Seedling Recruitment in Fragmented Populations of *Echinacea angustifolia*

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AMY BETH DYKSTRA

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Introduction

Human activities have resulted in the conversion of more than 20% of the world's land to agriculture and other human-dominated use (Hoekstra et al. 2005). Remaining native habitat often consists of small, isolated fragments existing in a matrix of altered land. Habitat fragmentation has multiple effects on native species, including reducing the size of remnant plant populations (Hobbs and Yates 2003). Small populations may experience genetic erosion due to increased inbreeding or increased genetic drift (Lande 1988; Ellstrand and Elam 1993; Young et al. 1996; Aguilar et al. 2008). Small populations are also more vulnerable than large populations to demographic and environmental stochasticity (Lande 1988; Lande 1993).

The tallgrass prairies of the American Great Plains are highly fragmented, with native habitat reduced to only 13% of historic cover (Samson et al. 2004). At the same time, grasslands are among the least protected biomes of the world (Hoekstra 2005). Samson and Knopf (1994) estimated that the tallgrass prairie in Minnesota had declined in area by 99.6%, with less than 1% protected. The conversion of prairies to agriculture continues to occur. The Environmental Working Group has estimated that more than 23 million acres (9.3 million hectares) of grassland, shrub land and wetlands were converted to crop land in the United States between 2008 and 2011 (Faber et al. 2012).

Despite ongoing conversion of tallgrass prairie, there has also been strong interest in ecological restoration since the 1980s (Anderson 2009). An important consideration for restorations is to obtain appropriately sourced germplasm (Broadhurst et al. 2008; Bischoff et al. 2010). Restoration guidelines commonly call for the use of locally sourced seed to maximize local adaptation and minimize outbreeding depression; however the degree of local adaptation and genetic divergence of native plant populations is often unknown (Rice and Emery 2003).

My research focuses on *Echinacea angustifolia*, a perennial forb native throughout the central plains of the United States, including the tallgrass prairies of Minnesota. This dissertation describes three research questions related to the effects of habitat fragmentation on *E. angustifolia*, with special attention paid to the seedling recruitment phase of the life cycle. My three research questions are outlined below.

In Chapter 1 I ask whether small remnant populations of *E. angustifolia* are declining relative to larger populations, and evaluate the importance of seedling recruitment to the population growth rate. I used population projection matrices, parameterized with data from two transition years (2009-2010 and 2010-2011). To assess the importance of population size on population growth rate, I divided the 2009-2010 transitions data into two subsets, one subset consisting of the 5 largest populations, and the other subset consisting of the remaining (smaller) populations in the study. I show that the small populations had significantly lower population growth rate than the large populations. Mortality was higher for seedlings than for other stages of the life cycle; however, survival of vegetative and reproductive plants made a greater contribution to population growth rate than did either emergence or survival of seedlings.

In Chapter 2 I ask whether genetic declines in remnant *E. angustifolia* populations can be remedied by outcrossing. I performed within-population and between-population crosses using six populations of varying size. Seed set, seedling emergence and survival were recorded for three years. Results indicate differential responses to cross type, depending on the populations represented and on the stage of the life cycle evaluated. Between-population crosses involving the smallest populations used in the study resulted in offspring with higher fitness than the offspring of within-population crosses (that is, heterosis), while other between-population crosses showed outbreeding depression compared to the within-population crosses. These results are consistent with a reduction in genetic diversity in the smallest populations.

In Chapter 3 I ask whether *E. angustifolia* populations exhibit local adaptation. I collected seeds from three widely-separated populations located along a 500-km transect from western South Dakota to western Minnesota. I sowed seeds from all three populations into experimental plots located near each source population. I counted emerged seedlings and censused for survival for two years. Seed source and sow site had significant effects on seedling emergence and survival, but no clear evidence of local adaptation among the three populations was observed. All three source populations had highest emergence in Minnesota and lowest emergence in western South Dakota. The western South Dakota and Minnesota-sourced seed had higher emergence than the central South Dakota seed at all three sowing sites. The western South Dakota seedlings had the highest survival and the Minnesota seedlings had the lowest survival at all three sites over the first growing season. Chapter 3 is essentially the same as a paper published in the Proceedings of the 22nd North American prairie conference (Dykstra and Shaw 2011). Ruth Shaw advised me on the experimental design, and helped with the sowing of the experimental plots. She also provided feedback on the analysis and on the manuscript. My contributions were to perform the experiment, collect the data, perform the analysis and write the manuscript.

My research contributes to our understanding of the effects of habitat fragmentation on a common prairie species. The results reported here suggest that small remnant populations of *E. angustifolia* have suffered demographic and genetic consequences of reduced population size. The smallest populations may benefit from artificial gene flow in the form of pollen or seed from other populations (genetic rescue). However, attempts at genetic rescue should be undertaken with caution, in spite of low apparent levels of local adaptation, since some between-population crosses have resulted in outbreeding depression.

Chapter 1. Demography of remnant *Echinacea angustifolia* populations in fragmented tallgrass prairie in Minnesota

Habitat fragmentation has reduced native tallgrass prairie populations to small, isolated remnants. Small populations may experience genetic and demographic consequences, leading to decreased reproduction and population growth. Matrix population models were used to estimate population growth rate (λ) for a fragmented *Echinacea angustifolia* population in west-central Minnesota over three years, and to compare λ between small and large population remnants. Using data pooled over all 14 population remnants, λ was 0.886 (95% confidence interval [0.854, 0.920] for 2009-2010 and 0.839 (95% CI [0.785, 0.908]) for 2010-2011; this difference in λ was not statistically significant. Elasticity analysis indicated that life history transitions making the largest contributions to changes in λ are stasis of small vegetative plants and flowering plants. A matrix population model representing the 5 largest populations had significantly higher λ (0.955; 95% CI [0.912, 0.999]) than the matrix representing the remaining 9 small populations (λ = 0.830; 95% CI [0.766, 0.910]). A life table response experiment (LTRE) indicated that the life history transitions contributing most to this significant difference in λ were increased fertility and generally higher stasis and growth of vegetative plants in large compared to small populations, offsetting decreased stasis of flowering plants and decreased proportion of large vegetative plants transitioning to flowering plants in large relative to small populations. This is one of very few studies to find a positive relationship between population size and growth rate.

Introduction

Land use changes, including conversion of land to agricultural use and urbanization, have resulted in the loss and fragmentation of much native habitat. Native populations in fragmented habitat are often reduced to small, isolated population remnants. These small populations may suffer genetic and demographic consequences (Lande 1988; Oostermeijer et al. 2003). Genetic consequences include inbreeding depression and decreased genetic diversity due to genetic drift (reviewed by Ellstrand and Elam 1993; Young et al. 1996). Demographic consequences of small population size include Allee effects (decreased reproduction at low population densities; Allee 1931; Stephens et al. 1999) and vulnerability to demographic and environmental stochasticity (Lande 1988; Oostermeijer et al. 2003). One potential cause of an Allee effect in plants is reduced availability of compatible mates in small populations of self-incompatible species (Byers 1995; Young and Pickup 2010).

Most studies of the effects of fragmentation on plants have focused on reproductive success (reviewed by Hobbs and Yates 2003; Aguilar et al. 2006). Population size has been shown to correlate with a variety of reproductive traits, such as seed production (Ågren 1996; Kery et al. 2000; Kery and Matthies 2004), seed set (Widén 1993; Young and Pickup 2010) and germination success (Menges 1991; Heschel and Paige 1995). Relatively few studies have examined the link between population size and population growth or viability, and published studies have produced mixed results. I am aware of two studies that have demonstrated a positive effect of population size on population growth rate in plant species (Fischer and Matthies 1998; Menges and Dolan

1998). Other researchers have found no clear effect of population size on population growth (Münzbergová 2006; Kolb et al. 2010; Csergő et al. 2011).

Matrix population models are commonly used to estimate population growth rates (reviewed by Crone et al. 2011). Age-classified matrix models were developed by Leslie (1945); Lefkovitch (1965) introduced stage-classified matrix models, which are often used for plant populations, since age may be a poor predictor of survival and reproduction probabilities (Harper 1977; Caswell 2001). A population projection matrix, $\mathbf{A}$, satisfies the equation $\mathbf{n}(t + 1) = \mathbf{An}(t)$, where $\mathbf{n}(t)$ is the vector of numbers of individuals in each class at time t, and $\mathbf{n}(t + 1)$ is the vector of class abundances at time $t + 1$. The matrix $\mathbf{A}$ can be used to calculate several demographic parameters. The dominant eigenvalue, λ, predicts population growth rate under the conditions that resulted in the transition rates that constitute the matrix (Caswell 2001). A growing population has $\lambda > 1$; while a declining population has $\lambda < 1$. Sensitivities of λ to incremental changes in the matrix elements can be derived from the matrix. Elasticities, proportional responses of λ to proportional changes in the matrix elements, allow comparison of the relative importance of each matrix element to λ (de Kroon et al. 2000). Several age-based statistics can also be calculated from stage-classified matrices (Cochrane and Ellner 1992).

Habitat fragmentation is particularly pronounced in the tall grass prairie of North America, where an estimated 82-99% of the original habitat has been converted to agriculture or urbanization (Samson and Knopf 1994). *Echinacea angustifolia* (Asteraceae) (narrow-leaved purple coneflower) is representative of many native prairie species in that it is long-lived, reproduces by seed, has little seed dispersion, is pollinated by generalist insects, and is self-incompatible (McGregor 1968; Wagenius 2004; Ault 2006). S. Wagenius, R. Shaw and others have established a long-term study of *E. angustifolia*, with the aim of elucidating the ecological and genetic effects of habitat fragmentation on this species. Demographic data have been collected for fourteen years on flowering individuals in a fragmented *E. angustifolia* population located in tall grass prairie remnants in Douglas County, Minnesota. Information on demography of pre-reproductive plants and natural seedling recruitment is more limited. In an experimental study of recruitment, seedling emergence was low (rarely exceeding 8% of achenes sown), and varied depending on vegetation type and burn treatment; seedling survival over five years was high (47-66%) in most treatments, but only 4% for unburned restored fields (Wagenius et al. 2012). Experimental evidence indicates that recruitment from dormant seeds is rare; few to no new seedlings emerged after the first growing season following sowing in three separate experiments (Dykstra and Shaw 2011; Wagenius et al. 2012; Chapter 2).

I use matrix population modeling to estimate population growth rate for fragmented native *E. angustifolia* populations located in Douglas County, Minnesota. I refer to the extant *E. angustifolia* plants in the various prairie remnants as “populations.” Molecular evidence suggests limited gene flow among these populations (Lonsdorf 2004), although there is evidence of greater gene dispersal between nearby remnants than among remnants geographically more widely separated (Ison 2010). I use matrix population models to compare demography across three years, and among large and small populations.

Methods

Study area and plant sampling

The study area is located in southwestern Douglas County, Minnesota, centered near 45°49'N, 95°42'30"W (Figure 1). I focused on 14 prairie remnants, the largest of which is Staffanson Prairie (SPP), a 38 hectare Nature Conservancy preserve. The number of *E. angustifolia* plants flowering at SPP was estimated at 5044, 1578, and 2262 in 1996, 1997 and 1998, respectively (Wagenius 2000). The other remnants in the study area are smaller in geographic area and contain *E. angustifolia* populations ranging in numbers of flowering plants from fewer than 5 to several hundred (Table 1).

New seedlings were identified in each remnant each spring from 2006-2010. In each population, 18 randomly selected plants known to have flowered the previous summer were identified as focal plants. Seedling searches were performed within circles with radius 41 cm around focal plants. For populations with fewer than 18 flowering plants, all plants that flowered the previous summer were used as focal plants, and the search radius was expanded to 50 cm. Seedling searches were also performed in two randomly-located circles in each remant. Seedlings were measured and mapped. A census of all seedlings was performed each year in late summer for all previously identified seedlings.

To estimate vital rates for juvenile and adult plants, I mapped 813 basal (non-reproductive) and 69 flowering plants in summer 2009. I used a stratified sampling approach to identify plants. First, I included all *E. angustifolia* plants found within the seedling search circles described above; these were areas with expected high densities of small plants. Second, I established five belt transects at random positions within each of eight remnants. Each transect was 0.5 m wide and 3-5 m long, depending on the dimensions of the *E. angustifolia* population. Each plant found within a seedling search circle or belt transect was scored as seedling (cotyledons visible), basal or flowering. In the seedling search circles, 65 seedlings, 624 basal plants and 38 flowering plants were identified, and in the belt transects, 0 seedlings, 189 basal plants and 31 flowering plants were found. For each individual, the number of rosettes and basal leaves were counted, and the length of the longest basal leaf was recorded.

In summer 2010, I performed a census of all plants mapped in 2009. For each individual, I recorded the status (basal, flowering or not present), rosette and leaf number, and length of the longest leaf. In summer 2011, a census was performed on a subset of the individuals mapped in 2009: the belt transects were not included and, in some populations, the census only included individuals located within circles where seedlings had been found in previous years.

Population projection matrices and analyses

As reflected by the categories used for data collection, *E. angustifolia* has 3 life cycle stages, seedlings, basal plants, and flowering plants. The basal plants vary widely in size, with basal leaf counts in this data set ranging from 1 to more than 50, and rosette counts ranging from 1 to 13. To identify a state variable that could be used to classify the basal plants into size classes (Morris and Doak 2002), I performed logistic regressions of survival as a function of basal leaf count, rosette count, length of the longest leaf, or a composite “size” variable defined as the product of leaf count and length of the longest leaf, using data from the 2009-2010 transitions, pooled across all populations. I also performed logistic regressions of flowering probability versus rosette count, leaf count, leaf length and size. Support for these models was compared using the Akaike Information Criterion (AIC) (Anderson *et al.* 2000). Each of the state variables tested were significant predictors of survival (Table 2; Figure 2) and flowering probabilities (Table 3; Figure 3). I used leaf number as the best overall predictor of both survival and flowering. Leaf number is also more consistently recorded in the field than is rosette number, since determining the number of rosettes can be somewhat subjective (personal observation).

To establish class boundaries for the basal plants, I applied an algorithm proposed by Vandermeer (1978) and modified by Moloney (1986). This algorithm is designed to minimize sampling error (i.e., if class boundaries are too narrow, classes with small sample sizes result) and distribution error (i.e., if class boundaries are too wide, individuals with different vital rates are lumped into one category). I constructed a stage- and size-based matrix population model (Caswell 2001) based on transitions from 2009-2010, using the following 6 classes: seedlings, basal plants with 1-3 leaves, basal plants with 4-6 leaves, basal plants with 7-10 leaves, basal plants with 11 or more leaves, and flowering plants. Since germination is rare more than a year after sowing (Chapter 2; Dykstra and Shaw 2011; Wagenius et al. 2012), no seed bank stage was included. To insure an adequate sample size, I set the lower boundary for the basal class with 11 or more leaves such that it included at least 50 individuals (Morris and Doak 2002).

The 2009-2010 matrix **A** consists of elements a_{ij} (the proportion of individuals in class j in 2009 that transitioned to class i in 2010). The dominant eigenvalue (λ), sensitivities and elasticities of λ to changes in each matrix element were calculated using the eigen.analysis function in the popbio package (Stubben and Milligan 2007) in R (R Development Core Team 2010).

I used bootstrap resampling (Caswell 2001; Stevens 2009) to obtain 95% confidence intervals for λ. I used two data sets for the construction of the resampled projection matrices, one data set for realized fertility of flowering plants with records for 187 seedling circles searched in 2010, and a second data set for transitions of plants of all stages, with records for 947 individuals. I generated bootstrap data sets with the same sample sizes as the original data sets by sampling with replacement from the fertility and transitions data sets. I repeated the process of generating bootstrap fertility and transitions data sets 3000 times, to produce 3000 bootstrap data sets (each including a fertility data set with a sample size of 187 and a transitions data set with a sample size of 947). These bootstrap data sets were used to obtain population projection matrices. The population growth rate (λ) was calculated for each of the 3000 resulting matrices. These values were used to obtain 95% confidence intervals for λ.

Several age-specific measures were calculated from matrix **A**. **A** was decomposed into **T**, the matrix of transition probabilities, and **F**, the fertility matrix (Caswell 2001). In this case, the only non-zero entry in **F** is f_{16}, the realized fertility of the 2009 flowering individuals. The fundamental matrix, **N**, was calculated from the formula $\mathbf{N} = (\mathbf{I} - \mathbf{T})^{-1}$, where **I** is the identity matrix (Caswell 2001). The elements of **N** are the expected values of the time spent in each class. **N**, as well as the variance of the time spent in each class, the mean time to death for each class, and the variance of time to death for each class, were calculated using the fundamental.matrix function in the popbio package (Stubben and Milligan 2007) in R (R Development Core Team 2010). I calculated age-specific survivorship for ages t = 1 to 60 years using the formula ($P[\eta_1 > t] \ldots P[\eta_6 > t]$) = $\mathbf{e}^{\mathrm{T}}\mathbf{T}^{t}$, where η_j is the time to death for class j, $\mathbf{e}^{\mathrm{T}}$ is the transpose of a column vector of ones and the entries of $\mathbf{T}^{t}$ give the probabilities of being in each class at time t, conditional on the starting class (Caswell 2001). The survivorship vector **s** is the sum of the first column of this matrix for each age t. Age-specific fertility was calculated from $\mathbf{\Phi}(t)$ = $\mathbf{F}\mathbf{T}^{t}(\mathrm{diag}(\mathbf{e}^{\mathrm{T}}\mathbf{T}^{t}))$, where $\mathrm{diag}(\mathbf{e}^{\mathrm{T}}\mathbf{T}^{t})$ is the matrix with the diagonal of $\mathbf{e}^{\mathrm{T}}\mathbf{T}^{t}$ on the diagonal and zeros elsewhere (Caswell 2001). The fertility vector **f** consists of the φ_{11} entry for each t. The net reproductive rate, R_0, that is, the mean number of offspring produced by a seedling during its lifetime, was derived from the matrix $\mathbf{R} = \mathbf{FN}$. Since **R** has only a single non-zero row, $R_0 = r_{11}$ (Caswell 2001).

I calculated two measures of generation time, T, the time required for a population to increase by a factor of R_0, and μ_1, the mean age at which a cohort of seedlings produces offspring. T was calculated with the equation $T = (\ln R_0)/(\ln \lambda)$ (Cochran and Ellner 1992; Caswell 2001). Following Caswell (2001), I calculated μ_1 as follows:

$$\mu_1 = \frac{\sum_t t\mathbf{s}(t)\mathbf{f}(t)}{\mathbf{s}(t)\mathbf{f}(t)}$$

To examine the effect of population size on λ, I divided the 2009-2010 transitions data into two subsets of approximately equal size, one including the five largest populations (SPP, LF, LC, Ri and ERi), with records for 494 individuals, and the other subset including the remaining nine populations, with records for 453 individuals. The fertility dataset was similarly divided. Population matrices were constructed for the small population and large population subsets; λ was calculated and bootstrapped 95%

confidence intervals were generated. To test the statistical significance of the difference in λ between the large population matrix and the small population matrix, I performed randomization testing as described by Caswell (2001). I generated 3000 permutations by drawing without replacement from the transitions and fertility datasets, such that individuals from the full dataset were randomly assigned to the “large population” or “small population” subsets in each permutation. Each permuted data set was then used to construct large population and small population matrices, and the difference in λ was calculated. Using the test statistic $\theta = |\lambda_L - \lambda_S|$, where λ_L is λ for the large populations and λ_S is λ for the small populations, I computed $P[\theta \geq \theta_{obs}|H_0]$, where θ_{obs} is the absolute value of the observed difference in λ and H_0 is the null hypothesis that differences in λ are due to chance.

To decompose the contributions of various life cycle classes to the observed difference in λ between large populations and small populations, I performed a life cycle response experiment (LTRE; Caswell 2001). “Experiment” is defined broadly to include observational differences between the large population “treatment” and small population “treatment.” Differences between the large population and small population were computed for each matrix element. These differences were then multiplied by the sensitivity of λ to a change in each matrix element, with the sensitivities computed from the matrix representing the average between the large population matrix and the small population matrix. The average matrix, $\hat{\mathbf{A}}$, was calculated as $\hat{\mathbf{A}} = (\mathbf{A}^L + \mathbf{A}^S)/2$, where $\mathbf{A}^L$ is the large population matrix and $\mathbf{A}^S$ is the small population matrix.

In addition to the projection matrix for 2009-2010, a 2010-2011 matrix was constructed, based on transitions for 489 individuals (43 seedlings, 409 basal plants, and 37 flowering plants). The value of λ was computed and 95% confidence intervals were generated as described for the 2009-2010 matrix. Age-specific measures were calculated as described for the 2009-2010 matrix. I used randomization testing to evaluate the statistical significance of the difference in λ between the 2009-2010 matrix and the 2010-2011 matrix. A LTRE was used to decompose the contributions of each matrix element to the difference in λ.

