

1 **Title:** Fitness and mating consequences of variation in male allocation in a wind pollinated plant

2 **Authors:** Abrar A. Aljiboury<sup>1</sup> and Jannice Friedman<sup>1,2</sup>

3

4 **Affiliations:** <sup>1</sup> Department of Biology, Syracuse University, Syracuse, NY, 13244 USA

5 <sup>2</sup> Department of Biology, Queen's University, Kingston, ON, K7L3N6, Canada

6

7 **Corresponding Author:** Jannice Friedman; 613.533.6398; [jannice.friedman@queensu.ca](mailto:jannice.friedman@queensu.ca)

8

9 **Keywords:** gain curves, protandry, mate diversity, monoecy, sex allocation, wind pollination

10 **Abstract:** In hermaphrodites, the allocation of resources to each sex function can influence  
11 fitness through reproductive success and mating success. In wind pollinated plants, sex allocation  
12 theory predicts that male fitness increases linearly with investment of resources into male  
13 function. However, there have been few empirical tests of this prediction. We experimentally  
14 manipulated allocation to male function in *Ambrosia artemisiifolia* (common ragweed) in a field  
15 experiment and measured mating success using genetic assays. We investigated the effects of  
16 various morphological traits and flowering phenology on male fitness, and on male and female  
17 mate diversity. Our results provide evidence for a linear relation between allocation to male  
18 function and fitness. We find earlier onset of male flowering time increases reproductive success,  
19 while later onset flowering time increases the probability of mating with diverse individuals.  
20 This research is among the first empirical studies testing the prediction of linear male fitness  
21 returns in wind pollinated plants. Our results provide insight into the large investment into male  
22 function by wind pollinated plants and temporal variation in sex allocation.

## 23 **Introduction**

24 Hermaphroditic organisms attain fitness through both female and male sex functions and they  
25 can maximize reproductive success through a wide variety of different strategies. Most flowering  
26 plants are hermaphroditic and because of vector-mediated gamete transfer, plant mating can be  
27 highly promiscuous with individuals mating with numerous sexual partners through either sex  
28 function. The ecological and genetic factors that determine reproductive success have been well  
29 documented for female function because of the relative ease of assessing fitness through  
30 maternal contribution. In contrast, success through male function has been more difficult to  
31 quantify because it relies on using molecular markers to identify paternity. With current  
32 molecular techniques, we are beginning to gain an understanding of male mating success,  
33 including who has mated with whom and how often (e.g. Tomaszewski et al. 2018; Christopher  
34 et al. 2019; Santos del Blanco et al. 2019).

35  
36 Sex allocation theory considers how the fitness acquired through each sex function varies with  
37 the investment of limited resources into female or male reproductive structures (Charnov 1982;  
38 Charlesworth 1991; Brunet 1992; Emms 1993). In general, hermaphroditic organisms should  
39 invest in both sex functions to the point where the marginal fitness returns are equal, at which  
40 point investment should turn to the sex function with the more linear, or less decelerating, sex  
41 function (Charlesworth 1991; Brunet 1992). The shape of the fitness gain curve is determined by  
42 a variety of intrinsic and extrinsic factors, including dispersal dynamics, biotic interactions,  
43 sibling competition, and mating patterns (Harder and Thomson 1989; Harder and Barrett 1995;  
44 Zhang 2006). While there has been extensive theoretical treatments of sex allocation theory,

45 empirical support for their predictions, particularly regarding fitness through male function,  
46 remains limited.

47

48 In sessile plants that rely on external vectors for transferring and receiving pollen, various  
49 features of the pollination environment can affect the shapes of fitness gain curves. Many animal  
50 pollinated plants likely have diminishing returns on investment in male function due to a variety  
51 of processes, including pollinators becoming saturated with pollen after visiting a plant (Lloyd  
52 1984), pollinator grooming removing pollen (Harder 1990), and deposition of related pollen  
53 causing local mate competition (Charnov 1982). In contrast, in wind pollinated plants the male  
54 gain curve is expected to be more linear (Burd and Allen 1988; Klinkhamer et al. 1997; Sakai  
55 and Sakai 2003). There are several explanations for this. First, it is unlikely that the air can  
56 become saturated with pollen, at least within the scope of biologically plausible pollen  
57 production. Second, once pollen is liberated from a plant, pollen loss from the airstream should  
58 be stochastic and independent of an individual plant (Niklas 1985). And finally, conspecific  
59 pollen captured by stigmas is likely proportional to its concentration in the air (although there  
60 might be subtle differences depending on pollen size (Paw U and Hotton 1989; Friedman and  
61 Harder 2005). Similar arguments have been made for the male gain curve in sessile spermcasting  
62 marine organisms (reviewed in Schärer 2009). Together these factors suggest that for a given  
63 individual, producing more pollen should result in siring more offspring (ie. linear relation  
64 between allocation to pollen production and fitness).

