Heterosis in crosses between remnant populations of a rare prairie forb: implications for restoration genetics

Isabelle A. Turner[1,2], Juan Diego Rojas-Gutierrez[1], Bob Easter[3], Christopher G. Oakley*[1]

[1] Department of Botany and Plant Pathology, and the Center for Plant Biology, Purdue University, West Lafayette, IN 47907, USA

[2] Present address: Department of Biology, Miami University, Oxford, OH 45056, USA

[3] NICHES Land Trust, Lafayette, IN 47905, USA

*Author for correspondence: oakleyc@purdue.edu

Running head: Heterosis in remnant populations of a rare prairie forb

ABSTRACT

- *Premise*

  Calls to adopt seed sourcing strategies in biological restoration that preserve local adaptation while maximizing genetic variation (e.g., regional admixture provenancing) are increasingly common. Heterosis, the increased fitness of progeny from between- relative to within-population crosses, could provide an added benefit under such strategies, but several open questions remain.

- *Methods*

  We quantified heterosis in crosses between three small remnant populations of a rare prairie forb. We measured early fitness components (seed number per fruit, germination, and juvenile survival) in a greenhouse. Adult fitness components (survival and reproduction) were quantified over two flowering seasons in two different environments: a field common garden simulating the initial stages of a restoration, and a greenhouse. Our approach is unique in estimating heterosis in an environment most relevant to the early stages of a restoration and in comparing heterosis under field and controlled conditions.

- *Key Results*

  The consequences of between-population crosses for cumulative fitness in the field were strongly positive in two of the populations (281% and 50% heterosis) and nearly neutral in a third. Heterosis was generally stronger when measured under field conditions, and stage specific patterns of heterosis varied among populations.

- *Conclusions*

Regional admixture provenancing in restorations should be beneficial for this species. We advocate for more research on heterosis in rare species, particularly in restorations. We also provide a cautionary note on how to calculate and report results for heterosis studies.

Key words: Fragmented populations, Genetic rescue, Heterosis, Prairie, Regional admixture provenancing, Restoration genetics, Revegetation, *Silene regia*

INTRODUCTION

Limited genetic variation needed to adapt to variable or changing conditions, and the negative fitness consequences of fixed deleterious recessive alleles, are major issues in conservation and restoration genetics (Frankham, 2005; Willi et al., 2006; Bell et al., 2019). One way to probe the relative extent of deleterious recessive alleles fixed within populations in nature is heterosis, the increased fitness in between- relative to within-population crosses (Fenster and Galloway, 2000; Paland and Schmid, 2003; Oakley and Winn, 2012; Lohr and Haag, 2015). Heterosis is common in crosses between natural populations (Fenster, 1991; van Treuren et al., 1993; Ouborg and Van Treuren, 1994; Luijten et al., 2002; Willi and Fischer, 2005; Pickup et al., 2012; Willi, 2013; Weisenberger et al., 2014; Koski et al., 2019; Oakley et al., 2019; Perrier et al., 2020; Söderquist et al., 2020; Thoen et al., 2025). Greater heterosis in small/less diverse compared to large/more diverse populations (Paland and Schmid, 2003; Escobar et al., 2008; Oakley and Winn, 2012; Lohr and Haag, 2015; Spigler et al., 2017) and in selfing compared to outcrossing populations (Busch, 2006; Oakley et al., 2015a) is consistent with a role of drift in fixing partly recessive deleterious alleles (Charlesworth, 2018). This suggests that heterosis may be particularly relevant in conservation and restoration of small remnant populations.

Some outstanding questions in the study of heterosis are how the magnitude depends on experimental assay conditions and to what extent it varies across the life cycle. The former is somewhat surprising given the considerable work on similar questions in inbreeding depression which is also due to partly recessive deleterious alleles. For example, work by Dudash (1990) demonstrated greater inbreeding depression in field compared to greenhouse conditions, and since then the environmental dependence of inbreeding depression has been well documented (reviewed in Fox and Reed, 2010; Cheptou and Donohue, 2011). Heterosis is often estimated in

the greenhouse, though studies estimating heterosis on plants transplanted into the field for at least part of the life cycle are becoming more common (Fenster, 1991; Ouborg and Van Treuren, 1994; Fenster and Galloway, 2000; Luijten et al., 2002; Wagenius et al., 2010; Maschinski et al., 2013; Weisenberger et al., 2014; Oakley et al., 2015a; Spigler et al., 2017; Oakley et al., 2019; Thoen et al., 2025). However, we are unaware of any comparison of heterosis in field vs. controlled conditions, though there are some comparisons in multiple environments (Fenster and Galloway, 2000; Willi et al., 2007; Li et al., 2018; Stojanova et al., 2021; Perrier et al., 2022). We note that heterosis differs from inbreeding depression in that it is caused by alleles fixed within populations rather than alleles segregating within populations (Whitlock et al., 2000; Whitlock, 2002; Charlesworth, 2018). Also, unlike inbreeding depression which can be comprised of both strongly deleterious and more mildly deleterious alleles (Charlesworth and Willis, 2009), heterosis is expected to be mostly due to mildly deleterious alleles (Whitlock et al., 2000; Glémin et al., 2003; Charlesworth, 2018). Heterosis may therefore be weak for individual fitness components, particularly early in the life cycle (c.f., Husband and Schemske, 1996; Perrier et al., 2020). Therefore, obtaining accurate estimates of heterosis may require estimates of fitness encompassing as many life stages as possible.

One context in which heterosis could be potentially useful is in restoration projects involving rare species. Restoration practitioners use seeds, or sometimes seedling plugs, to establish populations (Shaw et al., 2020), often including rare or threatened species that are dispersal- or seed-limited (Pywell et al., 2002; Seabloom et al., 2003; Clark et al., 2007). The method of seed sourcing and the genetics of the source populations can greatly influence establishment success (De Vitis et al., 2022). Traditionally it was thought that seeds should be sourced as locally as possible to minimize the risk of disrupting adaptation to local

environmental (extrinsic) conditions or outbreeding depression due to (intrinsic) genetic incompatibilities (McKay et al., 2005; Gann et al., 2019; Török et al., 2024). However, potential seed sources for restoration are often small remnant populations from which only a small number of individuals (sometimes only a single individual) are sampled (Godefroid et al., 2011). Thus, the founding population may harbor fixed deleterious mutations and have limited genetic variation to adapt to fluctuating or changing conditions, particularly in the face of climate change. Because of these risks, many authors have called for some version of seed sourcing strategies that increase genetic variation while also minimizing outbreeding depression and the loss of local adaptation (Broadhurst et al., 2008; Godefroid et al., 2011; Breed et al., 2013; Bucharova et al., 2019; St. Clair et al., 2020; Hoffmann et al., 2021; Stojanova et al., 2021; Jordan et al., 2023). One proposed strategy is "regional admixture provenancing" (Bucharova et al., 2019), where seed is sourced from multiple populations regionally to increase genetic variation. These populations would however come from similar habitat in the same eco-region, likely connected by gene flow prior to fragmentation, to minimize the risks of maladaptation and genetic incompatibilities. Additionally, heterosis following admixture in a restoration may provide at least a short term fitness boost that may aid establishment of rare species, analogous to the "catapult effect" proposed for the role of heterosis in the establishment of invasive species (Drake, 2006; Li et al., 2018). Despite the potential benefits, there are few empirical tests of the regional admixture provenancing approach (but see St. Clair et al., 2020) or the potential for heterosis during a newly established restoration.

Here we used within- and between- population crosses of three small remnant populations of a rare prairie forb, *Silene regia* Sims., to quantify heterosis for multiple fitness components in both a greenhouse experiment and a field experiment mimicking a newly

established restoration. We addressed the following questions: 1) What is the magnitude of heterosis (or conversely, outbreeding depression) under restoration conditions in the field, and does it vary among populations? 2) Is the magnitude of heterosis greater in a field common garden compared to controlled greenhouse conditions? and 3) How does the magnitude of heterosis vary across different life history stages? We expected to observe greater heterosis under field compared to greenhouse conditions, as well as greater heterosis in estimates of fitness incorporating later life history stages.

MATERIALS AND METHODS

Study system--

Silene regia (royal catchfly) is a tap-rooted perennial in the Caryophyllaceae family (Dolan, 1994). It is endemic to a dozen states in eastern North America and is thought to have been broadly distributed across prairies prior to the widespread conversion of this habitat to agriculture (Menges, 1991). Contemporary populations across much of the native range are small and isolated and exist as remnants in prairie cemeteries and along railroad rights-of-way and roadsides (Dolan, 1994). Remnant populations of *S. regia* are potentially at risk of genetic depletion (Menges and Dolan, 1998). This species does not vegetatively reproduce and achieves sexual reproduction via hummingbird pollination. It is self-compatible and some geitonogamous selfing may occur, but it is likely mostly outcrossing as the individual flowers are protandrous (Dolan, 1994; Menges, 1995).