Data for all size classes were only available for transitions from 2009-2010 and 2010-2011; however, data for transitions for all previously identified seedlings were available from 2007-2009. To assess how transitional rates observed in 2008-2009 would affect the population growth rate, I constructed a 2008-2009 population matrix using available transition data. Fertility (matrix element [1,6]) was calculated as the average number of seedlings present in August 2009 per seedling circle searched in spring 2009. Annual censuses of seedlings found from 2006-2008 provided transition rates for seedlings and small juvenile plants (matrix elements [2,1] and [2,2]). Demographic data for *E. angustifolia* individuals in SPP provided the rate of stasis of flowering plants (matrix element [6,6]. These demographic data do not include leaf counts. To obtain transition rates for flowering plants to basal plants (column 6, rows 2-5), I distributed individuals proportionally based on the matrix representing the average between the 2009-2010 and 2010-2011 matrices. I multiplied each corresponding matrix element from the average matrix by the ratio (total proportion transitioning from flowering to basal in the average matrix)/(total proportion transitioning from flowering to basal in 2008-2009).

Remaining matrix elements were parameterized as the average between the 2009-2010 and 2010-2011 matrices.

A 2007-2008 population matrix was parameterized using available data for 2007-2008 transitions. Fertility was calculated as the average number of seedlings per seedling circle searched in 2008; transition rates for 2007 seedlings were used to parameterize matrix column 1. Demographic data from SPP provided transition rates for flowering plants (column 6). Columns 2-5 were parameterized with average transition data for 2009-2010 and 2010-2011. In similar fashion, a 2006-2007 population matrix was constructed. Fertility was calculated as the average number of seedlings per seedling circle searched in 2007. Demographic data from SPP provided transition rates for flowering plants (column 6). All other matrix elements were parameterized with average transition data for 2009-2010 and 2010-2011.

Results

Results from the annual seedling searches are shown in Table 1 (lower panel). Realized fertility (seedlings present in August per seedling circle searched) was 0.60 seedlings per circle searched in 2007, 0.75 in 2008, 0.27 in 2009, 0.24 in 2010, and 0.88 in 2011.

The population projection matrix based on transitions from 2009 to 2010 is shown in Table 4(a). Based on this matrix, the dominant eigenvalue, λ, is equal to 0.886 (bootstrapped 95% confidence interval [0.854, 0.920]), implying population decline. Sensitivities of λ to changes in each matrix element are shown in Table 4(b). λ is most sensitive to changes in the proportion of 1-3 leaf basal plants that grow into larger basal plants or transition to flowering plants. Elasticities of λ to proportional changes in matrix elements are shown in Table 4(c). The highest elasticities are to changes in the proportion of 1-3 leaf basal plants that remain as 1-3 leaf plants, and to the proportion of flowering plants that remain as flowering plants.

The fundamental matrix based on the 2009-2010 projection matrix is shown in Table 5 (a). This matrix predicts the mean time spent in each class. Based on this table, a seedling will spend, on average, about 2.5 years as a 1-3 leaf basal plant, about 0.5 years as a 4-6 leaf basal plant, and has a low likelihood of growing larger than 7 leaves or flowering. In contrast, a basal plant with 1-3 leaves is expected to spend more time in each class, including nearly 4 years as a 1-3 leaf plant, due to the higher survival rate of 1-3 leaf basal plants than seedlings. The mean time to death for each class is the sum of the appropriate column of the fundamental matrix; thus, the mean time to death for seedlings is about 4.6 years, while the mean time to death for flowering plants is nearly 9.5 years (Table 5).

Age-specific survivorship, based on the 2009-2010 projection matrix, is shown in Figure 4. There is a very low probability of survival to age 30 years or more. Age-specific fertility, that is, the mean number of seedlings produced by an individual of a given age, is shown in Figure 5. Fertility is 0 for 1-year olds (seedlings), 0.003 for 2-year olds, 0.046 for 10-year olds, and continues to increase with age up to about age 20, when fertility is 0.055.

The net reproductive rate, R_0, based on the 2009-2010 projection matrix is 0.0805. R_0 is an estimate of the mean number of offspring that will be produced by a seedling by the end of its life, i.e., the per-generation growth rate or replacement rate (Caswell 2001).

An R_0 less than 1 indicates that members of the population are not replacing themselves (i.e., the population is declining). In contrast to R_0, λ is a prediction of the population growth rate per year. Generation times (T and μ_1) are shown in Table 6. T, the time required for a population to increase by R_0, is nearly twice the magnitude of μ_1, the mean age of the parents of the offspring produced by a cohort over its lifetime.

The matrix for the five largest populations had λ equal to 0.955 (95% confidence interval [0.912, 0.999]). λ for the small populations was 0.830 (95% confidence interval [0.766, 0.910]). Based on randomization testing, the difference in λ is statistically significant ($P < 0.001$). Differences in matrix elements and contributions of these matrix element differences to the difference in λ are shown in Figure 6. The greatest differences between matrix elements were the increased fertility and increased proportion of seedlings transitioning to 1-3 leaf basal plants in the large relative to the small populations (Figure 6a). However, the largest contributions to λ were the lower proportion of flowering plants that remained as flowering plants in the large populations relative to the small populations and the lower proportion of 7-10 leaf plants transitioning to flowering plants in large populations compared to small populations (Figure 6b). These negative contributions were offset by the increased fertility and increased proportion of flowering plants that transitioned to large ($\geq$ 11 leaves) basal plants in large relative to small populations, as well as generally larger proportions of basal plants remaining in the same size category or growing to a larger size in large relative to small populations (Figure 6b).

The population projection matrix constructed from 2010-2011 transitions (Table 7a) had λ equal to 0.839 (95% confidence interval [0.785,0.908]). Based on randomization testing, λ for the 2010-2011 matrix did not differ significantly from λ for the 2009-2010 matrix (*P* = 0.126). Differences between the two matrices as well as contributions to the difference in λ are shown in Figure 7. The marked increase in fecundity in 2011 compared to 2010 was offset by lower proportions of basal plants that transitioned to flowering plants, and lower proportions of flowering plants that remained as flowering plants or regressed to large basal plants from 2010-2011 compared to 2009-2010. Sensitivities and elasticities for the 2010-2011 matrix are shown in Tables 7b and 7c. As for the 2009-2010 matrix, λ for the 2010-2011 matrix is most sensitive to changes in the proportion of small (1-3 leaf) basal plants growing to larger classes or transitioning to flowering plants (Table 7b). Also similarly to the 2009-2010 matrix, the highest elasticity of λ for the 2010-2011 matrix is to changes in the proportion of small (1-3 leaf) basal plants remaining in the same size class (Table 7c). However, elasticity to the proportion of flowering plants that remain as flowering plants is lower for the 2010-2011 matrix (Table 7c) than for the 2009-2010 matrix (Table 4).

Transition rates for seedlings found in 2006-2008 (Table 8) and for flowering plants on a belt transect in Staffanson Prairie (Table 9) were used to parameterize population projection matrices for 2008-2009, 2007-2008, and 2006-2007. The population projection matrix parameterized with available transition data from 2008-2009 (Table 10) had λ equal to 0.846. The matrix parameterized with available 2007-2008 data (Table 11) had λ equal to 0.868. The matrix parameterized with available 2006-2007 data (not shown) had λ equal to 0.916.

Discussion

Habitat fragmentation has reduced *Echinacea angustifolia* to small remnant populations. Estimates of population growth rates (λ) based on demographic records from 2009-2011 predict population declines for these small populations. Furthermore, more rapid declines are predicted for the smallest populations than for the larger populations.

Analyses of each of the population projection matrices I constructed resulted in $\lambda < 1$, with confidence intervals not including 1, predicting population decline. In contrast, a study of five *E. angustifolia* populations in the Smoky Hills region of north-central Kansas showed essentially stable populations (λ ranged from 0.94 to 1.07, with confidence limits including 1 for all populations; Hurlburt 1999). The lower values of λ for Minnesota populations compared to Kansas populations may reflect greater fragmentation of the Minnesota tallgrass prairie compared to the mixed grass prairies in the Kansas study plot. Hurlburt (1999) chose a study site that contains “large areas of…native mixed-grass prairie rangeland,” and none of the populations in her study had fewer than 50 flowering *E. angustifolia*. However, population dynamics have been shown to vary substantially among populations and years for the same plant species (Bengtsson 1993; Horvitz and Schemske 1995; Buckley et al. 2010 and references therein). Moreover, the matrix models used by Hurlburt (1999) differ slightly from mine; for example, she included a “dormant” class, and two reproductive classes. Thus, it is difficult to judge whether the differences in λ among the Kansas and Minnesota populations are biologically meaningful.

My estimates of generation times (Table 6) also differ somewhat from those reported by Hurlburt (1999), who reported generation times ranging from 17-44 years. Generation times reported in Table 6 are consistent with an experimental seedling recruitment study, using achenes collected from the Minnesota study area; *E. angustifolia* recruits began to flower in their 7th to 10th years (Wagenius et al. 2012).

Elasticity analysis provides quantitative estimates of the relative contribution of each matrix element (i.e., life history transition) to changes in λ. Elasticity analysis for the 2009-2010 and 2010-2011 matrices showed that matrix elements representing stasis, i.e., individuals remaining in the same class, made the largest contributions to λ, with relatively low elasticity for fertility (Tables 4 and 7). The 2006-2007 matrix included the highest value for stasis of flowering individuals observed in this study, and a correspondingly high value of λ. It may be that *E. angustifolia* populations in the study area occasionally experience conditions conducive to high stasis for flowering plants and small basal plants, allowing for population growth rates ≥ 1.

High elasticities for stasis (particularly of large reproductive plants) have also been reported for other long-lived perennials (Charron & Gagnon 1991; Bengtsson 1993; Ehrlén 1995; Miller et al. 2007; Kolb et al. 2010). Oostermeijer et al. (1996) noted high elasticities for survival (defined as individuals remaining in the same size class or regressing to a smaller class) in declining populations of *Gentiana pneumonanthe,* while fecundity and growth had higher elasticities in growing populations. A similar result was reported for *Silene regia* (Menges and Dolan 1998). The high elasticities for stasis elements presented in Tables 4 and 7 are consistent with the declining population growth rate for both the 2009-2010 and 2010-2011 matrices.

An important result from this study is that λ is significantly smaller for small populations than for large populations, based on the 2009-2010 transitions. Higher fertility in the large populations vs. the small populations made an important contribution to the difference in λ (Figure 6). Lower fertility in small compared to large populations could be the result of a higher degree of relatedness among individuals in smaller populations than in larger populations, leading to a lower probability of receiving compatible pollen for individuals in small populations compared to large populations. Five studies of *E. angustifolia* populations in the same study area I used lend support to this explanation. Wagnenius (2000), using 7 allozyme loci, found lower genetic diversity in small compared to large populations. Ison (2010), using 8 microsatellite loci, documented increased pair-wise relatedness for small populations compared to large populations. Lower genetic diversity and higher relatedness in small relative to large populations could result in a smaller number of mating compatibility loci (S-alleles) in gene pools of small populations relative to large populations. This explanation of reduced compatibility in small populations is consistent with the finding that pollination limitation in *E. angustifolia* decreases with population size (Wagenius 2006), in spite of similar pollinator visitation rates in small and large populations (Wagenius and Lyon 2010). Moreover, mean pair-wise compatibility among *E. angustifolia* individuals increases with population size (Wagenius et al. 2007).

Lower genetic diversity and higher relatedness in small vs. large populations may also result in lower fitness of offspring from small populations, due to inbreeding depression. The increased proportions of basal plants exhibiting growth or stasis in large relative to small populations (Figure 6) provide evidence of reduced fitness in the smaller populations. Further evidence of decreased fitness in small relative to large populations was reported by Wagenius (2000), who collected achenes from 33 remnants in the study area, germinated the achenes under controlled laboratory conditions, and planted the emerged seedlings in a common garden field plot. He found that offspring of small populations had reduced vigor compared to offspring of large populations. Evidence of heterosis for between-population crosses involving small populations (Chapter 2) also suggests reduced fitness in offspring of small populations. Heterosis was evident for overall fitness over 3 years, as well as seed set. Thus, small *E. angustifolia* populations apparently suffer from inbreeding depression in addition to pollen limitation due to incompatibility.

Although studies have commonly shown a positive relationship between population size and reproductive success (e.g., Menges 1991; Widén 1993; Kery et al. 2000; Leimu et al. 2006), I know of only two other studies that have shown an effect of population size on population growth rate. Fischer and Matthies (1998) found that population growth rate (measured as the annual change in the number of flowering plants per population) increased with population size for the biennial *Gentianella germanica*. Menges and Dolan (1998) used matrix population models to study widely separated populations of *Silene regia*, a long-lived prairie perennial, and found that population size was correlated with λ, though fire management and geographic region were stronger predictors of λ. In contrast, Münzbergová (2006) found no significant effect of population size on λ in the rare grassland perennial, *Scorzonera hispanica*. However, Münzbergová constructed a mean matrix calculated from transitions of individuals in three of the largest populations in her study, then replaced only the reproductive values for matrices representing different populations. Based on her mean matrix, the highest elasticities were for stasis of large vegetative and flowering plants, while λ had low elasticity to proportional changes in fertility. As noted by the author, survival and growth transition rates may have differed for small populations. Thus, Münzbergová's population projection matrices may not have allowed detection of differences in λ related to population size. Kolb et al. (2010) used integral projection models to estimate population growth rates for 11 populations of *Phyteuma spicatum* in fragmented hardwood forests in Germany; they found no effect of population size on λ, though some vital rates were positively correlated with population size. No clear relationship between population size and λ was detected for *Trillium camschatcense* populations in fragmented broad-leaf forests in Japan (Tomimatsu and Ohara 2012), or for *Saponaria bellidifolia* populations in the southeastern Carpathians (Csergő et al. 2011).

Survival rates for each life cycle class can be determined by summing the appropriate column in the transition matrix. Mortality is highest for seedlings and decreases as plants increase in age, with very low mortality for flowering plants (Tables 4, 7-9). The high seedling mortality is reflected in the initial steep decline in the age-specific survivorship curve (Figure 4); probability of surviving to age 2 years or more is low, since individuals are unlikely to survive the seedling stage. High seedling mortality and low mortality for larger size classes is consistent with a finding of generally high 6-year survival rates for established *E. angustifolia* seedlings in a seedling recruitment experiment (Wagenius et al. 2012). High mortality of seedlings and low mortality for large, reproductive individuals have been commonly reported for other perennials (Ehrlén J. 1995; Horvitz and Schemske 1995; Walck et al. 2002).

Although it is impossible to predict long-term population viability with only two years of transitions data (Crone et al. 2011), my research suggests that small populations of *E. angustifolia* are vulnerable to population decline. The LTRE comparing the small population matrix with the large population matrix, coupled with elasticity analysis of the population matrices, suggest that management practices that enhance recruitment of new seedlings, survival and growth of basal plants could make positive contributions to population growth. One such practice is controlled burning, which increases seedling emergence and survival (Wagenius et al. 2012).

Table 1. Upper panel: number of flowering plants in 13 populations of *Echinacea angustifolia* in Douglas County, Minnesota, 2007-2010. Lower panel: number of seedlings identified in spring searches, 2007-2012. See text for seedling search methods.

Population	2007	2008	2009	2010	Average
SPP*	68	216	89	222	148.8
LF	399	607	321	490	454.3
LC	191	135	126	178	157.5
Ri	95	126	39	204	116.0
ERi	82	59	78	126	86.3
KJs	93	67	41	99	75.0
SAP	29	48	55	83	53.8
NWLF	23	49	29	72	43.3
EELR	45	46	25	55	42.8
Ness	23	15	11	16	16.3
ETH	4	6	7	11	7.0
Randt	7	3	4	4	4.5
SGC	1	4	4	5	3.5
LiH	5	5	0	3	2.7

*Flowering plants in a belt transect at SPP; estimated total flowering plants for this remnant were 5044, 1578 and 2262 in 1996, 1997 and 1998, respectively.

Population	2007	2008	2009	2010	2011	2012
SPP	1	32	4	20	26	4
LF	9	49	13	0	0	0
LC	10	5	8	8	1	2
Ri	7	8	2	15	7	14
ERi	19	44	18	2	20	26
KJs	13	14	24	6	44	33
SAP	13	12	22	1	19	11
NWLF	1	0	2	0	1	4
EELR	11	15	5	2	0	102
Ness	26	80	5	0	3	0
ETH	0	0	7	0	4	0
Randt	0	5	1	1	0	NS
SGC	NS	0	0	0	2	0
LiH	0	1	0	NS	0	NS

NS indicates population not searched this year

Table 2. Results of logistic regressions of survival versus rosette number, leaf number, length of the longest leaf, or size (= leaf number * length of longest leaf).

Predictor	b_0	b_1	P	AIC
Rosette number	0.8253	0.5282	<0.0001	763.32
Leaf number	0.9539	0.1698	<0.0001	751.55
Leaf length	0.9834	0.0459	<0.0001	765.06
Size	1.2859	0.003893	0.0004	769.00

b_0 and b_1 are coefficients in the logistic regression, probability of survival versus the given predictor. P values are for the Chi-squared test comparing the model including the given term with the null model. AIC is a version of Akaike's Information Criterion, minus twice the maximized log-likelihood plus twice the number of coefficients.

Table 3. Results of logistic regressions of flowering versus rosette number, leaf number, length of the longest leaf, or size (=leaf number * length of longest leaf).

Predictor	b_0	b_1	P	AIC
Rosette number	-3.1007	0.4252	<0.0001	453.99
Leaf number	-3.3081	0.1634	<0.0001	409.85
Leaf length	-4.1593	0.1130	<0.0001	423.71
Size	-3.1737	0.0078	<0.0001	402.55

b_0 and b_1 are coefficients in the logistic regression, probability of flowering versus the given predictor. P values are for the Chi-squared test comparing the model including the given term with the null model. AIC is a version of Akaike's Information Criterion, minus twice the maximized log-likelihood plus twice the number of coefficients.

Table 4. (a) Population projection matrix based on *E. angustifolia* transitions from 2009-2010. Column labels indicate the status of plants in 2009; row labels indicate status in 2010: seedlings, basal plants with 1-3 leaves (B1), basal with 4-6 leaves (B2), basal with 7-10 leaves (B3), basal with greater than 11 leaves (B4), flowering. Fertility of flowering plants, matrix element [1,6], is the average number of seedlings present in August 2010 per seedling circle searched. All other matrix elements [*a*, *b*] represent the proportion of plants in stage *b* in 2009 that transitioned to stage *a* in 2010. Columns do not sum to 1 due to mortality. (b) Matrix of sensitivities based on the population projection matrix shown in (a). Each matrix element is the sensitivity of λ to a change in the corresponding element of the population projection matrix. (c) Matrix of elasticities. Elasticity is the proportional response of λ to a proportional change in the corresponding population projection matrix element.

(a)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.241
B1	0.677	0.663	0.270	0.062	0.076	0.087
B2	0.000	0.083	0.405	0.141	0.061	0.145
B3	0.000	0.008	0.098	0.344	0.121	0.087
B4	0.000	0.000	0.006	0.078	0.242	0.174
Flowering	0.000	0.010	0.123	0.312	0.455	0.464

(b)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.075
B1	0.027	0.250	0.089	0.043	0.032	0.098
B2	0.000	0.509	0.181	0.087	0.066	0.200
B3	0.000	0.743	0.265	0.127	0.097	0.292
B4	0.000	0.000	0.282	0.135	0.103	0.310
Flowering	0.000	0.814	0.290	0.139	0.106	0.319

(c)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.00	0.000	0.000	0.000	0.000	0.020
B1	0.02	0.187	0.027	0.003	0.003	0.010
B2	0.00	0.048	0.083	0.014	0.005	0.033
B3	0.00	0.006	0.029	0.049	0.013	0.029
B4	0.00	0.000	0.002	0.012	0.028	0.061
Flowering	0.00	0.009	0.040	0.049	0.054	0.167

Table 5. (a) Fundamental matrix (**N**), or mean time (number of years) spent in each class, based on the 2009-2010 population projection matrix shown in Table 4. **N** is calculated from the formula $\mathbf{N} = (\mathbf{I} - \mathbf{T})^{-1}$, where **I** is the identity matrix and **T** is the transition matrix. (b) Variance of time spent in each class, calculated as $\mathbf{V} = (2\mathbf{N}_{dg} - \mathbf{I})\,\mathbf{N} - \mathbf{N} \circ \mathbf{N}$, where $\mathbf{N}_{dg}$ is the matrix with the diagonal of **N** on its diagonal and zeros elsewhere and $\mathbf{N} \circ \mathbf{N}$ is the Hadamard product, obtained by element-by element multiplication. (c) Mean time to death, $E(\eta_j)$, the column sum of the j^{th} column of **N**, and variance of time to death, $V(\eta_j)$, where $(V(\eta_1) \ldots V(\eta_6)) = \mathbf{e}^T(2\mathbf{N}^2 - \mathbf{N}) - \mathbf{e}^T\mathbf{N} \circ \mathbf{e}^T\mathbf{N}$, where $\mathbf{e}^T$ is the transpose of a column vector of ones. All formulas from Caswell (2001).

