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1 Article

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3 **Is plant fitness proportional to seed set? An experiment and a spatial model**

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21 3 Figures

22 Online Appendices A, B and C

23

24

25 ABSTRACT: Individual differences in fecundity often serve as proxies for differences in  
26 overall fitness, especially when it is difficult to track the fate of an individual's offspring  
27 to reproductive maturity. Using fecundity may be biased, however, if density-dependent  
28 interactions between siblings affect survival and reproduction of offspring from high- and  
29 low-fecundity parents differently. To test for such density-dependent effects in plants, we  
30 sowed seeds of the wildflower *Ipomopsis aggregata* (scarlet gilia) to mimic partially-  
31 overlapping seed shadows of pairs of plants, one of which produced twice as many seeds.  
32 We tested for differences in offspring success using a genetic marker to track offspring to  
33 flowering multiple years later. Without density dependence the high-fecundity parent  
34 should produce twice as many surviving offspring. We also developed a model that  
35 considered the geometry of seed shadows and assumed limited survivors so that number  
36 of juvenile recruits is proportional to area. Rather than a ratio of 2:1 offspring success  
37 from high- vs. low-fecundity parents, our model predicted a ratio of 1.42 to 1, which  
38 would translate into weaker selection. Empirical ratios of juvenile offspring and of  
39 flowers produced conformed well to the model's prediction. Extending the model shows  
40 how spatial relationships of parents and seed dispersal patterns modify inferences about  
41 relative fitness based solely on fecundity.

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43

## Introduction

44 Fitness depends upon survival and fertility at each age or stage of the life cycle. A rich  
45 theoretical literature relates overall measures of individual fitness, such as the total  
46 contribution of offspring or finite rate of increase, to these age- or stage-specific  
47 components (McGraw and Caswell 1996; Moorad 2014; Shaw et al. 2008). Estimating  
48 overall fitness is, however, challenging under natural conditions. It is not surprising, then,  
49 that most empirical studies of natural selection on a trait rely on measuring a component  
50 of individual fitness rather than a more integrated measure (98% of studies according to  
51 Kingsolver et al. 2012). For example, in flowering plants the majority of phenotypic  
52 selection estimates on floral traits are based on pollen import, fruit production, or seed  
53 production (review in Harder and Johnson 2009), with a smaller number also using an  
54 estimate of male fecundity (e.g., Conner et al. 1996; Meléndez-Ackerman and Campbell  
55 1998; Stanton et al. 1986).

56 A trait can, however, have multiple fitness effects that extend even beyond the  
57 portion of the lifecycle when the trait is expressed. For example, fecundity need not  
58 capture all of the fitness effects of a reproductive trait, which can include viability earlier  
59 in the life cycle (Mojica and Kelly 2010). Thus many investigators have also examined  
60 fitness effects of floral traits that occur outside of the time window of pollination and  
61 seed maturation. Some such studies of self-compatible species have examined the  
62 influence of traits, such as floral display size, not only on selfing rate during seed  
63 formation, but also on expression of inbreeding depression in offspring (review by  
64 Barrett and Harder 1996). Other studies have examined pleiotropic effects of floral traits  
65 on defense against herbivores (Kessler et al. 2013; Strauss and Whittall 2006).

66           One stage of the life cycle has been almost completely ignored in estimating  
67 individual fitness in plants, and that is the difficult-to-characterize transition between  
68 seed input to the soil and seedling emergence. Even in cases where investigators have  
69 measured fitness over the entire lifetime from seed to seed (e.g. Campbell et al. 2008),  
70 they have not generally tracked the fate of individual seeds in a way that allows for  
71 natural seed dispersion and spatially-explicit interactions between seeds.

72           In contrast, the gap between seeds and seedlings has been explored at the  
73 population level in studies of average fitness that do not assign offspring to particular  
74 parents (e.g., Bricker and Maron 2012; Bricker et al. 2010; Ehrlén 2002; Feldman and  
75 Morris 2011; Shefferson et al. 2014; see Clark et al. 2007 for review). In some cases,  
76 these studies have demonstrated density dependence in survival of seeds to seedlings,  
77 given natural seed dispersal. For example, in *Ipomopsis aggregata*, the species we  
78 address in this study, a higher production of seeds did not lead to an exactly equal  
79 increment in the numbers of seed offspring that reach reproductive maturity. We showed  
80 similar levels of population-wide density dependence both with an experiment in which  
81 we sowed seeds into plots and followed their fates en masse (Price et al. 2008), and in an  
82 observational demographic study in which we estimated seed rain in natural populations  
83 and related the numbers of plants that reach maturity to the density of this seed input  
84 (Waser et al. 2010).

85           Such studies at the level of entire populations cannot tell us, however, whether the  
86 relative fecundities of individuals suffice as a proxy for relative fitness at the individual  
87 level. The overall success of offspring might rise (or fall) across an entire population as a  
88 function of increasing average fecundity for the population in ways that do not exactly

89 reflect effects of variation in individual parental fecundity. A variety of mechanisms  
90 could differentially influence individual fitness during the gap between generations  
91 (Metcalf and Pavard 2006). The novel mechanism we focus on is the spatial distribution  
92 of offspring and how it affects interactions between siblings. It is possible for the number  
93 of juveniles recruited to be independent of the number of seeds set in a population  
94 (extreme density dependence at the population level), and yet for an individual plant that  
95 sets twice as many seeds as its neighbor to contribute twice as many of those juveniles as  
96 its low-fecundity neighbor (absence of density dependence at the individual level). This  
97 situation could occur if the density of juveniles is constant over space, but neighboring  
98 individuals overlap in dispersal of their seed offspring such that the high-fecundity parent  
99 has twice as many chances for a successful juvenile recruit. Conversely, an individual  
100 that sets twice as many seeds may not contribute twice as many offspring to the next  
101 generation if competition occurs primarily among siblings. The relative importance of  
102 sibling interactions, and thus whether differences in seed production translate into  
103 proportional differences in overall fitness, is likely to depend upon how widely seed  
104 offspring are dispersed.

