2005), increased survival of more northern genotypes at early stages of out-
planting could have obvious impacts.

This study highlights the advantages in continuous monitoring of restoration
sites with a diverse genetic makeup. While a site may be planted with a multitude of
genetically distinct individuals, overall diversity may decrease in time as a result of
varying degrees of fitness. Our results suggest that differences in mortality due to
genotype can be highly variable. Thus, caution must be placed to ensure that the final
genetic composition of a site is indeed the same as to that which was initially planted.

Our results suggest that temperature, elevation, and precipitation may all act as
selection pressures on genotype. Previous studies have provided evidence that genotype
has a propensity to determine traits and mortality relative to environmental factors. This
data adds to the growing body of evidence that genotypic variance between populations
determines fitness relative to the environmental stress of a geographic area.

**Future Study**

Differential survival among genotypes may lead to unexpected consequences. In
the three plots examined at Cibola National Wildlife Refuge, a post-planting mortality
rate of 31.55% was determined. Genetic variation is clearly important to the larger
community and ecosystem (see Whitham et al. 2006), but this same variation could also
result in accidental selection for genotypes from more northern environments if
restoration cuttings are not closely monitored. In a changing global climate, we should also consider that genotypes most adapted for an altered future climate may not be the most locally adapted genotypes, since local genotypes may be adapted to the current climate, and not the future climate.

Our study looked at how genetics influence mortality. However, quantitative differences between genotypes expressed in terms of genetic distance and specific traits may further advance our understanding of restoration success and genetic diversity. Although we can say that specific genotypes are different, we cannot easily quantify the specific amount of genetic difference between genotypes. Current research is using Amplified Fragment Length Polymorphisms (AFLPs; Allen and Honchack, unpublished data; Whitham et al. 2006) to look to determine inter-genome differences. Using AFLP data, it would be possible to measure genetic distance between genotypes from across the geographic range of *P. fremontii*, and determine more precisely the amount of variance in mortality explained by genetics.

Our study provides a foundation for these future efforts by showing that genetic variation clearly matters to restoration success in *P. fremontii* forests. As such, it is likely that restoration efforts often result in a genetic population significantly different from the original out-plants. Selection on traits among genotypes could affect the genetic face of future riparian forests along restored southwestern riparian areas.
Acknowledgements

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Figure Legends

Figure 1: Geographic dispersal of Cibola transplants

Figure 2: Variation in mean mortality rates categorized by genotype

Figure 3: Environmental variables as predictors of mortality

Table 1: AIC table ranking significance of factors in model

<table>
<thead>
<tr>
<th>Model</th>
<th>AICc</th>
<th>DeltaAICc</th>
<th>Lik-Model</th>
<th>wi (~probabilities)</th>
<th>Evid. Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>altitude</td>
<td>-55.85</td>
<td>0</td>
<td>1</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>temp</td>
<td>-55.06</td>
<td>0.78</td>
<td>0.68</td>
<td>0.25</td>
<td>1.48</td>
</tr>
<tr>
<td>precip</td>
<td>-54.71</td>
<td>1.14</td>
<td>0.57</td>
<td>0.21</td>
<td>1.77</td>
</tr>
<tr>
<td>intercept</td>
<td>-52.13</td>
<td>3.72</td>
<td>0.16</td>
<td>0.06</td>
<td>6.41</td>
</tr>
<tr>
<td>diameter</td>
<td>-49.97</td>
<td>5.87</td>
<td>0.05</td>
<td>0.02</td>
<td>18.86</td>
</tr>
</tbody>
</table>

Note: Row titles reflect the predictive variables in the models. The “intercept” model has no predictive variables, and thus functions as a “null” model. Models are ranked from the best (top) to worst (bottom) model based on ΔAICc values (based on Akaike’s Information Criterion for small sample size (AICc)) which reflect an index of amount of information lost when approximating truth with the model. However, as a general rule, models with delta AICc within 2 of each other are indistinguishable in terms of fit. Letters denote statistically indistinguishable groups. Altitude is the best model for predicting mortality according to all model selection criteria, including: the ΔAICc values (lowest), the likelihood value for the best model
(LIKELIHOOD; 1.0), the Akaike weight of evidence ($w_i$ closest to 1), and the evidence ratio (EVID. RATIO; next best model has a 1:1.5 chance of being better, given the data). This is reflected by the delta AIC, which suggests this model is statistically indistinguishable from the next three. The size of the cutting (diameter) is a far worse predictor of mortality than the location the cutting came from.

Figure 1
Figure 2
Figure 3