(a)	Seedling	B1	B2	B3	B4	Flowering
Seedling	1.000	0.000	0.000	0.000	0.000	0.000
B1	2.538	3.749	2.646	2.421	2.487	2.522
B2	0.483	0.713	2.584	1.520	1.437	1.527
B3	0.164	0.243	0.675	2.237	0.977	0.902
B4	0.098	0.144	0.407	0.785	2.047	0.925
Flowering	0.335	0.494	1.377	2.360	2.678	3.569

(b)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.000
B1	10.050	10.305	10.191	9.870	9.975	10.027
B2	1.780	2.465	4.094	4.026	3.926	4.034
B3	0.544	0.785	1.890	2.768	2.440	2.320
B4	0.293	0.426	1.093	1.813	2.144	2.006
Flowering	1.941	2.789	6.554	8.914	9.264	9.166

(c)	Seedling	B1	B2	B3	B4	Flowering
$E(\eta_j)$	4.617	5.344	7.689	9.323	9.627	9.443
$V(\eta_j)$	29.11	33.78	47.63	54.41	54.60	53.82

Table 6. Generation times, in years, based on the 2009-2010 projection matrix and the 2010-2011 projection matrix. T is the time required for a population to increase by a factor of R_0 (the per-generation growth rate). μ_1 is the mean age at which a cohort of seedlings produces offspring, calculated following Caswell (2001) and following Cochran and Ellner (1992).

Projection matrix	T	μ_1
2009-2010	20.9	11.2
2010-2011	16.5	8.8

Table 7. Population projection matrix based on *E. angustifolia* transitions from 2010-2011 (a) and sensitivities (b) and elasticities (c) based on the matrix. Column labels indicate the status of plants in 2010; row labels indicate status in 2011; abbreviations as in Table 4. Fertility of flowering plants, population projection matrix element [1,6], is the average number of seedlings present in August 2011 per seedling circle searched. All other matrix elements [*a*, *b*] represent the proportion of plants in stage *b* in 2010 that transitioned to stage *a* in 2011. Columns do not sum to 1 due to mortality.

(a)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.884
B1	0.674	0.707	0.184	0.062	0.000	0.135
B2	0.000	0.039	0.490	0.125	0.333	0.270
B3	0.000	0.012	0.061	0.438	0.222	0.189
B4	0.000	0.000	0.020	0.188	0.333	0.000
Flowering	0.000	0.003	0.061	0.125	0.000	0.351

(b)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.024
B1	0.032	0.371	0.108	0.056	0.000	0.030
B2	0.000	0.749	0.219	0.112	0.050	0.061
B3	0.000	1.263	0.368	0.189	0.085	0.102
B4	0.000	0.000	0.306	0.157	0.071	0.000
Flowering	0.000	1.548	0.452	0.232	0.000	0.126

(c)	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.025
B1	0.025	0.313	0.024	0.004	0.000	0.005
B2	0.000	0.035	0.128	0.017	0.020	0.020
B3	0.000	0.018	0.027	0.099	0.023	0.023
B4	0.000	0.000	0.007	0.035	0.028	0.000
Flowering	0.000	0.006	0.033	0.035	0.000	0.053

Table 8. Transition rates for plants initially found in seedling searches, 2006-2008.

2007-2008 transitions	Seedlings (n=121)	1-leaf basal (n=15)
1-lf basal	0.455	0.533
2-leaf basal	0.207	0.267
3-leaf basal	0.017	0
4-leaf basal	0.017	0

2008-2009 transitions	Seedlings (n=154)	1-leaf basal (n=62)	2-leaf basal (n=29)	3-leaf basal (n=2)	4-leaf basal (n=2)
1-leaf basal	0.344	0.274	0.138	0	0
2-leaf basal	0.117	0.081	0.379	0.5	0.5
3-leaf basal	0.013	0.032	0.034	0	0
4-leaf basal	0	0	0.034	0	0

Table 9. Number of flowering plants within a belt transect in Staffanson Prairie, 2006-2009, and the proportion of these that transitioned to basal plants or flowering plants the following summer.

	2006 (n=68)	2007 (n=216)	2008 (n=89)	2009 (n=222)
Basal	0.294	0.759	0.404	0.486
Flowering	0.691	0.241	0.596	0.455

Table 10. Population projection matrix based on available transitions from 2008-2009. Fertility of flowering plants, matrix element [1,6], is the average number of seedlings present in August 2009 per seedling circle searched in 2008. Survival of seedlings, matrix element [2,1], is the proportion of 2008 seedlings that were 1-3 leaf basal plants in 2009. Matrix element [2,2] is the proportion of 1-3 leaf basal plants in 2008 that remained as 1-3 leaf basal plants in 2009. Matrix element [6,6] is the proportion of 2008 flowering plants that flowered again in 2009. Elements 2-5 in column 6 are the proportion of 2008 flowering plants that were basal in 2009, distributed proportionally based on the matrix representing the average between the 2009-2010 and 2010-2011 matrices. All other matrix elements represent the average between the 2009-2010 and 2010-2011 matrices. Classes as in Table 4.

	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.284
B1	0.560	0.471	0.227	0.062	0.038	0.079
B2	0.000	0.061	0.447	0.133	0.197	0.147
B3	0.000	0.010	0.080	0.391	0.172	0.098
B4	0.000	0.000	0.013	0.133	0.288	0.062
Flowering	0.00	0.006	0.092	0.219	0.227	0.596

Table 11. Population projection matrix based on available transitions from 2007-2008. Fertility of flowering plants, matrix element [1,6], is the average number of seedlings present in August 2008 per seedling circle searched in 2007. Survival of seedlings, matrix element [2,1], is the proportion of 2007 seedlings that were 1-3 leaf basal plants in 2008. Matrix element [6,6] is the proportion of 2007 flowering plants that flowered again in 2008. Elements 2-5 in column 6 are the proportion of 2007 flowering plants that were basal in 2008, distributed proportionally based on the matrix representing the average between the 2009-2010 and 2010-2011 matrices. All other matrix elements represent the average between the 2009-2010 and 2010-2011 matrices. Classes as in Table 4.

	Seedling	B1	B2	B3	B4	Flowering
Seedling	0.000	0.000	0.000	0.000	0.000	0.756
B1	0.726	0.685	0.227	0.062	0.038	0.147
B2	0.000	0.061	0.447	0.133	0.197	0.275
B3	0.000	0.010	0.080	0.391	0.172	0.183
B4	0.000	0.000	0.013	0.133	0.288	0.115
Flowering	0.000	0.006	0.092	0.219	0.227	0.242

Figure 1. Map of study area in southwest Douglas County, Minnesota. *Echinacea angustifolia* populations as in Table 1.

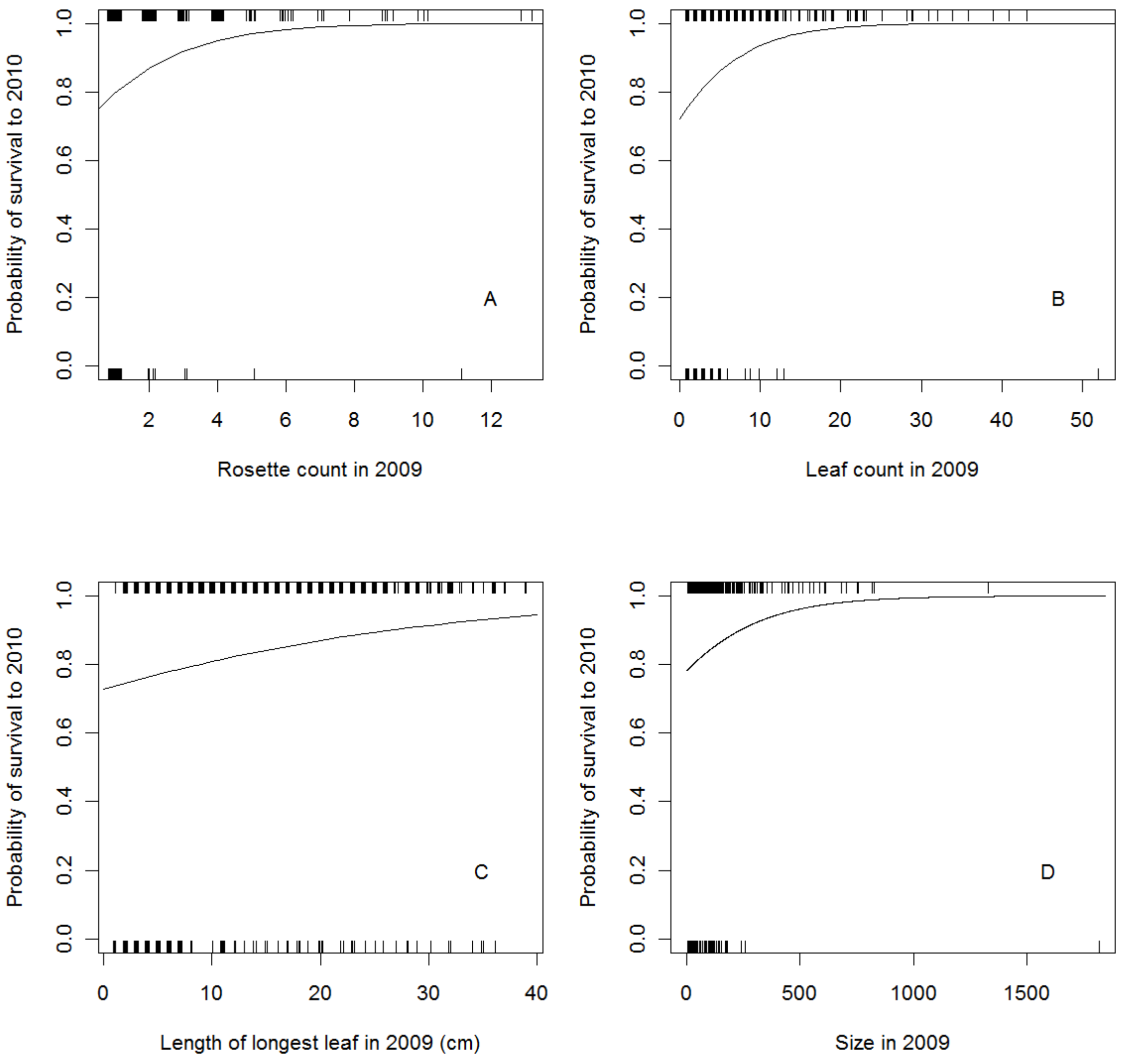

Figure 2. Fate in 2010 of *Echinacea angustifolia* plants that were basal in 2009. Each short vertical line along the horizontal axes represents a plant with the given size in 2009 that either survived (y=1) or died (y=0); x-values are jittered. Best fit line is from the logistic regression, survival vs. rosette count (panel A), vs. leaf count (panel B), vs. length of length of longest leaf (panel C), or vs. size (panel D), where size is the number of leaves multiplied by length of the longest leaf. For regression coefficients, see Table 1.

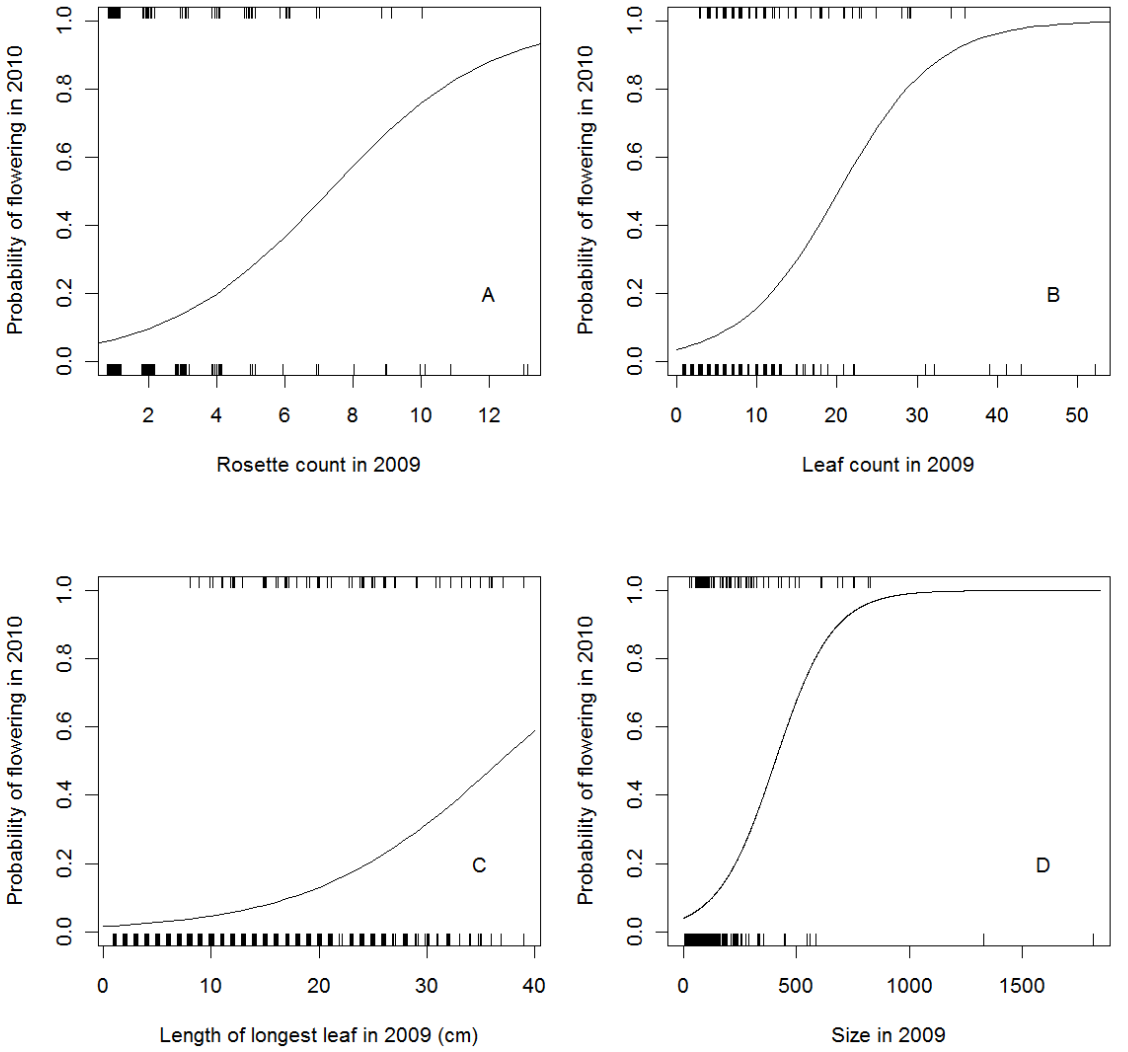

Figure 3. Flowering probability in 2010 for *E. angustifolia* plants that were basal in 2009. Each short vertical line along the horizontal axes represents a plant with the given size that either flowered (y=1) or did not flower (y=0); x-values are jittered. Best fit line is from the logistic regression, probability of flowering vs. rosette count (panel A), vs. leaf count (panel B), vs. length of longest leaf (panel C), or vs. size (panel D); size as in Figure 1. For regression coefficients, see Table 2.

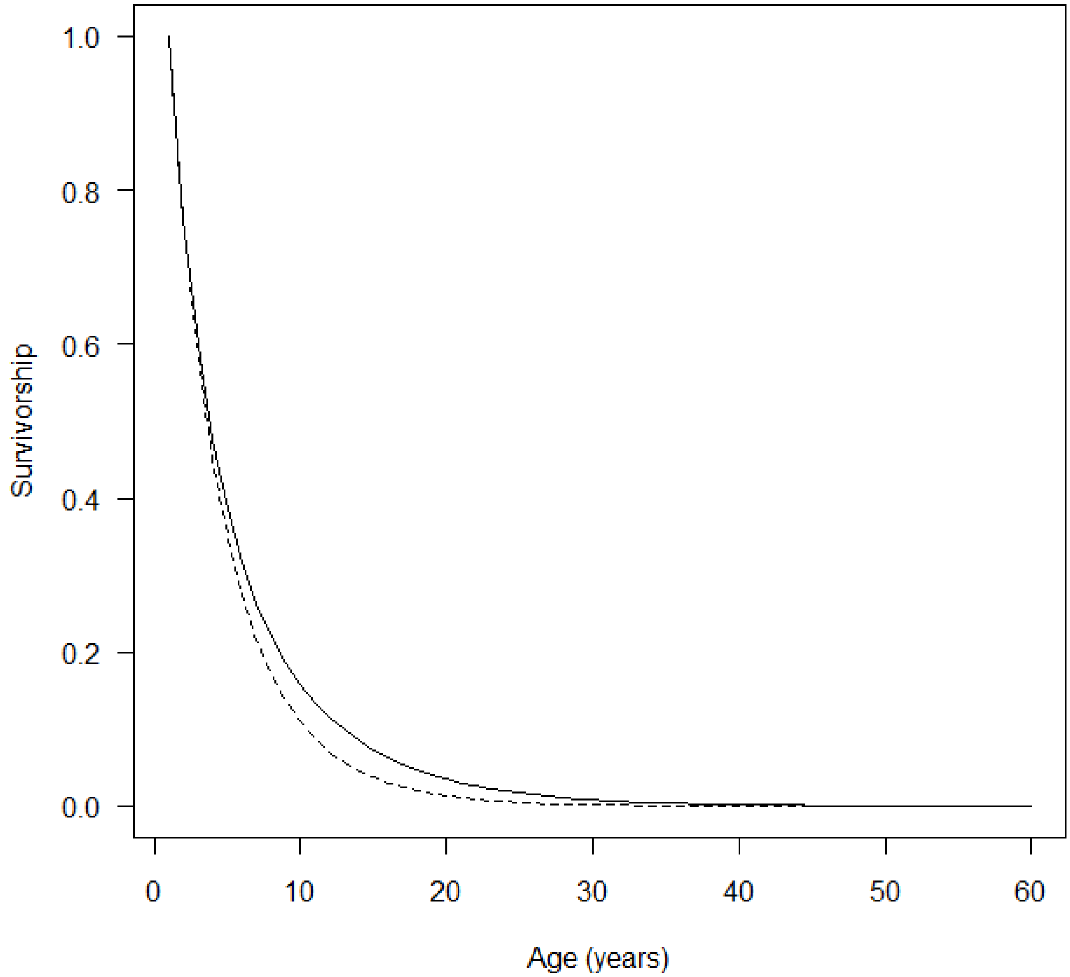

Figure 4. Age-specific survivorship (probability of survival to age *t*) based on the 2009-2010 population projection matrix (solid curve) and the 2010-2011 projection matrix (dashed line). Survivorship for age *t* is the sum of the first column of the matrix calculated from $\mathbf{e}^{T}\mathbf{T}^{t}$, where $\mathbf{e}^{T}$ is the transpose of a column vector of ones, **T** is the matrix of transition probabilities from the population projection matrix, and the entries of $\mathbf{T}^{t}$ give the probabilities of being in each class at time *t*, conditional on the starting class.

