

**The conservation value of small population remnants: variability in inbreeding depression
and heterosis of a perennial herb, the narrow-leaved purple coneflower (*Echinacea
angustifolia*)**

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Running head: Heterosis in fragmented populations

Abstract

Anthropogenically fragmented populations may have reduced fitness due to loss of genetic diversity and inbreeding. The extent of such fitness losses due to fragmentation and potential gains from conservation actions are infrequently assessed together empirically. Controlled crosses within and among populations can identify whether populations are at risk of inbreeding depression and whether interpopulation crossing alleviates fitness loss. Because fitness depends on environment and life stage, studies quantifying cumulative fitness over a large portion of the lifecycle in conditions that mimic natural environments are most informative. To assess fitness consequences of habitat fragmentation, we leveraged controlled within-family, within-population, and between-population crosses to quantify inbreeding depression and heterosis in seven populations of *Echinacea angustifolia* within a 6400-hectare area. We then assessed cumulative offspring fitness after 14 years of growth in a natural experimental plot (N = 1136). Mean fitness of progeny from within-population crosses varied considerably, indicating genetic differentiation among source populations, even though these sites are all less than 9 km apart. The fitness consequences of within-family and between-population crosses varied in magnitude and direction. Only one of the seven populations showed inbreeding depression of high effect, while four populations showed substantial heterosis. Outbreeding depression was rare and slight. Our findings indicate that local crossings between isolated populations yield unpredictable fitness consequences ranging from slight decreases to substantial increases. Interestingly, inbreeding depression and heterosis did not relate closely to population size, suggesting that all fragmented populations could contribute to conservation goals as either pollen recipients or donors.

Keywords: Habitat fragmentation, outbreeding depression, multi-year study, genetic rescue,
Aster model, Asteraceae

Introduction

Mating patterns can profoundly affect progeny fitness and, accordingly, population growth rates. For example, mating between close relatives may reduce mean progeny fitness, i.e., inbreeding depression (Charlesworth and Charlesworth 1987; Keller and Waller 2002). On the other hand, mating between more distantly related individuals (e.g., between members of different populations) has yielded mixed results: offspring may exhibit reduced or greater mean fitness than their counterparts produced by random mating within populations (outbreeding depression and heterosis, respectively; Edmands 1999; Frankham et al. 2011). As conservationists strive to avert extirpation of populations, managing inbreeding and outbreeding depression can be crucial for maintaining population viability (O’Grady et al. 2006; Nonaka et al. 2019). Conversely, heterosis may be leveraged to rescue inbred populations from extinction (Willi et al. 2007; Frankham 2015; Whitely et al. 2015). With 28% of species threatened with extinction worldwide (IUCN 2022), deducing the extent to which natural populations express inbreeding depression and outbreeding depression or heterosis contributes critical insights into ways to preserve biodiversity.

Deleterious alleles that are partially recessive constitute a part of a population’s genetic load and are the primary contributors to inbreeding depression and heterosis (Falconer and Mackay 1996; Whitlock et al. 2000; Keller and Waller 2002; Hedrick and Garcia-Dorado 2016; Bertorelle et al. 2022). Inbreeding increases homozygosity, including that of deleterious recessive alleles, such that, in the homozygous state, they affect phenotype and reduce fitness (Charlesworth and Willis 2009; Bertorelle et al. 2022). To the extent that two populations harbor

distinct sets of recessive deleterious alleles, mating between them increases heterozygosity, masking effects of those alleles and leading to heterosis for offspring, as shown theoretically (genetic rescue, Whitlock et al. 2000; Keller and Waller 2002). Crossing between populations may, however, impair fitness (i.e., outbreeding depression; Edmands and Timmerman 2003; Frankham et al. 2011). Outbreeding depression is considered most likely for populations that are strongly genetically diverged (Frankham et al. 2011). Thus, for populations occupying similar environments near to each other and that may recently have been connected by gene flow, between-population mating is not expected to result in outbreeding depression.

Determining populations at risk of inbreeding depression or candidates for heterotic crosses has long been a goal for conservation biologists; theoretical expectations and empirical evidence imply that inbreeding depression and heterosis of natural populations vary in relation to population size. The greater influence of genetic drift on smaller populations tends to result in extreme allele frequencies (including fixation), such that further inbreeding has little effect on genotype frequencies and mean fitness, resulting in low estimates of inbreeding depression (Bataillon and Kirkpatrick 2000; Glémin 2003; Hedrick and Garcia-Dorado 2016; Bertorelle et al. 2022). Moreover, homozygosity of small populations potentiates greater heterosis compared to larger populations (Whitlock et al. 2000; Oakley and Winn 2012). Indeed, empirical studies in non-fragmented populations generally support these expectations (Angeloni et al. 2011; Oakley and Winn 2012; Frankham 2015; Lohr and Haag 2015). Consequently, large populations are often preferred for conservation because of their high genetic diversity and higher mean fitness, which enhances their likelihood of persistence. These genetically diverse populations have been shown to produce greater heterosis than small populations when donating pollen to low fitness populations, implicating their potential for the genetic rescue of other populations (Willi et al.

2007; Pickup et al. 2013). In addition to empirical evidence, models predict these trends for populations at mutation-selection-drift balance (e.g., Bataillon and Kirkpatrick 2000; Whitlock et al. 2000; Hedrick and Garcia-Dorado 2016). However, this assumption may not hold for populations in recently fragmented habitat (Lopez et al. 2009; Spigler et al. 2016).

