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BIPARENTAL INBREEDING AND INTERREMNANT MATING IN A PERENNIAL PRAIRIE PLANT: FITNESS CONSEQUENCES FOR PROGENY IN THEIR FIRST EIGHT YEARS

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Despite fundamental importance to population dynamics, mating system evolution, and conservation management, the fitness consequences of breeding patterns in natural settings are rarely directly and rigorously evaluated. We experimentally crossed *Echinacea angustifolia*, a widespread, perennial prairie plant undergoing radical changes in distribution and abundance due to habitat fragmentation. We quantified the effects of both biparental inbreeding and crossing between remnant populations on progeny survival and reproduction in the field over the first eight years. Lifetime fitness is notoriously difficult to assess particularly for iteroparous species because of the long sequence and episodic nature of selection events. Even with fitness data in hand, analysis is typically plagued by nonnormal distributions of overall fitness that violate the assumptions of the usual parametric statistical approaches. We applied aster modeling, which integrates the measurements of separate, sequential, nonnormally distributed annual fitness components, and estimated current biparental inbreeding depression at 68% in progeny of sibling-mating. The effect of between-remnant crossing on fitness was negligible. Given that relatedness among individuals in remnant populations is already high and dispersal very limited, inbreeding depression may profoundly affect future dynamics and persistence of these populations, as well as their genetic composition.

KEY WORDS: **Aster life-history analysis, *Echinacea angustifolia*, genetic rescue, habitat fragmentation, inbreeding depression, outbreeding depression, restoration, self-incompatibility, tallgrass prairie.**

Inbreeding and its impairment of fitness (inbreeding depression) can influence both population dynamics and mating system evolution (Charlesworth and Charlesworth 1987; Uyenoyama et al. 1993; Byers and Waller 1999; Keller and Waller 2002). Inbreeding may assume special significance in recently fragmented landscapes, where once-continuous native populations have been dissected into physically disconnected population remnants. Increasing genetic drift resulting from reduced population size can decrease genetic diversity and heterozygosity (Hartl and Clark 1989; Whitlock et al. 2000; Glémin et al. 2003). Studies that correlate fitness with population size or genetic diversity suggest that inbreeding depression has resulted from increased drift in smaller populations (Ouborg and Van Treuren 1994; Heschel and Paige 1995; Paland and Schmid 2003). Beyond this, habitat fragmentation alters mating patterns, especially in immobile plants, by changing the number and spatial relationships of individuals within populations, the availability of mates, the composition of pollinator communities, and pollinator behavior (Rathcke and Jules 1993; Mustajarvi et al. 2001; Wagenius et al. 2007). These changes may increase the degree of relatedness between mates and thus the expression of inbreeding depression (Levin and Kerster 1968; Keller and Waller 2002). Inbreeding effects on viability and fecundity can directly affect population dynamics (reviewed by Crnokrak and Roff 1999; Keller and Waller 2002), and evidence also indicates that natural levels of inbreeding depression can ultimately influence population persistence—a major conservation concern (Mills and Smouse 1994; Newman and Pilson 1997; Saccheri et al. 1998; Oostermeijer 2000; Brook et al. 2002).

In contrast to the abundance of data on inbreeding and its consequences, the consequences of more genetically or spatially distant crossing, for instance between populations, have been less well studied, and outcomes vary strikingly. Concordant with the finding that inbreeding tends to reduce individual fitness and the expectation that members of a single small population may be closely related, some studies have demonstrated heterosis, with fitness of progeny from interpopulation mating exceeding that of progeny from random mating within populations (e.g., Richards 2000; Ebert et al. 2002; Luijten et al. 2002; Paland and Schmid 2003). Other studies, however, have documented outbreeding depression, that is, reduction in seed production or offspring fitness with crossing at various distances, even down to 10 m (e.g., Waser and Price 1994; Edmands 1999; Montalvo and Ellstrand 2001; Quilichini et al. 2001; Heiser and Shaw 2006).

Outbreeding depression can impact population dynamics or population persistence, whether it is due to disruption of epistatic gene expression or due to dilution of locally adapted genotypes (Hufford and Mazer 2003; Tallmon et al. 2004). Conversely, matings between populations can contribute disproportionately to recruitment and, hence, can result in a population "rescue effect" (Richards 2000; Ebert et al. 2002). Habitat fragmentation has been shown to increase the incidence of long-distance interpopulation matings in some systems, notably remnant populations of tropical trees (Dick et al. 2003). Restorations and other projects involving human-mediated movement of seeds are also likely to increase the incidence of interpopulation matings (Hufford and Mazer 2003). Because the occurrence and magnitude of outbreeding depression varies considerably, even among replicate studies of the same populations (Waser et al. 2000), generalizations about the demographic impact of this phenomenon have not emerged.