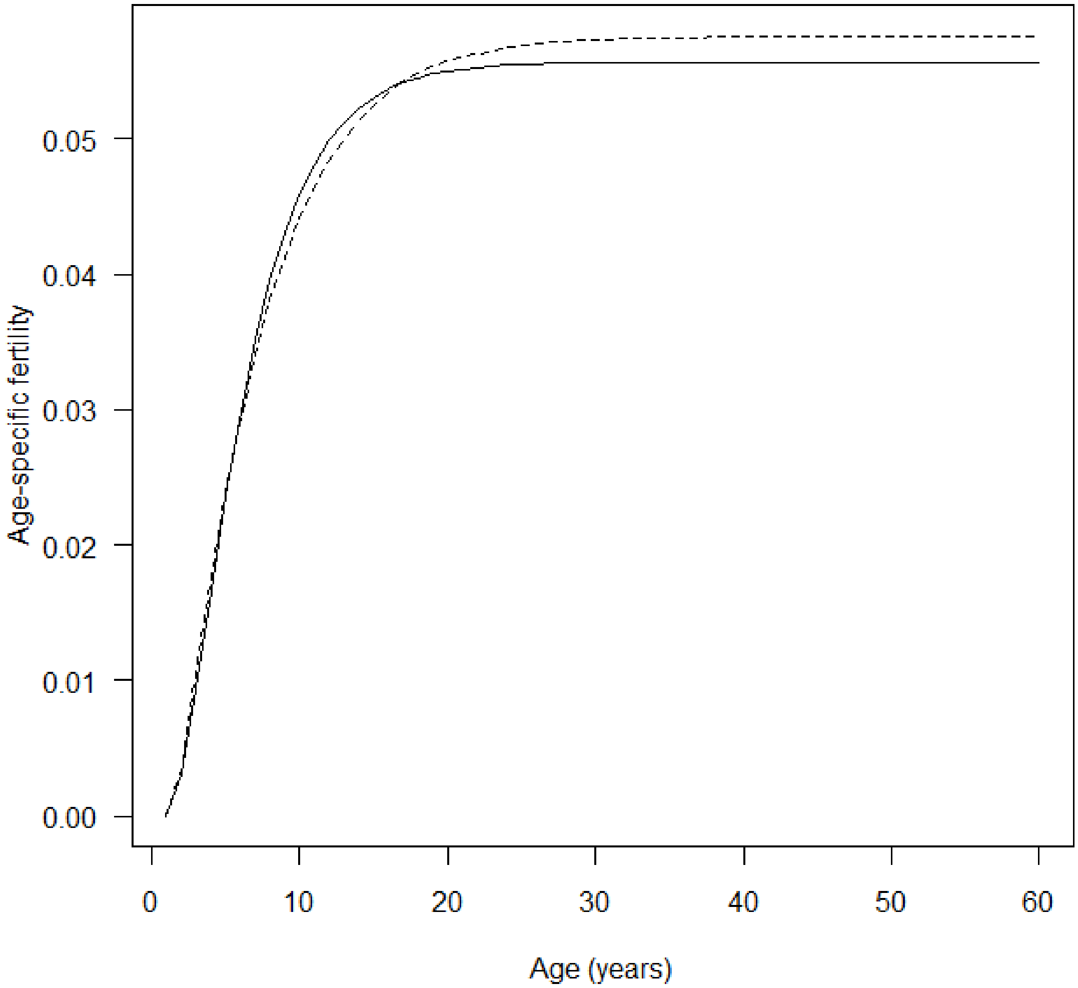

Figure 5. Age-specific fertility based on the 2009-2010 population projection matrix (solid line) and the 2010-2011 projection matrix (dashed line). Fertility at age *t*, that is, the mean number of seedlings produced by an individual of age *t*, is the φ_{11} entry of the matrix $\mathbf{\Phi}(t) = \mathbf{F}\mathbf{T}^t(\mathrm{diag}(\mathbf{e}^{\mathrm{T}}\mathbf{T}^t))$, where $\mathbf{e}^{\mathrm{T}}$ is the transpose of a column vector of ones, diag(**X**) is the matrix with the diagonal of **X** on its diagonal and zeros elsewhere, **F** is the fertility matrix, **T** is the matrix of transition probabilities, and the entries of $\mathbf{T}^t$ give the probabilities of being in each class at time *t*, conditional on the starting class (Caswell 2001).

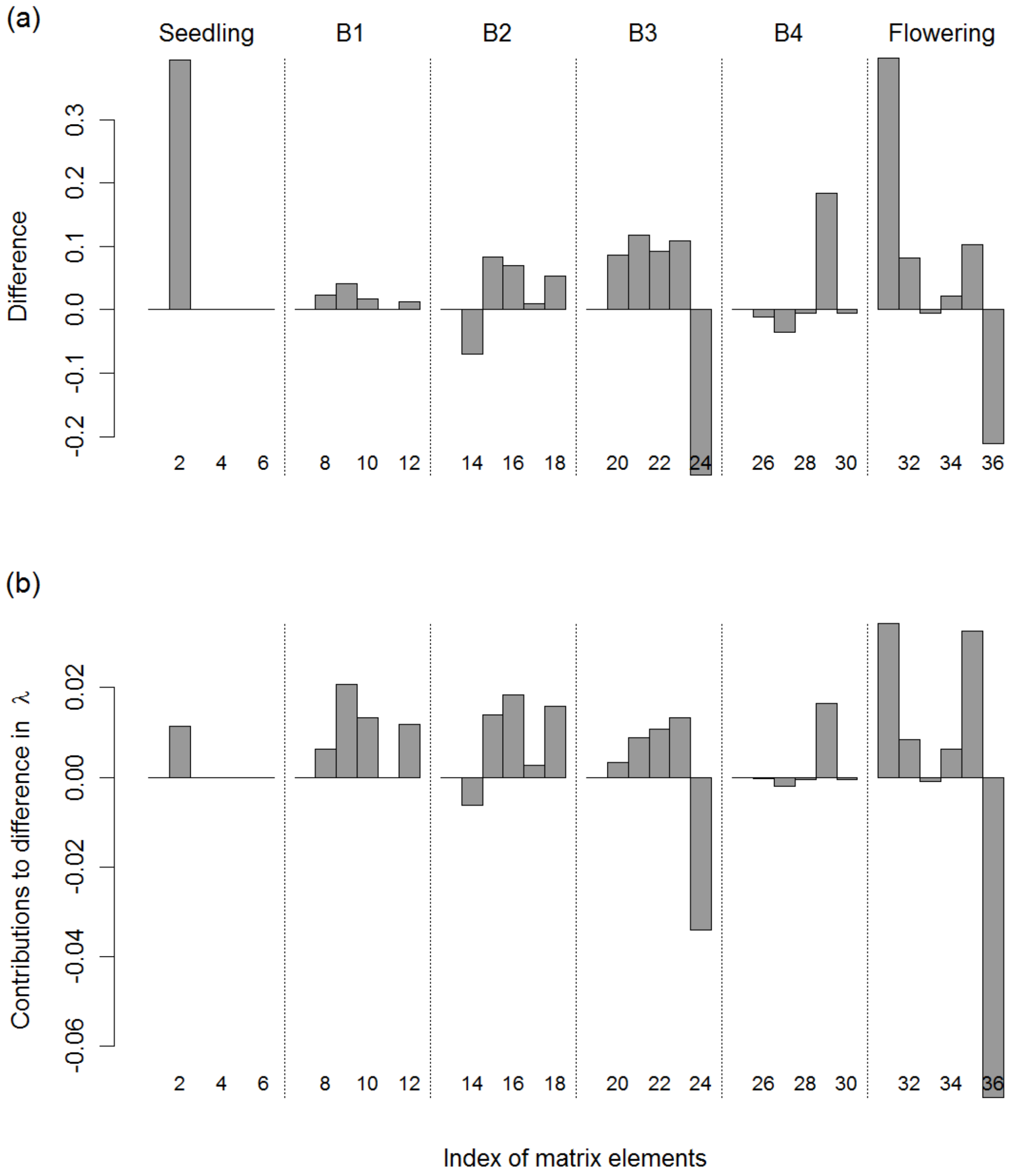

Figure 6. Differences in matrix elements between large population matrix and small population matrix (a; elements of the small population matrix are subtracted from elements of the large population matrix) and contributions of matrix element differences to the difference in λ between the large population matrix and the small population matrix (b). The 36 elements of the 6 x 6 matrix are represented in column order along the x-axis: fates of seedlings (elements 1-6), fates of 1-3 leaf basal plants (elements 7-12),…fates of

basal plants with 11 or more leaves (elements 25-30), fertility of flowering plants (element 31), fates of flowering plants (elements 32-36); classes include seedlings, 1-3 leaf basal plants (B1), 4-6 leaf basal plants (B2), 7-10 leaf basal plants (B3), basal plants with 11 or more leaves (B4), and flowering plants.

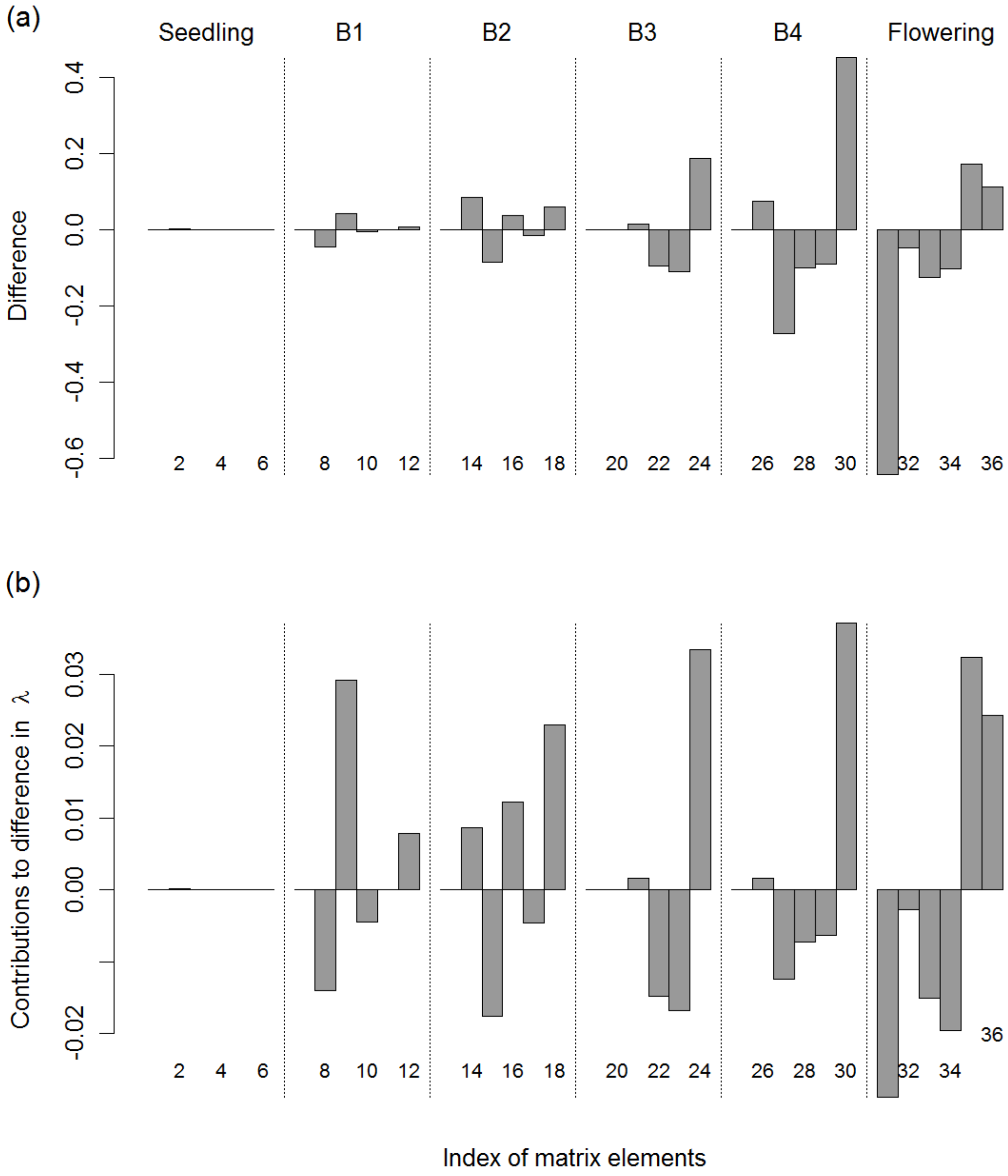

Figure 7. Differences in matrix elements between 2009-2010 matrix and 2010-2011 matrix (a; elements of the 2010-2011 matrix are subtracted from elements of the 2009-2010 matrix) and contributions of matrix element differences to the difference in λ between the 2009-2010 matrix and 2010-2011 matrix (b). The 36 elements of the 6 x 6 matrix are represented in column order along the x-axis: fates of seedlings (1-6), fates of

1-3 leaf basal plants (7-12),…fates of basal plants with 11 or more leaves (25-30), fertility of flowering plants (31), fates of flowering plants (32-36); classes as in Figure 6.

Chapter 2. Heterosis and outbreeding depression in crosses among fragmented populations of *Echinacea angustifolia*

Habitat fragmentation in the tallgrass prairies of North America has reduced native species to small, isolated populations. These small, isolated populations may experience genetic erosion due to genetic drift and inbreeding depression. Genetic diversity may be increased by introducing genetic material (e.g., pollen) from neighboring populations; however, outcrossing among populations also carries the risk of outbreeding depression (decreased fitness of offspring of between-population vs. within-population matings). Within-population and between-population crosses were performed among 6 populations of *Echinacea angustifolia* in west-central Minnesota to evaluate the relative potential for heterosis (increased fitness of offspring of between-population vs. within-population crosses) and outbreeding depression. Effects of crosses varied depending on the maternal and paternal populations involved, and on the fitness trait assessed. Heterosis was observed for 1 maternal population for seed set, 1 population for overall fitness, and 2 populations for fitness excluding seed set (i.e., seedling emergence and survival); outbreeding depression was observed for 2 populations for seed set, 2 populations for overall fitness, and 1 population for fitness excluding seed set. No clear evidence of a relationship between maternal population size and seed set or overall fitness was found; however, the strongest evidence for heterosis was observed for the two smallest populations in the study. Managers should use caution when introducing genetic

material from foreign populations (i.e., genetic rescue) due to the risk of outbreeding depression.

Introduction

Human activities (e.g., logging of forests, conversion of land to agricultural use, urbanization) have caused the widespread destruction of terrestrial ecosystems. The remaining native habitat often consists of small, isolated populations of plant and animal species. These fragmented populations may experience increased inter-population genetic divergence, and individual population remnants may suffer genetic erosion due to genetic drift, inbreeding, and reduced gene flow among populations, as has often been noted (Young et al. 1996; Aguilar et al. 2008). The decreased effective population size (N_e) of fragmented populations can lead to decreased fitness (Ellstrand and Elam 1993) and an increased probability of extinction (Newman and Pilson 1997). These genetic consequences of habitat fragmentation apply to both rare and common plant species (Honnay and Jacquemyn 2007).

The genetic diversity of a population can be increased by introducing genetic material (e.g., pollen) from a neighboring population. This induced gene flow may increase the average fitness of the recipient population (i.e., reduce inbreeding depression) in a process known as genetic rescue (Thrall et al. 1998; Richards 2000; Tallmon et al. 2004). However, hybridization between populations may instead result in decreased fitness of the recipient population, or outbreeding depression (Price and Waser 1979; Shields 1982; Ellstrand and Elam 1993; Edmands 2007), due to dilution of locally adapted genomes or disruption of coadapted gene complexes. Thus, an important consideration for plant population restoration and conservation is to assess the degree of inbreeding depression in fragmented populations, and evaluate whether outcrossing leads to increased population fitness or outbreeding depression (Fenster and Dudash 1994; Keller and Waller 2002; Edmands 2007).

A number of studies have used controlled crosses to evaluate the effects of interpopulation vs. intrapopulation crosses. Many studies have documented fitness gains in the hybrid offspring (i.e., heterosis; Moll 1965; Woessner 1972; Levin and Bulinska-Radomska 1988; Fenster 1991; van Treuren et al. 1993; Hauser and Loeschcke 1994; Ouborg and van Treuren 1994; Heschel and Paige 1995; Fenster and Galloway 2000; Sheridan and Karowe 2000; Luitjen et al. 2002; Willi and Fischer 2005; Bossuyt 2007, Willi et al. 2007; Bermingham and Brody 2011; Finger et al. 2011). Other experiments have shown reduced fitness in offspring of interpopulation vs. intrapopulation crosses (i.e., outbreeding depression; Sobrevila 1988; Fischer and Matthies 1997; Montalvo and Ellstrand 2001; Heiser and Shaw 2006; Vandepitte et al. 2010; Becker et al. 2011), while still other studies have documented both heterosis and outbreeding depression (Price and Waser 1979; Waser and Price 1989; Waser et al. 2000; Galloway and Etterson 2005; Busch 2006; Grindeland 2008). Outbreeding depression may be evident over distances as small as 10-100 meters (Price and Waser 1979; Waser and Price 1989; Grindeland 2008). Alternatively, heterosis may be observed for crosses at distances up to thousands of kilometers (Moll et al. 1965; Fenster and Galloway 2000). The variation in observed heterosis and/or outbreeding depression in different studies may reflect differences in mating distances, degree of inbreeding of observed populations, traits measured, or mechanisms of inbreeding or outbreeding depression (Hufford and Mazer 2003).

Echinacea angustifolia (Asteraceae; narrow-leaved purple coneflower) is a long-lived perennial native to the tall grass prairie of North America. Habitat fragmentation is particularly pronounced in this ecosystem, where an estimated 82-99% of the original habitat has been converted to agriculture or urbanization (Samson and Knopf, 1994), and remaining prairie is usually confined to small fragments. *E. angustifolia* is representative of many native prairie species in that it reproduces soley by seed, has limited seed dispersal, is pollinated by generalist insects, and is self-incompatible (Wagenius 2000, 2004; Ault 2006). Plants grow from a taproot, annually producing basal rosettes, and rarely flower before their seventh year (Wagenius et al. 2012). Once a plant begins to produce flowers, it may not flower each growing season (Wagenius 2006). Each season, a flowering plant may produce one or more capitulate inflorescences (heads). Seeds are dispersed by gravity; achenes drop from heads on persistent stalks, or are dislodged when the flowering stalks break and the heads strike the ground.

Molecular analyses of fragmented *E. angustifolia* populations in Douglas County, Minnesota have indicated generally high genetic diversity in all populations (e.g., mean observed heterozygosity across loci ranging from 0.640 to 0.778; Ison 2010), but a lower genetic diversity in small populations compared to large ones (Wagenius 2000; Lonsdorf 2004; Ison 2010). Furthermore, offspring of plants from small populations tend to be smaller than offspring of plants from larger populations (Wagenius 2000). Wagenius et al. (2010) report a significant reduction in fitness for offspring of sibling-mating, but no significant effect of between-remnant crosses, compared to within-remnant (non-sibling) crosses, though survival of between-remnant seedlings exceeded that of both within-remnant and sibling-mated crosses. However, the seeds from these crosses were germinated under conditions designed to promote emergence; it is unknown whether progeny of between-remnant and within-remnant crosses would exhibit differential success in field emergence and early survival. Furthermore, the experimental design of Wagenius et al. (2010) was insufficient to test for effects of population identity or size.

I describe an experiment designed to investigate the effects of within-population vs. between-population crosses on seedling recruitment in a fragmented *E. angustifolia* metapopulation. I refer to individual demes as "populations." I asked whether inter-population crosses would exhibit heterosis or outbreeding depression compared to intra-population crosses, and compared the results among 6 maternal populations of varying size. A secondary aim was to determine whether the population size of the pollen donors makes a difference in the outcrosses; I asked whether pollen from a large population produces progeny that outperform progeny sired by pollen from a small population in between-population crosses.

Methods

Experimental design

In July-August 2008, I performed within-population crosses and between-population crosses using plants representing six populations of *E. angustifolia*. The parental plants used in this experiment were collected as seeds from remnant prairie populations of various sizes located within a 6400-hectare study area in southwestern Douglas County, MN, in 1995 and 1996. The seeds were germinated in controlled laboratory conditions, and then planted as seedlings into randomized locations within an experimental field located within the study area in Douglas County (Wagenius 2006;

Wagenius et al. 2010). Thus, the parental plants used in this crossing experiment had grown to maturity under similar environmental conditions. My goal was to use 20 flowering plants from each population as maternal plants, and an additional 5 plants from each population as pollen donors. The realized crossing design was unbalanced due to insufficient numbers of flowering plants for 3 populations (Table 12). For example, only ten individuals from the Aanenson (AA) population flowered. Consequently, I randomly assigned 8 of the AA plants as maternal plants, and 2 as pollen donors.

Each flowering plant produced one or more capitulate inflorescences (hereafter "heads"). On each maternal plant, the first head to exhibit mature male florets was designated for use in the primary crosses. Half of the florets in each head received pollen from a pollen donor of the same population (within-population cross), while the other half of the head was outcrossed to a pollen donor from a different population (between-population cross). For the AA, Lf, Ness, NWLF and SAP maternal plants, the inter-population pollen donor was a SPP plant; to obtain interpopulation crosses on SPP maternal plants, pollen from Lf donors was applied. The rationale for using primarily SPP pollen was that the SPP remnant population is by far the largest *E. angustifolia* population in our study area. Consequently, SPP plants could be expected to exhibit the greatest genetic diversity of the populations available for this experiment. Additionally, because of its size, SPP may be more likely than smaller remnants to export pollen to neighboring populations.