We selected three remnant focal populations in different counties in western Indiana (Tip = Tippecanoe; Fou = Fountain; Ver = Vermillion). Exact locations of these populations are not provided because this species is critically imperiled in Indiana (NatureServe, 2024), but these are

136 distinct populations, all separated by at least 14 km. These three populations include all known
137 existing remnants within a 55 km radius, and there may be as few as 10 extant remnant
138 populations in the entire state of Indiana (B. Easter, pers. obs.). While including more
139 populations could have broadened our scope of inference, we restricted the sampling to remnants
140 from the same local area as would be advised under regional admixture provenancing. The
141 smallest population by census size was Tip (10 flowering individuals) from a ~0.5 ha remnant,
142 which has likely been small for an extended period (B. Easter, pers. obs.). The largest population
143 was Ver (~50 flowering individuals) from a ~0.4 ha remnant, and population size reductions are
144 likely more recent at this site (B. Easter, pers. obs.). The size of Fou, from a ~0.4 ha remnant,
145 was intermediate to the other populations (~20 flowering individuals). While there is some
146 variation in census size among these remnants, they are all quite small and are thus all expected
147 to have limited genetic variation and harbor fixed deleterious mutations.
148
149 ***Generation of seeds for the fitness assays--***
150 Bulk seed was collected from each population in September 2019. Our goal was to characterize
151 population level heterosis, and the small number of individuals in these populations precluded
152 experimental designs accounting for distinct maternal lineages. We collected seeds from most of
153 the reproductive individuals in the two smaller populations and from a haphazard subset of
154 approximately 20 individuals in the largest population, Ver.
155 In early October 2019, seeds from each population were surface sterilized in a 10%
156 bleach solution for 10 minutes, followed by three rinses in deionized-autoclaved water. The
157 seeds were then placed into moist paper towels in bags and cold stratified in the dark at 4°C for
158 10, 30, or 60 days. Different stratification periods were used because 60 days would be ideal for

159 germination, but starting the plants sooner was preferrable due to logistical constraints. Because
160 of low overall germination we included individual maternal plants arising from all three seed
161 stratification treatments. While there was a significant effect of seed stratification treatment (for
162 seeds that produced the parental plants) on seed number per fruit following controlled crosses,
163 this treatment (involving the generation prior to the experimental assay) did not qualitatively
164 affect the results for either the effects of cross type or the interaction between maternal
165 population and cross type (compare Table S1 to Table 1) so we omitted this term from
166 subsequent analyses.

167 After stratification, we sowed seeds (5 per cell) into a 72-cell flat insert (cell volume = 70
168 ml, Greenhouse Megastore, Danville, Illinois, USA), filled with a 3:1 mixture of MetroMix
169 potting soil (Sun Gro Horticulture, Agawam, Massachusetts, USA) and autoclaved sand. A total
170 of 30-50 seeds were sown for each population-stratification treatment combination. The seeds
171 were then germinated in an incubator (Percival scientific, Perry, Iowa, USA) set to a constant
172 27°C and a 12-hour photoperiod. After approximately 4 weeks, cells were thinned to one
173 seedling per cell, and the flat was moved to a heated greenhouse (minimum: 20°C) with
174 supplemental lighting (12-hour photoperiod). Plants were grown there for approximately 6
175 weeks, watering as needed. Plants were then moved to a large refrigerator (4°C) with lights (10-
176 hour photoperiod) for approximately one month to synchronize flowering. After the end of this
177 vernalization period in mid-January 2020, plants were moved back to the greenhouse and
178 transplanted into 17 cm circular pots (volume = 2.4 l, Greenhouse Megastore, Danville, Illinois,
179 USA) filled with the same soil mixture. Plants were watered as needed and fertilized monthly
180 with ¼ strength Hoagland's solution.

181 Once plants had begun to flower (early March 2020), we performed controlled hand
182 pollinations within and between populations in all possible combinations and crossing directions.
183 In mid-March, because of COVID related shutdowns, the plants (19, 13, and 10 individuals from
184 Fou, Tip, and Ver respectively) were moved to a private residence and grown on light racks with
185 a 14-hour photoperiod for the duration of the hand pollinations. Most of the pollinations were
186 performed from mid-March through the end of June 2020. Before each pollination we identified
187 ovule donor flowers several days prior to anthesis and removed the upper part of the corolla
188 tube, filaments, and anthers with scissors and forceps. Pollinations were completed when the
189 stigma lobes of the ovule donor flowers appeared to be receptive (usually 3-4 days later) by
190 removing stamens from a pollen donor flower with forceps and brushing the dehisced anthers
191 against the stigma. Each pollination was tracked with a unique color combination of embroidery
192 floss tied around the pedicel. Pollinations were done haphazardly based on flower availability,
193 but we attempted to balance the cross types done for a given maternal population on a given day
194 to minimize confounding treatment effects with age effects. Because we started with bulked seed
195 collections and were interested in population level estimates of heterosis, and because of
196 practical constraints of the number of receptive flowers per day on a given plant, we did not
197 execute a balanced crossing design at the level of individual plants. However, most individual
198 plants participated as both ovule and pollen donors, with population mean averages of 5-8
199 flowers as ovule donors and 4-7 flowers as pollen donors for between population crosses per
200 plant. For within population crosses, each individual plant contributed an average (by
201 population) of 3-4 flowers as ovule donors and 2-3 flowers as pollen donors.
202 The number of hand pollinations per maternal population ranged from 39-58 (mean = 46)
203 for within-population (WIN) crosses and 85-102 (mean = 91) for between-population (BET)

204 crosses, for a grand total of 411 crosses. The approximately two-fold greater number of hand
205 pollinations for between-population crosses is because each maternal population was crossed to
206 two different paternal populations, and we wanted sufficient power to test for interactions
207 between maternal and paternal populations.

208 In addition to hand pollinations, we performed control emasculations where flowers were
209 emasculated but no pollen was applied to the stigma lobes. Of the 69 control emasculations, less
210 than 3% (2 emasculations) resulted in a fruit, giving us confidence that the vast majority of the
211 pollinations performed were successful crosses between the intended pollen and ovule donors.
212

213 ***Early fitness components--***

214 We collected fruits of all successful pollinations to be used in subsequent fitness assays. This
215 species required an extended period between pollination and fruit maturity (mean = 56.8 days,
216 SD =10.4). There were no patterns in fruit set or the rate of fruit development with respect to
217 cross type, but fruits of Tip developed on average about 5 days faster than fruits from the other
218 two populations. For each of 17-39 WIN fruits and 45-71 BET fruits per maternal population
219 (Table S2), we counted the number of seeds per fruit, excluding small, shriveled seeds presumed
220 to be inviable.

221 We selected a subset of the viable seeds for each population and cross type, prioritizing
222 fruits with enough viable seeds for germination assays and the goal of proportionally balanced
223 sample sizes. This subset included approximately 300 seeds each of the WIN and the two BET
224 combinations for populations Tip and Ver and approximately double those numbers for Fou
225 (Table S2). These seeds were cold stratified as previously described for a period of 7 weeks
226 starting in mid-November of 2020. Seeds were then sown into 72 cell flat inserts filled with the

same soil mix (5 flats in total) with 7-12 (mean = 9.96, SD = 0.34) seeds per cell in a randomized block design. The number of cells per flat varied by maternal population with an average of 6 and 12 for WIN and BET crosses respectively from Tip and Ver, and approximately double the sample size for each cross type from Fou (Table S2). We recorded germination daily for a period of 24 days starting in mid-January 2021. Most seeds germinated within the first week, and only 5 new germinants were recorded during the second half of the scoring period.

Once seeds had germinated and seedlings had two true leaves, they were transplanted into individual cells of 72 cell flat inserts and moved to the greenhouse. The seedlings were transplanted into an incomplete randomized block design with a total of 30 flats (blocks). The number of cells per flat varied by maternal population, with an average of 5-6 for WIN and 10 for BET crosses from Tip and Ver, and an average of 11 WIN and 24 BET crosses from Fou. Any seedlings that died within the first week were replaced with another seedling of the same population-cross type. This mortality was likely due to transplant shock, and these individuals were omitted from subsequent analyses. Once roots started to poke through the bottom of the cells, individuals were transplanted first into 6 cm square (volume = 250 ml, Greenhouse Megastore, Danville, Illinois, USA) pots filled with the same soil mixture. Plants were watered as needed and fertilized twice with ¼ strength Hoagland's solution. Cumulative juvenile survival was recorded up to approximately seven weeks after stratification, excluding individuals that died within one week of the initial transplant. Surviving plants that were established in the 6 cm pots were then randomly divided into two experiments: one in the greenhouse and one in a field common garden simulating a new restoration.