These intra- and inter-population genetic processes are of critical importance in relation to anthropogenic habitat fragmentation and conversion, which seriously threaten global biodiversity (Fahrig 2003) by extirpating populations or reducing the populations that persist to extremely low numbers, i.e. severe bottlenecks (Young et al. 1996; Cozzolino et al. 2003; Jump and Peñuelas 2003). Theory suggests that the consequent inbreeding can purge their genetic load (Lopez et al. 2009; Grossen et al. 2020), but this process is slow and not guaranteed; indeed, in especially small populations, fixation of deleterious alleles is expected (Glémin 2003; Hedrick and Garcia-Dorado 2016). Even if there is (partial) purging, this can take numerous generations and does not restore fitness to the original level. Thus, long-lived, self-incompatible species with few generations since habitat fragmentation may have low population mean fitness because their mating system does not effectively purge their genetic load. Moreover, continued disturbance of already fragmented habitat (e.g., due to ongoing human activities that continue to reduce effective population size intermittently) may preclude purging (Spigler et al. 2016). It is thus critical to evaluate mean fitness of population remnants and assess whether a) they may undergo fitness declines from further inbreeding and b) interpopulation crossing may enhance their persistence.

Experiments comparing the mean fitness of progeny from controlled crosses that differ in the resulting degrees of inbreeding can elucidate population responses to inbreeding or intercrossing (Keller and Waller 2002). Many studies have employed this approach, but most

plant studies focus on species capable of selfing, rather than self-incompatible species (per sample sizes in Angeloni et al. 2011; Frankham 2015). Moreover, few studies have tracked cumulative fitness across the life cycle of long-lived species in an environment that mimics natural conditions (i.e., an experimental plot in natural field habitat; Angeloni et al. 2011; Frankham 2015). Because fitness varies with age and environment (Cheptou and Donahue 2011; Sandner et al. 2021), inbreeding depression and heterosis are estimated most realistically under natural conditions and cumulatively across the life cycle.

Few studies have, moreover, quantified the degree of inbreeding depression and heterosis in recently fragmented habitat, especially in long-lived species soon after fragmentation (but see Willi et al. 2007; Wagenius et al. 2010; Sletvold et al. 2012; Pickup et al. 2013; Spigler et al. 2016). Here, we leverage an experiment spanning 25 years to study fitness consequences of biparental inbreeding and heterosis in a self-incompatible, long-lived prairie plant, *Echinacea angustifolia*. This study expands on the work of Wagenius et al. (2010), which detected pronounced inbreeding depression, but no heterosis in an 8-year experiment on this species. We ask: 1) how strong are biparental inbreeding depression and heterosis or outbreeding depression? 2) Do the effects of inbreeding and crossing between populations vary among source populations? We discuss the results in the context of the source population characteristics, population size and disturbance history. We expected inbreeding depression, especially in the larger populations. Both because our study area is small, spanning less than 9 km, and because major habitat conversion began less than 10 generations ago, we did not expect outbreeding depression; rather, heterotic outcomes of crossing were more likely. Further, we expected smaller and more disturbed remnant populations to show lower mean fitness and inbreeding depression, along with greater heterosis, compared to larger and less disturbed populations.

Methods

Study system

Echinacea angustifolia (family Asteraceae; hereafter, *Echinacea*) is a long-lived herbaceous plant native to tall- and mixed-grass prairie of the Great Plains. Like many plants characteristic of this biome, it is tap rooted and only reproduces by seed (Leuszler et al. 1996). *Echinacea* is hermaphroditic, but does not reproduce via selfing due to sporophytic self-incompatibility; it is mainly pollinated by native ground-nesting bees (Wagenius and Lyon 2010). Dense seeds within fruits (2-5 mg), are gravity dispersed, such that seedlings typically emerge within a meter of maternal plants (Dykstra 2013; Richardson et al. 2024; Waananen et al. 2024). Individuals take multiple years to reach reproductive stage; when planted in the field as greenhouse-started seedlings, individuals rarely flower before their third year. In a recent study, fewer than 1% of seedlings that germinated in natural conditions flowered within their first 8 years (Richardson et al. 2024; Waananen et al. 2024). Hurlburt (1999) indirectly estimated the generation time of *Echinacea* at 24 years.

Our study site in west-central Minnesota, USA (near 45°49' N, 95°43' W) encompasses 6400 ha of agriculturally dominated landscape (Figure S1). This land was historically extensive, continuous, tallgrass prairie, but Europeans settling in the late-19th century began its massive conversion to agriculture, leaving small prairie fragments. Within the study area, over 45 remnant patches contain *Echinacea angustifolia*. In this study, we consider those growing in each remnant a population; microsatellite marker variation (presumed neutral) indicates moderate genetic differentiation among them (Ison 2010), and they differ in mean fitness (Geyer et al. 2007). Many populations persist in prairie remnants between roadways and agricultural fields and are frequently subject to anthropogenic disturbances, such as mowing and trampling.

These disturbances often remove inflorescence buds from bolting plants, resulting in a reduced breeding population most years. A few populations persist in large swaths of relatively undisturbed prairie and are managed with prescribed burns. Some populations have been extirpated in the last 25 years (SW, *pers obs*).

Experimental plot and crossing design

In 1995 and 1996, we collected seed from seven representative remnant populations (Figure S1; See Supplementary material for detailed site descriptions). In spring of 1996 and 1997, we germinated seeds using a modified method of Feghahati and Reese (1994) and then planted individual seedlings into randomly chosen locations in an area of a formerly cultivated field now dominated with prairie plants. We manage this plot by removing select weeds and burning it approximately biannually. Details about this plot, "P1," are in Reed (2022) and references within.