To avoid confounding potential differences in resource allocation among multiple heads on a single maternal plant with the effects of crosses within and between populations, I applied both treatments to a single head, with half the head assigned to each. I randomly assigned either the east or west half of each head to receive intra-population pollen; the other half of the head received inter-population pollen. I painted bracts subtending florets to identify the borders of half heads and signify assigned pollen source. For individuals that produced more than one inflorescence, a second head was used for a secondary cross to a distinct other population, NWLF. NWLF is a much smaller remnant population than SPP (Table 12).

Seed heads were collected at maturity, and transferred to a laboratory at the University of Minnesota. The heads were dissected to separate the achenes resulting from each cross. Field notes were used to exclude achenes resulting from florets that were not pollinated. The achenes from each primary (within-population or between-population) cross were divided evenly into three separate coin envelopes. The achenes from each envelope were individually weighed, using an automated apparatus at the Chicago Botanic Garden, to estimate seed set, that is, the number of achenes in each envelope that contained seeds. Achenes from each envelope were then sown into the experimental plot.

Heads from the secondary crosses (between-population using NWLF pollen) were handled differently. All of the florets on each secondary head were pollinated with NWLF pollen; thus the secondary crosses generally resulted in a greater number of achenes than the primary crosses. Consequently, the achenes from each secondary cross were divided into four envelopes; achenes in three envelopes were sown into the experimental plot, and one envelope was retained so that individual seed masses could be obtained subsequent to sowing.

Achenes were sown into an experimental plot in the Hegg Lake Wildlife Management Area (WMA), a prairie restoration in Douglas County, MN, in November

2008. Three blocks were established; each block consisted of 9 rows, with each row containing 23 sowing positions at 0.5 m intervals. Nails were driven into the ground at 1-meter intervals in each row, to mark sowing positions. Each of the three envelopes representing each cross was randomly assigned to one of the blocks and a random sowing position within a grid in its designated block.The achenes from an envelope were scattered in a row at the designated sowing position (either in a row to the west of a nail, or in a row to the west of the half-meter mark between nails). There were 93 within-population crosses, 93 between-population crosses using SPP or Lf pollen, and 40 secondary between-population crosses using NWLF pollen, for a total of 226 crosses. In some cases, phenological differences of pollen donors and maternal plants had prevented successful pollinations. A few seed heads contained no viable achenes. There were a few pollination errors. Consequently, the total number of successful crosses was reduced to 207 (Table 12). Altogether 15,491 achenes were sown.

I searched for seedlings in May 2009, and performed a census of seedlings in August 2009. A second census, as well as a search for new seedlings, was performed in June 2010. Additional censuses were performed in August 2010 and July 2011. At each census, I counted the number of leaves and measured the length of the longest leaf for each seedling.

Analysis

To determine which achenes were likely to contain seeds, I made a histogram of achene masses, and used the natural break in the bimodal distribution as the critical mass demarcating unfilled and filled achenes (“seeds”; Wagenius and Lyon 2010).

To analyze seed set, I performed a logistic regression of seed counts as a proportion of the number of achenes weighed, using binomial family generalized linear models (GLM) with logit link function, implemented in R (R Development Core Team 2010). This is a weighted regression, using individual sample sizes (number of achenes in each envelope) as weights. To compensate for overdispersion (residual deviance >> residual df), I used quasi-binomial errors (Crawley 2007).

To assess the effect of cross type and maternal population on overall seedling performance, I used aster models, implemented in R (R Development Core Team 2010). I used the number of achenes sown at each sowing position as the root node for the aster models (Geyer et al. 2007). For analyses of the primary crosses, I used the following life cycle nodes: number of seeds, number of emerged seedlings in May 2009, number of seedlings present during survival censuses in August 2009, June 2010, August 2010 and July 2011, and total leaf number in July 2011 (Figure 8). For analyses including the secondary crosses, I excluded the seeds node, since achenes from these crosses were not weighed before sowing. The zero-truncated Poisson family was used for the leaf number in 2011 node of the aster models; the Bernoulli family was used for all other nodes. Leaf number in 2011 was used as a proxy for overall fitness, since leaf number is correlated with survival for basal *E. angustifolia* plants (chapter 1). All factors were tested as affecting leaf number, in order to directly evaluate their effect on this measure of overall fitness (Geyer et al. 2007). I used unconditional aster models; thus the effects of the variables at the level of leaf number propagate back to the earlier stages of the life cycle.

I first tested the effect of the spatial position (CGRow and CGPos) of maternal plants in their experimental common garden plot, as well as block and sowing position

(HLRow and HLPos) in the seedling plot located at Hegg Lake WMA. I started with a full model including all 5 spatial positioning variables, CGRow, CGPos, HLRow, HLPos and Block (Table 13). I tested each of the five variables by removing them, one at a time, from the full model. I also tested each spatial variable by adding it to a null model. Once I determined a suitable subset of the above factors to include in models (Block and HLRow), I subsequently tested the factors of primary scientific interest, cross type and maternal population.

To determine whether the significant effects at leaf number could be due to differences in seed production, I fit aster models with the effects modeled at the seed node, as well as at both the leaf number and seed nodes. For consistency, the spatial terms, Block and HLRow, modeled as affecting leaf number, were included in each model. I compared the full model (with effects modeled at both seed and leaf number) with models only including effects modeled at leaf number or at seed.

To evaluate the effects of maternal population, cross type or their 2-way interaction on life cycle stages later than seeds (Figure 8), I fit additional aster models testing the factors as affecting each node of the life cycle. I used sets of nested models for model comparison. In each set of nested models, the largest model tested the variables as affecting both nodes of interest, the middle model tested the variables as affecting only one of the nodes, and the smallest model contained only the spatial terms.

As an alternative approach to determining whether the significant effects at leaf number were attributable solely to differences in seed set, I fit unconditional aster models that used “seeds” as the root, and included only 6 life cycle stages. I modeled the variables of scientific interest (cross type and maternal population) as affecting leaf number. I also fit 6-node aster models with the variables modeled as affecting each of the other nodes of the life cycle. These models using seeds as root tested for the effects of maternal population and cross type on seedling emergence, survival and growth.

For the 7-node aster models described above (models using achene number as root), I obtained predicted values for each combination of maternal population and cross type in the experiment, with the root value specified as 25 total achenes, the average number of achenes per envelope across the experiment. For 6-node aster models, the root value was specified as 4 seeds. The spatial predictors were assigned as Block B, and HLRow 15, and a value of 1 was assigned for each node of the life cycle graph. I used the *predict* function in aster, implemented in R (R Development Core Team 2010), to make unconditional estimates of the total leaf number (i.e., overall fitness) that would result if each envelope contained 25 achenes, based on the fitted aster models.

Results

Achene weights, seed set and seedling emergence

The distribution of achene weights was bimodal, with the majority of achenes having a mass smaller than 2 mg (Figure 9). Achenes with a mass greater than 2.0 mg were designated as seeds. By this criterion, one of the 515 primary cross envelopes sown contained 0 seeds, but 1 seedling emerged in the corresponding sowing position. This envelope contained 5 achenes, with masses ranging from 0.40 mg to 0.55 mg, plus one achene that was not weighed (generally, achenes that were not weighed were very light and thus did not easily slide into the automated balance). This envelope was excluded from further analyses. When I re-defined seeds as achenes weighing more than 2.2 mg, an additional sowing position with 1 observed seedling was associated with an envelope containing 0 seeds. Therefore, I used a critical mass of 2.0 mg for further analyses. By this criterion, seed set (proportion of achenes designated as seeds) ranged from 0 to 0.885 across the envelopes.

The logistic regression of seed counts as a proportion of the number of achenes weighed revealed a significant interaction of maternal population and cross type (Table 14). With the two-way interaction excluded from the model, there was a significant main effect of maternal population but cross type was ns (Table 14). The significant main effect of maternal population was characterized by a relatively high seed set for the Ness and SAP populations, and a relatively low seed set for Lf (Figure 10), compared to the other 3 populations. The significant interaction effect indicates differential performance of the three cross types among the various populations, with the populations evenly split with respect to relative performance of within-population vs. between-population primary crosses. Moreover, the within-population cross resulted in a greater proportion of filled achenes relative to either between-population cross for one maternal population, Ness. In contrast, the between-population cross involving the large population pollen donor (SPP) produced the highest seed set for maternal populations AA and SAP (Figure 10). However, the between-population cross with Lf as pollen donor had the lowest proportion of filled achenes for maternal population SPP (Figure 10).

To assess the sensitivity of these findings to the choice of cut-off weights for seeds, I also analyzed seed set using alternative minimum seed weights. Logistic regressions using seeds defined as achenes weighing more than 1.6 mg, 1.8 mg, 2.2 mg or 2.4 mg were qualitatively similar to the regression using 2 mg as the critical mass for seeds. The regression using a critical mass of 1.4 mg was also similar except that the maternal population:cross type interaction was only marginally significant ($p = 0.0643$).

Altogether 395 seedlings were found in May 2009. An additional 43 seedlings were found during the August 2009 census. These additional seedlings may have been overlooked in May, or may have germinated later in the season. Ten additional seedlings were found during the 2010 censuses, only one of which was positively identified as a new germinant by the presence of cotyledons.

Aster models: primary crosses

In aster models testing the effects of the spatial positioning variables as affecting leaf number in 2011, both Block and HLRow were significant in (Table 13). The other 3 spatial variables, HLPos, CGRow and CGPos were ns, regardless of whether they were added to a smaller model or removed from a larger model. I retained both Block and HLRow, tested as affecting leaf number, in all subsequent aster models.

To evaluate the effects of maternal population and cross type for the primary crosses, I used unconditional 7-node aster models (i.e., using achene number as root and all 7 life cycle stages, Figure 8). When the factors were modeled as affecting leaf number, there was no significant effect of the maternal population:cross type interaction (Table 15). There was a significant main effect of maternal population, but cross type was ns. However, when the factors were modeled as affecting the seed node (i.e., seed set), there was a highly significant maternal population:cross type interaction as well as a significant main effect of maternal population, though still no significant main effect of cross type (Table 16). The maternal population term, tested as affecting leaf number, did not significantly improve a model including the variables tested as affecting seed set, suggesting that the signifanct effects of maternal population and maternal population:cross type on seed set account for the significant effect of maternal population on leaf number in 2011. Similarly, maternal population had a significant effect when tested as affecting each of the other stages of the life cycle; however, there was no significant main effect of maternal population at any life cycle stage, over and above its effect on seed set.

I used nested models to compare the effects of maternal population and cross type on each stage of the life cycle. When maternal population, cross type and maternal population:cross type, tested as affecting survival to 2011, were added to the model with the 3 terms tested as affecting seed set, there was a significant difference between the two models (Table 17). Table 16 indicates that this significant difference was due to a significant effect of the maternal population:cross type interaction tested as affecting survival to 2011, suggesting that the significant effect of the maternal population:cross type interaction on survival was not fully accounted for by the effects of maternal population and cross type on seed set. Based on the model testing the effects of the factors as affecting both seed set and survival to 2011, there was evidence of increased overall fitness for the between-population cross relative to the within-population cross for NWLF, the smallest population in the study; in contrast, the within-population crosses had higher fitness than the between-population cross for SPP, the largest population (Figure 11). The mean leaf numbers shown in Figure 11 are unconditional predictions, accounting for seed set, seedling emergence, survival and growth.

As an alternative approach to evaluating the effects of maternal population and cross type on seedling emergence and survival, I fit aster models that used seeds (rather than achenes) as the root node, and thus included only 6 of the life cycle nodes (Figure 8). For 6-node aster models testing the terms as affecting leaf number in 2011, the maternal population:cross type interaction was ns, as was maternal population and cross type. However, there was a significant maternal population:cross type interaction when the factors were tested as affecting survival to August 2009, June 2010, August 2010, or July 2011. Model selection using nested models indicated that the model that best fit the data included the factors tested as affecting both survival to 2011 and survival to August 2009 (Table 18; Appendix A). Based on the simplified model, there was evidence for increased overall fitness for between-population vs. within-population crosses (i.e., heterosis) for NWLF and Ness, the two smallest populations in the study. In contrast, there was decreased overall fitness for the between-population vs. within-population crosses (i.e., outbreeding depression) for AA (Figure 12).

Aster models including secondary crosses

To evaluate the effects of maternal population and cross type for the secondary crosses, I used unconditional aster models that excluded the seeds node (Figure 8), since achenes resulting from the secondary crosses were not weighed to assess seed set. When the factors were tested as affecting leaf number in 2011, there was a significant main effect of maternal population and a significant maternal population:cross type interaction; cross type was ns (Table 19). Significant effects of maternal population and the 2-way interaction were also found when the factors were tested as affecting other stages of the life cycle. Using nested models, I determined that the minimal adequate model tested maternal population, cross type and the maternal population:cross type interaction as affecting both the seedling stage (seedlings) and survival to August 2009, plus maternal population tested as affecting either survival to August 2010 or survival to 2011 (Appendix B). As for models based exclusively on the primary crosses, offspring of the between-population cross (SPP pollen) showed greater fitness than those of the within-population cross for NWLF and Ness (i.e., heterosis; Figure 13). The reciprocal cross, with SPP as maternal population and NWLF as pollen donor, also exhibits a trend toward greater fitness for the between-population cross. However, the cross between SPP as maternal population and Lf as pollen donor has lower fitness than the SPP within-population cross (i.e., outbreeding depression; Figure 13). The between-population cross with NWLF pollen shows lower fitness than the within-population cross for AA and SAP.

Discussion

Intra-population and interpopulation crosses can be used to assess fitness differences among populations and the potential for genetic rescue in populations exhibiting genetic decline. The results reported here indicate differential responses to crossing within and between populations of *E. angustifolia*, depending on the maternal and paternal populations involved, and on the stage of the life cycle at which fitness was evaluated. Maternal populations differed in the effect of pollen source on the fitness of the mating, whether the fitness measure included seed set or began with seed germination. Outcrossing effects ranged from 54% outbreeding depression to heterosis as great as 95%.

At the seed set stage, there is evidence for heterosis for the AA-SPP cross but not for the AA-NWLF cross, and a similar trend for the SAP maternal population (Figure 10). Evidence of outbreeding depression was observed for the Ness-SPP, Ness-NWLF and SPP-Lf crosses (Figure 10). Seed set in this experiment ranged from 0 to 0.885 across all cross types. Wagenius (2000) reported a similar range for 130 open-pollinated *E. angustifolia* plants in prairie remnants within the same study area used for this experiment. The large proportion of unfilled achenes (Figure 10) may have resulted in part from failed fertilization due to pollen incompatibility. Another factor that may have affected pollination success in the experiment described here is pollen viability. Pollen used in crosses was sometimes collected one or more days before hand pollinations were attempted.

Turning to overall fitness, evidence for heterosis was noted for the NWLF-SPP cross, but outbreeding depression occurred for the SPP-Lf cross (Figure 11). For Ness and AA, the relationship between intra- and inter-population crosses for seed set is reversed for overall fitness; Ness shows a trend toward heterosis for overall fitness, while AA shows a trend toward outbreeding depression (compare Figures 10 and 11). Clear differences were observed among the inter-population crosses using NWLF, SPP and Lf pollen. The AA-SPP cross had higher seed set and greater overall fitness than the AA-NWLF cross (Figures 10 and 13). In contrast, the SPP-Lf cross had lower seed set and lower overall fitness than the SPP-NWLF cross (Figures 10 and 13).

Aster models that use seeds rather than achenes as the root node illustrate the effects of maternal population and cross type on fitness excluding the role of variation in seed set, which could have been affected by differences in the compatibility or viability of the pollen samples. The trend for outbreeding depression in overall fitness in the SPP-Lf cross is weaker when seed set is excluded (compare Figures 11 and 12), suggesting that the decreased overall fitness for the SPP-Lf vs. the SPP within-population cross is largely the result of low seed set. In contrast, there is evidence of heterosis in seedling emergence and survival for the NWLF-SPP and Ness-SPP interpopulation crosses (Figure 12), independent of seed set effects. For the AA-SPP cross, there is evidence of outbreeding depression for seedling emergence and survival (Figure 12), rather than the heterosis exhibited for seed set (Figure 10). Further evidence that population and cross type affect fitness beyond their effects on seed set is found in the significant effects of these factors on survival to August 2010 or survival to 2011 (Table 17).

Other researchers have found varying levels of heterosis or outbreeding depression among different populations or for different life cycle traits. Waser et al. (2000) report varying responses among offspring of *Ipomopsis aggregata* maternal parents crossed over distances of 1, 10 and 100 meters, with many sibships exhibiting outbreeding depression for the long-distance cross while others displayed heterosis. Crosses among populations of *Silene alba* resulted in heterosis for isolated populations but not for more centrally located populations (Richards 2000). Busch (2006) performed intra- and interpopulation crosses using five large, self-incompatible and three small, self-compatible populations of *Leavenworthia alabamica*. He observed heterosis for one trait for one of the self-compatible populations and outbreeding depression for two other traits for another self-compatible population; heterosis was observed for one trait each for two of the self-incompatible populations. There are several possible explanations for these variable results. Heterosis may result from the masking of deleterious recessive alleles, which could have variable expression at different stages of the life cycle. Populations have different genetic histories; each population is likely to have a different suite of alleles at high frequency, leading to differential responses to between-population crosses. Different populations may also have developed different coadapted gene complexes, which may be disrupted by inter-population hybridizations, resulting in variable expression of outbreeding depression.

Although most of the maternal populations in this study did not serve as pollen donors, there are two reciprocal crosses, the NWLF-SPP and the Lf-SPP crosses. For the Lf-SPP cross, both seed set and overall fitness were similar regardless of the direction of the cross; both seed set and overall fitness were similar to the Lf within-population cross and lower than the SPP within-population cross values (Figures 10 and 13). Seed set for the NWLF-SPP cross was similar regardless of the direction of the cross, and was approximately equal to either within-population cross (Figure 10). In contrast, overall fitness for this cross was higher when SPP rather than NWLF served as the maternal population (Figure 13). The differences in fitness for the SPP-NWLF crosses may be due to maternally inherited genes, which may differ between the two populations.

This study covered a small portion of the life cycle of *E. angustifolia*. Additional evidence of heterosis or outbreeding depression may emerge at later stages of survival, or in reproductive output. Wagenius et al. (2010) document increasing fitness differences among sibling mated, within-population and between-population crosses as later-stage survival and seed production were included in analyses. Moreover, outbreeding depression may be more pronounced in the F2 and subsequent generations than in F1 offspring, due to disruptions of positive epistatic interactions among parental alleles at different loci and decreasing heterozygosity following the F1 generation (Tallmon et al. 2004; Edmands 2007). C. Ridley has begun an experiment to investigate fitness in the F2 for the crosses described in Wagenius et al. (2010).

Maternal population had a significant main effect on seed set (Table 14) and on nearly every stage of the life cycle in aster models (e.g., Tables 15, 16, 19). Systematic effects of maternal environment were minimized by using parents grown in randomized locations within a common garden field plot; thus the significant effect of maternal population is suggestive of genetic differences among populations. There is no clear evidence that maternal population size is positively correlated with seed set or overall fitness for within-population crosses (Figures 10-12). In fact, the highest seed set was exhibited by Ness, one of the 2 smallest populations in the study (Figure 10), while relatively low seed set and overall fitness were exhibited by Lf, the second largest population (Figures 10, 11 and 13). However, the strongest evidence of heterosis was exhibited by NWLF and Ness, the two smallest populations in the study (Figures 11, 12 and 13). Small populations are more likely to suffer from loss of genetic diversity than are the larger populations in the study. Van Treuren et al. (1993) and Hauser and Loeschcke (1994) also found significant differences among maternal populations, but no clear relationship between population size and pollination treatment effects. Other studies (Ouberg and van Treuren 1994; Heschel and Paige 1995; Paland and Schmid 2003; Willi and Fischer 2005; Willi et al. 2007) document heterosis for inter-population crosses involving small populations, but less or no heterosis for large maternal populations.

This experiment was not designed to evaluate the seed bank of *E. angustifolia*. However, the paucity of new seedlings found in the 2010 censuses suggests that recruitment of new seedlings from the seed bank is minimal. This result is consistent with previous results of experimental seedling recruitment in *E. angustifolia* (Wagenius et al. 2012, Dykstra and Shaw 2011).

This experiment contributes to a growing body of literature investigating heterosis and outbreeding depression resulting from intra-specific crosses. It is tempting to suggest that small populations of *E. angustifolia* may be good candidates for genetic rescue, since heterosis was detected in between-population crosses of the two smallest populations in my study. However, the expression of outbreeding depression for some populations at various stages in the life cycle suggests that artificial gene flow should be undertaken with caution. Hedrick and Fredrickson (2010) suggest that genetic rescue should not be attempted unless there is evidence of low fitness of the fragmented population, and the risk of outbreeding depression is low. Further research is needed in this system to evaluate the risk of outbreeding depression in later stages in the life cycle, and in generations beyond the F1.