Further impeding generalization, fitness effects of inbreeding and outbreeding depend on the environment in which plants are grown (Dudash 1990; Hauser and Loeschcke 1996). Some have identified stressful or natural environments as likely to exacerbate inbreeding depression compared to benign or controlled environments (Crnokrak and Roff 1999; Keller and Waller 2002; Armbruster and Reed 2005; but see Waller et al. 2008). In contrast, Ronce et al. (2009) have shown that an extreme environment can ameliorate inbreeding depression and even result in outbreeding depression in a population subject to stabilizing selection. In perennial species, fitness effects of inbreeding and outbreeding may also differ among growing seasons both because the environment can change dramatically within the lifetime of an individual and because of differences in the action of genes expressed at different developmental stages. Habitat fragmentation can also alter the biotic and abiotic environment in different ways from remnant to remnant, and some of these changes (in fire frequency and pollinator activity, for instance) greatly affect plant fitness (Leach and Givnish 1996; Aguilar et al. 2006). In addition, environmental quality may vary with population size. It is therefore important in evaluating effects of inbreeding and outbreeding on fitness in nature to conduct experiments under shared, natural conditions to avoid confounding environment and crossing treatment.

This study focuses on a widespread purple coneflower, *Echinacea angustifolia* (Asteraceae) (hereafter *Echinacea*), which exhibits self-incompatibility (SI) and perenniality. Neither trait is well represented in studies of effects of breeding patterns on progeny fitness (but see Price and Waser 1979; Johnston 1992; Montalvo 1994; Koelewijn et al. 1999; Luijten et al. 2002; Glémin et al. 2006), whereas they are better represented in studies of reproductive success (e.g., seed set) of matings at varying distances (e.g., Campbell and Waser 1989; Byers and Meagher 1992; DeMauro 1993; Moran-Palma and Snow 1997; Campbell and Husband 2007). SI has been implicated in reducing reproductive success in many plants (Aguilar et al. 2006), including *Echinacea*. In our study area, as in much of the range of this species, habitat fragmentation has reduced the density of flowering conspecifics and the proportion of nearby compatible mates (i.e., individuals that do not share an identical allele at the *S* mate recognition locus) resulting in reduced mating success (Wagenius 2006; Wagenius et al. 2007). Also, seed dispersal is limited and fine-scale spatial genetic structure suggests that, in smaller remnants, average relatedness of pairs of plants separated up to 20 m is between half-siblings and first cousins, whereas no neighbor relatedness is evident in the largest remnant comprising several thousand *Echinacea* plants (Wagenius 2000). Consequently, the frequency of mating between close relatives has likely increased following fragmentation. In sporophytic SI systems, at least 25% of full-sibling pairs and at least 37.5% of half-sibling pairs are expected to be mating compatible. High genetic load is expected in SI species, because deleterious recessive mutations are rarely expressed and purged, especially in historically large populations. Therefore, inbreeding depression is predicted to be more severe in SI plants like *Echinacea* compared to species with some degree of selfing (Vogler et al. 1999). Furthermore, Glémin et al. (2001) have developed a theory showing that SI systems can increase inbreeding depression in small populations. Outbreeding vigor or depression in interpopulation crosses is less predictable, on the other hand, and will reflect the extent of genetic differentiation between remnants resulting from either drift or local adaptation. The frequency of interremnant matings is also less predictable.

In this article, we present results on the effects of mating within families, within remnants, and between remnants on progeny fitness. The three types of crosses, carried out under natural conditions, are likely representative of mating events in fragmented prairie habitat. We measured survival and reproductive output of *Echinacea* individuals planted in a common natural environment, over their first eight years. This phase is a critical stage in plant establishment, growth, and onset of flowering, but it may be a fraction of the life span of many individuals. We evaluated the effect of crosstype on the survival of progeny through eight years, their flowering, and their total fitness, that is, the expected number of achenes produced per individual taking survival into account. We discuss how mating patterns may ultimately influence the persistence and numerical dynamics of population remnants, as well as their genetic composition.

Materials and Methods

STUDY SYSTEM

Echinacea, the narrow-leaved purple coneflower, is a herbaceous perennial native to the tallgrass prairie and plains of North America. Its characteristics typify much of the prairie flora. Plants are long-lived, rarely flowering before their third year and not necessarily every year thereafter. Spring burns promote summer flowering. Plants have a sporophytic SI system and are pollinated by native generalist insects (De Nettancourt 1977; Wagenius and Lyon (2010) in Press). *Echinacea* has a large taproot from which it resprouts in spring, but it does not reproduce or spread vegetatively; each new plant arises solely from seed. The seeds are borne singly in dense achenes (3–6 mg), which have no specialized mechanisms to enhance dispersal by wind or animals. Like those of most summer flowering prairie plants, *Echinacea* seeds are dormant over the winter and require specific conditions to break dormancy (Baskin et al. 1992; Feghahati and Reese 1994; Macchia et al. 2001). There is no evidence for a persistent seed bank (S. Wagenius and R. G. Shaw, unpubl. data).

Echinacea is common in undisturbed tallgrass prairie and the Great Plains west of the Mississippi River, ranging from Texas to Canada. Today, tallgrass prairie remnants are sparsely scattered and may harbor thousands of *Echinacea* individuals or as few as one (Wagenius 2006). In our 6400 ha study area, located in rural western Minnesota (centered near 45°49′N, 95°42.5′W), the entire landscape, apart from lakes and wetlands, apparently offered suitable habitat for a continuously distributed population of this plant prior to European settlement, which began in the 1870s. Now, 45 remnants of the population persist. The largest is on a 45 ha virgin prairie preserve owned and managed by The Nature Conservancy with 1000–6000 flowering plants per year. The smallest remnants have 0–4 flowering plants per year (Wagenius 2006). Most prairie fragments remain because they are inaccessible to farm machinery or are otherwise unsuitable for agricultural production.