105         Here we address the question: under what circumstances does making more seeds  
106 of similar size make an individual plant more fit in equal proportion? We address this  
107 question using a field experiment and a model of the spatial relationships of plants and  
108 their seed shadows. For the experiment, we sowed seeds of two known genotypes of *I.*  
109 *aggregata* into natural subalpine meadows in a fashion that mimicked overlapping seed  
110 shadows of pairs of neighboring plants, one with twice the fecundity of the other. We  
111 subsequently followed the success of seed offspring of each pair of parents over multiple

112 years until the offspring had all died. The model in turn considers geometry of the seed  
113 shadows and assumes that offspring success is limited by a constant density of sites  
114 suitable for juvenile recruitment. The experiment revealed that recruitment of offspring  
115 from high- and low-fecundity parents occurred in an average ratio of 1.4 to 1 rather than  
116 2 to 1, and that final total flower production in progenies of the two types of parents  
117 averaged 1.5 to 1, both of which agree well with the relative success of 1.42 to 1  
118 predicted by the model. We then extend the model to consider spatial relationships of  
119 neighboring plants and their seed shadows other than those simulated by the experiment.  
120 We derive some general conclusions about how the spatial overlap of parental seed  
121 shadows affects the relationship between overall relative fitness and fecundity.

## 122 **Methods**

### 123 *Study System*

124 *Ipomopsis aggregata* Pursh (V. Grant) subsp. *aggregata* (Polemoniaceae) is a  
125 monocarpic perennial wildflower common in montane meadows of the western United  
126 States. It has been studied extensively near the Rocky Mountain Biological Laboratory  
127 (RMBL; 2900 m elevation) in west-central Colorado. In this area, the species generally  
128 flowers from late June through August, and seeds are shed from mid-August to mid-  
129 September. An individual plant can produce between 0 - 1200 seeds (mean = 61 seeds in  
130 Campbell 1989a). Seeds are gravity-dispersed and fall close to the maternal parent. Most  
131 seedlings germinate after snowmelt in late May or early June of the year after they are  
132 shed. Plants grow for several years as vegetative rosettes and eventually flower once, set  
133 seed, and die; very few flower more than once and those usually have been damaged as  
134 vegetative rosettes (Brody et al. 2007). Most surviving individuals flower in the third or

135 fourth growing season after they germinate, but some take as long as a decade (Campbell  
136 1997).

137 Previous demographic work with *I. aggregata* detected population-level density  
138 dependence at several life stages after seed germination. When seed input densities were  
139 varied experimentally over a 10-fold range from 33 to 333 seeds/m<sup>2</sup>, seedling emergence  
140 increased linearly with seed input, but rosette size before flowering and flower  
141 production decreased with seed input density. As a result, the finite rate of population  
142 increase decreased nonlinearly with seed input density to a plateau at densities above  
143 about 125 seeds/m<sup>2</sup> (Price et al. 2008). A study of natural variation in seed rain produced  
144 quantitatively similar nonlinear relationships due to density dependence after the  
145 germination stage in survival and flower production (Waser et al. 2010). At even higher  
146 seed input densities (2400 seeds/m<sup>2</sup>), density dependence was detected in survival to  
147 flowering for *Ipomopsis aggregata* ssp. *candida* by (Juenger and Bergelson 2000).

#### 148 *Experimental Approach*

149 Population-wide density dependence detected in these previous studies invites the  
150 question of whether the fitness of neighboring *I. aggregata* plants that produce different  
151 numbers of seeds is proportional to their fecundity. To explore this question, we sowed  
152 seeds of known genotypes into natural meadows to mimic partially- overlapping seed  
153 shadows of pairs of maternal plants that differed two-fold in fecundity. We then mapped  
154 emerging seedlings and, when those individuals (hereafter “recruits”) were large enough,  
155 genotyped a sample of leaf material to assign parentage to the high-fecundity mother, the  
156 low-fecundity mother, or to some other source. We censused the recruits annually to  
157 assess their survival and reproductive success.



158 Production of Known-Genotype Offspring

159           To produce offspring of known genotype, we collected flower bud tissue in 1999  
160 from 52-61 bolting *I. aggregata* individuals growing within an area of 10 m radius in  
161 each of three meadows near the RMBL. We genotyped a total of 169 individuals based  
162 on alleles at the 6PGD-2 enzyme locus. Two alleles at this locus, designated “Medium”  
163 and “Slow”, are present at high frequencies in populations of *I. aggregata* near the  
164 RMBL (Campbell 1991a) and are easily scored with starch-gel electrophoresis (Campbell  
165 1991a; Campbell et al. 2003; Campbell and Dooley 1992). From the 169 genotyped  
166 individuals, we chose homozygous Medium (MM) and homozygous Slow (SS)  
167 individuals in each meadow that could be crossed to produce homozygous offspring. We  
168 potted these plants (23 MM and 17 SS in total) and brought them into a screenhouse at  
169 the RMBL.

170           As new flowers opened on each plant and stigmas became receptive, we used a  
171 clean wooden toothpick to cross-pollinate with pollen from two donors from the same  
172 meadow that had the same (homozygous) genotype, alternating which donor’s pollen was  
173 applied first. We used multiple donors because *Ipomopsis* fruits are typically multiply-  
174 sired (Campbell 1998). We did not emasculate recipient flowers because *I. aggregata* is  
175 self-sterile (Waser and Price 1991). Plants were hand-pollinated and watered every other  
176 day, and fertilized once per week using dilute (0.13%) 20-20-20 NPK fertilizer. As fruits  
177 matured, seeds from each plant were collected and stored in coin envelopes.