Table 12. Realized experimental design. Population size and number of individuals used for each cross. Maternal populations designated as Northwest Landfill (NWLF), Nessman (Ness), Aanensen (AA), Steven's Approach (SAP), Landfill (Lf) and Staffanson Prairie Preserve (SPP), listed in order of increasing size. Population sizes are average number of flowering plants observed from 1996-1998 (Wagenius 2000).

Maternal population	Population size	Within-population	Between-population (SPP)	Between-population (Lf)	Between-population (NWLF)
NWLF	13	19	20	–	–
Ness	15	8	10	1	4
AA	25	8	8	–	6
SAP	37	11	13	–	7
Lf	101	20	20	–	6
SPP	2961	16	–	18	12

Table 13. Tests for unconditional aster model comparison for spatial variables. The model formulae are given above and the analysis of deviance below. Deviance is double the log likelihood ratio. *p* values are for the chi-square test of the null hypothesis that a model is not significantly different from the model listed in the line above. The full model included Block, HLRow and HLPos (spatial position of seedlings in the experimental plot at Hegg Lake), CGPos and CGRow (spatial position of maternal plants in their experimental plot), modeled as affecting leaf number in 2011 (lfnum), the final node of the life cycle graph. resp is the response vector; varb is the vector of life cycle nodes, as in Figure 8.

Model 1: resp ~ varb
Model 2: resp ~ varb + (HLRow):lfnum
Model 3: resp ~ varb + (Block + HLRow):lfnum
Model 4: resp ~ varb + (Block + HLRow + HLPos):lfnum
Model 5: resp ~ varb + (Block + HLRow + HLPos + CGPos):lfnum
Model 6: resp ~ varb + (Block + HLRow + HLPos + CGPos + CGRow):lfnum

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	7	21212			
2	8	21204	1	8.0536	0.0045
3	10	21196	2	7.6812	0.0215
4	11	21195	1	1.4989	0.2208
5	12	21194	1	0.9661	0.3256
6	13	21193	1	0.5709	0.4499

Table 14. Likelihood ratio tests for stepwise model simplification of binomial GLM models for seed set, using backward elimination. The model formulae are given above and the analysis of deviance below (deviance is the likelihood ratio test statistic). *p* values are for the *F*-test of the null hypothesis that a given model is not significantly different from the model listed in the previous line. The response term, seedprop, includes both counts of "seeds" (achenes with a mass > 0.002 g) and counts of achenes with mass < 0.002 g (i.e., statistical successes and failures). Predictor variables include cross type (crossclass; 3 levels: within-population, between-population-large population pollen donor or between-population-small population pollen donor) and matpop (maternal population, 6 levels as in Table 12).

Model 1: seedprop ~ 1
Model 2: seedprop ~ crossclass
Model 3: seedprop ~ matpop + crossclass
Model 4: seedprop ~ matpop + crossclass + matpop:crossclass

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	549	3140.8			
2	547	3138.5	2	2.362	0.7780
3	542	2879.5	5	258.940	< 0.0001
4	533	2787.2	9	92.348	0.0219

Table 15. Tests for model comparison of 7-node unconditional aster models evaluating the primary crosses. The factors were tested as affecting leaf number in 2011 (lfnum). The model formulae are given above and the analysis of deviance below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a model is not significantly different from the model in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8), Block and HLRow as in Table 13, and lfnum, matpop and crossclass as in Table 14.

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow + matpop):lfnum
Model 3: resp ~ varb + (Block + HLRow + matpop + crossclass):lfnum
Model 4: resp ~ varb + (Block + HLRow + matpop + crossclass + matpop:crossclass):lfnum

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	10	16087			
2	15	16075	5	12.5066	0.0285
3	16	16075	1	0.2119	0.6453
4	21	16070	5	5.0369	0.4114

Table 16. Tests for model comparison of 7-node unconditional aster models evaluating the primary crosses, with factors tested as affecting the seed stage and survival to 2011 stage (surv2011) of the life cycle. The model formulae are given above and the analysis of deviance below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a model is not significantly different from the model in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8), Block and HLRow as in Table 13, and lfnum, matpop and crossclass as in Table 14.

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + crossclass:seed
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass):seed
Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed
Model 5: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + crossclass:surv2011
Model 6: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + (matpop + crossclass):surv2011
Model 7: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	10	16087			
2	11	16087	1	0.743	0.3887
3	16	15833	5	253.517	< 0.0001
4	21	15755	5	78.100	< 0.0001
5	22	15754	1	0.557	0.4553
6	27	15748	5	6.592	0.2528
7	32	15734	5	13.417	0.0198

Table 17. Tests for aster model comparison of two sets of nested models. The model formulae are given above and the analysis of deviance tables below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a given model is not significantly different from the model listed in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8), Block and HLRow (spatial variables as in Table 13), seed (seeds level of varb), lfnum (leaf number level of varb), surv2011 (survival to July 2011 node of varb), matpop (maternal population, 6 levels as in Table 12) and crossclass (3 levels as in Table 14).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	10	16087			
2	21	15755	11	332.36	< 0.0001
3	32	15734	11	20.57	0.0382

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	10	16087			
2	21	16064	11	23.98	0.0128
3	32	15734	11	328.95	< 0.0001

Table 18. Tests for model comparison of two sets of nested 6-node aster models. The model formulae are given above and the analysis of deviance tables below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a given model is not significantly different from the model listed in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8 but not including the seeds node), Block and HLRow (spatial variables as in Table 13), lfnum (leaf number level of varb), surv2011 (survival to July 2011 node of varb), Aug09 (survival to August 2009 node of varb), matpop (maternal population, 6 levels as in Table 12) and crossclass (3 levels as in Table 14).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2907.1	11	21.268	0.0307
3	31	2885.1	11	22.028	0.0242

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2909.5	11	18.875	0.0634
3	31	2885.1	11	24.422	0.0111

Table 19. Tests for model comparison of aster models evaluating both primary and secondary crosses, with factors tested as affecting leaf number in 2011 (lfnum). The model formulae are given above and the analysis of deviance below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a model is not significantly different from the model in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8 but excluding seeds), Block and HLRow as in Table 13, and lfnum, matpop and crossclass as in Table 14.

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + matpop:lfnum
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass):lfnum
Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):lfnum

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	14	5219.8	5	15.3324	0.0090
3	16	5219.5	2	0.3466	0.8409
4	25	5202.3	9	17.1388	0.0466

$$\begin{tikzcd}
\text{achenes} \arrow[r] & \text{seeds} \arrow[r] & \text{seedlings} \arrow[r] & \text{survAug09} \arrow[r] & \text{survJun10} \arrow[r] & \text{survAug10} \arrow[r] & \text{surv2011} \arrow[d] \\
 & & & & & & \text{lfnum}
\end{tikzcd}$$

Figure 8. Life cycle graph. Stages are achenes (number of achenes sown), seeds (number of achenes with a mass greater than 2 mg), seedlings (number of emerged seedlings in May 2009), survAug09 (surviving seedlings in August 2009), survJun10 (surviving plants in June 2010), survAug10 (surviving plants in August 2010), surv2011 (surviving plants in July 2011) and lfnum (total leaf number of all surviving plants in July 2011).

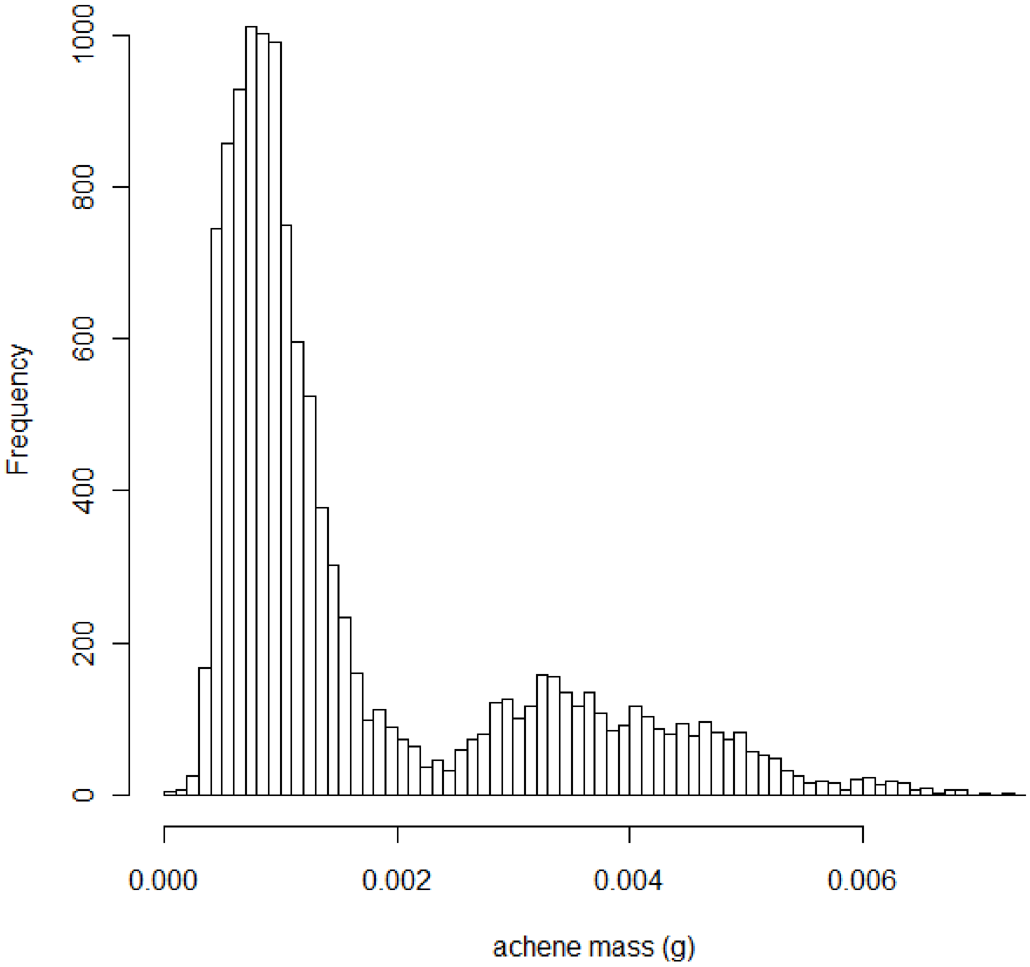

Figure 9. Histogram of achene mass. 3 achenes with mass > 0.008 g were omitted from this histogram.

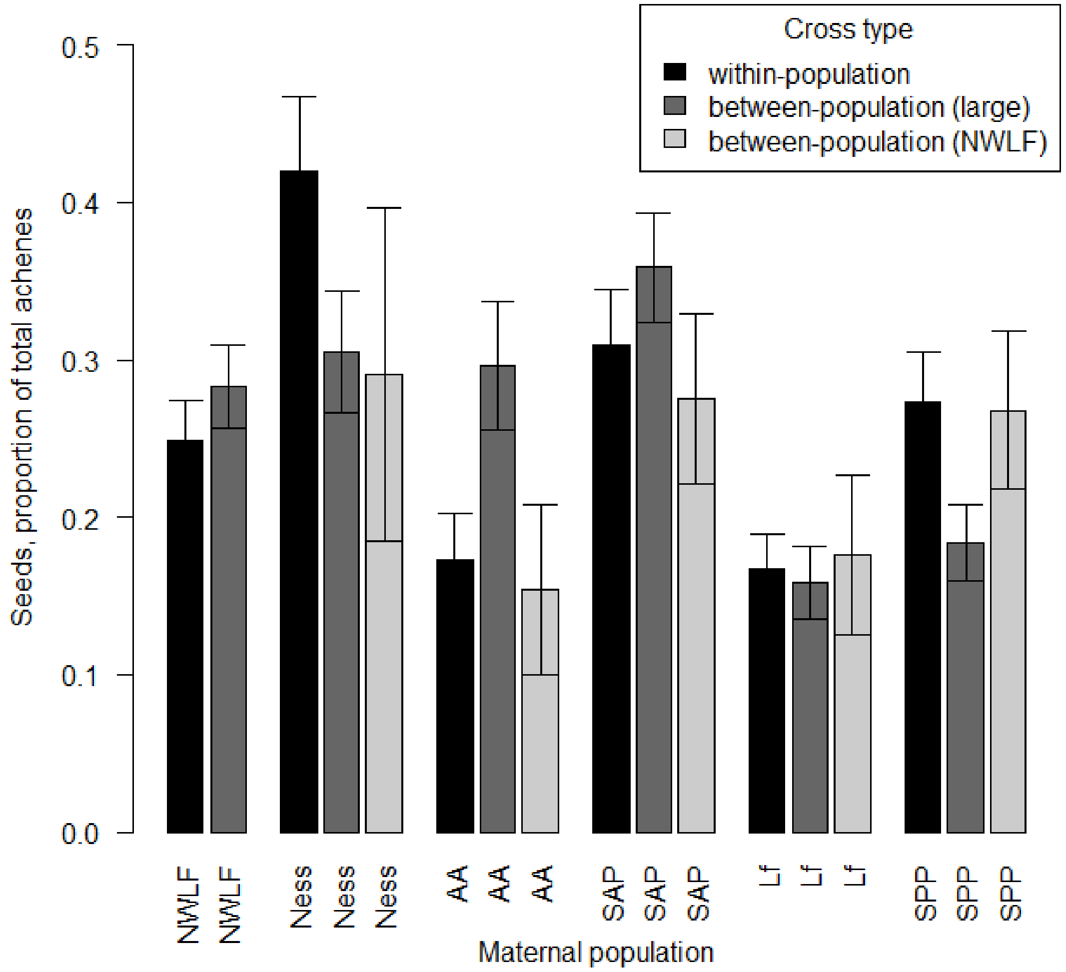

Figure 10. Estimated proportion of seeds (achenes with a mass greater than 2 mg), predicted from the binomial family GLM with maternal population (6 levels as in Table 12), cross type (3 levels: within-population [black bars], between-population using pollen from a large population [dark gray bars], and between-population using a pollen donor from a small population [light gray bars]) and the maternal population:cross type interaction. Maternal populations NWLF, Ness, AA, SAP, Lf and SPP are shown ordered from smallest to largest (left to right) on the x-axis. Lf served as pollen donor for the between-population (large population) cross for the SPP maternal population; SPP was the pollen donor population for all other between-population (large) crosses.

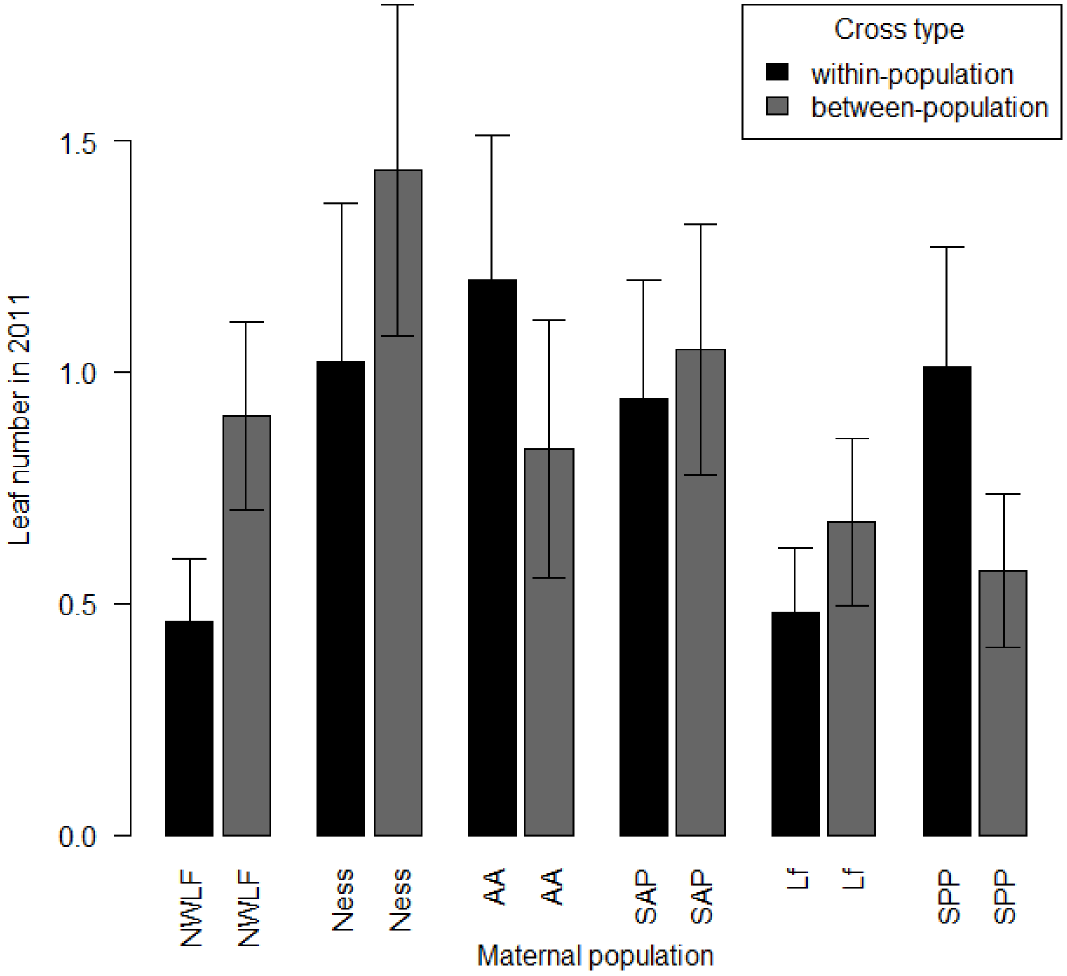

Figure 11. Mean leaf number in July 2011 predicted for 25 achenes sown in fall 2008, based on the aster model with formula resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seed + (matpop + crossclass + matpop:crossclass): surv2011 (see Table 16). Predictions are unconditional, taking into account both seedling emergence and survival. Maternal populations NWLF, Ness, AA, SAP, Lf and SPP are shown ordered from smallest to largest (left to right) on the x-axis. Lf served as pollen donor for the between-population cross for the SPP maternal population; SPP was the pollen donor population for all other between-population crosses.

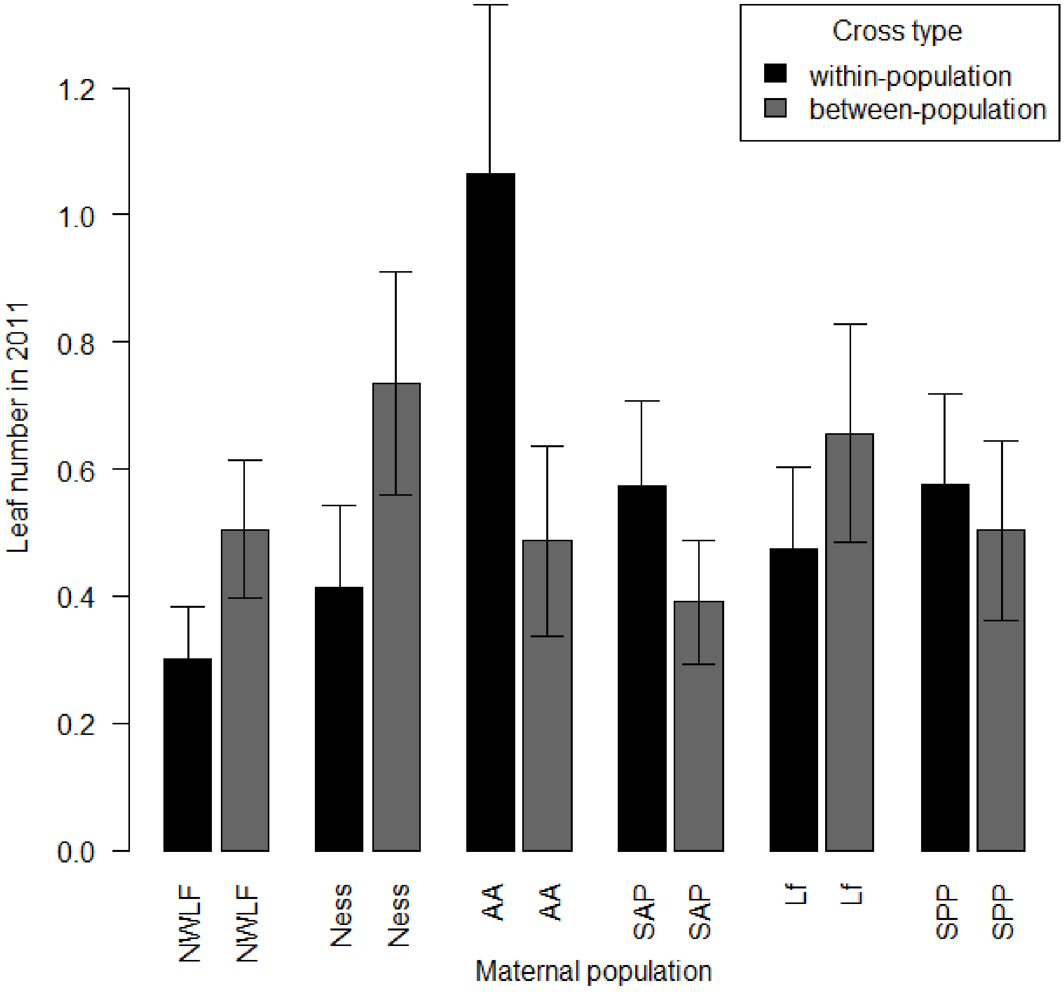

Figure 12. Mean leaf number in July 2011 predicted for 4 seeds sown in fall 2008, based on the 6-node aster model with formula resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + matpop:surv2011 (see Appendix A). Predictions are unconditional, taking into account both seedling emergence and survival. Maternal populations NWLF, Ness, AA, SAP, Lf and SPP are shown ordered from smallest to largest (left to right) on the x-axis. Lf served as pollen donor for the between-population cross for the SPP maternal population; SPP was the pollen donor population for all other between-population crosses.