ESTABLISHMENT OF PARENTAL PLANTS IN COMMON GARDEN EXPERIMENT

In autumn 1995, we randomly collected open-pollinated seed heads (capitula) from plants in five small remnants and from belt transects through two larger remnant populations in the study area. These remnant populations are separated by 2.3–7.8 km (mean 4.8 km) and had from about two dozen to 2000 flowering plants in 1995 (Wagenius 2006). We dried the seed heads at room temperature and removed achenes, which we stored in coin envelopes at room temperature. In spring 1996, we germinated seeds using a protocol modified from Feghahati and Reese (1994; see Wagenius 2004) and grew them in randomly arranged cone-tainers (Stuewe and Sons, Inc., Corvallis, OR) filled with sterilized soil from an old field within the study area. We established a common garden experiment in 1996 in an old field that was abandoned from agriculture in 1989 and planted with perennial grasses (*Bromus inermis* and *Dactylis glomerata*) and legumes (*Medicago sativa*, *Melilotus alba*, and *M. officinalis*). In addition, *Euphorbia esula*, *Carduus acanthoides*, *Taraxacum officinale*, *Symphyotrichum laeve*, *Solidago rigida*, and *S. canadensis* now grow abundantly. We prepared the site by mowing and broadcasting seeds of two native perennial grasses (*Andropogon gerardii* and *Sorghastrum nutans*).

We transplanted 625 2–4 month old plants into the experimental plot in summer 1996 with 1 m spacing within and between rows. Ongoing maintenance of the experiment has included biennial prescribed spring burning starting in 1998, annual broadcast seeding of two native perennial grasses (*Bouteloua curtipendula* and *Schizachyrium scoparium*) starting in 2000, annual mowing of narrow walking paths between rows, continuous removing of pocket gophers, and periodic trimming or pulling of the following plants: *C. acanthoides*, *Melilotus* spp., *Toxicodendron rydbergii*, *Fraxinus pennsylvanica*, *Salix* spp., and *Rhus glabra*.

CROSSING DESIGN

In 1999, 10 plants from five different remnants flowered for the first time; in 2000, five plants flowered again and 88 plants from six remnants flowered for the first time. We used 59 of these plants as parents to generate three groups of offspring (“crosstypes”) by crossing individuals originating (1) from different remnants, (2) from different maternal plants within the same remnant, or (3) from the same maternal plant. These groups will be referred to as between-remnant, within-remnant, and inbred crosses.

In 1999, we attempted reciprocal crosses among all 10 flowering plants. In 2000, we attempted crosses among plants that had siblings flowering at the same time. We attempted all reciprocal crosses within sibling groups (inbred crosstype) and random matings among all other plants (between- and within-remnant crosstypes). We chose mates randomly from among plants that were both presenting fresh styles and shedding pollen on a given day. We attempted 229 unique combinations of sires and dams.

The crossing procedure followed a three-day cycle as detailed by Wagenius et al. (2007). In brief, flower heads were enclosed in mesh bags to exclude pollinators. For each cross, five to six bracts subtending recipient florets were painted in a color representing a particular pollen donor, and pollen was transferred the following day. The realized crossing design was unbalanced due to mating incompatibilities and asynchronous flowering. Mating incompatibility due to the sporophytic SI system was present in 32% of the attempted inbred crosses and in 14% of attempted random matings. One parental plant was incompatible in all crosses attempted, thus the parental generation comprised 58 individuals. We harvested seed heads each fall and individually removed all achenes from painted bracts and stored them at room temperature on post-it notes (3 M, St Paul, MN). The crosses produced 1077 achenes yielding 139 germinants from 40 crosses in 1999 and 418 germinants from 147 crosses in 2000. There were 323, 95, and 139 germinants in the between, within, and inbred crosstypes, respectively ($n = 557$).

TRANSPLANTATION AND ANNUAL MEASUREMENT OF PLANTS

We germinated progeny achenes as we did the parents, except that we used slotted and labeled blotter paper (Anchor Paper Company, St. Paul, MN) to keep track of individual achenes. Germinants emerged for a two-week period starting 20 February 2001. We assessed germination daily and removed an individual when its radicle emerged at least 1 mm. All assessments and measurements were "blind" in the sense that the measurer identified the seed, seedling, or plant by a randomly assigned number. Each seed with an emerged radicle (germinant) was placed in a randomly determined plug on a 12×24 plug tray (Landmark plastic, Akron, OH). Plugs were 2 cm square on top and tapering below, 4.5 cm deep, and filled with Sunshine plug mix (Sun Gro Horticulture, Vancouver, BC). Germinants were not placed in edge plugs. We rotated and watered trays from below three times a week and kept them in a growth chamber at 25°C with 16 h of fluorescent light per day. Seedling mortality was assessed regularly and we determined whether each individual survived to produce cotyledons (stage lds1) or produced a true leaf (lds2).

Seedling survival and stage (cotyledons or true leaf) were recorded regularly until the day of transplantation into the experimental plot. During 13–22 March 2001, we transplanted seedlings to cone-tainers (Stuewe and Sons, Inc., Corvallis, OR). During 15–19 May 2001, we planted 508 seedlings at 50 cm spacing into the common garden experimental plot in four rows adjacent to the rows of the parental plants. We watered the plants for one week after planting.