178 Planting to Mimic High- and Low-fecundity Parents

179           At the end of the 1999 flowering season, we selected, from among the pool of  
180 sufficiently fecund parents, two MM and two SS parents from each of two meadows and

181 four of each genotype from the third meadow. We paired each MM with a sympatric SS  
182 parent to form eight MM-SS pairs. We originally used an additional meadow with two  
183 pairs of parents to generate a total sample of 10 pairs but later abandoned that meadow  
184 because seedling emergence was poor. Within each of the eight pairs we assigned one  
185 plant to be “high-fecundity” and the other to be “low-fecundity”, and we counted out 300  
186 and 150 seeds respectively from the envelopes for these two plants. The only exception  
187 was one pair in which the high-fecundity parent only produced 217 seeds; in that case we  
188 used 109 seeds from the low-fecundity parent. MM and SS genotypes were equally  
189 represented in the high- and low- fecundity treatments in each meadow. Because we  
190 assigned high- and low-fecundity treatments independently of the true fecundity of each  
191 parent, we avoided any tradeoff between seed number and seed size. Seed mass was  
192 similar between the high- and low-fecundity treatments in any case (means across ten  
193 pairs of parents = 1.13 mg and 0.98 mg, respectively, randomized block ANOVA,  $F_{1,9} =$   
194 1.67,  $P = 0.23$ ). We next established 1-2 pairs of plots within each meadow within 10 m  
195 of the cluster of source plants. Each 3.5 m  $\times$  3.0 m plot was gridded with steel nails  
196 placed in the ground at 0.5 m intervals. These reference nails allowed us to map and  
197 relocate *I. aggregata* plants within the plots. Existing natural vegetation within each plot  
198 was left intact except that after sowing seeds we removed flowering *I. aggregata*  
199 individuals from within each plot and 1.5 m beyond its perimeter in 1999 and 2000. Few  
200 seeds naturally fall beyond this distance and dormancy of *I. aggregata* seeds is rare (1-  
201 4%; Campbell 1997).

202 We sowed seeds from one pair of high- and low-fecundity parents into each plot,  
203 mimicking natural seed shadows from two plants that were 0.5 m apart in the center of

204 the plot. This distance is a typical spacing for flowering individuals near the RMBL;  
205 nearest-neighbor distance averaged 0.37 m with a range of 0 (within measurement error)  
206 to 1.71 m in the central part of a population near one of our sites in 1987 (Campbell  
207 1991a). We characterized natural seed shadows in two ways: by placing plastic sheets  
208 sprayed with sticky Tanglefoot (Tanglefoot Company, Grand Rapids, MI 49504) around  
209 12 flowering *I. aggregata* plants in 1995 and mapping the distribution of dispersed seeds;  
210 and by observing the spatial distribution of seedlings that emerged in 1997 around 6  
211 isolated plants that had flowered in 1996. For plants on level ground, < 2% of seeds or  
212 seedlings were found >1.5 m from the parent. Of the seeds or seedlings within 1.5 m of  
213 each parent, 53% on average were found within 0.5 m, 33% between 0.5 m and 1.0 m,  
214 and the remaining 14% between 1.0 m and 1.5 m. To mimic this pattern, for each parent  
215 in our experiment we scattered 53% of seeds evenly within a circular wire frame of  
216 radius 0.5 m centered on the imaginary location of that parent within a plot, 33% between  
217 frames of 0.5 m and 1.0 m radius, and the remaining 14% between frames of 1.0 m and  
218 1.5 m radius. Figure 1a shows the expected numbers of seeds from each parent that fell  
219 into various sectors of the overlapping seed shadows.

## 220 Offspring Censuses

221 Most *I. aggregata* seedlings that emerged in the seeded plots appeared in the  
222 spring of 2000, and a much smaller number in 2001. We returned every summer from  
223 2000 to 2008 to census all individual recruits in the 2000 and 2001 seedling cohorts as  
224 well as pre-existing individuals in the plots. To do this we used 1 m<sup>2</sup> quadrats subdivided  
225 by fishing line into 10 cm × 10 cm squares and positioned them over the reference nails,  
226 allowing us to characterize the coordinates of each plant. In June of each year, when

227 flowering individuals had begun to bolt, we recorded presence or absence of each  
228 mapped individual recruit, its stage (seedling, vegetative rosette, or flowering), and the  
229 size of each rosette (number of leaves and maximum leaf length). At the end of each  
230 season in August we recorded the cumulative number of flowers produced by those  
231 individuals that had flowered.

232         Seedlings could not be genotyped immediately upon emergence, since sampling  
233 tissue required removing 1-2 leaves. Instead, we waited up to two years until plants from  
234 the 2000 and 2001 seedling cohorts had successfully established and had formed sizeable  
235 rosettes that could withstand removal of leaf tissue. We genotyped each recruit at least  
236 twice. A plant was assigned to the high- or low-fecundity treatment only if there was  
237 agreement in gel scores and if the genotype was MM or SS.

238         In all, we successfully genotyped 245 recruits. Of these, 185 were either MM or  
239 SS genotypes and 60 were other genotypes that could not be assigned to one of the two  
240 parents whose seeds we had sown. This large number of “impossible” genotypes was  
241 unexpected for two reasons. First, we had removed all flowering stalks within each plot  
242 and for 1.5 m around it in 1999 and 2000, under the assumption that by doing so we were  
243 eliminating virtually all seed dispersal into the plots. Second, previous studies indicated  
244 that very few seeds of *I. aggregata* remain dormant in the soil beyond the first summer  
245 after they are shed. Regardless of the cause, the existence of these genotypes indicates  
246 some seed flow into the plots or some unusual dormancy. These volunteer seeds in turn  
247 imply that we could have wrongly assigned some MM or SS individuals as offspring of  
248 our experimental parents, when in fact they came from other sources, representing what  
249 we term “cryptic seed flow”. We therefore corrected the numbers of individuals assigned

250 to experimental parents to account for cryptic seed flow (calculations in online Appendix  
251 A).