In 2005, plants flowering in these 1995 and 1996 cohorts were selected for experimental crossing to assess mean fitness of progeny for three different crosstypes. Plants from each source remnant were crossed to siblings (Inbred; I), to plants chosen at random from representatives of the same source remnant, with the restriction that they were not maternal siblings (Within; W), and to plants from another remnant (Between; B). We randomly selected individuals from each population to serve as pollen recipients (maternal plants) and donors (sires). For each instance of pollen transfer, we collected pollen from a designated sire and deposited it onto five receptive styles within a row on the head of the designated maternal plant. To retain the identity of the sire crossed to each floret, we painted the floret's subtending bract with a color particular to that sire. Each maternal plant received pollen from three sires, one sire from each of the three crosstypes; in other words, nearly all maternal plants received pollen in I, W, and B crosses, and thus, each

population had multiple maternal plants representing each crosstype (Tables S1 and S2). Occasionally, crosses between plants were either incompatible or maternal plants had an insufficient number of receptive styles to receive pollen from three pollen donors. In these instances, we selected additional plants as pollen recipients. Between 6-10 maternal plants and 6-8 paternal plants were used per population (Table S1). In total, we made 134 unique crosses with 57 pollen recipients and 50 pollen donors. We were able to perform crosses between nearly all pairs of populations (besides for Eri and SPP; Tables S1 and S2). In most cases, each between-population cross pair was represented by one pollen donor and one pollen recipient (Table S2), and thus, we do not interpret results from individual population pairs. However, for each population, multiple different individuals served as pollen donors or recipients in B crosses; results of between-population crosses thus represent the fitness of outcrossing to other populations, generally. The physical crossing procedure is detailed in Wagenius et al. (2007), which reports on a distinct study of inbreeding and outcrossing.

In spring 2006, we germinated seeds from these hand-crosses, using the method described by Wagenius et al. (2010). A total of 1251 germinants were randomly assigned locations in plug trays (Landmark plastic, Akron, OH # P-288SQD) and grown in a greenhouse. We planted the 1136 of the 1142 surviving seedlings into randomly assigned locations in a 1m x 0.5m grid in the experimental plot; the identities of six seedlings were lost during planting; they are excluded from further consideration here. In summer 2006 and each year thereafter through 2019 (totaling 14 years), we visited the location of each plant to assess its survival, flowering status (whether or not a plant produced pollen), and reproductive effort (number of heads produced). We harvested each flowering head in the fall and took them to the Chicago Botanic Garden, where volunteers cleaned seed heads and counted all fruits on every head. In 2019, 24%

of the cohort remained alive, and 64% of living plants had not yet flowered for the first time. Only 3% of plants that died before 2019 flowered.

Statistical framework

To compare cumulative fitness of progeny among three crosstypes and seven remnant source populations across multiple life history stages for *Echinacea* seedlings planted in the experimental plot, we used unconditional aster models (Geyer et al. 2007; Shaw et al. 2008). Unconditional aster models jointly analyze the multiple fitness components specified in a graphical model to estimate cumulative fitness. The graphical model demonstrates dependence of later fitness components on earlier components, while modeling each stage with an appropriate statistical distribution (Geyer et al. 2007). We modeled each fitness component in each observation year separately; we modeled survival and incidence of flowering with Bernoulli distributions and head count as 0-truncated Poisson (Figure 1). Due to high variability in fruit production, we modeled yearly fruit count with a 0-truncated negative binomial distribution, for which we estimated a dispersion coefficient from the maximum likelihood for our most parsimonious model (2.802 for paternal and 2.823 for maternal population models; see below). In some years (years 5, 12, and 14), all plants that flowered produced one head, so the incidence of flowering and head count are equal for all individuals (i.e. are perfectly collinear, 1 in both cases). In these years, we collapsed the annual flowering and head count fitness components to a single node. In our analyses, we produce estimates of cumulative fruit counts after 14 years (Figure 1). In the main text, we report absolute differences in fitness among crosstypes. All analyses were performed in R version 4.0.2 and with the *aster* package, version 1.3 (Shaw et al. 2008).

Effects of crosstype and population on fitness

We used a set of nested models to test for effects of crosstype and source population on cumulative fruit count. Our *Sub* model, which corresponds to the null hypothesis that cumulative fruit count over the study period does not differ among crosstypes and source populations, includes indicator variables to specify elements of the graphical model. The *Sub* model also includes continuous x, y covariates for each plant's location within the experimental plot to account for spatial variation in fitness (Table S3). Our *Crosstype* model included the same predictors as the *Sub* model and also included effects of crosstype (B, W, and I) on cumulative fruit count (Table S3). We also fit a *Pop* model to test for a difference in fitness among populations without regard to crosstype (Table S3). Our *Crosstype + Pop* model corresponds to the hypothesis that fitness varied among source populations and crosstype (Table S3). Finally, we fit a model with an interaction of crosstype and source population (*Crosstype:Pop*) which allows the magnitude and direction of the effects of crosstype on cumulative fruit count to differ among source populations (Table 1; Table S3).

To test for differences in fitness of *Echinacea* among crosstypes and source populations, we compared nested models using likelihood ratio tests. For estimates of mean fitness, we calculated predictions of the mean and standard error of cumulative fruit count for each experimental group for a plant located in the middle of the experimental plot from our best-supported model; these are interpreted as unconditional estimates of cumulative fruit count up to 2019 per seedling planted in the experimental plot in 2006 (14 years).

When assessing fitness differences among crosstypes for different populations, I and W crosses can be ascribed to a single population, the source of both maternal and paternal plants. However, for B crosses, maternal and paternal plants were drawn from different source populations. Although the B crosses were nearly fully factorial with respect to population, for

each population pairing, there were only 1 or 2 pollen recipients or donors crossed with the other population. Additionally, these crosses were not reciprocal at the level of individual parents (e.g., the individual pollen donor for SAP to SPP was not the pollen recipient from SPP to SAP). For this reason, we fit two separate sets of models with maternal and paternal source population, respectively, as predictors of progeny fitness. Models using paternal source population assess the effect of the pollen donor's population on fitness, whereas those using maternal source population model the effect of the pollen recipient's population on fitness. Fitness of between-population crosses, for either the paternal or maternal model, are thus the mean progeny fitness when the focal population is crossed to any other population (not one specific population). When showing results for maternal and paternal models (e.g., Figure 2), we present the mean of between-population crosses averaging the estimates from the maternal and paternal models. In these cases, the mean predicted fitness is the mean from both models, while the error was calculated as $\sqrt{((SE_{pat})^2 + (SE_{mat})^2)/2}$. We quantify inbreeding depression and heterosis as proportional differences in cumulative fruit count among progeny groups; decreases from W to I crosstypes and increases from W to B crosstypes, respectively.