We annually assessed further survival, size, and flowering status in July or August. Plant size (rosette and leaf number) was measured and evaluated; analyses of size have been presented in Shaw et al. (2008). In 2004, individuals began to flower, and we counted the heads each plant produced. Each year, all seed heads were removed from plants as they matured but before dispersing achenes (mid-August through September). On several occasions, achenes had dispersed prior to capitulum removal, and in these cases we determined how many had fallen by counting the gaps in the head. Heads were collected in small paper bags and kept dry at room temperature. In winter, we removed all achenes from each receptacle, spread them out in a flat-bottomed glass dish and scanned the contents with a flatbed scanner. We then counted the number of achenes per image. Achene count per plant per year is the sum of all collected and dispersed achenes from all heads and closely represents each plant's annual ovule production. Plants that produced no achenes in one year were scored as not flowering that year.

STATISTICAL ANALYSIS

We compared the survival of plants in the three different crosstypes over eight years (2001–2008). We constructed nested models to account for the variation in survival at 11 stages (three prior to transplantation and eight annual field assessments), each modeled as Bernoulli (0 or 1) (Fig. 1A) and compared them using a likelihood ratio test. We conducted this and all other statistical analyses with the software package R (R Core Development Team 2008) using aster analysis (Geyer et al. 2007; Shaw et al. 2008). Aster analysis is a likelihood approach that takes into account the dependence of an individual's observed fitness component in any observation period on its state (in the case of the survival analysis, alive or dead) in the previous observation period. The model corresponding to the null hypothesis of no difference in survival among crosstypes (Model "Null survival") included the plant location (row and position within a row) and the year the cross was made (1999 or 2000, called crossyear) as predictor variables. In the second model (Model "Crosstype survival"), we added crosstype as a predictor. In addition, we obtained maximum likelihood estimates of the expected probability of survival for each crosstype over each interval. These estimates, and all parameter estimates, account for the three covariates by reporting values for a hypothetical individual in a middle row, at the median position, and generated in crossyear 2000 (Shaw et al. 2007).

We also used unconditional aster models to obtain an integrated measure of fitness, including survival (lds01–s08) and annual counts of flower heads and achenes produced, as a basis for comparing the crosstypes with respect to their timing of first flowering and, of primary interest, their overall fitness. In

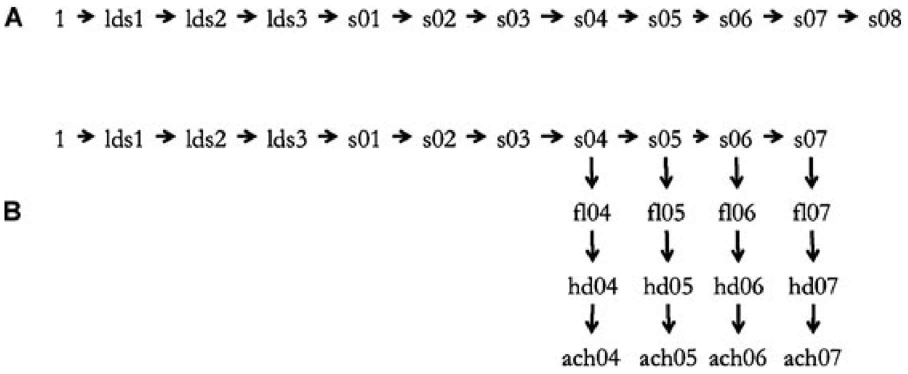

Figure 1. **Graphical models for aster analyses of (A) survival and (B) total fitness. Arrows point from prior to later nodes. lds*i* represents three intervals in the growth chamber: lds1 is survival to cotyledon emergence, lds2 is survival to production of true leaf, and lds3 is survival to transplantation to the field. s0*i* represents survival in the field from 2001 to 2008, fl0*i* whether an individual flowered in 2004 to 2007, hd0*i* the number of flowering heads produced by a flowering plant in 2004 to 2007, and ach0*i* the number of achenes produced by a plant in a given year.**

each case, we used the full graphical model including mortality at all 10 intervals and fecundity in the first four years of flowering (2004–2007) (Fig. 1B). In addition to addressing dependence of one stage on another, aster allows for specification of a suitable distribution for each component of fitness, leading to an analysis of total fitness that obviates dependence on the assumption, typically violated with fitness data, of normally distributed residuals (Shaw et al. 2008). We modeled the transitions between survival nodes and between survival and flowering nodes as Bernoulli. Annual head counts and achene counts per plant were modeled as zero-truncated Poisson (Fig. 1B). To evaluate differences in the early onset of reproduction, we compared the model that included planting row, position within row, and crossyear as effects on all components of fitness (Model "Null fitness") with one that added crosstype as a predictor for total head count in 2004 and 2005 (Model "Crosstype early flowering").