252 From data on the life histories of offspring assigned to each parent, we then  
253 determined the number of seed offspring that recruited (germinated and survived to be  
254 genotyped), the number that survived to flower, and their fecundity (estimated as the  
255 number of flowers they ultimately produced). These numbers were calculated with and  
256 without the correction for cryptic seed flow. We then compared each of these measures of  
257 offspring success against expectations from two models for the relative success of high-  
258 and low-fecundity neighbors—one model that assumes no density dependence, and an  
259 alternative model that assumes a simple, “safe-site” form of density dependence as  
260 described in the next section.

### 261 *Models*

#### 262 Expected Relative Offspring Success under Two Alternative Models

263 If there were no density dependence we would expect each seed to have equal  
264 success in establishment, survival, and flowering, in which case the high-fecundity  
265 parents in our experiment would produce twice the number of successful offspring on  
266 average as the low-fecundity parents. Alternatively, suppose that there is a uniform  
267 density of “safe sites” suitable for seed germination and recruitment of juvenile plants  
268 throughout the area in which seeds fall, and that only one juvenile can occupy each safe  
269 site. Under this simple density-dependent model, the numbers of successful offspring in  
270 various sectors of the seed shadows of two parents (Fig. 1) would be proportional to the  
271 areas of those sectors. The expected ratio of successful offspring from the high vs. low  
272 fecundity parent would be less than 2 to 1 because offspring of the high-fecundity parent

273 experience greater competition with siblings than do offspring of the low-fecundity  
274 parent. At one extreme where seed shadows do not overlap at all, both parents would  
275 have the same number of successful progeny. Given the actual geometry of seed shadows  
276 used in the experiment this model predicts a 1.42:1 overall ratio of successful offspring  
277 from high- vs. low-fecundity parents (derivation in online Appendix B1).

#### 278 Extension of the Spatial “Safe Site” Model

279 We extended the spatial model in three ways. First, we explored the effect of seed  
280 dispersal, by comparing results under the pattern of seed dispersal actually observed for *I.*  
281 *aggregata* to results with highly localized seed dispersal and with even dispersion over  
282 the full seed shadow with a radius of 1.5 m (online Appendix B2). Second, we considered  
283 effects of the distance between adult plants relative to the size of their combined seed  
284 shadows (online Appendix B3). Third, we explored the effect of including more than  
285 two parents, by allowing for more than two overlapping seed shadows (online Appendix  
286 B4 and Fig. B1, B2).

#### 287 *Statistical Comparison of Experimental Results with Model Predictions*

288 We tested for density dependence in offspring success by comparing recruits per  
289 seed input and number of flowers produced per recruit between the high-fecundity and  
290 low-fecundity treatments. Since each of the eight plots was sown with seeds from one  
291 high-fecundity and one low-fecundity parent, we used a randomized block analysis of  
292 variance with parent fecundity as a fixed effect and plot as a random effect (Proc Mixed  
293 in SAS version 9.3). Residuals for both dependent variables were normally distributed  
294 based on Shapiro-Wilks tests. Density dependence of survival from recruit to flowering  
295 was also tested by using the R function `nnd` (R ver. 3.3.1) to determine the distance to the

296 nearest neighbor, and then using a logistic regression with a binomial distribution to test  
297 for the effect of nnd on survival (Proc Genmod in SAS 9.3).

298 To compare experimental results with model predictions, we calculated the ratio  
299 of recruits produced by the high-fecundity and low-fecundity parents for each plot, and  
300 used the eight values to determine the standard error around the mean relative fitness for  
301 comparison with the expected value of 1.42 from the model described in online Appendix  
302 A. The ratios were calculated in two ways: (1) assuming that all of the MM and SS  
303 recruits were indeed offspring of the experimental parents; and (2) incorporating cryptic  
304 seed flow. For the latter, we calculated cryptic seed flow rates separately by meadow and  
305 subtracted the appropriate rate from the observed offspring success in each plot. We also  
306 regressed the number of recruits from the high-fecundity parent on the number of recruits  
307 from the low-fecundity parent in the same plot, and tested the linear hypothesis that the  
308 slope is equal to 2 and the linear hypothesis that the slope is equal to 1.42. Ratios of  
309 successful recruits were also calculated for total survivors to flowering and for total  
310 flowers produced by offspring of the two types of parents.

## 311 **Results**

312 From the 3476 seeds sown into the eight plots, a total of 185 seedlings emerged in  
313 2000 or 2001 and survived to be genotyped as MM or SS one to two years later. Data are  
314 available in the Dryad Digital Repository (<http://dx.doi.org/>), and spatial maps of these  
315 recruits are shown in online Fig. C1. This number corresponds to an overall  
316 “recruitment” of 5.3%. For genotyped offspring, percent recruitment was higher on  
317 average for seeds from low-fecundity than from high-fecundity parents (mean = 6.9% vs.  
318 4.4%, randomized block ANOVA on proportion,  $F_{1,7} = 8.83$ ,  $P = 0.0208$ ). Out of the 185

319 recruits, 47 plants survived to flower. Age at flowering ranged from 2 to 7 years with a  
320 mean of 3.7 years. The probability of surviving to flower did not depend significantly on  
321 distance to the nearest neighbor plant, (logistic regression slope of logit on distance =  
322 0.0215, Wald  $\chi^2 = 2.68$ ,  $P = 0.1017$ ). Furthermore, the number of flowers eventually  
323 produced per recruit, a statistic that combines survival to flowering and number of  
324 flowers per survivor, did not differ detectably between low- and high-fecundity parents  
325 (means = 17.4 vs. 10.8;  $F_{1,7} = 2.17$ ,  $P = 0.1839$ ). Thus, there was no strong evidence for  
326 additional density-dependent effects among siblings beyond the stage of seedling  
327 establishment.