Results

Variability among populations

Crosstype and population both affected fitness of *Echinacea*. We quantified fitness as total fruits produced per seedling transplanted through 14 years (i.e., cumulative fruit count). Among our seven populations, mean fitness ranged from 10 to 50 fruits (Figure 2). In addition to this considerable population effect, the magnitudes and directions of crosstypes effects also varied among populations (Figure 2; Figure S2). The *Crosstype:Pop* model was the best-fitting model for both the maternal and paternal source population analyses (Table 1).

276 *Inbreeding depression*

277 Only one population, NWLF, clearly exhibited inbreeding depression, where cumulative
278 fruit count of progeny of random W crosses produced six times as many fruits as those from
279 inbred crosses, 72 compared to 12 (Figure 2). At all other populations, the absolute difference in
280 cumulative fruit counts between progeny of W and I crosses did not exceed 13 (Figure 2). Across
281 all populations (i.e., the *Crosstype* model), progeny of W crosses produced 32 fruits compared to
282 23 from progeny of I crosses (Figure 3), i.e., cumulative fitness of I plants was 28% lower than
283 that of W plants. Interestingly, I plants had greater survival than W plants through 2019 (24% vs
284 20%, respectively) and a slightly greater percentage of I plants flowered compared to W plants
285 (9% vs 8%; Figure 4).

286 *Heterosis*

287 We detected substantial heterosis; specifically, four populations showed clear heterosis in
288 combined estimates from maternal and paternal source population models (Figure 2). Progeny of
289 B crosses for these four populations had cumulative fruit counts more than double that of those
290 from W crosses from the same population (ERI = 166% increase, NESS = 306%, SPP = 149%,
291 SAP = 400%). In these four populations, cumulative fruit count was greater for B offspring by a
292 minimum of 25 fruits (Figure 2). The fitness of B and W offspring were similar (or slightly
293 heterotic) in the three populations that did not show clear heterosis; thus, no populations showed
294 clear outbreeding depression (Figure 2). One of these three populations, LF, displayed some
295 heterosis, offspring from its B crosses produced 24 more fruits than from its W crosses (88%
296 increase; Figure 2). NWLF and AA were notable in that they were the populations where fitness
297 of W plants was the highest, and B crosses produced only a minor increase (NWLF) or decrease
298 (AA) in offspring fitness.

In simpler statistical models that do not include population of origin, substantial heterosis is apparent, with B plants producing 60 fruits compared to 32 for W plants (Figure 3). In addition to higher cumulative fitness, models show that B crosses have 30% survival compared to 20% from W crosses and boast a greater rate of flowering (16% vs 8%, respectively; Figure 4). These simpler models are inferior to models that include population.

When considering the effect of maternal and paternal source population on heterosis separately, evidence for heterosis persists for most populations (Figure S2). Two populations (SAP and NESS) show heterosis of strong effect in both crossing directions, and three populations (SPP, LF, and ERI) exhibit heterosis in one crossing direction (Figure S2). The remaining two populations, AA and NWLF, have no heterosis in either direction (Figure S2). We note that for AA in the paternal model, the fitness of W crosses was estimated as 26 fruits greater than B crosses (Figure S2).

Discussion

Fitness in *Echinacea*, as measured by cumulative fruit production over 14 years, varied among crosstypes and populations. Moreover and of particular importance, we found that populations differed in their responses to the crossing treatments (Pop x Crossing treatment interaction). This result makes clear that it is not valid to generalize about effects of inbreeding and population intercrossing, even for close populations within a small study area, as in this study (Figure S1). Both the magnitude and direction of effects of inbreeding and crossing differed strikingly among populations (Figures 2, 3, S2), and appeared to be independent of population size and disturbance history (Figures 2, S5, S6).

Variable inbreeding depression among populations

In three of our seven study populations, the mean fitness of inbreds equaled or exceeded the corresponding mean of offspring from random mating; the remaining four populations showed fitness declines with inbreeding, but for only one of these did inbreeding reduce mean fitness substantially (Figure 2). Because populations mostly exhibited heterosis, low inbreeding depression is likely not due to purging (discussed below), but rather, to genetic drift, which results in (near) fixation of deleterious alleles within populations. On the other hand, considerable inbreeding depression in NWLF largely accounted for overall inbreeding depression when source population was not considered in the *Crosstype* model (Figure 3). Contemporary gene flow between remnants that masks deleterious mutations and increases mean population fitness may have generated this result (Whitlock et al. 2000; Roze and Rousset 2004). Some remnant populations are within the realized range of foraging native solitary bees, the primary pollinators of *Echinacea* (Leuszler et al. 1996; Kendall et al. 2022). NWLF is within 1 km of populations that are not included in this study, and the high fitness of W crosses and high inbreeding depression is consistent with pollen import raising the mean population fitness. On the other hand, because habitat fragmentation is recent relative to the generation time of this species, fitness variation may be influenced strongly by pre-fragmentation distributions and the timing of fragmentation, in addition to (or, instead of) contemporary mating patterns and population size. Determining the roles of contemporary or historic factors in fitness differentiation is vital to understand how fitness varies over a fragmented landscape.