Greenhouse experiment--

250 In late March 2021, seedlings were transplanted into 11 cm square (volume = 1L, Greenhouse
251 Megastore, Danville, Illinois, USA) pots filled with the same potting mix in an incomplete
252 randomized block design consisting of 48 flats each containing 7-8 pots. Each maternal
253 population cross type combination was represented by an average of 1.3-2.6 plants in at least 19
254 different flats (blocks). The total size of the greenhouse experiment was 354 plants with sample
255 sizes for between population crosses being approximately double that of within population
256 crosses, and sample sizes for Fou being approximately double that of the other populations
257 (Table S2). Plants were grown for approximately 18 months, until mid-October of 2022, which
258 included two flowering periods. During this time plants were watered as needed and fertilized
259 once with Osmocote timed release 15-9-12 fertilizer at ½ the recommended dose after the first
260 flowering period was over.
261 For each plant in the greenhouse experiment we scored adult fitness as the cumulative
262 number of flowers produced over ~18 months (two flowering seasons) including zeros for plants
263 that died prior to reproducing or that did not reproduce. We additionally quantified two
264 components of this composite measure of adult fitness separately: combined cumulative
265 probability of survival and reproduction (did a plant reproduce or not over the entire course of
266 the experiment), and cumulative fecundity (total flower number over the entire course of the
267 experiment) of reproducing plants.
268
269 ***Field experiment--***
270 We established a field common garden experiment to quantify adult fitness components under
271 more realistic restoration conditions. The site was located on the property of Arbor America LLC
272 in West Point, Indiana (Tippecanoe County) USA. This location is within the extant range of *S.*

regia, and the field common garden experiment was planted on Coloma Sand (Mixed, mesic Lamellic Udipsamments), a soil type and series similar to the well-drained sites where *S. regia* naturally occurs (ISEE Network, 2024). The plot had previously been treated to establish a prairie strip with an application of Roundup in May 2020 to remove the turf, followed by controlled burning approximately 1 month later, and another application of Roundup after approximately 2 more months. Herbicide applications and controlled burns are typical of establishing grassland restorations and help to promote native forb establishment (Török et al., 2024). In early March 2021, the plot was hand seeded with a short stature, high-diversity prairie mix from Spence Restoration Nursery (Muncie, IN, USA).

In early April 2021, seedlings were transplanted into a randomized complete block design consisting of six blocks containing 120 plants each. In total there were 720 plants, with approximately double the sample size of the greenhouse experiment for each maternal population-cross type combination (Table S2). Each block was a rectangular grid of 3x40 plants with 30 cm spacing between the plants. Plants were hand watered for the first week only, and plants that died during this first week were considered to have succumbed to transplant shock and were replaced. In July of both 2021 and 2022, other vegetation within 10 cm of the base of experimental plants was clipped once to facilitate locating plants for the post-reproductive censuses. While the conditions of the experimental plot closely mimic the conditions during the initial phase of a restoration, we acknowledge that this species would likely be sown as seeds in an actual restoration. Given the extremely low rates of field establishment from seed for this species (Menges, 1995) and these populations in particular (< 2%, C. Oakley, unpublished data), and the effort to create enough seeds via experimental crosses, starting these plants in the field as seedlings was a necessary compromise.

In late October 2021, we recorded whether each plant was still alive and had reproduced, and for plants that had reproduced we counted the number of fruits. Only about 11% of all plants had reproduced in the first year. We re-censused the same fitness components in October 2022. We estimated adult fitness as the total number of fruits (sum over both years) produced per individual transplanted, including zeros for plants that died before reproducing or surviving plants that did not reproduce. We additionally calculated the combined cumulative (across both years) probability of survival and reproduction for each plant and cumulative fecundity (total number of fruits across both years) for each reproducing plant.

Statistical analyses and estimates of heterosis--

We analyzed each fitness component using ANOVA. We tested for the effects of maternal population (Fou, Tip, and Ver), paternal population, and their interaction. For all fitness components except seed number per fruit from the initial crosses (where there was no blocking possible), we included a term for block in the analysis to account for micro-spatial variation within the experiment. All effects were treated as fixed effects because the limited number of populations and blocks preclude treating them as a truly random sample. Inspection of residuals indicated no major issues with normality or heteroscedasticity for many fitness components, and for these we used normal error distributions. Juvenile survival and the combined probability of survival and reproduction in both the greenhouse and field experiments required a generalized linear model with a binomial error distribution. For each model with a significant interaction between maternal and paternal populations or significant main effects for both maternal and paternal population, we used linear contrasts to test *a priori* hypotheses. We first contrasted BET

318 crosses to WIN crosses (over all populations) to test if there was significant overall heterosis (or
319 outbreeding depression).

320 We then performed two different sets of contrasts to test for heterosis (or outbreeding
321 depression) separately by maternal population. First, we contrasted BET crosses to WIN crosses
322 for each maternal population. This is the traditional approach to investigating heterosis within
323 maternal populations. However, this approach may lead to misleading conclusions if progeny
324 from WIN crosses from different populations have very different mean fitness. To illustrate this
325 issue, consider a hypothetical example where population A has very low within population
326 fitness and population B has very high within population fitness. With purely additive effects
327 (i.e. no dominance complementation or heterosis) the cross between these two populations
328 should have fitness equal to the mean of the two parents, a.k.a. the midparent value (Lynch and
329 Walsh, 1998; Demuth and Wade, 2006; Oakley et al., 2015b). Comparing BET crosses to WIN
330 crosses for population A could lead to the mistaken interpretation of heterosis, and a similar
331 mistaken interpretation of outbreeding depression could result for the comparison in population
332 B. To more accurately test for heterosis or outbreeding depression within maternal populations
333 while accounting for additive differences in mean population fitness we performed contrasts of
334 BET crosses for a given population to WIN crosses pooled over all populations (because all
335 populations were crossed in all combinations). In discussing the results, we primarily focus on
336 these corrected contrasts but point out cases where results for the two methods differ notably. All
337 statistical analyses were performed in JMP Pro v. 16.1.0 (JMP, 1989-2021).

338 For each fitness component and composite estimate of fitness we calculated estimates of
339 heterosis separately for each population using means for each maternal population-cross type
340 combination averaging over the block means (Table S2). For the traditional approach we

341 calculated the percent increase in fitness of between-population crosses relative to within-
342 population crosses ([(BET-WIN)/WIN]*100), where positive values indicate heterosis and
343 negative values indicate outbreeding depression. To account for differences in mean WIN fitness
344 among populations, we pooled WIN crosses from all three populations for the denominator
345 ([(BET-WIN_{pool})/WIN_{pool}]*100). We additionally calculated heterosis relative to the midparent
346 value for each pair of maternal and paternal populations. However, relative fitness of BET
347 crosses for different paternal populations were always of the same sign (i.e. variation in the
348 degree of heterosis or outbreeding depression for both populations, but never contrasting
349 directions of effects; Figure S1). For simplicity, we therefore focus on the estimates pooling
350 within population crosses across populations but estimates for all three approaches can be found
351 in Table S3.
352 For cumulative fitness, calculated separately for the greenhouse and field experiments,
353 we used the product of four estimates: 1) seed number per fruit, 2) proportion germination, 3)
354 proportion juvenile survival, and 4) adult fitness. Because this multiplicative measure of
355 cumulative fitness yields only one estimate per cross type per population, we could not directly
356 test the effects of population, cross type, and their interaction as with the fitness components.
357 Other authors have approached this problem by using replicate line level estimates of
358 multiplicative fitness (see for e.g., Dudash, 1990), but we were unable to use this approach both
359 because of our initial bulked seed collection and because not all cross types were performed on
360 all plants.
361
362 **RESULTS**
363 ***Early fitness components--***