328         Accordingly, the high-fecundity parents did not produce twice as many successful  
329 offspring as the low-fecundity parents. The actual ratio for recruits with SS or MM  
330 genotype averaged 1.40 to 1 across the 8 plots (Fig. 2). This ratio was 1.31:1 (80 low-  
331 fecundity recruits and 105 high-fecundity recruits) when we summed the total numbers of  
332 recruits across all plots rather than averaging ratios across plots.

333         Cryptic seed flow ranged from 7.0% to 19.7% across the three meadows (online  
334 Appendix A). Subtracting recruits at those rates from the observed numbers of MM and  
335 SS recruits in each plot caused us to attribute 26 recruits (13 of each treatment) to cryptic  
336 seed flow. Taking cryptic seed flow into account changes the overall fitness ratio only  
337 modestly, from 1.31:1 to a new value of 1.37:1.

338         These observed measures for components of relative fitness are all very close to  
339 the predicted value of 1.42:1 under the model that assumes a fixed density of safe sites  
340 and that duplicates the size of seed shadows used in the experiment (Fig. 1; online  
341 Appendix 2A). Indeed, a regression of recruits from the high-fecundity parent on recruits



342 from the low-fecundity parent produces a slope indistinguishable from 1.42 ( $P = 0.616$ )  
343 and different from 2.0 ( $P = 0.0002$ ). As the model illustrates, it is only in the central  
344 portions of the overlapping seed shadows (those sectors that symmetrically overlap the  
345 midline between the positions of the parents) that the expected ratio of seeds from the  
346 high-fecundity and low-fecundity parents is equal to 2:1. In outer sectors of the seed  
347 shadows, the expected ratio deviates from 2:1. Thus the relative strength of sibling  
348 competition increases for each parent in sectors skewed toward its side of the midline, but  
349 is higher overall for the high-fecundity parent. Sibling competition for limited safe sites  
350 ameliorates parental fecundity advantage, reducing it from 2:1 to 1.42:1. The relatively  
351 small numbers of recruits prevented us from doing a finer-scale comparison with model  
352 predictions on a sector by sector basis.

353         The ratio of survivors to flowering from high fecundity versus low fecundity  
354 parents averaged 1.1:1, and the final ratio of total flowers from high fecundity versus low  
355 fecundity parents, a more complete assessment of overall success that includes both  
356 parental fecundity and offspring success, averaged 1.5:1 (Fig. 2). Both of these latter  
357 ratios showed high variation due to the small sample size of offspring that survived to  
358 flower. The ratio of total flowers produced was indistinguishable from either 1.42:1 or  
359 2.0:1 ( $P > 0.05$ ), the contrasting expectations under the model that assumes a fixed  
360 density of safe sites and hence density dependence vs. the model that assumes no density  
361 dependence.

362         Extending the density-dependent spatial model showed that sibling competition  
363 for safe sites is more severe as seed dispersal becomes more localized and as the relative  
364 distance between the parents increases. Those conditions lead to lower ratios of offspring

365 success from a high-fecundity than a low-fecundity parent (Fig. 3). At the extreme, when  
366 seed shadows do not overlap at all, as long as number of recruits remains proportional to  
367 area, a high-fecundity parent has the same fitness as a low-fecundity parent, despite  
368 producing more seeds (Fig. 3). At the other extreme, when seed shadows are completely  
369 overlapping, fitness is expected to be proportional to fecundity. As seed shadows move  
370 from non-overlapping to completely-overlapping, a parent with twice the fecundity of  
371 another experiences an accelerating increase in relative fitness from 1:1 to 2:1 compared  
372 to the low fecundity parent, even though its absolute fitness declines by 1/3 due to  
373 sharing a fixed number of safe sites for recruitment. If a parent has a seed shadow  
374 overlapping partially with multiple seed shadows instead of only one other seed shadow,  
375 relative fitness is expected to more closely follow relative fecundity as the offspring  
376 experience less sibling competition (online Appendix Fig. B2).

377

### **Discussion**

378 Many studies of selection on reproductive traits use fecundity as an estimate of  
379 fitness, but the extent to which an individual fitness component, such as fecundity,  
380 correlates with overall fitness is rarely investigated (Kingsolver et al. 2012). That the  
381 assumption of direct proportionality between fecundity and longer-term fitness can fail  
382 was pointed out for plant-herbivore interactions by (Harper 1977), who stressed that  
383 density-dependent processes, such as safe-site limitation, could modify the differential  
384 success of individuals measured at the time of reproduction. Here we showed that  
385 individual differences in plant fecundity, such as those that depend on a floral trait, can  
386 be partially offset by post-dispersal density dependence, so that overall fitness need not  
387 be exactly proportional to seed set. In our experiment, successful per-capita offspring

388 recruitment was higher on average for seeds from low-fecundity *I. aggregata* parents, and  
389 a 2-fold difference in seed set translated into a smaller difference (1.4 to 1.5 fold) in  
390 established vegetative rosettes and offspring flower production.

391 Previous studies showed that population growth rate is linked to seed production  
392 in multiple species, including *I. aggregata* (Price et al. 2008). To our knowledge,  
393 however, the results presented here are the first study of links between seed production,  
394 offspring survival and reproduction at the level of individuals within populations of  
395 plants with overlapping seed shadows under natural conditions. A population-level  
396 approach can tell us how sensitive population growth rate is to fecundity versus survival,  
397 and how a decline in pollination or an increase in seed predation may affect the  
398 persistence of a particular plant species (Castro et al. 2015; Weber and Kolb 2011). But a  
399 population-level approach cannot tell us whether individual fecundity is necessarily an  
400 accurate measure of individual fitness, which is critical to an unbiased estimate of  
401 selection.