Limited inbreeding depression in our study differs strikingly from the findings of a similar study in a nearby common garden experiment. Wagenius et al. (2010) planted 557 seedlings in 2001 in an experimental plot ~40m from the plot in this study. This study also performed B, W, and I crosses (N = 323 B, 95 W, and 139 I) but did not have adequate

representation of populations to test for cross by population effects. They found fitness of within-population crosses was nearly three times that of sibling matings in *Echinacea* after eight years of growth. We analyzed our data after eight years of growth in the plot to determine how much the expression of inbreeding depression and heterosis change across the life cycle (Table S4; Figures S3 and S4). We found inbreeding depression was weaker after 8 years when compared to 14 years (fitness increased 27% vs 40% from I to W crosses, respectively), contrasting the strong inbreeding depression after 8 years in Wagenius et al. (2010). Further, W crosses in Wagenius et al. (2010) produced 90 fruits per plant after 8 years, compared to just 16 fruits after 8 years in this study. Thus, differences between studies resulted from differences in environment, rather than the timing of inbreeding depression, per se. Inbreeding depression is environment dependent such that it increases with overall variance in fitness (Sandner et al. 2021). Whether temporal or spatial differences in environment caused differences between studies is unclear, but exploring fitness expression across a wide temporal and spatial scale surely yields a broader perspective on the manifestation of inbreeding depression, especially for long-lived species.

Heterosis among populations

Clear heterosis in four of seven populations and a high overall degree of heterosis (with B crosses having, on average, almost double fitness of W; Figure 2), is consistent with genetic differentiation of our study populations, but not differentiation that produced outbreeding depression (Frankham et al. 2011; Frankham 2015). In our study, populations were at most 9 km away from one another, and fragmentation began within the last 140 years, meaning fewer than ~10 generations have elapsed since fragmentation (Hurlburt 1999). Proximity and relatively recent fragmentation of populations makes it unlikely that there has been considerable genetic differentiation due to local adaptation. While heterosis has often been found over short distances

in anthropogenically fragmented systems (e.g., Willi et al. 2007; Pickup et al. 2013; Spigler et al. 2016; reviewed in Frankham 2015), it is not universal. Heiser and Shaw (2006) found outbreeding depression in crosses of *Calylophus serrulatus* among populations 20 km apart in fragmented prairie habitat. This finding may be due to the less typical genetic system of this species, a permanent translocation heterozygote, whose chromosomes do not recombine. Sletvold et al. (2012) found outbreeding depression between populations of 1.6 km from one another. However, these populations did not exist in fragmented habitat. Though we found heterosis in fragmented populations of *Echinacea*, this body of research shows that the unique genetic systems and demographic histories of populations may play a large role in genetic rescue.

Because the majority of our populations showed heterosis and little inbreeding depression, we expect most populations did not purge their genetic load. Because *Echinacea* is strictly self-incompatible, it cannot purge through selfing (Glémin 2003). In the future, populations of *Echinacea* could purge via small population size (Glémin 2003; Hedrick and Garcia-Dorado 2016). Indeed, Grossen et al. (2020) found a multi-generational bottleneck of alpine ibex was sufficient to purge deleterious mutations of large effect, suggesting sustained bottlenecks may improve population fitness via purging, even in self-incompatible species (but note many deleterious mutations of small effect may still hamper maximum fitness; Grossen et al. 2020). In our study, the population AA notably exhibited fitness consistent with purging (high fitness of W crosses, and no inbreeding depression or heterosis). Perhaps this population had a unique demographic history that lended itself to purging (i.e., an extreme bottleneck or founder event; Grossen et al. 2020; Bertorelle et al. 2022). Nevertheless, AA uniquely showing characteristics consistent with purging emphasizes the chaotic nature of habitat fragmentation.

But we note that, even in this instance, between-population crosses were still not a detriment to fitness.

Wagenius et al. (2010) did not detect heterosis or outbreeding depression after 8 years of growth. In the present study, there was a little difference in heterosis when we compared data from 8 to 14 years (B fitness was 82% higher than W after 8 years, and 85% after 14 years; Figures 3 and S3). We once again highlight the importance of tracking fitness in a variety of environmental conditions, as this likely caused differences in heterosis between our studies. None of the between population pairings of *Echinacea* exhibited outbreeding depression. This indicates that, at the spatial scale of this study, inter-remnant crossing may be an effective tool with little genetic risk for the conservation of remnant populations.

Idiosyncrasy of intercross parent and population characteristics

Low representation of individual parents for each between-population cross pair could contribute to the idiosyncratic trends in fitness for between-population crosses (Figure S1; Tables S1 and S2). However, despite low familial representation in individual pairs of between-population crosses, each source population had multiple maternal and paternal plants involved in between-population crosses, and thus, maternal and paternal fitness of between-population crosses represents the fitness of a single population when outcrossed to any other population. Aside from limitations due to familial sample size, differences in offspring fitness between crossing directions could represent cytoplasmic maternal effects (Roach and Wulff 1987) or an interaction between cytoplasmic and nuclear genomes. Oakley et al. (2015) crossed *Arabidopsis thaliana* populations that spanned the European continent and found the crossing direction impacted fitness, with some crosses having nearly twice the fitness of the reciprocal, even after minimizing environmental maternal effects in the greenhouse (as here, with the parental

generation grown in the experimental plot). Though our crosses were performed over much smaller distances, individual maternal effects may have contributed additional variation to our results.