364 In general, heterosis was weak and/or non-significant for early fitness components (Fig. 1,
365 Tables 1&S3). For seed number per fruit and germination there were genetically based
366 differences among maternal populations (Table 1), with Fou having greater overall seed number
367 per fruit and germination than the other two populations (Fig. S2; Table S2). There was no
368 overall heterosis for either fitness component (BET_{pool} vs. WIN_{pool} contrast in Table 1). However,
369 there was suggestive evidence of heterosis in germination for Fou (Table 1). On average,
370 estimates of population level heterosis (or outbreeding depression) for seed number per fruit
371 ranged from -14% to 41%, and estimates of heterosis (or outbreeding depression) for
372 germination ranged from -7% to 23% (Table S3). For juvenile survival there was a significant
373 interaction between maternal and paternal population (Table 1) and significant overall heterosis
374 of about 6%. Pairwise contrasts of cross types separately by maternal population indicated
375 significant heterosis (13%) only for Fou (Fig. 1, Tables 1&S3).
376
377 ***Greenhouse experiment--***
378 For adult fitness (flowers per seedling planted) in the greenhouse experiment, there were no
379 significant effects for any of the model terms (Fig. 2; Table 2). On average, relative fitness
380 estimates (Table S3) ranged from modest heterosis (32%) in Ver to weak outbreeding depression
381 in Fou (-3%) and Tip (-6%). There was limited evidence of significant effects for fitness
382 components (Fig. 2; Table 2). However, there was a suggestive interaction between maternal and
383 paternal population for fecundity (Table 2) with about 19% overall heterosis. This was most
384 evident in Ver with 36% heterosis (Figs. 2&S3; Table S3). For the combined probability of
385 survival and reproduction there was weak outbreeding depression on average for all populations
386 -3% to -13% (Table S3). For this experiment, the three WIN crosses had similar mean fitness,

387 and thus calculations of heterosis were qualitatively similar between the different approaches
388 (Figs. 2&S3; Table S3).
389
390 ***Field experiment--***
391 For adult fitness (fruits per seedling planted) in the field, there were significant effects of both
392 maternal and paternal population (Table 3), indicating genetically based differentiation in mean
393 fitness among populations. Most notably there was four-fold variation in mean fitness of progeny
394 from WIN crosses, ranging from 2 fruits per seedling planted for Tip, to 3 for Fou, and 8 for Ver
395 (Fig. S4; Table S2). Additionally, there was a significant interaction between maternal and
396 paternal populations which could indicate overall heterosis (or outbreeding depression) and/or
397 variation among populations in the degree of heterosis of BET crosses (Table 3). Indeed,
398 contrasts revealed significant overall heterosis (BET_{pool} vs. WIN_{pool}) of about 45%. Contrasts of
399 BET crosses accounting for additive differences between populations (BET vs. WIN_{pool})
400 indicated significant heterosis only for Fou (Fig. 3; Table 3) of about 78% (Table S3). At least on
401 average, there was also heterosis for Tip (20%) and Ver (36%) (Tables 3&S3).
402 The methodology for testing/calculating relative fitness separately for maternal
403 populations had a considerable effect on adult fitness for some populations (Tables 3&S3). This
404 is likely due to the extent of population differentiation in mean fitness of progeny from within-
405 population crosses, which had not been observed for other fitness components thus far. Using the
406 traditional approach, we would conclude significant heterosis of 160% for Tip (Tip: BET vs.
407 WIN contrast Table 3) compared to the non-significant 20% heterosis above (Table S3). The
408 results for Ver do not qualitatively change between approaches but there is a reversal in the sign
409 of effects between the two approaches, -26% outbreeding depression with the traditional

410 approach compared to 36% heterosis above (Table S3). Both approaches indicate significant
411 heterosis for Fou (Table S3).
412 There was also significant variation among populations and population combinations for
413 individual fitness components. For the combined probability of survival and reproduction there
414 were significant effects of both maternal and paternal populations. As with adult fitness, the
415 probability of surviving and reproducing for WIN crosses in Ver was much greater (about 1.6-
416 fold) than for the other populations (Fig. S4; Table S2). There was no significant overall
417 heterosis (about 10% on average) for this fitness component, but there was a suggestive effect of
418 heterosis (23%) in Fou (Fig. 3; Tables 3&S3). For fecundity, there was a significant effect of
419 paternal population and a suggestive effect for the interaction between maternal and paternal
420 populations (Table 3). There was significant overall heterosis for fecundity of about 38% with
421 heterosis in all populations (29 to 55%), at least on average, significantly so in Fou (Fig. 3;
422 Tables 3&S3).
423 As with adult fitness, the methodology for testing/calculating relative fitness for the
424 fitness components had a strong influence on the results. For probability of survival and
425 reproduction, the traditional approach would suggest highly significant heterosis for Fou (Fou:
426 BET vs. WIN contrast Table 3; Table S3) of a much greater magnitude (89% compared to 23%
427 above) and highly significant outbreeding depression (-34% compared to an average of 11%
428 heterosis for the corrected comparison) for Ver (Tables 3&S3). For fecundity, both methods
429 yielded qualitatively similar results, likely because of the more limited variation among maternal
430 populations in mean fecundity.
431
432 ***Cumulative fitness--***

Estimates of cumulative heterosis across all measured life stages were substantial in both greenhouse and field experiments, but they were generally stronger in the field experiment. For the greenhouse experiment there was about 35% heterosis overall, and within populations heterosis was 92% for Fou and 35% for Ver, and there was weak (and non-significant based on results for fitness components) outbreeding depression (-23%) for Tip (Table S3). Using the traditional comparison suggested less heterosis for Fou and more for Ver (Table S3). For the field common garden experiment, heterosis was 112% over all populations. All three populations exhibited heterosis for cumulative fitness with considerable variation in magnitude among populations, from weak (6%) in Tip to modest (50%) in Ver to very strong (281%) in Fou (Table S3). Using the traditional comparisons would cause the false conclusion of very strong heterosis (131%) in Tip and weak outbreeding depression (-4%) in Ver, but estimates for Fou were similar for both methods (Table S3).

DISCUSSION

Seed sourcing strategies for restoration such as regional admixture provenancing have been suggested to help maintain both local adaptation and amounts of genetic variation within restored populations. Heterosis may provide an added short-term benefit to such strategies. To our knowledge, our study is the first to quantify heterosis for multiple fitness components in a newly established restoration. Averaged over all populations, the consequences of between population crosses for cumulative fitness was strongly positive (112% overall heterosis) in the field, suggesting that regional admixture provenancing should provide at least a short-term fitness advantage for this species. Estimates of heterosis from the field were greater than those from the greenhouse (35% overall heterosis). Additionally, life-stage specific estimates of both

456 mean population fitness and heterosis varied among populations, particularly in the field
457 experiment. This suggests that while there is an overall benefit of crosses between populations
458 under realistic field conditions, relative fitness outcomes can depend on the populations involved
459 and the environmental conditions of the fitness assay.

460 Results from the field experiment simulating early restoration conditions is most directly
461 relevant for restoration practitioners. Over all populations there was significant heterosis for both
462 fruits per seedling planted (45%) and fecundity (38%), and on average there was consistently
463 increased relative fitness for these measures (ranging from 20-78%) within populations (Table
464 S3). Heterosis for the combined probability of survival and reproduction was weaker (10%
465 overall), and none of the results for individual populations were significant. Thus, admixture of
466 these populations in field restorations are likely to result in a net positive effect, at least for
467 reproductive fitness, despite some among population variation in the degree of heterosis.

468 The among population variation in mean fitness in the field experiment is important for
469 the calculation and interpretation of heterosis for individual populations, and also has direct
470 implications for restoration potential. For example, Ver had much greater survival and adult
471 fitness of WIN crosses compared to other populations (Fig. S4). This highlights the importance
472 of using the mid-parent value as the frame of reference in calculating heterosis to avoid
473 confounding additive and non-additive effects (field results for Ver; Table S3). Estimating
474 heterosis as a proportional deviation from the mid-parental value is commonly used for natural
475 populations with predominantly selfing mating systems (Lynch and Walsh, 1998; Oakley et al.,
476 2019; Clo et al., 2021), but it should be broadly applicable to other systems. Alternatively,
477 studies where different population categories (e.g. small vs. large) are represented by many
478 populations (Paland and Schmid, 2003; Oakley and Winn, 2012; Willi, 2013) or where each

479 maternal population is crossed with many paternal populations (Busch, 2006; Weisenberger et
480 al., 2014; Spigler et al., 2017; Thoen et al., 2025) achieve a similar end. These approaches
481 minimize the influence of individual populations with very high or very low mean within
482 population fitness by averaging over many populations. The use of such approaches to minimize
483 confounding additive and dominance effects can also be important in greenhouse studies if the
484 parental populations differ in mean fitness (Rojas-Gutierrez et al., 2026). The greater mean
485 fitness within Ver may be because it is the largest of the three populations and more recently
486 reduced in size and may therefore harbor fewer fixed deleterious recessive alleles. The low mean
487 adult fitness and fitness components within Tip for the field experiment (Fig. S4) was also
488 notable, especially because the origin of this population was geographically closest to the
489 planting site. This was the smallest population and may therefore suffer the most from fixed
490 deleterious alleles, representing a situation in which the most "local" population may not always
491 be the "best" source material for a restoration (Pizza et al., 2023).