402 Our spatial model predicted that individual differences in fecundity can be  
403 partially offset by sibling competition, and our field experiment showed good agreement  
404 with the quantitative prediction from a spatial model that included the particulars of  
405 spacing between parents and extent of seed dispersal. The experiment showed that such  
406 modifications of fitness can happen, but two caveats are in order. The first is that a  
407 limited and uniform distribution of safe sites for offspring recruitment is not the only  
408 mechanism of density dependence that could operate in the *I. aggregata* system. The  
409 density dependence could be driven by competition for renewable resources, in which  
410 case its magnitude could change as resources become sufficiently abundant to allow all

411 seeds to survive, rather than by physical characteristics of specific microsites for  
412 seedlings. In principle, density-dependent attack by shared enemies such as herbivores or  
413 pathogens could also yield a similar outcome. Pre-dispersal density dependence in the  
414 form of a tradeoff between seed number and seed size is theoretically possible but was  
415 not detected at the maternal plant level in a previous study (Waser et al. 1995). The  
416 second caveat is that the exact quantitative result we obtained may, or may not, be a good  
417 representation for natural populations of the species. We attempted to choose a realistic  
418 spacing of 0.5 m between parents, but actual spacing can vary from 0 m to 1.7 m  
419 (Campbell 1991a), and the quantitative effect of sibling competition is sensitive to  
420 spacing (Fig. 3), other aspects of the spatial geometry of the populations, and genetic  
421 diversity of seeds, as argued below. Furthermore, density-dependent effects on offspring  
422 success could vary with temporal variation in environmental conditions. Here we saw  
423 strong effects on survival of recruits to establishment for seeds that germinated in 2000 or  
424 2001, whereas in an earlier study of seeds that germinated in 1996, we saw density-  
425 dependent effect on the number of flowers produced when offspring of these seeds  
426 matured, but not on survival to flowering (Waser et al. 2010). As our population-level  
427 studies have never detected density dependence at the seedling germination stage (Waser  
428 et al. 2010), it is likely that the density dependence we witnessed here occurred between  
429 germination and establishment of the recruits.

430        Changing a 2:1 ratio in fitness to a 1.5:1 ratio could have a large impact on the  
431 rate of evolution because it reduces the strength of natural selection by 1/3. For a scenario  
432 of a single gene trait and complete dominance with two phenotypes corresponding to  
433 high- and low-fecundity parents, the selection coefficient would change from 0.5 to 0.33.

434 At a point where the allele frequencies are equal at 0.5, including the effects of post-  
435 dispersal density dependence would cause the deleterious allele frequency to be 0.045  
436 less rather than 0.071 less in the next generation (following equation 3.6b in Hedrick  
437 2000). A similar reduction by 1/3 holds for a standardized linear selection differential on  
438 a quantitative trait, assuming that the density dependence is linear. As one example from  
439 *Ipomopsis aggregata*, plants that bloomed earlier in 1986 produced more seeds, and  
440 incorporating the density dependence found here would reduce the standardized selection  
441 differential through female function from -0.44 (Campbell 1991b) to -0.29, if we ignore  
442 the caveats in the preceding paragraph. Selection through male function is likely to be  
443 less affected by post-dispersal density dependence, as pollen from a given individual is  
444 typically spread among many seed-producing mates (Campbell 1989b; 1998). Thus, the  
445 effects of sibling interactions on selection should be greatest for traits that are under  
446 selection through female function only, such as the proportion of time that *I. aggregata*  
447 flowers spend in the female phase (Campbell 1989b).

448 Our generalized model illustrates conditions under which fitness estimates based  
449 only on fecundity are likely to be inaccurate because of post-dispersal processes. First,  
450 offspring success must be density dependent. Second, seed dispersal must be limited so  
451 that siblings interact with each other. Furthermore, for interactions to be stronger within  
452 rather than among sibships, neighboring parents need to be far enough apart that their  
453 seed shadows do not completely overlap. The degree to which fecundity differences  
454 determine relative fitness of parents in our model erodes rapidly and non-linearly as the  
455 overlap between parental seed shadows decreases, eventually disappearing completely  
456 when seed shadows do not overlap at all (Fig. 3). The model also shows that the

457 fecundity advantage erodes more quickly as seed dispersal becomes more localized.  
458 These effects are lessened, however, when parental seed shadows overlap with those of  
459 more neighboring plants.

460         In addition to the geometry of seed shadows, we could expect the relative strength  
461 of sibling interactions to depend on the level of genetic diversity of seeds produced by a  
462 single parent. Genetic diversity can decrease sibling interactions through a sampling  
463 effect, through niche partitioning, or through decreased enemy sharing (Hughes et al.  
464 2008; Price and Waser 1982). For example, in *Eucalyptus*, offspring fitness increased  
465 with the incidence of multiple paternity (Breed et al. 2014). *Ipomopsis aggregata* exhibits  
466 high multiple paternity, with a single fruit averaging 4 pollen donors (Campbell 1998),  
467 more than the 2 pollen donors we used for each experimental plant in this study. The  
468 number of donors at the plant level is likely to be even higher. Thus, sibling interactions  
469 may be weaker than those we infer to have occurred in our experiment, which would tend  
470 to ameliorate the relative strength of sibling competition compared to non-sibling  
471 competition and make relative fitness resemble fecundity differences more closely.  
472 Multiple paternity is quite high in *Ipomopsis* compared to other species that have been  
473 studied (reviewed in Mitchell et al. 2013), suggesting that effects of sibling interaction  
474 may be more important for other plant species with lower pollen carryover (and hence  
475 lower multiple paternity), at least those that have no specialized means of longer-distance  
476 seed dispersal.