Population size and disturbance history had no apparent effect on fitness in *Echinacea*. We defined population size as the mean number of flowering plants from 1995-1997 (when seeds were initially collected), and we distinguished high disturbance populations as those where all or almost all plants are in a road right of way adjacent to an agricultural field and thus subject to decapitation, trampling, scraping, and herbicide application, resulting from activities related to road maintenance and agriculture. Such activities reduce the number of successfully flowering individuals in most years. Our cursory post-hoc tests found the magnitude of inbreeding depression and heterosis (expressed as relative performance values; Ågren and Schemske 1993) was not significantly associated with population size or disturbance ($P > 0.37$ in all cases; Figures S5 and S6). Given that *Echinacea* has had few generations since fragmentation, effects of population size and disturbance on allelic diversity may not have compounded enough to support predictions (e.g., Bataillon and Kirkpatrick 2000; Whitlock et al. 2000; Lopez et al. 2009; Spigler et al. 2016), especially given the limited number of populations studied. While the transient nature of population genetics following fragmentation (e.g., Lopez et al. 2009) makes predicting inbreeding depression and heterosis difficult for long-lived species, there are cases where heterosis in fragmented systems follows predicted trends. Willi et al. (2007) and Pickup et al. (2013) found that smaller populations in fragmented environments benefit more from outbreeding than larger populations, which each study ascribed to small populations being more inbred. In our study, heterosis did not correspond to population size or degree of disturbance, but

populations with higher mean fitness did have low heterosis, consistent with the idea that more fit populations may benefit less from outcrossing.

Conservation implications

We saw considerable fitness gains from outcrossing between populations, though the magnitude of heterosis varied among populations. We highlight that, here, the populations intercrossed lie at most 9 km apart. For substantially differentiated populations, pollen import may disrupt local adaptation and lower average population fitness. At short geographic distances, environmental autocorrelation and gene flow decrease the likelihood that populations are differentiated in a way that induces outbreeding depression (Frankham 2015; but see Sletvold et al. 2012). Consequently, we emphasize that our results should not be generalized to scales beyond locally fragmented populations for species that have had a limited number of generations since fragmentation. Additionally, we were unable to investigate the F2 generation of our crosses, but the breakage of coadapted gene complexes in the F2 generation may reduce fitness and should not be discounted (Edmands 1999; Fenster and Galloway 2000). Nevertheless, oft-overlooked small populations may provide a two-fold contribution to species conservation: 1) they can produce heterosis when crossed to other populations, and 2) they may serve as intermediary populations to mediate gene flow between populations, which would reduce likelihood of future genetic differentiation and outbreeding depression. Small populations are often considered targets for conservation because of their high predicted extinction risk from mechanisms such as genetic drift and demographic stochasticity (Richards 2000; Wootton and Pfister 2013; Hufbauer et al. 2015). Here, we recommend that small populations may serve as valuable resources for conservation, that may be especially beneficial in fragmented habitat to bolster the fitness of other populations.

Conclusions

Anthropogenic habitat fragmentation is broadly considered a threat to the persistence of many plant species. In our study, variable heterosis, but no outbreeding depression, suggests spatial genetic structure due to fragmentation could be utilized to improve fitness of populations through human assisted short-distance seed movement, transplants, or hand crosses. Interestingly, heterosis did not vary in relation to population size, suggesting all populations, including small ones, in fragmented environments may serve as valuable donors of genetic material (e.g., pollen and seeds) to other populations. However, striking differences in our results compared with Wagenius et al. (2010) implicate the importance of assessing fitness across environments and substantial portions of the life cycle. Conserving all populations may be critical to ensure high fitness in future generations through genetic rescue.

Data availability statement

We have deposited the primary data underlying these analyses as follows:

- Longitudinal fitness data in the experimental plot: Dryad

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Table 1. Model comparisons to partition effects of crosstype and paternal source population (top) and maternal source population (bottom) on fitness (cumulative fruit count). All test statistics and P-values were obtained from likelihood ratio tests. *Sub* model designates no effects of crosstype or population on fitness and *Crosstype* designates an effect of crosstype (between, within, inbred) on fitness. *Crosstype + Pop* and *Crosstype:Pop* models specify additive and interactive effects of crosstype and source population on fitness, respectively. Df is degrees of freedom. Based on tests, the *Crosstype:Pop* model best fits the data when both maternal and paternal source population are used. P-values < 0.05 are bolded.

Paternal source population:						
Model name	Model df	Model deviance	Nested model	Test df	Test deviance	Test P-value
<i>Sub</i>	43	15,596	-	-	-	-
<i>Crosstype</i>	45	15,576	<i>Sub</i>	2	20.2	< 0.001
<i>Pop</i>	49	15,583	<i>Sub</i>	6	13.5	0.036
<i>Crosstype + Pop</i>	51	15,562	<i>Pop</i>	2	20.6	< 0.001
<i>Crosstype + Pop</i>	51	15,562	<i>Crosstype</i>	6	13.8	0.031
<i>Crosstype:Pop</i>	63	15,539	<i>Crosstype + Pop</i>	12	23.2	0.026
Maternal source population:						
Model name	Model df	Model deviance	Nested model	Test df	Test deviance	Test P-value
<i>Sub</i>	43	15,646	-	-	-	-
<i>Crosstype</i>	45	15,625	<i>Sub</i>	2	20.3	< 0.001
<i>Pop</i>	49	15,629	<i>Sub</i>	6	16.4	0.012
<i>Crosstype + Pop</i>	51	15,607	<i>Pop</i>	2	22.2	< 0.001
<i>Crosstype + Pop</i>	51	15,607	<i>Crosstype</i>	6	18.3	0.006
<i>Crosstype:Pop</i>	63	15,583	<i>Crosstype + Pop</i>	12	23.8	0.022