492 Field experiments of heterosis are logistically challenging but are increasingly more
493 common, and like our study are often based on transplanted seedlings (Fenster, 1991; Ouborg
494 and Van Treuren, 1994; Wagenius et al., 2010; Weisenberger et al., 2014; Oakley et al., 2015a;
495 Spigler et al., 2017; Oakley et al., 2019; Thoen et al., 2025). We limit our discussion to studies of
496 heterosis in rare or recently fragmented species because of the expected context dependency of
497 heterosis on population size, genetic distance between parents, and spatial scale of the field
498 experiment. Our overall conclusions agree with an exceptional 14-year field study that found
499 inter-population variation in heterosis and limited evidence for outbreeding depression of seven
500 fragmented populations of *Echinacea angustifolia* (Thoen et al., 2025). An 8-year field study in
501 the same species conducted near the Thoen et al. (2025) study also found no evidence for

502 outbreeding depression, but curiously did not find any heterosis (Wagenius et al., 2010), which
503 Thoen et al. (2025) attribute to environment dependent expression of heterosis. Generally
504 speaking, other field studies in rare taxa also find heterosis and little evidence for outbreeding
505 depression (Weisenberger et al., 2014), though the magnitude of heterosis seems to depend on
506 population characteristics (Pickup et al., 2012) and temporal environmental variation
507 (Maschinski et al., 2013).

508 Comparisons between field- and greenhouse-based estimates of heterosis are rare in the
509 literature, though some studies have indicated that heterosis may be stronger under more
510 stressful conditions (Maschinski et al., 2013; Hahn and Rieseberg, 2017; Stojanova et al., 2021).
511 Our results indicate that heterosis is greater in field compared to greenhouse conditions for fruits
512 per seedling planted (adult fitness) and both of its component parts, combined probability of
513 survival and reproduction and fecundity. We also found stronger average heterosis in the field
514 compared to greenhouse for cumulative fitness. Stronger heterosis under presumably more
515 variable and stronger selection pressures in the field compared to the greenhouse is in agreement
516 with results from many, though not all, similar comparisons for inbreeding depression (Dudash,
517 1990; Fox and Reed, 2010; Cheptou and Donohue, 2011).

518 In addition to the comparison between field and greenhouse, we were interested in how
519 heterosis might change across different life stages. If heterosis is caused by many mildly
520 deleterious alleles, we would expect to see greater heterosis in later life history stages or in
521 estimates that integrate multiple fitness components (van Treuren et al., 1993; Willi et al., 2007;
522 Escobar et al., 2008; Oakley et al., 2015a; Spigler et al., 2017; Perrier et al., 2020; Thoen et al.,
523 2025) as is seen for inbreeding depression in highly selfing species (Husband and Schemske,
524 1996). In general, the results for heterosis over all populations follow such a pattern, with no

525 significant heterosis for the earliest two fitness components, seed number per fruit and
526 germination, and very modest heterosis for seedling survival. It is important to note that in our
527 study all early components of fitness were measured in the greenhouse. Similar experiments are
528 rarely started from seed (but see Luijten et al., 2002), likely due to low field germination rates
529 (e.g. Thoen et al., 2025) as expected for *S. regia* (Menges, 1995). Strong heterosis for early
530 fitness components has been reported for some species even in controlled environment
531 conditions (Busch, 2006; Oakley et al., 2015a; Söderquist et al., 2020), but outbreeding
532 depression for early fitness components is also possible (Sletvold et al., 2012). For later life
533 stages in the greenhouse there was stronger overall heterosis for fecundity (19%), though not for
534 the combined probability of survival and reproduction or adult fitness. Incorporating additional
535 years of fecundity estimates would likely increase overall heterosis in both the greenhouse and
536 field environments. Thus, our measures of adult fitness components may underestimate overall
537 heterosis.

538 In conclusion, the heterosis reported here for crosses between these small remnant
539 populations in conditions representative of a newly established restoration strongly suggest that
540 regional admixture provenancing would be a beneficial strategy for this species. The short-term
541 fitness boost provided by heterosis is in addition to any potential long-term benefits of increased
542 genetic variation necessary to adapt to environmental heterogeneity and climate change. We
543 agree with previous authors that outbreeding depression is unlikely to have serious negative
544 consequences for restoration and conservation efforts (Frankham, 2015; Ralls et al., 2018; Bell et
545 al., 2019; Hoffmann et al., 2021; Jordan et al., 2023) if appropriate guidelines are followed
546 (Frankham et al., 2011; Weeks et al., 2011). Even if there is some outbreeding depression in
547 recombinant generations in this system, selection is expected to quickly remove deleterious

548 combinations (Luijten et al., 2002; Erickson and Fenster, 2006; Aitkin and Whitlock, 2013;
549 Hamilton and Miller, 2016). In future studies of heterosis, we suggest crossing designs and/or
550 analytical approaches (i.e. comparisons to mid-parent value for paired population designs) to
551 interpret dominance effects correctly accounting for differences in mean fitness between
552 populations. Furthermore, we suggest that it is important to report mean values for each cross
553 type for each population in addition to estimates of heterosis/outbreeding depression. Many open
554 questions about heterosis remain, and we agree with Menges (2008) that restorations should be
555 seen as experimental opportunities in addition to their other benefits.
556

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564

565 **AUTHOR CONTRIBUTIONS**

566 I.A.T. and C.G.O. conceived of and designed the study. B.E. provided the seeds and established
567 the field plot. I.A.T. and C.G.O. conducted the experiment and collected the data. C.G.O.,
568 J.D.R.G. and I.A.T. analyzed the data. J.D.R.G. produced the figures. I.A.T. and C.G.O. drafted
569 the manuscript. All authors contributed to revising the manuscript.
570

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764

Table 1. ANOVA results for early fitness components for maternal and paternal populations and their interaction and block (where appropriate). Models for both seed number per fruit and percent germination (per cell) employed normal error distributions (denominator degrees of freedom in parentheses). The model for juvenile survival employed a binomial error distribution. Contrasts between cross types within a maternal population are only reported for models with a *P* value < 0.1 for either the interaction between maternal population and paternal population, or both maternal and paternal populations. For the contrasts, WIN = within-population cross, BET = between-population cross, the subscript pool indicates that the contrast involves all individuals of a given cross type (pooled over populations). Contrasts that start with a population code followed by a colon contain at least one cross type specific to that maternal population.

	Seed number per fruit (Denom. DF = 229)		Germination (Denom. DF = 346)		Juvenile Survival	
	F	*P*	*F*	*P*	χ^2	*P*
Maternal population	17.9	**<0.001**	9.1	**<0.001**	5.6	0.060[†]
Paternal population	0.5	0.635	2.4	0.097[†]	6.0	**0.049**
Mat. Pop * Pat. Pop	0.2	0.840	0.9	0.437	14.5	**0.006**
Block	n/a	n/a	4.6	**0.001**	65.3	**<0.001**
Contrast	*t*	*P*	*t*	*P*	χ^2	*P*
BET_{pool} vs. WIN_{pool}	n/a	n/a	0.3	0.736	5.7	**0.017**
Fou: BET vs. WIN_{pool}	n/a	n/a	3.1	**0.002**	21.4	**<0.001**
Tip: BET vs. WIN_{pool}	n/a	n/a	-1.0	0.312	0.7	0.408
Ver: BET vs. WIN_{pool}	n/a	n/a	-0.8	0.410	0.2	0.672
Fou: BET vs. WIN	n/a	n/a	1.8	0.079[†]	30.6	**<0.001**
Tip: BET vs. WIN	n/a	n/a	-1.3	0.195	0.0	-
Ver: BET vs. WIN	n/a	n/a	0.6	0.565	0.0	-

Table 2. ANOVA results for the greenhouse experiment. The model for the combined probability of survival and reproduction employed a binomial error distribution, whereas the models for fecundity (flowers per reproducing plant) and flowers per seedling planted (including zeros) employed normal error distributions. Contrasts between cross types within a maternal population are only reported for models with a *P* value < 0.1 for either the interaction between maternal population and paternal population, or both maternal and paternal populations. For the contrasts, WIN = within-population cross, BET = between-population cross, the subscript pool indicates that the contrast involves all individuals of a given cross type (pooled over populations), and contrasts that start with a population code followed by a colon contain at least one cross type specific to that maternal population.