To test the effect of crosstype on total fitness, we compared Model Null fitness with one that also included crosstype as a predictor variable for the summed achene counts from 2004–2007 (Model "Crosstype fitness"). Such a model leads to estimates of the differences among crosstypes in cumulative achene count, our best measure of total fitness to date, which may depend in part on differences in life history at earlier stages (e.g., survival to flowering), as accommodated by aster modeling (Shaw et al. 2008). For both early flowering and total fitness, we compared the fit of nested models using likelihood ratio tests (Table 1). We calculated

Table 1. **Model comparisons to test for effects of crosstype on (A) survival and (B) early flowering and total fitness. The model formulae are given above and the analysis of deviance below; deviance is double the log likelihood ratio. "Graph nodes" refers to the aster graphs specified in Figure 1. To account for spatial and temporal heterogeneity, all models include fixed effects of crossyear (1999 or 2000) and spatial location (row and position within row) in the experimental plot. In the case of early flowering and total fitness, comparisons are made with Null fitness. Parameter estimates for models Crosstype survival and Crosstype fitness are shown in Figures 2 and 5, respectively.**

Model name	Model df	Model deviance	Test df	Test deviance	Test *P*-value
(A) Survival					
Null survival: Response=Graph nodes+row+position+crossyear Crosstype survival: Response=Graph nodes+row+position+crossyear+crosstype					
Null survival	16	1924.5	–	–	
Crosstype survival	18	1899.8	2	24.7	<0.0001
(B) Early flowering and total fitness					
Null fitness: Response=Graph nodes+row+position+crossyear Crosstype early flowering: Response=Graph nodes+row+position+crossyear+crosstype∗head number 04/05 Crosstype fitness: Response=Graph nodes+row+position+crossyear+crosstype∗achene number					
Null fitness	26	370521	–	–	
Crosstype early flowering	28	370524	2	2	0.33
Crosstype fitness	28	370543	2	22	<0.0001

maximum likelihood estimates of cumulative achene production for an individual of each crosstype using Model Crosstype fitness. We note that, in the absence of information on achene production through pollen export for this hermaphroditic species, we focus on individuals' reproductive output via female function. Thus, our fitness comparisons assume that variation in paternal function is uncorrelated with maternal function. Preliminary analyses revealed that the realized experimental design was insufficient to test for effects of the size or identity of source population; an experiment to address these issues is underway.

We present estimates of inbreeding load based on comparisons of cumulative achene production for crosses between siblings relative to crosses at random within remnants (as noninbred reference). The inbreeding load quantifies the linear decline in the logarithm of fitness with inbreeding level (see e.g., Keller and Waller 2002, Box 3) and is useful when comparing studies, because it takes the degree of inbreeding into account. When the fitness measure is survival, the inbreeding load is often referred to as the number of lethal equivalents, the number of alleles per gamete, each of which is lethal in homozygous state (Morton et al. 1956).

Results

SURVIVAL

By 2008, the seventh year after transplantation to the field, raw survival for the entire experiment was approximately 50%. For inbred plants from sibling-matings, survival was 37%, whereas survival for within- and between-remnant crosses was 46% and 57%, respectively. Thus, within-remnant inbreeding depression is 20% with respect to survival alone. Model comparisons demonstrate that the differences in survival among the crosstypes are highly significant (Table 1). At every interval, progeny of crosses between remnants had the highest estimated probability of being alive; inbred plants always had the lowest estimated rate of survival (Fig. 2).

FLOWERING

In 2004, 21 of the 400 surviving plants flowered for the first time. In 2005, 15 of the 384 survivors flowered. For 12 of these 15 plants it was their first year of flowering. Although the percentage of living plants in flower in the first two years differed among crosstypes (Fig. 3), this difference in early onset of flowering was not significant (Table 1).

The proportion of living plants that had not yet flowered by the end of 2008 was 49.8%, 41.5%, and 58.2% for between-remnant, within-remnant, and inbred crosses, respectively. The mean age of first flowering among the plants that had already flowered was similar among crosstypes, ranging from 5.19 to 5.43 years. If all remaining living plants flowered in 2009, the mean ages of first flowering would be 6.59, 6.39, and 6.93 for between-remnant, within-remnant, and inbred crosstypes.

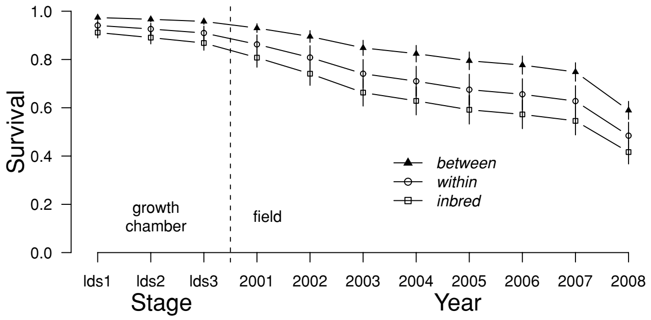

Figure 2. Expected survival for three crosstype over all intervals where survival was assessed. The crossing treatments are "between": Between-remnants, randomly-mated; "within": Within-remnants, randomly-mated; and "inbred": Within-remnants, sibling-mated. Values plotted are maximum likelihood estimates ±1 SE following model Crosstype survival in Table 1, accounting for spatial position in experimental plot and year of crossing (n = 557 germinants).