477         Overall, female fitness differences based on fecundity would be most likely  
478 reduced by sibling interactions in plant populations with (1) low adult density; (2) low  
479 seed dispersal; and (3) little multiple paternity. If sibling interactions are facilitative,

480 rather than competitive, fitness differences could be magnified rather than reduced by  
481 seed shadow overlap. Such positive interactions have been reported in situations where  
482 neighboring plants ameliorate a stressful environment (Butterfield and Callaway 2013),  
483 for example by acting as nurse plants that provide shade, or by facilitating attractiveness  
484 to pollinators. Consider the frequently correlated traits of plant height and flower number.  
485 Higher flower number of a parent often results in more seeds (Harder and Johnson 2009),  
486 and the resulting seedlings, if they inherit taller stature, are likely to better shade one  
487 another, reducing water loss and perhaps increasing survival. In such a scenario, patterns  
488 in offspring survival could enhance fitness differences seen in seed set. Taller plants that  
489 produce more flowers might also disperse seeds farther, as is seen across species for  
490 wind-dispersed seeds (Bullock et al. 2017). If so, we would expect a negative correlation  
491 between fecundity and the relative strength of sibling interactions which could impose  
492 selection favoring traits that increase dispersal, if sibling interactions are primarily  
493 competitive, or traits that decrease dispersal if sibling interactions are facilitative.

494         In conclusion, our model and field experiment show that the fitness benefit of  
495 high fecundity may be altered by density-dependent offspring success, such that relative  
496 fecundity may not be a completely accurate measure of overall fitness. We recognize that  
497 the use of genetic markers to measure offspring success and overall plant fitness directly  
498 is not widely practical. Still, future investigators of selection on plant reproductive traits  
499 may wish to consider the likelihood that interactions among siblings could alter fitness  
500 based solely on fecundity, especially if they are studying a system that combines low  
501 adult density, restricted seed dispersal, and low genetic diversity of seeds. Extending  
502 theoretical models to consider an even wider range of conditions for fitness modification

503 due to spatial distribution of parents and offspring dispersal would also be well  
504 worthwhile.

505  
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514

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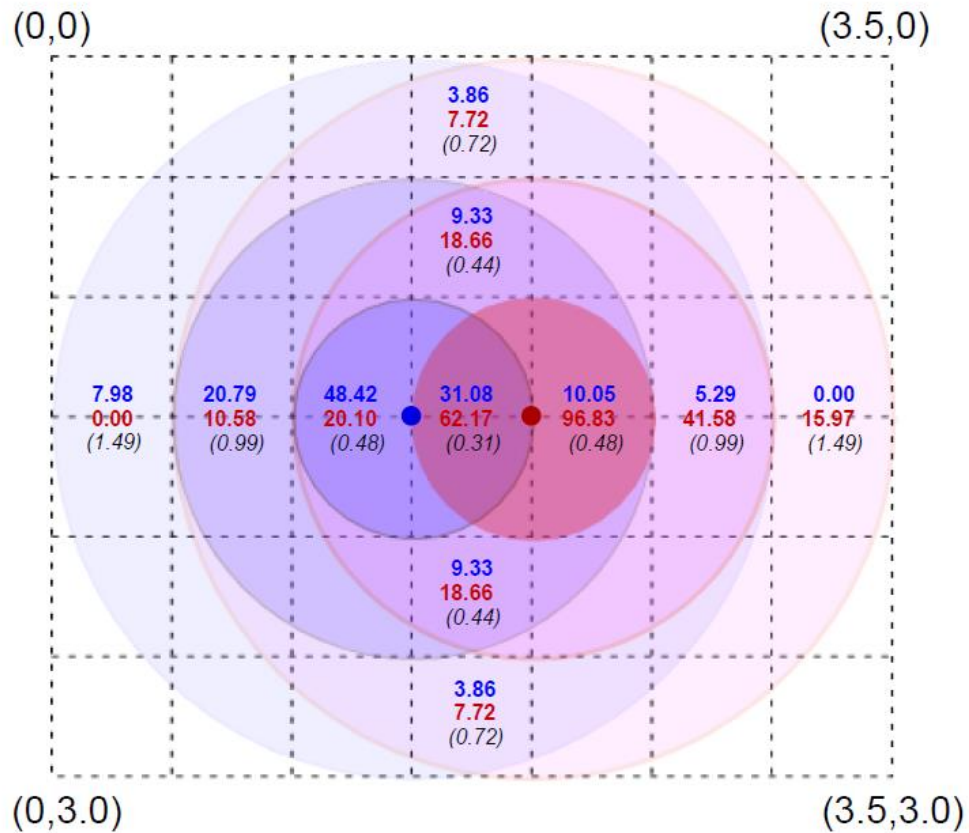
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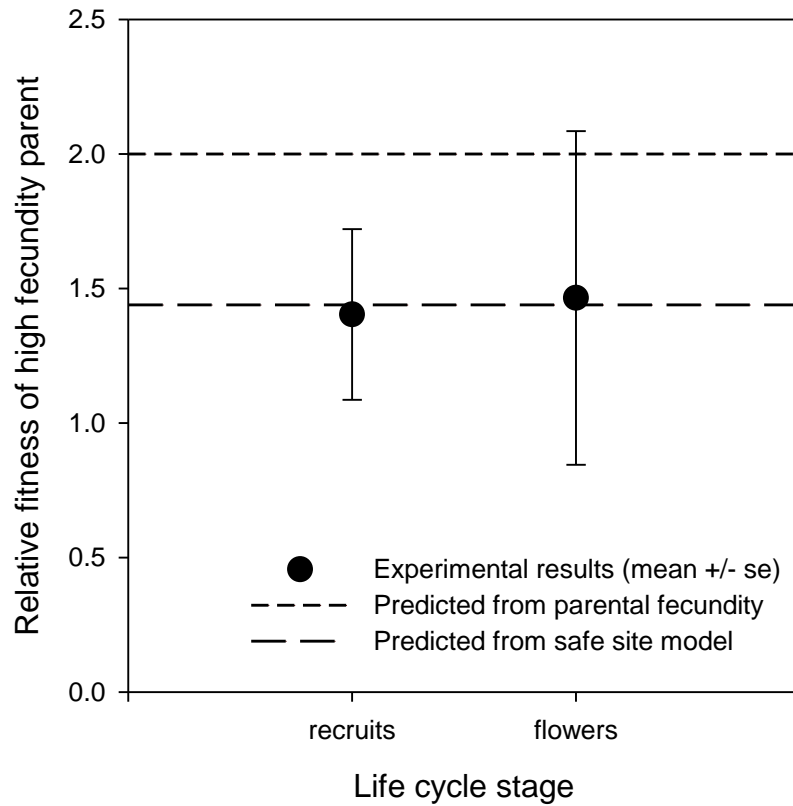
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628 **Figure 1.** Diagram of the experimental seed sowing experiment showing the expected  
 629 numbers of seeds from high-fecundity (in red) and low-fecundity (in blue) parents that  
 630 would land in each of 11 sectors within overlapping seed shadows of the parents,  
 631 assuming uniform distribution of seeds within each annulus of a given seed shadow. The  
 632 sown plots are 3.5 m wide and 3.0 m tall; the origin (0,0) is in the upper left corner of  
 633 each plot and the positions of hypothetical high-fecundity (red dot) and low-fecundity  
 634 (blue dot) seed parents are  $(x = 1.5, y = 1.5)$  and  $(2.0, 1.5)$ , respectively. The total area of  
 635 each parent's seed shadow is  $7.0686 \text{ m}^2$ , and the areas in  $\text{m}^2$  of each overlapping and non-