	Survival and reproduction		Fecundity (Denom. DF = 239)		Flowers per seedling planted	
	χ^2	*P*	*F*	*P*	*F*	*P*
Maternal population	0.8	0.675	1.4	0.250	1.6	0.194
Paternal population	1.9	0.380	0.8	0.459	1.6	0.208
Mat. Pop * Pat. Pop	3.5	0.483	2.1	0.080†	1.1	0.372
Block	45.0	0.554	1.3	0.142	1.2	0.202
Contrast	χ^2	*P*	*t*	*P*	*t*	*P*
BET_{pool} vs. WIN_{pool}	n/a	n/a	2.5	**0.015**	n/a	n/a
Fou: BET vs. WIN_{pool}	n/a	n/a	1.6	0.248	n/a	n/a
Tip: BET vs. WIN_{pool}	n/a	n/a	1.2	0.235	n/a	n/a
Ver: BET vs. WIN_{pool}	n/a	n/a	3.2	**0.002**	n/a	n/a
Fou: BET vs. WIN	n/a	n/a	1.0	0.300	n/a	n/a
Tip: BET vs. WIN	n/a	n/a	0.5	0.599	n/a	n/a
Ver: BET vs. WIN	n/a	n/a	2.5	**0.014**	n/a	n/a

Table 3. ANOVA results for the field experiment. The model for the combined probability of survival and reproduction employed a binomial error distribution, whereas the models for fecundity (flowers per reproducing plant) and fruits per seedling planted (including zeros) employed normal error distributions. Contrasts between cross types within a maternal population are only reported for models with a *P* value < 0.1 for either the interaction between maternal population and paternal population, or both maternal and paternal populations. For the contrasts, WIN = within-population cross, BET = between-population cross, the subscript pool indicates that the contrast involves all individuals of a given cross type (pooled over populations), and contrasts that start with a population code followed by a colon contain at least one cross type specific to that maternal population.

	Survival and reproduction		Fecundity (Denom. DF = 258)		Fruits per seedling planted	
	χ^2	*P*	*F*	*P*	*F*	*P*
Maternal population	10.9	**0.004**	1.1	0.313	3.3	**0.038**
Paternal population	21.7	**<0.001**	4.4	**0.014**	10.9	**<0.001**
Mat. Pop * Pat. Pop	4.7	0.315	2.1	0.080†	2.4	**0.049**
Block	23.5	**<0.001**	1.3	0.267	4.6	**<0.001**
Contrast	χ^2	*P*	*t*	*P*	*t*	*P*
BET_{pool} vs. WIN_{pool}	1.3	0.263	2.4	**0.019**	2.3	**0.024**
Fou: BET vs. WIN_{pool}	3.7	0.054†	3.0	**0.003**	3.6	**<0.001**
Tip: BET vs. WIN_{pool}	0.0	0.944	1.5	0.123	0.7	0.465
Ver: BET vs. WIN_{pool}	0.9	0.338	1.3	0.194	1.3	0.187
Fou: BET vs. WIN	15.3	**<0.001**	1.7	0.098†	4.2	**<0.001**
Tip: BET vs. WIN	2.3	0.131	1.9	0.067†	2.0	**0.044**
Ver: BET vs. WIN	7.3	**<0.001**	0.7	0.676	-1.4	0.167

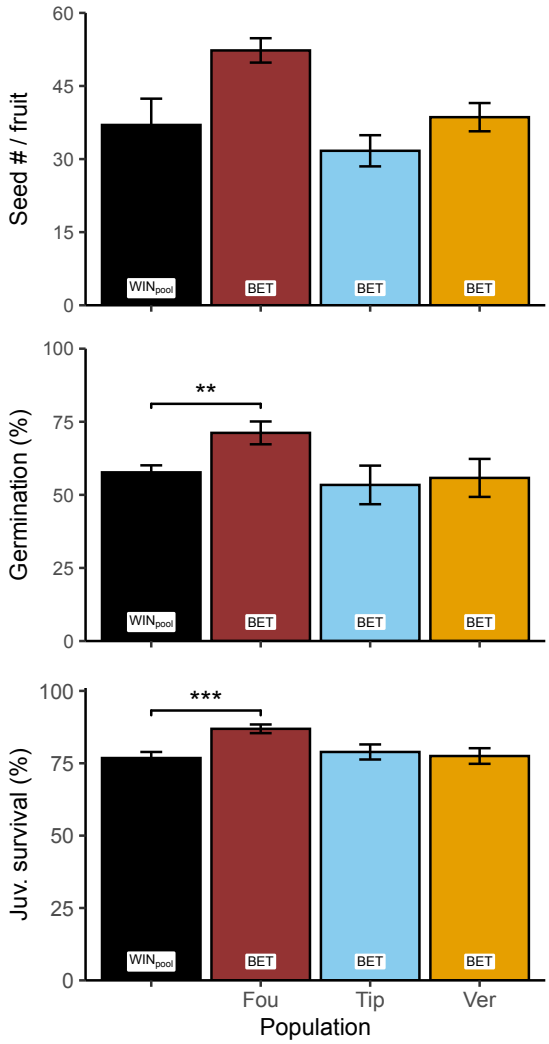

Figure 1. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN_{pool} = within-population crosses pooled over populations, BET = between-population cross) for seed number per fruit of the hand pollinations, germination, and juvenile survival. Means and standard errors were calculated as the mean of block means. Significant contrasts of BET vs. WIN_{pool} are indicated where appropriate. ** $P < 0.01$, *** $P < 0.001$ (see Table 1).

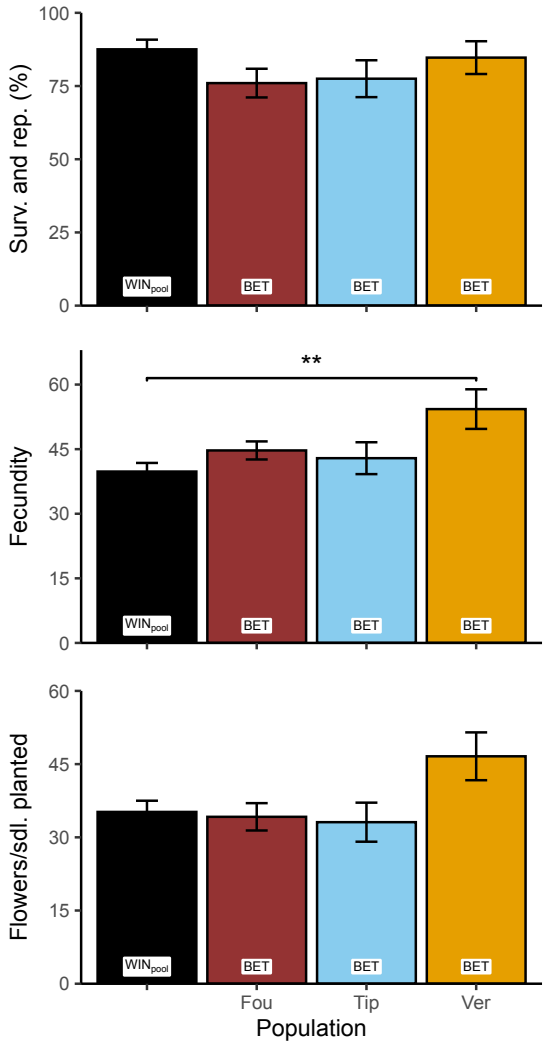

Figure 2. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN_{pool} = within-population crosses pooled over populations, BET = between-population cross) for the greenhouse experiment. Means and standard errors were calculated as the mean of block means. Significant contrasts of BET vs. WIN_{pool} are indicated where appropriate. ** $P < 0.01$ (see Table 2).

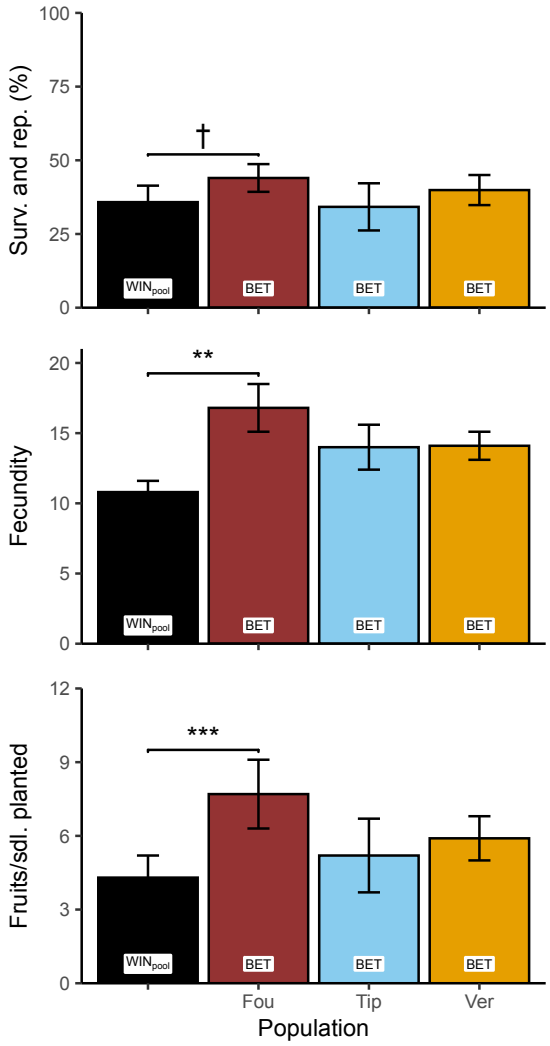

Figure 3. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN_{pool} = within-population crosses pooled over populations, BET = between-population cross) for the field common garden experiment. Means and standard errors were calculated as the mean of block means. Significant contrasts of BET vs. WIN_{pool} are indicated where appropriate. † $0.10 > P > 0.05$, ** $P < 0.01$, *** $P < 0.001$ (see Table 3).