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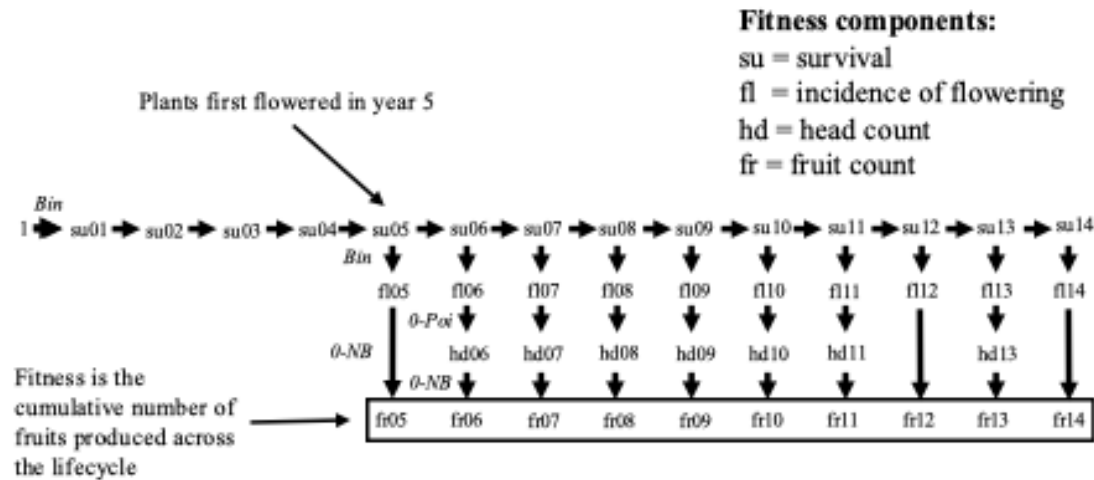
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Table and Figure legends



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684 Figure 1. Graphical model for aster model of plants grown in the experimental plot for the

685 analysis of crosstype and source population. The root node specifies a seedling planted in the

686 experimental plot. Distribution abbreviations: Bin = binomial, 0-NB = 0-truncated negative

687 binomial, 0-Poi = 0-truncated Poisson.

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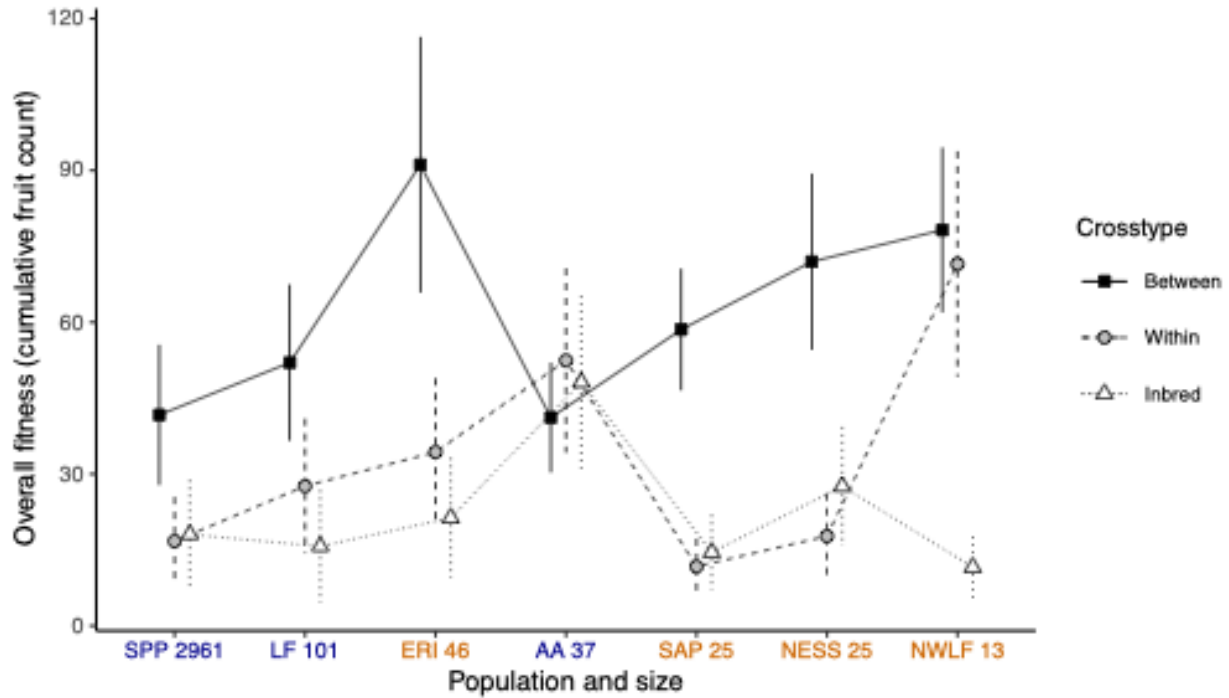


Figure 2. Expected cumulative fruit counts after 14 years among maternal and paternal source population and crosstype. Numbers next to population indicate the mean number of flowering plants from 1995-1997. Blue and orange mark low- and high- disturbance populations, respectively. Cumulative fruit counts are maximum likelihood estimates (± 1 SE) from the minimal adequate *Crosstype:Pop* model (Table 1). Expected cumulative fruit counts for between-population crosses are averaged from separate models that assess fitness from the perspective of maternal and population (for separate models, see Figure S2). Cumulative fruit count estimates indicate the expected number of fruits produced per seedling planted in the experimental plot after 14 years of growth. For true heterosis, fitness of between-population crosses should be greater than for within-population crosses, whereas for inbreeding depression, the fitness of within-population crosses should exceed that of inbred crosses.