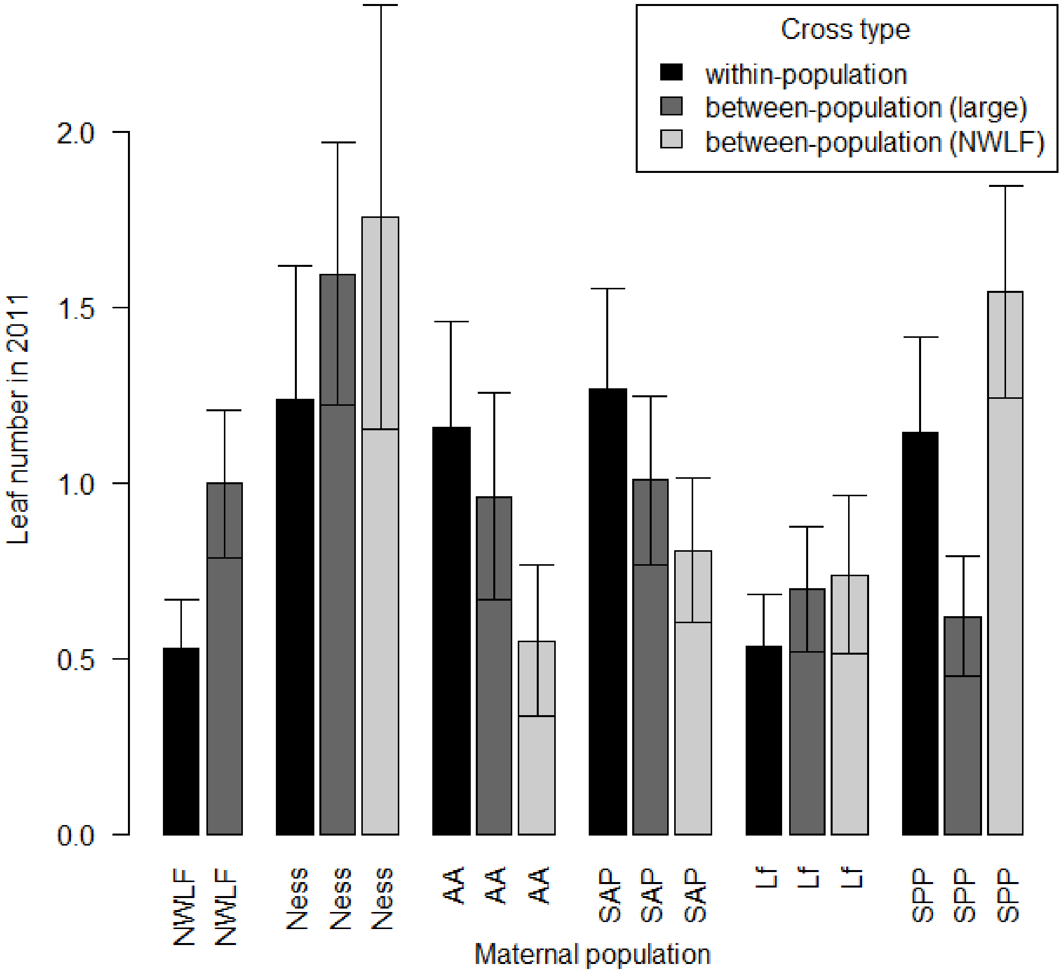

Figure 13. Mean leaf number in 2011 predicted for 25 achenes sown in fall 2008, based on the aster model with formula, resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):seedlings + matpop:surv2011 (Appendix B). Predictions are unconditional, taking into account both seedling emergence and survival through six life cycle stages: seedlings, survAug09, survJun10, survAug09, surv2011 and lfnum (Figure 8). Maternal populations NWLF, Ness, AA, SAP, Lf and SPP are shown ordered from smallest to largest (left to right) on the x-axis. Lf served as pollen donor for the between-

population (large population) cross for the SPP maternal population; SPP was the pollen donor population for all other between-population (large) crosses.

Chapter 3. Local adaptation in seedling recruitment of narrow-leaved purple coneflower.

Restoration guidelines often call for locally sourced seed, to reduce the likelihood of introducing maladapted genotypes and to avoid outcrossing depression. However, few empirical studies focus on the degree of local adaptation of native plants. To evaluate local adaptation in narrow-leaved purple coneflower (*Echinacea angustifolia*), a native perennial commonly used in prairie restorations, seeds were collected at three sites along a 500-km transect from northwestern South Dakota to west-central Minnesota. Collecting from locations at the same latitude allowed sampling of populations along a moisture gradient, while controlling for daylength and mean annual temperature. The seeds were reciprocally sown into prairie restorations located near each source population. Seedling emergence was lowest in the western South Dakota plot and highest in the Minnesota plot for all three source populations, corresponding to expectations given the moisture gradient. Moreover, seedling emergence was higher for the western South Dakota and Minnesota seeds than for the central South Dakota seeds in all three experimental plots. Survival to the end of the first growing season ranged from <50% at the central South Dakota plot to >80% in western South Dakota. Survival was highest for western South Dakota seedlings and lowest for Minnesota seedlings at all three experimental sites. No evidence for local adaptation was observed at this early stage in the life cycle of *E. angustifolia*, though local adaptation may become evident at later life stages.

Introduction

Human activities (e.g., logging of forests, conversion of land to agricultural use, urbanization) have resulted in widespread conversion of terrestrial ecosystems. Temperate grasslands and savannas have been identified as a critically endangered biome due to extensive habitat conversion accompanied by limited habitat protection (Hoekstra et al. 2005). Less than 5% of the original habitat remains in the northern and central tall-grass prairies of North America (Samson et al. 2004). In response to this extensive habitat conversion, there is a need to conserve extant prairie, and there is increasing interest in restoring native habitat (Anderson 2009).

Restoration guidelines often recommend using local germplasm. For example, entities using funding from Minnesota's Outdoor Heritage Fund for restoration projects are directed to "plant vegetation or sow seed only of ecotypes native to Minnesota, and preferably of the local ecotype, using a high diversity of species originating from as close to the restoration site as possible" (Laws of Minnesota 2009). The rationale for using local seed is to introduce plants that are well adapted to local environmental conditions, and to avoid genetic contamination of extant native populations. Reciprocal transplant studies have shown that plant populations are often adapted to local environmental conditions (Rehfeldt et al. 1999, Etterson 2004, Hufford et al. 2008), suggesting that establishment or persistence of individuals translocated outside their ranges may be reduced, and populations that do become established may have reduced fitness compared to populations growing in regions to which they are adapted. In a recent review of reciprocal transplant studies, Hereford (2009) found an overall frequency for local adaptation (defined as equal or higher fitness of the local population compared to foreign populations) of 0.71. Similarly, Leimu and Fischer (2008) found that plants have a homesite advantage in 71% of cases analyzed. However, in only about 45% of population pairs did performance of the local population exceed that of the foreign one at both sites in a reciprocal transplant (Leimu and Fischer 2008). Additionally, nearby populations in fragmented habitat may not contain sufficient genetic diversity for successful reintroductions or to allow for evolutionary response to changing climatic conditions (Broadhurst et al. 2008).

We report on a reciprocal transplant experiment designed to test whether *Echinacea angustifolia* (narrow-leaved purple coneflower) exhibits local adaptation. *E. angustifolia* is a perennial native to the tall and mixed grass prairies of North America. *E. angustifolia* is representative of many native prairie species in that it is long-lived, reproduces by seed, has limited seed dispersal, is pollinated by generalist insects, and is self-incompatible (Wagenius 2000, 2004; Ault 2006). It is commonly used in restorations.

Genetic differentiation both within and among populations of *E. angustifolia* has been demonstrated using isozyme markers (Baskauf et al. 1994), random amplified polymorphic DNA (RAPD) markers (Kapteyn et al. 2002) and amplified fragment length polymorphism (AFLP) markers (Kim et al. 2004, Still et al. 2005). In the latter study, Still et al. (2005) detected increased genetic divergence with geographic distance for *E. angustifolia* populations located along a 1500-km north-south transect from North Dakota to Oklahoma. In spite of this molecular evidence of genetic differentiation, it is not known whether adaptive differentiation has occured, since differentiation in neutral markers is not well correlated with variation in selectively important traits (Reed and Frankham 2001, McKay and Latta 2002).

Methods

Experimental Design

Seeds were collected from native *Echinacea angustifolia* populations in three locations along an east-west line. Source populations were located in the Grand River National Grassland in western South Dakota (latitude 45° 40' N, longitude 102° 10' W), and The Nature Conservancy's Samuel H. Ordway Prairie in central South Dakota (45° 43' N, 99° 5' W) and Staffanson Prairie in western Minnesota (45° 45' N, 95° 44' W). The aim was to sample along an aridity gradient, while minimizing temperature and daylength variation that would accompany a north-south transect. Average monthly temperature maxima and minima are similar for the three collection sites. Average annual precipitation is approximately 40 cm for the western South Dakota site, 50 cm for the central South Dakota site and 62 cm for the Minnesota site, based on data from the High Plains Regional Climate Center, University of Nebraska, Lincoln (http://www.hprcc.unl.edu/).

Following the protocols suggested by the Seeds of Success program of the Chicago Botanic Garden (http://www.cbgseedbank.org/), we collected capitula (seed heads) from 50 randomly selected individuals of each population. Mature capitula were collected near the end of the growing season (September 2008), and processed in the lab. Achenes from each individual were counted and divided into six lots. *E. angustifolia* capitula contain from 0 to more than 200 achenes (personal observation); achene lots consisted of 0 to more than 30 achenes. A total of 21,678 achenes were sown. One achene lot from each capitulum was sown into each of two blocks in experimental plots located near each collection site. In each block, achene lots were sown into random locations within a grid. Thus, each of the three experimental plots consists of two blocks, each with 150 sowing positions (six rows, each with 25 positions at 0.5 m intervals). Achenes were scattered in a line directly west of each sowing position. Small brooms were used to brush away litter so that achenes could be placed directly on the soil. Experimental plots were sown in November 2008; sowing in the fall allowed for cold stratification *in situ*, and simulated natural conditions for seed germination (Ault 2006).

The experimental plots are located in the Grand River National Grassland (45° 40' N, 102° 10' W, managed by the US Forest Service), the Perch Lake Waterfowl Production Area (45° 38' N, 99° 12' W, managed by the South Dakota Department of Environment and Natural Resources) and the Hegg Lake Wildlife Management Area (45° 47' N, 95° 40' W, managed by the Minnesota Department of Natural Resources). All three plots are on previously tilled land that has been restored to grassland. The predominant grass at Hegg Lake and Perch Lake is smooth brome (*Bromis inermis*), while crested wheat grass (*Agropyron cristatum*) dominates at the Grand River site. The experimental plots at Grand River and Hegg Lake had been burned in spring 2008; these two plots had experienced one season of re-growth prior to our fall 2008 sowing. The plot at Perch Lake had taller grass and more litter than the other two plots.

In May 2009, all seedlings that emerged were counted and their positions marked with toothpicks. In August 2009, a second census was performed, to assess seedling survival. A third search was performed in May 2010 to census the survivors and identify new germinants. In each survival census, we counted all plants present, including those with wilted or dead leaves.

The central South Dakota experimental plot was inadvertently sprayed with a combination of Tordon® and Telar® herbicides on August 4, 2009. We performed the second census on this plot on August 9-10, 2009. Although some of the *E. angustifolia* plants were beginning to wilt, the second census likely captured seedling survival up until the point of herbicide application.

Data Analysis

We analyzed the proportion of achenes sown that emerged as seedlings, using a binomial family generalized linear model (GLM), implemented in R (R Development Core Team 2009), with seed source and sowing site as predictors. The residual deviance of the full model exceeded the residual degrees of freedom, indicating overdispersion; therefore, the quasibinomial family was used for the analysis (Crawley 2007). Emergence as a proportion of achenes sown was estimated from the simplest model found to adequately fit the data. As an alternative approach, we performed a mixed effects GLM, using the R function glmer in package lme4, to account for the spatial structure in the experimental design. Block was designated as a random effect, while seed source and sow site were fixed effects. The results of the mixed effects models were qualitatively similar to the GLM models, and we here report only the results from the GLM using the quasibinomial.

Seedling survival was analyzed with a binomial family GLM, using seed source and sowing site as predictors. As in the emergence analysis, the residuals indicated overdispersion; accordingly, the quasibinomial family was used for further analysis. Estimates of the proportion of emerged seedlings that survived to late in the first growing season, and to the second season, were estimated from the simplest models that adequately fit the data. A mixed effects GLM was also performed, with qualitatively similar results to the GLM models. We here report only the results from the GLM using the quasibinomial.

Results

Seedling emergence was low (less than 15%) at all three sowing sites for all three source populations (Figure 14). There was no significant seed source × sow site interaction, but the main effects of seed source and sow site were highly significant (Table 20). Emergence was lower for the central South Dakota seeds than for the western South Dakota or Minnesota seeds at all three experimental plots (Figure 14). Seeds from all three sources had the highest emergence at the Minnesota site and the lowest emergence at the western South Dakota site (Figure 14). A small number of new seedlings (six at the Minnesota plot and nine at the central South Dakota plot) had emerged in spring 2010. The total number of new seedlings represented only 0.12% of the total number of achenes sown.

Survival over the first growing season (through August 2009) ranged from approximately 50% at the central South Dakota plot to approximately 80% at the western South Dakota site. The seed source × sow site interaction was ns, but main effects of seed source and sow site were significant (Table 21). The significant source effect reflects a higher survival rate for western South Dakota-sourced seedlings and a lower survival for the Minnesota-sourced seedlings at all three sowing sites (Figure 15). The significant sow site effect reflected a much lower survival in the central South Dakota site compared to the western South Dakota and Minnesota sites.

For the analysis of survival to the second growing season (through May 2010), there was no significant seed source × sow site interaction. The main effect of seed source was also ns, but the main effect of sow site was highly significant ($P < 0.0001$), reflecting the fact that there were no surviving seedlings at the central South Dakota experimental plot, while survival at the Minnesota and western South Dakota plots was relatively high. When the central South Dakota plot was excluded from the analysis, the main effects of seed source and sow site were both ns, as was the two-way interaction term. Excluding the central South Dakota plot, the mean proportion of 2009 seedlings that survived to May 2010 was 0.75.

Discussion

Our results do not indicate that the *Echinacea angustifolia* populations we sampled are locally adapted, as measured by seedling emergence or early seedling survival. Evidence of local adaptation may emerge as longer-term survival and fecundity are considered. The magnitude of local adaptation tends to be smaller when fitness is measured as viability than when fitness is measured as fecundity or a composite of viability and fecundity (Hereford 2009).

The significant effect of seed source observed in both emergence and survival could reflect genetic differences among the populations sampled. Alternatively, maternal environmental effects may have influenced the emergence or early survival of the *E. angustifolia* seedlings (Roach and Wulff 1987). In particular, the low seedling emergence for the central South Dakota population relative to the other two populations may be at least partially attributable to collection of immature seeds of the central South Dakota population. We noted during collections that at least three of the central South Dakota seed heads were collected from green stems. The *E. angustifolia* seed heads matured earlier in western South Dakota than in central South Dakota or Minnesota in 2008 (personal observation). Collections were made on September 6 at the western South Dakota site, September 7 at the central South Dakota site, and September 20 at the Minnesota site. Since maternal effects often decrease at later life stages (Roach and Wulff 1987, Zeiter and Stampfli 2008), a signal of local adaptation may become evident when long-term fitness is assessed.

For all three source populations, seedling emergence was lowest in western South Dakota and highest in Minnesota (Figure 14). The increase in emergence from west to east did not seem to be directly attributable to the precipitation gradient; total precipitation from the time of seed sowing until the first seedling census (December 2008-May 2009) was approximately equal at the western South Dakota (20 cm) and central South Dakota (21 cm) sites, and lower at the Minnesota site (12 cm). Seeds were exposed to a suite of biotic and abiotic environmental conditions at each site (e.g., diversity and abundance of herbivores and competitors, soil characteristics, snow cover, relative humidity, etc.). The sowing sites were selected as representative of potential restoration sites as close to the seed source populations as possible, with the aim of testing for adaptation to the entire suite of local conditions.

Survival over the first growing season was lower in the central South Dakota site than in western South Dakota or Minnesota (Figure 15). This reduced survival in central South Dakota may have been due to higher competition for light or other resources at that site relative to the western South Dakota and Minnesota sites, which had been burned in the spring prior to the fall sowing, reducing grass cover compared to the central South Dakota site (personal observation). Our results agree well with a 10-year recruitment experiment (Wagenius et al. 2012) showing that survival of *E. angustifolia* seedlings was enhanced by burn treatments.

The western South Dakota population had the highest survival and the Minnesota population had the lowest survival at all three sites over the first growing season (Figure 15). However, this trend was not observed in survival to the second growing season, as all three populations had equally high proportion surviving (mean 0.75).

The initial analysis of survival to the second growing season indicated a significant main effect of sowing site, driven entirely by the mortality of all *E. angustifolia* seedlings at the central South Dakota site, which was sprayed with herbicide in August 2009. When the central South Dakota site was removed from the analysis, the main effect of seed source was no longer significant, indicating that there were no significant differences in survival among the 3 source populations or between the 2 remaining sites.

Although this study did not provide evidence of local adaptation in *E. angustifolia*, we do not suggest that seed provenance is an unimportant consideration for restoration. As mentioned above, non-local populations may have reduced fecundity or long-term viability compared to local plants. Additionally, crosses between local and non-local genotypes may result in out-crossing depression (Hufford and Mazer 2003, Cremieux et al. 2010). Wagenius et al. (2010) performed between-population and within-population crosses for remnant *E. angustifolia* populations in Douglas County, MN. They report a non-significant decrease in fitness for the between-population crosses compared to the within-population crosses. Between-population crosses for more distant populations may exhibit more severe out-crossing depression.

Table 20. Likelihood ratio tests for step-wise model simplification using backward elimination for the seedling emergence analysis. Deviance is the likelihood ratio test statistic. P values are for the F-test of the null hypothesis that a model simplified by removing the listed interaction or factor is not significantly different from the model including the listed term. The full model included main effects of seed source and sow site as well as the seed source × sow site interaction. After model simplification, the minimal adequate model included main effects of seed source and sow site.

Interaction or factor	*df*	Deviance	*P*
seed source × sow site	4	5.88	0.7263
seed source	2	314.4	<0.0001
sow site	2	256.84	<0.0001

Table 21. Likelihood ratio tests for step-wise model simplification using backward elimination for the analysis of seedling survival to the end of the first growing season. Deviance is the likelihood ratio test statistic. P values are for the F-test of the null hypothesis that a model simplified by removing the listed interaction or factor is not significantly different from the model including the listed term. The full model included main effects of seed source and sow site as well as the seed source × sow site interaction. After model simplification, the minimal adequate model included main effects of seed source and sow site.