TOTAL FITNESS

The distribution of total number of achenes per individual by the end of the seventh year is not amenable to conventional parametric statistical analysis (Fig. 4). Total fitness ranged from zero to 3065 achenes, with a modal value of zero in each crosstype. Individuals with zero achenes include those that died without flowering 196 of 557 (35%) and living individuals that had not yet flowered 235 of 557 (42%). The inbred crosstype had the greatest proportion of individuals that died without flowering 75 of 139 (54%), the lowest proportion of individuals that had flowered 14 of 139 (10%), and the lowest proportion of individuals that were alive but had not yet flowered 50 of 139 (36%). Compared to the progeny of random crosses within remnants, those from crosses between

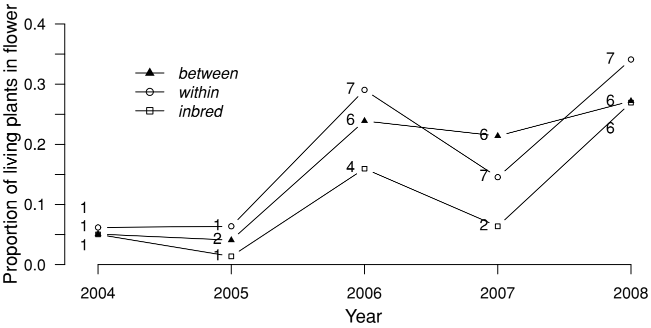

Figure 3. Proportion of live plants in flower within each crosstype. Numbers indicate the maximum number of heads produced per plant for each crosstype in each year. The mode of head-count for every year was 1. No plants flowered before 2004. Burns occurred in spring 2004, 2006, and 2008. Crosstypes are labeled as in Figure 2.

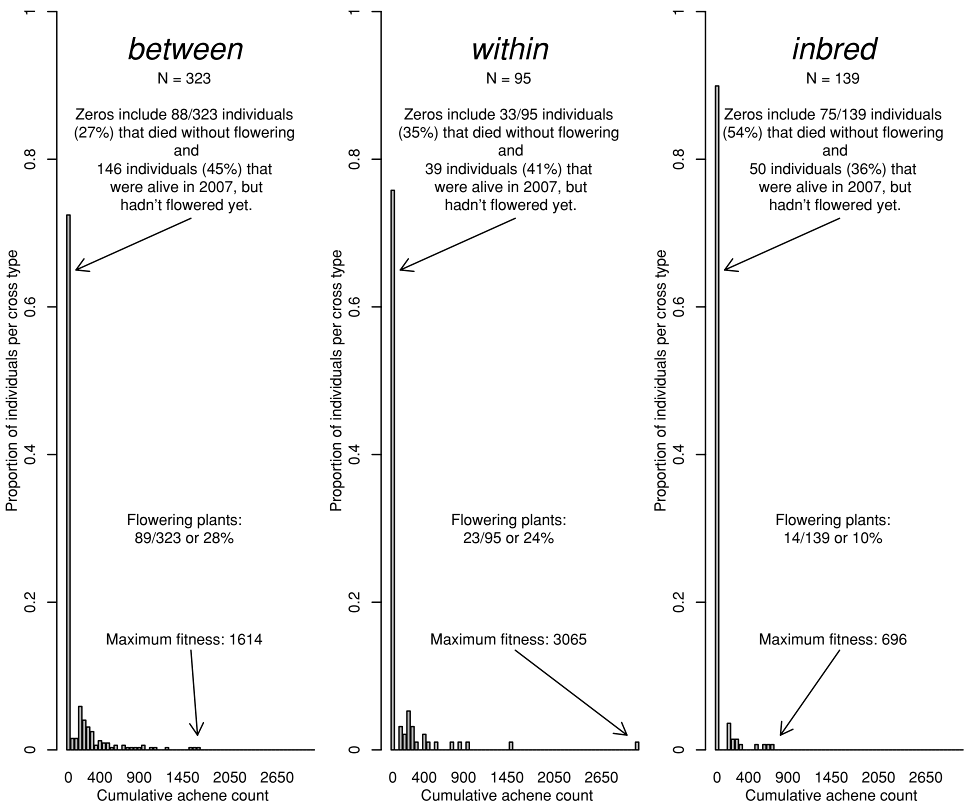

Figure 4. **The distributions of total fitness (cumulative achene count) per crosstype (labeled as in Fig. 2). Each bar shows the proportion of individuals per crosstype having total fitness within one of the following bins: 0, 1–51, 51–100, 101–150, etc. Total fitness is the count of all achenes (ovules) produced per individual that germinated in spring 2001 (n = 557) through the flowering season of summer 2007. Note that the zeros include both individuals that died before flowering and individuals that were alive in 2007 but had not yet flowered.**

remnants had a lower proportion of plants that died without flowering (27% compared to 35%) and a greater proportion that had flowered (28% compared to 24%).

Aster analysis, which appropriately models the fitness distribution (see methods), revealed a highly significant effect of crosstype on total fitness, represented by cumulative achene count (Crosstype fitness vs. Null fitness, $P < 0.0001$) (Table 1). Estimated total fitness in the first seven years was 68% lower in the inbred crosstype than in the within-remnant crosstype (Fig. 5). Biparental inbreeding depression of 68% represents an inbreeding load of 4.5–9 alleles per gamete (Keller and Waller 2002), assuming full- and half-sibling parents, respectively. Total fitness in the between-remnant crosstype, was lower than in the within-remnant crosstype, but not significantly so.