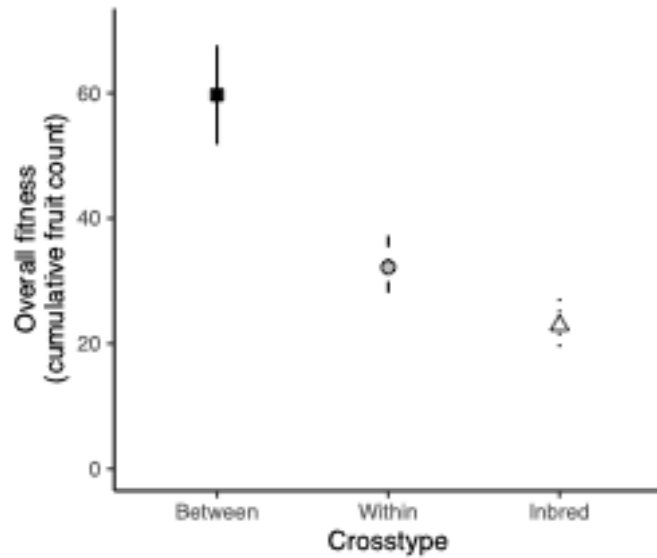


Figure 3. Expected cumulative fruit counts after 14 years of growth in the experimental plot between three crosstypes. Cumulative fruit counts are maximum likelihood estimates (± 1 SE) from the *Crosstype* model, which has less support than the *Crosstype:Pop* model (Table 1). Cumulative fruit count estimates indicate the expected number of fruits produced per seedling planted in the experimental plot after 14 years of growth.

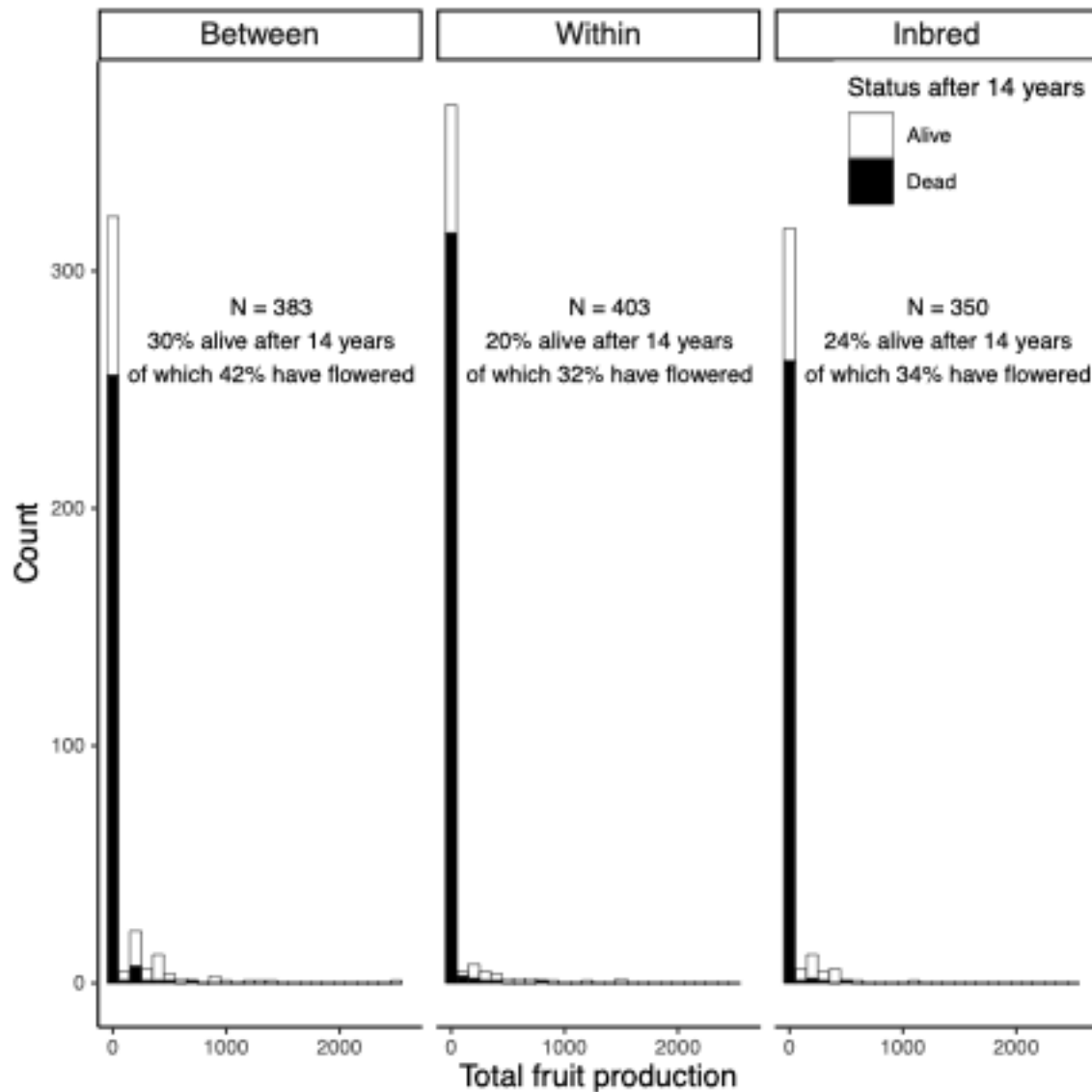


Figure 4. Distribution of individual fitness (cumulative fruit count) among cross types after 14 years of growth in the experimental plot. Cumulative fruit counts and survival are observed values from plants (i.e., they are not predicted values).

Table 1. Model comparisons to partition effects of crosstype and paternal source population (top) and maternal source population (bottom) on fitness (cumulative fruit count). All test statistics and P-values were obtained from likelihood ratio tests. *Sub* model designates no effects of

717 crosstype or population on fitness and *Crosstype* designates an effect of crosstype (between,
718 within, inbred) on fitness. *Crosstype + Pop* and *Crosstype:Pop* models specify additive and
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721 paternal source population are used. P-values < 0.05 are bolded.

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