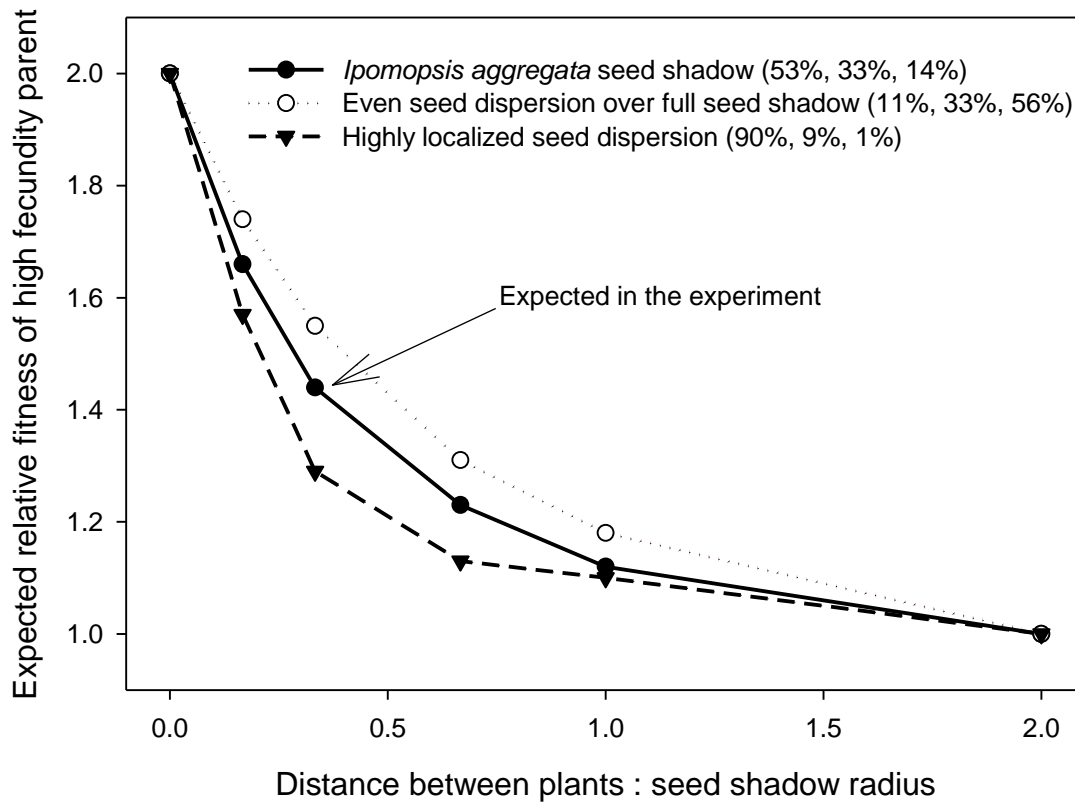
636 overlapping sector of the two seed shadows are given in black, in italics within  
637 parentheses.



638

639 **Figure 2.** Actual relative fitness of the high-fecundity parent shown as mean and standard  
 640 error across 8 sites and compared with predicted values based just on parental fecundity  
 641 or the safe site model. Results are based on number of recruits that survived to a size at  
 642 which we could genotype them (ignoring cryptic seed flow) and total flowers produced  
 643 by these offspring.





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645

646

647 **Figure 3.** Results of the generalized spatial model of competition for safe sites. Relative

648 fitness of the high-fecundity parent is plotted against the distance between adult plants

649 compared to the radius of the entire seed shadow. Three types of seed shadows are

650 shown: (i) as estimated for *I. aggregata* for the three distance classes; (ii) seeds dispersed

651 evenly over the entire seed shadow regardless of distance class; and (iii) highly-localized

652 seed dispersal.

653