Discussion

We have demonstrated extremely severe inbreeding depression in the self-incompatible, perennial plant *Echinacea*. The experiment, conducted under natural field conditions that are representative of restored prairie habitats in the area, and the analysis via aster, which integrates measures of fitness over seven years, have yielded unusually realistic and comprehensive measures of inbreeding depression for a long-lived species. Crosses made between sibling parents resulted in offspring with a 20% reduced chance of survival after eight years, reduced flowering, and altogether 68% lower fitness at the end of seven years as compared to offspring from crosses between individuals at random within a remnant population. In crosses made between individuals from different remnant populations, we found little indication of either heterosis or outbreeding depression.

Crosses between siblings, such as those experimentally generated here, are likely to be common in natural remnant populations of *Echinacea*. In this experiment, 68% of crosses attempted between siblings and 86% of crosses between all other pairs were mating compatible. Limited pollen dispersal and the tendency for plants in close proximity to be related jointly promote crossing between relatives (Wagenius 2000; Wagenius et al. 2007), entailing the genetically based fitness costs demonstrated here. In addition, *Echinacea* plants growing in small remnants incur a further fitness cost; seed set is reduced by pollen limitation resulting from spatial isolation and a paucity of compatible mates (Wagenius 2006). Mate scarcity along with reduced seed set has been documented in many SI plants (e.g., Byers and Meagher

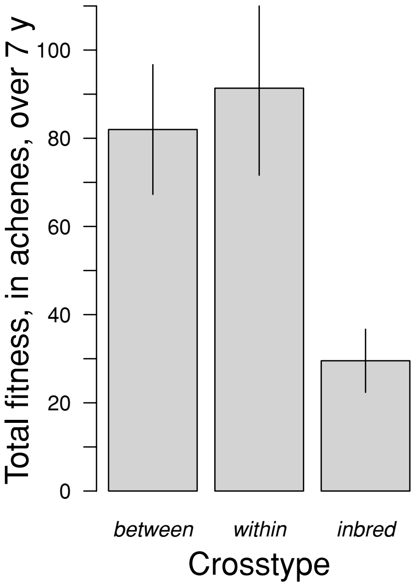

Figure 5. Expected total progeny fitness through seven years for each crosstype (labeled as in Fig. 2), estimated as cumulative achene count in 2007 per germinant in 2001. Values plotted represent maximum likelihood estimates ±1 SE following model Crosstype fitness in Table 1, accounting for spatial position in experimental plot and year of crossing (n = 557 germinants). The difference in fitness of the inbred crosstypes relative to within remnant crosstype represents biparental inbreeding depression (68%).

1992; DeMauro 1993; Pickup and Young 2008), but far fewer studies have investigated effects of breeding patterns on progeny fitness (but see Byers 1998 and Colling et al. 2004).

We previously presented results from this experiment for survival and vegetative size through 2005, using the same statistical approach (Shaw et al. 2008). Now that we have survival data through 2008 and reproduction data through 2007, it is instructive to compare results to examine the consistency of inbreeding effects through time. Our earlier analyses revealed that crosstype significantly affected survival and size. We measured a 7–10% reduction in survival of inbred plants compared to outbred plants in the growth chamber before transplantation to the field and, taking this difference into account, ~40% reduced size (i.e., rosette number) for inbred versus outbred plants after five years in the field (Shaw et al. 2008). With the additional years of fitness records, we observe dramatically more severe inbreeding depression: 20% considering survival only, and 68% including reproduction.

Thus, the expression of inbreeding depression is substantial at early stages and increases in later stages, in conjunction with reproduction, in accordance with the generalization Husband and Schemske (1996) drew from their survey of available studies. We note that analysis of rosette number as a fitness proxy for the 2008 data shows inbreeding depression roughly comparable to that expressed in 2005 (~36%; results not included). Studies of inbreeding that are limited to early life measurements of perennial plants, as many are for logistical reasons, may greatly underestimate the degree of lifetime inbreeding effects in populations. We emphasize that aster modeling allows us to obtain comprehensive, statistically sound estimates of fitness and, hence, an integrated measure of inbreeding depression over multiple stages of the life span.

Inbreeding depression is nearly ubiquitous in plant and animal systems. Inbreeding depression has been detected by relating fitness components to inbreeding coefficients determined from pedigrees or inferred from molecular marker heterozygosity (but see Balloux et al. 2004; Slate et al. 2004) in a wide variety of species (reviewed in Keller and Waller 2002). Few studies, however, directly estimate the severity of inbreeding depression under natural conditions and over an extended period of the life span. Studies of coniferous trees have demonstrated severe inbreeding depression both in early life-history traits (Sorensen 1969; Karkkainen et al. 1996; Remington and O'Malley 2000) and in survival over two decades (Koelewijn et al. 1999). Moreover, similar to our findings, the magnitude of inbreeding depression (in survival) appears to increase in *Pinus sylvestris* such that inbred offspring suffer increasingly harsh mortality selection compared to open-pollinated (and presumed outbred) offspring as they age (Koelewijn et al. 1999). Such a trend could result from initial differences in plant size that disadvantage inbred plants and may result in slower growth and greater mortality of inbreds during subsequent competition (Schmitt and Ehrhardt 1990). Annual leaf counts indicate that surviving inbred *Echinacea* plants do tend to be smaller than outbred plants, although only slightly and not compoundingly so (results not shown).