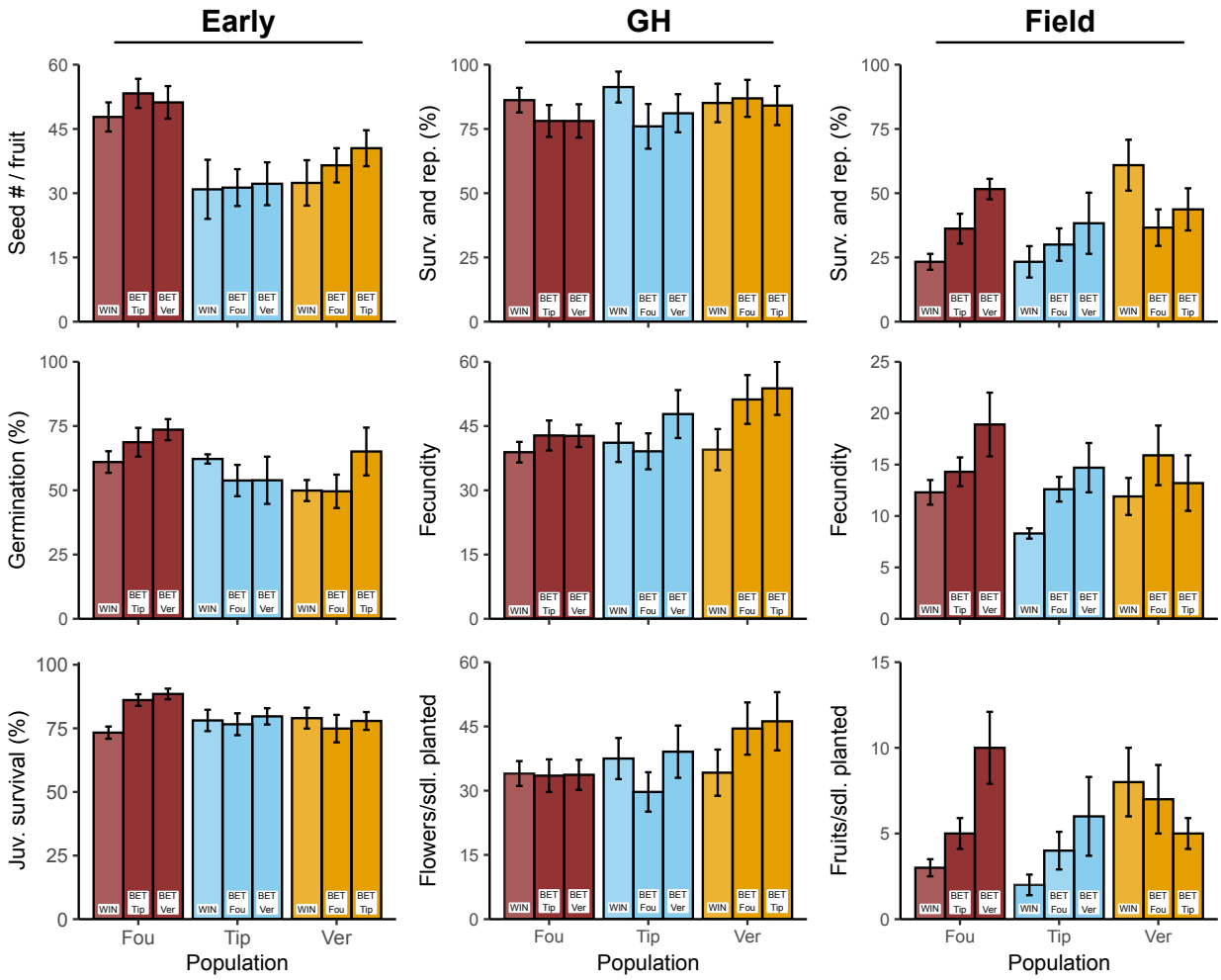

Figure S1. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN = within-population cross, BET = between-population cross) separately by the two paternal populations for between-population crosses. Means and standard errors were calculated as the mean of block means, except for seed number per fruit which are the raw means and SE.

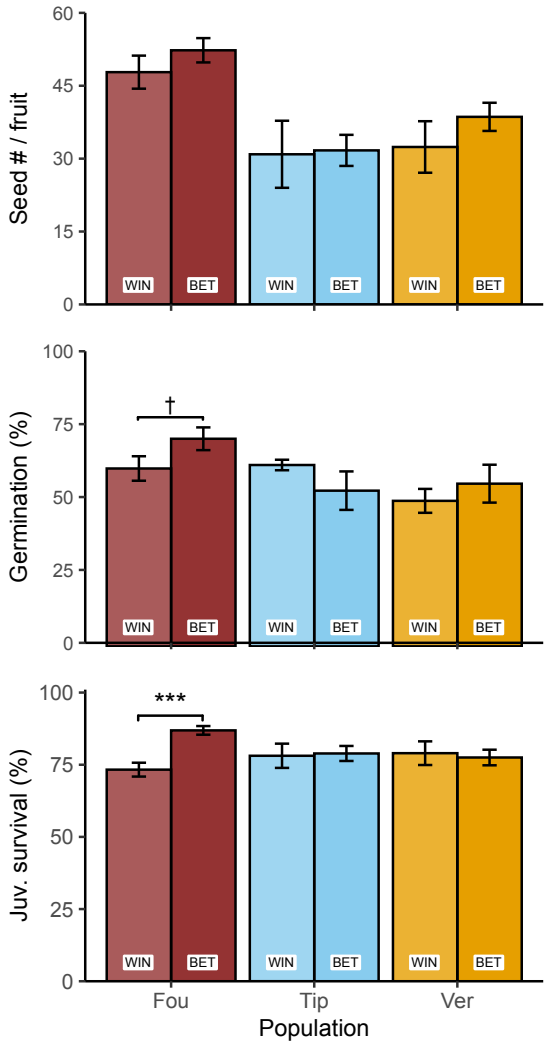

Figure S2. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN = within-population cross, BET = between-population cross) for seed number per fruit of the hand pollinations, germination, and juvenile survival. Means and standard errors were calculated as the mean of block means, except for seed number per fruit which are the raw means and SE. Within a population, significant contrasts of BET vs. WIN are indicated where appropriate. † 0.10 > P > 0.05, *** $P < 0.001$ (see Table 1).

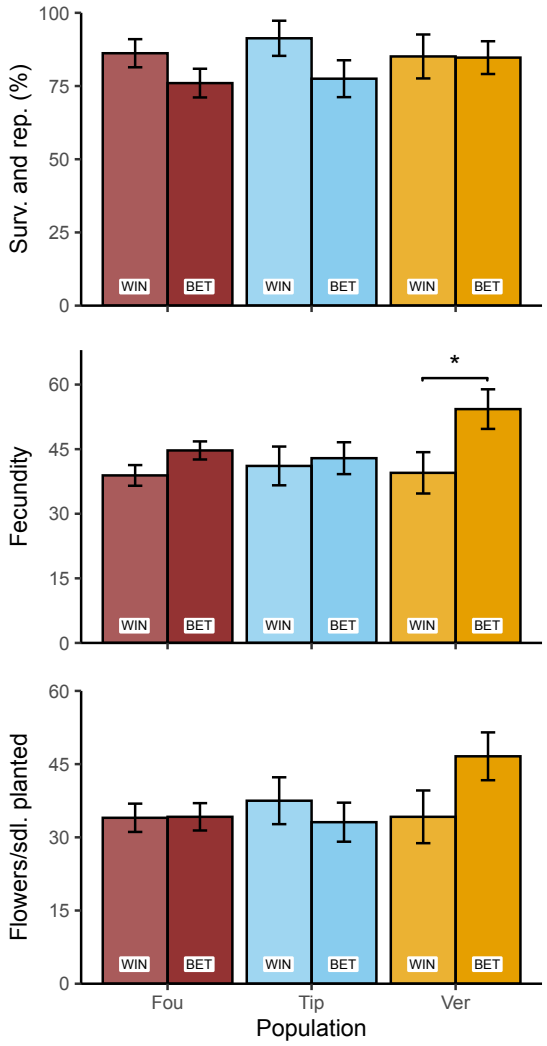

Figure S3. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN = within-population cross, BET = between-population cross) for the greenhouse experiment. Means and standard errors were calculated as the mean of block means. Within a population, significant contrasts of BET vs. WIN are indicated where appropriate. * $P < 0.05$ (see Table 2).