Interaction or factor	*df*	Deviance	*P*
seed source × sow site	4	4.93	0.5388
seed source	2	9.99	0.04391
sow site	2	132.98	<0.0001

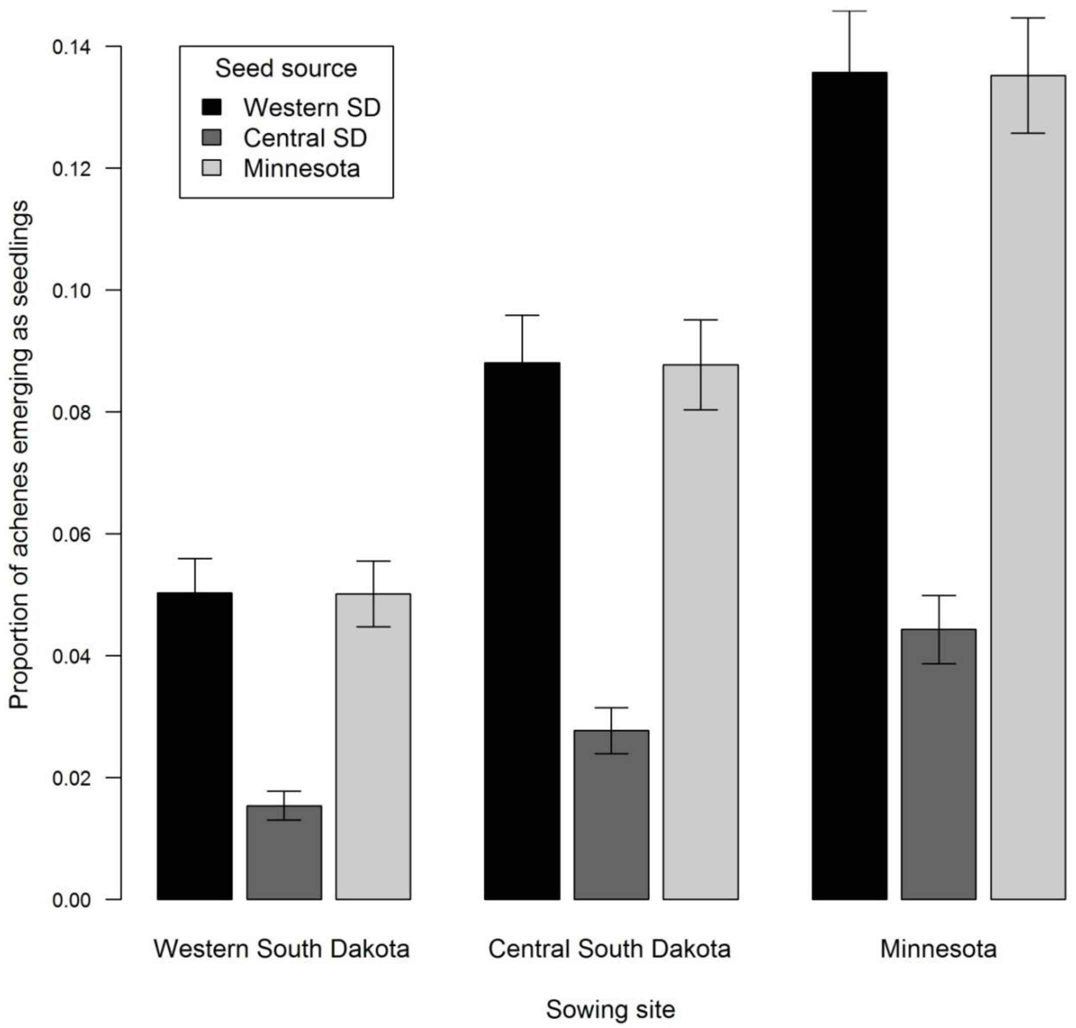

Figure 14. Mean (+/- 1 SE) proportion of *Echinacea angustifolia* achenes sown in fall 2008 that emerged as seedlings in spring 2009. Means were predicted from a binomial family generalized linear model that included main effects of seed source and sowing site. Seeds (achenes) were collected from the Grand River National Grassland in western South Dakota (“Western SD”), Samuel H. Ordway Prairie in central South Dakota (“Central SD”) and Staffanson Prairie in western Minnesota (“Minnesota”). Sowing sites were located in the Grand River National Grassland (“Western South Dakota”), Perch Lake Waterfowl Production Area (“Central South Dakota”) and Hegg Lake Wildlife Management Area (“Minnesota”).

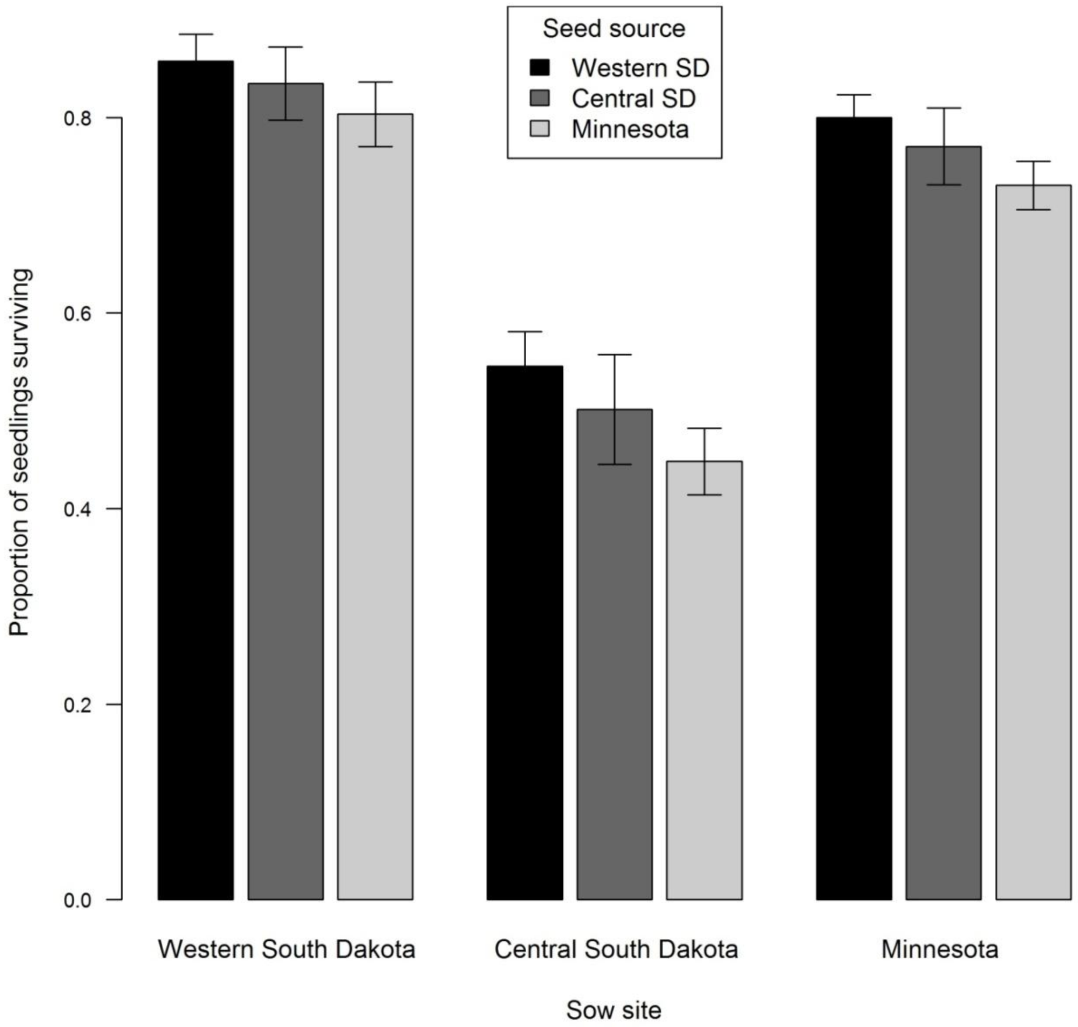

Figure 15. Mean (+/- 1 SE) proportion of seedlings that emerged in 2009 surviving until late summer 2009. Means were predicted from a generalized linear model that included main effects of seed source and sow site. Seeds were collected from the Grand River National Grassland in northwestern South Dakota (“Western SD”), Samuel H. Ordway Prairie in central South Dakota (“Central SD”) and Staffanson Prairie in western Minnesota (“Minnesota”). Sow sites were located in the Grand River National Grassland (“Western South Dakota”), Perch Lake Waterfowl Production Area (“Central South Dakota”) and Hegg Lake Wildlife Management Area (“Minnesota”).

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Appendix A. Model selection for 6-node aster models, primary crosses

I used two sets of nested models in addition to Table 18. For each set, the model formulae are given above and the analysis of deviance tables below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a given model is not significantly different from the model listed in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8 but not including the seeds node), Block and HLRow (spatial variables as in Table 13), lfnum (leaf number level of varb), surv2011 (survival to July 2011 node of varb), Jun10 (survival to June 2010), Aug10 (survival to August 2010) matpop (maternal population, 6 levels as in Table 1) and crossclass (3 levels as in Table 14).

Table A1. Set 1

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Jun10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2907.1	11	21.268	0.0307
3	31	2889.6	11	17.532	0.0931

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Jun10
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Jun10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2909.6	11	18.780	0.0652
3	31	2889.6	11	20.021	0.0451

Set 1 shows that testing the terms as affecting survival to June 2010 (Jun10) does not significantly improve a null model or the model with the terms tested as affecting survival to 2011 (surv2011).

Table A2. Set 2

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2907.1	11	21.268	0.0307
3	31	2896.8	11	10.319	0.5020

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug10
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	20	2908.8	11	19.630	0.0507
3	31	2896.8	11	11.957	0.3669

Set 2 indicates that testing the factors as affecting survival to August 2010 (Aug10) only marginally improves the null model, and does not significantly improve a model with the terms tested as affecting survival to 2011 (surv2011).

Model simplification

After selecting a model that tested the terms as affecting both survival to 2011 and survival to August 2009, I performed model simplification by removing terms one-by-one from the largest model. This process resulted in the selection of two alternative models, Model 5 in Table A3 and Model 5 in Table A4. Since these two models are not nested, their statistical significance cannot be compared using the *anova* command in R. I suggest that these 2 models are approximately equivalent, statistically. Predicted values for both of these models were qualitatively similar (Figure 12 and Figure A1).

Table A3.

Model 1: resp ~ varb + (Block + HLRow):lfnum

Model 2: resp ~ varb + (Block + HLRow):lfnum + matpop:surv2011

Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass):surv2011

Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011

Model 5: resp ~ varb + (Block + HLRow):lfnum + matpop:Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model 6: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass):Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model 7: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	14	2921.8	5	6.5870	0.2532
3	15	2921.2	1	0.6041	0.4370
4	20	2907.1	5	14.0772	0.0151
5	25	2893.2	5	13.9178	0.0161
6	26	2892.1	1	1.0914	0.2962
7	31	2885.1	5	7.0191	0.2192

Table A4.

Model 1: resp ~ varb + (Block + HLRow):lfnum

Model 2: resp ~ varb + (Block + HLRow):lfnum + matpop:Aug09

Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass):Aug09

Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09

Model 5: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + matpop:surv2011

Model 6: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + (matpop + crossclass):surv2011

Model 7: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):Aug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	2928.4			
2	14	2924.1	5	4.2664	0.5117
3	15	2924.1	1	0.0692	0.7925
4	20	2909.5	5	14.5389	0.0125
5	25	2893.3	5	16.2646	0.0061
6	26	2891.7	1	1.5609	0.2115
7	31	2885.1	5	6.5967	0.2524

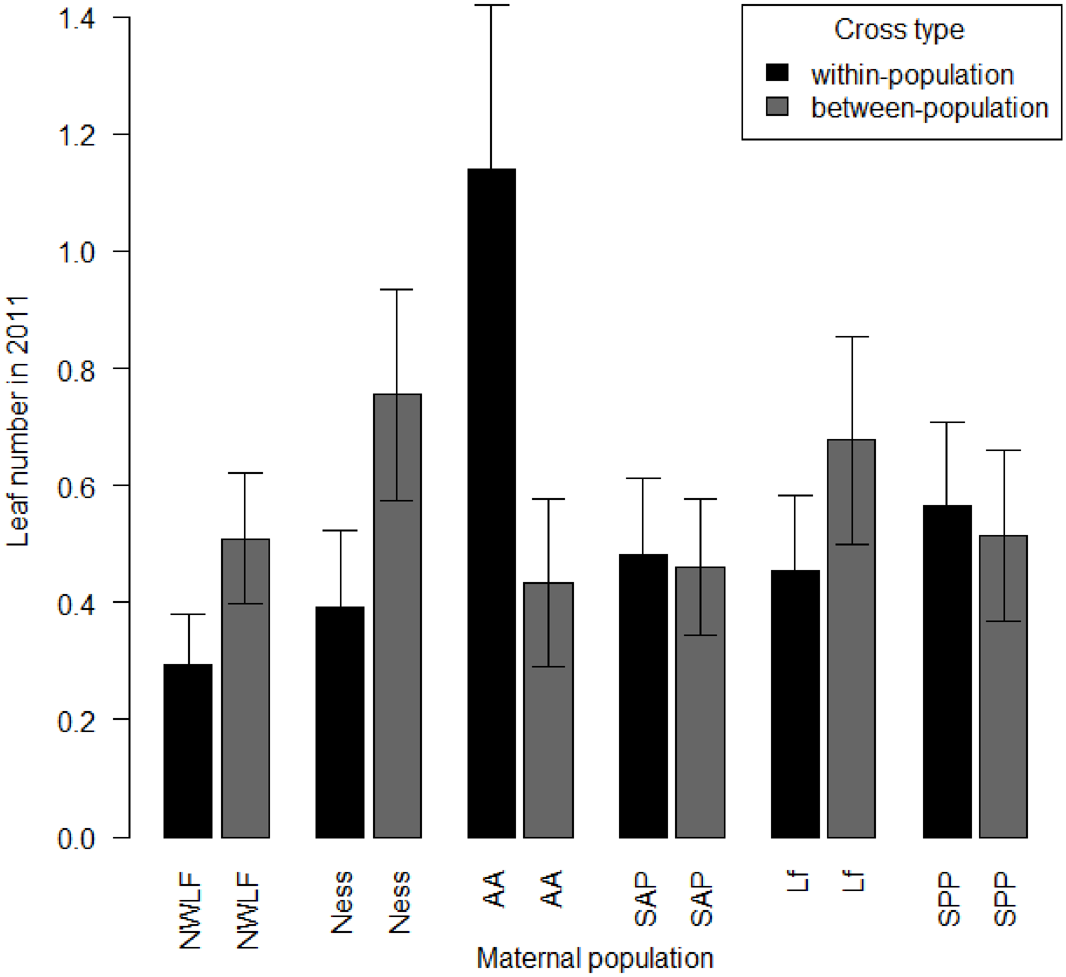

Figure A1. Mean leaf number in July 2011 predicted for 4 seeds sown in fall 2008, based on the 6-node aster model with formula resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011 + matpop:Aug09 (see Table A3). Predictions are unconditional, taking into account both seedling emergence and survival. Maternal populations NWLF, Ness, AA, SAP, Lf and SPP are shown ordered from smallest to largest (left to right) on the x-axis.

Appendix B. Model selection for aster models including primary and secondary crosses

I used sets of nested models to compare models testing the factors as affecting different stages of the life cycle. For each set, the model formulae are given above and the analysis of deviance tables below (deviance is twice log likelihood). *p* values are for the chi-square test of the null hypothesis that a given model is not significantly different from the model listed in the preceding line. Terms include resp (response vector), varb (vector of life cycle nodes as shown in Figure 8 but excluding the seeds node), Block and HLRow (spatial variables as in Table 13), seedlings, survAug09, survJun10, survAug10, surv2011 and lfnum (life cycle stages as in Figure 8), matpop (maternal population, 6 levels as in Table 1) and crossclass (3 levels as in Table 14).

The first set of models (Table B1) indicates that a model testing the factors as affecting both seedlings and leaf number in 2011 (lfnum) is not significantly different from a model that tests the factors as affecting seedlings only. In contrast, testing the factors as affecting survival to August 2009 (survAug09) and seedlings significantly improves the model testing the factors as affecting seedlings only (Table B2). Testing the factors as affecting survival to June 2010 (survJun10) and survival to August 2009 does not significantly improve a model testing the factors as affecting survival to August 2009 only (Table B3). Testing the factors as affecting survival to August 2010 (survAug10) and survival to August 2009 significantly improves a model testing the factors as affecting survival to August 2009 only (Table B4). Similarly, testing the factors as affecting survival to August 2011 (surv2011) and survival to August 2009 significantly improves the model testing the factors as affecting survival to August 2009 only (Table B5). Based on these sets of nested models, it is apparent that a minimal adequate model must test the effects of the factors as affecting some combination of seedlings, survival to August 2009, survAug10 and survival to 2011.

The set of models shown in Table B6 indicates that adding the factors tested as affecting survival to 2011 does not significantly improve the model testing them as affecting seedlings, survival to August 2009, and survAug10; similarly, adding the factors tested as affecting survAug10 does not significantly improve the model testing them as affecting seedlings, survival to August 2009, and survival to 2011. Thus the minimal adequate model should test the factors as affecting seedlings and survival to August 2009, and either survAug10 or survival to 2011. Model simplification results in two alternative models (Table B7). Neither model may be further simplified without significant loss of information. Predicted values for the two models are qualitatively and quantitatively similar; I here only present predicted values for the model testing the three factors as affecting both seedlings and survival to August 2009 and maternal population as affecting survival to 2011 (Figure 13).

Table B1. Set of models testing the factors as affecting seedlings and leaf number in 2011 (lfnum).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):lfnum
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):lfnum + (matpop + crossclass + matpop:crossclass):seedlings

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5202.3	16	32.818	0.0078
3	41	5169.7	16	32.600	0.0083

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):lfnum + (matpop + crossclass + matpop:crossclass):seedlings

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5187.0	16	48.135	< 0.0001
3	41	5169.7	16	17.283	0.3675

Table B2. Set of models testing the factors as affecting seedlings and survival to August 2009 (survAug09).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):seedlings

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5190.2	16	44.890	0.0001
3	41	5156.1	16	34.136	0.0052

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):seedlings

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5187.0	16	48.135	<0.0001
3	41	5156.1	16	30.891	0.0139

Table B3. Set of models testing the factors as affecting survival to August 2009 (survAug09) and survival to June 2010 (survJun10).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survJun10

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5190.2	16	44.890	0.0001
3	41	5167.1	16	23.162	0.1095

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survJun10
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survJun10

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5192.3	16	42.801	0.0003
3	41	5167.1	16	25.251	0.0655

Table B4. Set of models testing the factors as affecting survival to August 2009 (survAug09) and survival to August 2010 (survAug10).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5190.2	16	44.890	0.0001
3	41	5161.1	16	29.113	0.0232

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug10
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5193.8	16	41.356	0.0005
3	41	5161.1	16	32.647	0.0082

Table B5 Set of models testing the factors as affecting survival to August 2009 (survAug09) and survival to 2011 (surv2011).

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5190.2	16	44.890	0.0001
3	41	5156.3	16	33.923	0.0056

Model 1: resp ~ varb + (Block + HLRow):lfnum
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	9	5235.1			
2	25	5193.8	16	41.332	0.0005
3	41	5156.3	16	37.481	0.0018

Table B6. Sets of models testing the factors as affecting seedlings, survival to August 2009 (survAug09), survival to August 2010 (survAug10), and survival to 2011 (surv2011).

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10

Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	57	5127			
2	73	5109	16	18.025	0.3224

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	57	5122.2			
2	73	5109.0	16	13.215	0.657

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	57	5143.1			
2	73	5109.0	16	34.136	0.0052

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):Aug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	57	5136.4			
2	73	5109.0	16	27.407	0.0372

Table B7. Comparisons of models testing the factors as affecting both seedlings and survival to August 2009 (survAug09), and as affecting either survival to August 2010 (survAug10; above) or survival to 2011 (surv2011; below)

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop):survAug10
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass):survAug10
Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):survAug10

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	41	5156.1			
2	46	5141.7	5	14.367	0.0134
3	48	5141.5	2	0.286	0.8668
4	57	5127.0	9	14.460	0.1069

Model 1: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09
Model 2: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop):surv2011
Model 3: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass):surv2011
Model 4: resp ~ varb + (Block + HLRow):lfnum + (matpop + crossclass + matpop:crossclass):seedlings + (matpop + crossclass + matpop:crossclass):survAug09 + (matpop + crossclass + matpop:crossclass):surv2011

Model	Model df	Model Deviance	Test df	Test Deviance	Test *p*-value
1	41	5156.1			
2	46	5138.7	5	17.3737	0.0038
3	48	5136.9	2	1.8246	0.4016
4	57	5122.2	9	14.7249	0.0988