In the herbaceous, outcrossing perennial *Arnica montana*, a plant whose natural and recent history of reduced range and fragmentation in Europe is similar to that of *Echinacea*, Luijten et al. (2002) compared the consequences of selfing, intra- and interpopulation crossing. As in our experiment, the progeny were transplanted as seedlings into the field; in addition, a subset was sown into the field as seeds. In this largely self-incompatible plant, selfing resulted in dramatic reduction in seed production (73% less than interpopulation crosses). This represents an inbreeding load of 2.6 alleles per gamete. Thereafter, no inbreeding depression was detectable in terms of survival or the probability of reaching reproductive maturity over the first three years in the field, although slight inbreeding depression was detected for growth. Effects of biparental inbreeding were not evaluated. In contrast to our results for crosses between remnants, Luijten et al. (2002) found that progeny of interpopulation crosses were more likely to flower than those from intrapopulation crosses, evidencing heterosis.

Glémin et al. (2006) demonstrated considerable inbreeding depression in survival over two years for two populations of the self-incompatible, perennial *Brassica insularis* growing in controlled conditions. By circumventing the SI mechanism, they generated selfed progeny and estimated 38–49% inbreeding depression (genetic load of 1–1.4 alleles per gamete). Several species of herbaceous, self-compatible perennial plants also show high levels of inbreeding depression. Both *Lobelia* and *Aquilegia* exhibit ∼50% inbreeding depression, ranging up to 83% in one population of *Lobelia*, in selfed versus outcrossed progeny over three years in the field (Johnston 1992; Montalvo 1994). These values represent a genetic load of 1.5–3.5.

In our study, *Echinacea* exhibits 68% inbreeding depression in progeny of crosses between siblings drawn from open-pollinated capitula (i.e., half to full siblings) versus outcrossed progeny, which corresponds to a genetic load of 4.5–9 alleles per gamete. Based on this amount of load, we would expect 90–99% inbreeding depression in selfed individuals. This finding conforms to the theoretical expectation that purging of deleterious recessive alleles is relatively inefficient for a self-incompatible species (Vogler et al. 1999). This amount of inbreeding depression is also expected to impede the evolution of selfing (Uyenoyama et al. 1993).

Fragmentation is expected to alter the genetics and evolution of remnant populations in many ways by limiting interremnant gene flow, which can allow divergence of populations both by natural selection and genetic drift. In the joint analysis of survival and reproduction, we found no evidence for heterosis, as would be expected under divergence by drift, or outbreeding depression, as may be expected under divergence by either natural selection or drift, in crosses of *Echinacea* between remnants. It is possible that there has been insufficient time for substantial divergence due to the relatively recent occurrence of fragmentation and the long generation time of the plant. Interestingly, we found ∼20% higher survival for progeny of crosses between remnants than within, whereas these two progeny groups were nearly equal in total fitness. Thus, progeny from within-remnant crosses "made up" the fitness deficit in reduced survival via greater reproduction. An alternate view of our results, therefore, is that there is weak heterosis for survival, coupled with outbreeding depression for reproduction. Such inconsistent responses of traits to interpopulation mating are found in other systems (e.g., Sheridan and Karowe 2000; Caño et al. 2008). Given that the majority of plants from between- and within-remnant crosses are still alive, it remains an open question whether their lifetime fitness will eventually differ. As noted previously, obtaining lifetime fitness measurements requires patience. To gauge the life span of our study population, we used a rough projection method that assumes future mortality and its variability will mirror the patterns of the past four years (10,000 simulations in which annual mortality was determined by randomly selecting an observed experiment-wide mortality rate of 3–22%). On this basis, we expect 5% of the original transplants will still be alive in 2034 (2026–2043 90% bootstrap CI). This indication of life span accords with estimates of generation time obtained for four populations of *Echinacea* in Kansas, ranging from 17 to 44 years (Hurlburt 1999), using the method of Orive (1993).

During restoration, the decision is often weighed whether to use seeds from more than one source population. The likely influence of heterosis and outbreeding depression on population dynamics should be considered carefully, given that generalizations about their prevalence and magnitude have not emerged. Our experiment suggests that their effects on individual fitness of *Echinacea* in the first generation may be balanced on the small geographic scale considered here. Other studies have documented outbreeding depression on even finer spatial scales, including Waser et al. (2000) for self-incompatible *Ipomopsis aggregata* and Quilichini et al. (2001) and Heiser and Shaw (2006) for two highly inbred species. Other factors, including the size and characteristics of remnants used in restoration, the expression of heterosis or outbreeding depression later in the life cycle, and hybrid breakdown in advanced generations, may also impact the dynamics of populations restored using multiple seed sources, each of which is currently under study in our system.

One of the ultimate goals of this research is to predict the persistence and spread of the remnant populations, given their current demography and genetic composition and the feedbacks between the two as the populations continues to evolve. The results of this study suggest that biparental inbreeding depression will play an important role in population growth rates considering the magnitude of its effect on both survival and reproduction. Together with our ongoing studies of recruitment under natural conditions, our detailed and accumulating knowledge of the timing and magnitude of inbreeding and outbreeding effects will illuminate the interplay of genetics and demography in population persistence or extinction.

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