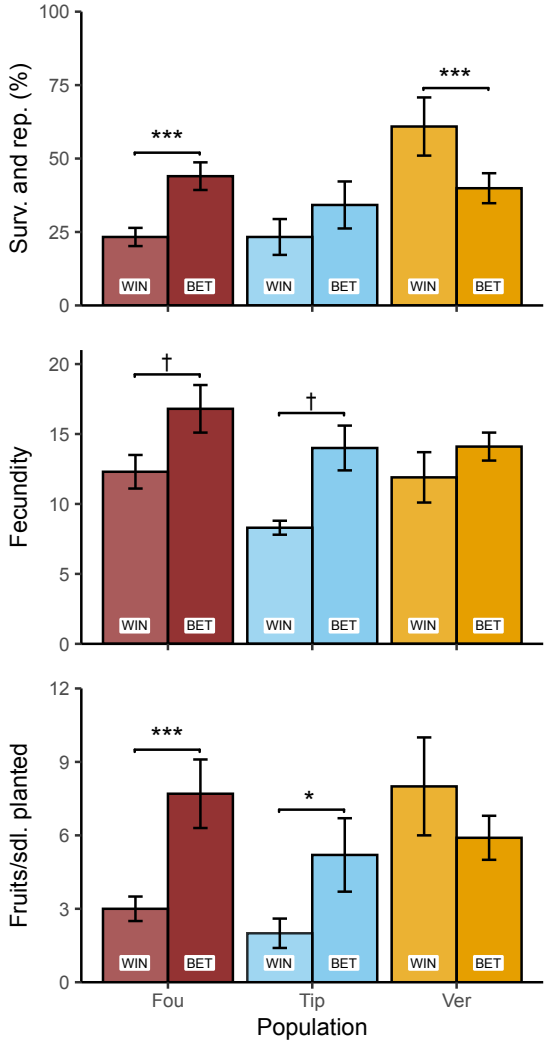

Figure S4. Mean fitness components for each maternal population (Fou, Tip, and Ver) and cross type (WIN = within-population cross, BET = between-population cross) for the field common garden experiment. Means and standard errors were calculated as the mean of block means. Within a population, significant contrasts of BET vs. WIN are indicated where appropriate. † $0.10 > P > 0.05$, *** $P < 0.001$ (see Table 3).

Table S1. ANOVA results for early fitness components for the effects of maternal stratification treatment (10, 30, or 60 days), maternal and paternal populations and their interaction, and block (where appropriate). Models for both seed number per fruit and percent germination (per cell) employed normal error distributions (denominator degrees of freedom in parentheses). The model for juvenile survival employed a binomial error distribution. Contrasts between cross types within a maternal population are only reported for models with a significant interaction between maternal population and paternal population or significant main effects for both of those terms. For the contrasts, WIN = within-population cross, BET = between-population cross, the subscript pool indicates that the contrast involves all individuals of a given cross type (pooled over populations), and contrasts that start with a population code followed by a colon contain at least one cross type specific to that maternal population. Model results were similar to models without incorporating effects of maternal stratification treatment (compare with Table 1) so we proceed with the simpler model throughout the results.

	Seed number per fruit (Denom. DF = 227)		Germination (Denom. DF = 344)		Juvenile Survival	
	F	*P*	*F*	*P*	χ^2	*P*
Maternal population	13.1	**<0.001**	6.4	**0.002**	5.3	0.070†
Paternal population	1.0	0.366	2.7	0.071†	6.3	**0.044**
Mat. Pop * Pat. Pop	0.3	0.877	1.1	0.345	14.6	**0.006**
Mat. Strat. Trt.	13.5	**<0.001**	2.0	0.132	0.4	0.804
Block	-	-	4.5	**0.002**	64.9	**<0.001**
Contrast	*t*	*P*	*t*	*P*	χ^2	*P*
BET_{pool} vs. WIN_{pool}	-	-	0.3	0.758	6.0	**0.015**
Fou: BET vs. WIN_{pool}	-	-	2.8	**0.005**	21.5	**<0.001**
Tip: BET vs. WIN_{pool}	-	-	-1.0	0.305	0.5	0.458
Ver: BET vs. WIN_{pool}	-	-	-0.6	0.520	0.3	0.577
Fou: BET vs. WIN	-	-	1.9	0.064†	30.9	**<0.001**
Tip: BET vs. WIN	-	-	-1.5	0.142	0.0	-
Ver: BET vs. WIN	-	-	0.6	0.525	0.0	-

Table S2. Sample sizes and mean values (averaged over block where appropriate) for fitness components for each maternal population and cross type. For seed number per fruit, sample size indicates the number of fruits. For germination, mean is the percent germination per cell (usually starting with 10 seeds) averaged over five flats, and sample size represents the total number of cells over all flats. For juvenile survival, the mean percent survival over the means of the 30 flats is given, and sample size represents the total number of seedlings over all flats. For the greenhouse experiment, sample sizes are the total number of plants at the start of the experiment over all 48 flats, and means are the mean over flat means. For the field experiment, sample sizes are the total number of plants at the start of the experiment over all 6 blocks, and means are the mean over block means. For fecundity in both experiments, sample sizes are the number of plants that reproduced.

	Fou				Tip				Ver			
	BET		WIN		BET		WIN		BET		WIN	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Early												
Seed # / fruit	71	52.3	39	47.8	45	31.7	17	30.9	47	38.6	19	32.4
Germination (%)	120	71.2	60	61.0	60	53.4	30	62.2	59	55.8	30	49.9
Juv. survival (%)	723	86.9	337	73.3	307	78.9	170	78.1	291	77.5	149	79.0
Greenhouse												
Surv. and rep. (%)	118	76.0	59	86.2	59	77.5	30	91.3	59	84.7	29	85.1
Fecundity	112	44.7	55	38.9	53	42.9	28	41.1	55	54.3	26	39.5
Flowers/sdl. planted	118	34.2	59	34.0	59	33.1	30	37.5	59	46.6	29	34.2
Field												
Surv. and rep. (%)	239	44.0	120	23.3	120	34.2	60	23.3	118	39.9	59	60.9
Fecundity	105	16.8	28	12.3	41	14.0	14	8.3	47	14.1	36	11.9
Fruits/sdl. planted	239	7.7	120	3.0	120	5.2	60	2.0	118	5.9	59	8.0

Table S3. Estimates of heterosis (outbreeding depression for negative values) for each maternal population for fitness components and composite fitness estimates.

	Fou_{pool}	**Tip_{pool}**	**Ver_{pool}**	Fou	Tip	Ver	Fou*Tip	Fou*Ver	Tip*Fou	Tip*Ver	Ver*Fou	Ver*Tip
Early												
Seed # / fruit	**41**	**-14**	**4**	9	3	19	35	28	-20	1	-9	28
Germination (%)	**23**	**-7**	**-3**	17	-14	12	12	33	-13	-4	-11	16
Juv. survival (%)	**13**	**3**	**1**	19	1	-2	14	16	1	1	-2	-1
Greenhouse												
Surv. and rep. (%)	**-13**	**-11**	**-3**	-12	-15	0	-12	-9	-14	-8	2	-5
Fecundity	**12**	**8**	**36**	15	4	37	7	9	-2	19	31	34
Flowers/sdl. planted	**-3**	**-6**	**32**	1	-12	36	-6	-1	-17	9	31	29
Field												
Surv. and rep. (%)	**23**	**-5**	**11**	89	47	-34	55	23	29	-9	-13	4
Fecundity	**55**	**29**	**30**	37	69	18	39	56	22	46	31	30
Fruits/sdl. planted	**78**	**20**	**36**	157	160	-26	111	85	61	27	22	2
Cumulative												
Greenhouse	**92**	**-23**	**35**	52	-21	78	61	95	-42	8	5	90
Field	**281**	**6**	**50**	289	131	-4	262	265	14	26	-2	50