65

66 Sex allocation theory also explains shifts in relative resource allocation to female or male  
67 function with plant size (Klinkhamer et al. 1997; Zhang 2006). Larger and taller plants often

68 have more resources to invest in gamete production leading to greater fecundity. This can  
69 produce a “budget” effect of plant size on sex allocation. Generally, access to more resources is  
70 associated with greater relative allocation to female function (Lloyd 1984; Korpelainen 1998;  
71 Chen et al. 2017), because of the greater cost associated with producing seeds and fruit. In  
72 addition to the budget effect, size can also have a direct effect on fitness, where larger plants  
73 have greater fitness than small plants for a given investment in reproduction. For example, in  
74 wind pollinated plants, the physical placement of male flowers at higher positions or at the tips  
75 of long branches, results in more effective pollen dispersal and greater siring success (Young and  
76 Schmitt 1995; Tonnabel et al. 2019a). The influence of budget and direct effects of plant size on  
77 sex function may be aligned, but the underlying mechanism might be through selection on  
78 fecundity or through access to more mates.

79  
80 Male mating with more than one female partner is almost ubiquitous in the flowering plants  
81 although whether this arises as a by-product of selection on siring success in general, or on  
82 outcross mate diversity in particular is not clear (Pannell and Labouche 2013). Mating with  
83 multiple partners can provide an advantage— regardless of any increase in total fecundity—by  
84 increasing the genetic diversity of offspring (Barrett and Harder 2017). Higher mate diversity  
85 almost certainly increases the variance in offspring genotypes and may decrease the variance in  
86 final offspring number (for example, by minimizing the risk of unsuccessful pairings) which  
87 raises the probability of successfully leaving offspring (Gillespie 1974; 1977). Producing a set of  
88 genetically diverse offspring increases the probability of generating a “winning” phenotype  
89 (Williams 1975; Maynard Smith 1976). Increasing the variance in offspring genotypes is  
90 especially beneficial in heterogeneous environments if it produces genotypes that succeed in

91 different conditions, akin to bet-hedging strategies (Antonovics and Ellstrand 1984; Simons  
92 2011). Finally, greater mate diversity may reduce sibling competition between related sibs  
93 (Karron and Marshall 1990). From the maternal perspective, a common measure of mate  
94 diversity is to determine the genetic contribution of different fathers to the seeds on a plant  
95 (correlated paternity,  $r_p$ ; Ritland 2002). This measure can be extended to consider the entire  
96 mating portfolio of a plant (Barrett and Harder 2017) through both male and female function,  
97 although a full quantification of mate diversity is rare (but see Tomaszewski et al. 2018;  
98 Christopher et al. 2019).

99

100 Mating opportunities between plants are influenced by the timing of female and male function  
101 (Lloyd 1980). The vast majority of studies on the evolution of flowering time have focused on  
102 animal pollinated plants, and fitness through female function (Christopher et al. 2020), but of  
103 course selection through the two sex functions need not be in harmony (Delph and Ashman  
104 2006). Selection on male flowering time may be driven by mating opportunity or through genetic  
105 covariation with other traits (Austen and Weis 2016a). Furthermore, many hermaphroditic and  
106 monocious plants have some temporal separation in the onset of female and male function  
107 (dichogamy; Bertin and Newman 1993) that leads to a shift in the mating environment (the  
108 relative abundance of female and male phase flowers) through time. For example, in protandrous  
109 plants, the floral sex ratio shifts from male- to female-dominated during a population's flowering  
110 season (Brunet and Charlesworth 1995; Brookes and Jesson 2010). This means that in general,  
111 early male-phase flowers encounter pollen competition for few ovules compared with later male-  
112 phase flowers that have access to more ovules (Nakamura et al. 1989; Stanton 1994). This effect

113 is weakened if protandry in incomplete and flowering duration is long with substantial overlap in  
114 sex function.

115

116 Our primary goal in this study is to use a manipulative field experiment to evaluate the effect of  
117 allocation to male function on siring success and mate diversity in a wind pollinated herbaceous  
118 plant. We also set out to determine if there were additional benefits accrued through plant size  
119 (height, width, and plant biomass) on siring success; and to determine the effect of flowering  
120 time on mating opportunities and reproductive success through male function. We used the  
121 monoecious herb, *Ambrosia artemisiifolia* (common ragweed), a weedy annual plant that is  
122 known to produce a prodigious amount of allergenic pollen. It has been well established in *A.*  
123 *artemisiifolia* that sex allocation is both size and resource dependent, where taller plants and  
124 those with high light availability invest more in male function (McKone and Tonkyn 1986;  
125 Ackerly and Jasiński 1990; Traveset, 1992; Paquin and Aarssen 2004; Friedman and Barrett  
126 2011a; Nakahara et al. 2018). To achieve our aims we experimentally manipulated allocation to  
127 male function to limit confounding sex allocation with overall condition or plant size and to  
128 produce individuals with a range of different male allocation patterns (Emms 1993, Schärer  
129 2009). After allowing plants to naturally wind pollinate, we used genetic markers to estimate the  
130 progeny sired by each plant. This study represents the first attempt to estimate the male gain  
131 curve in a wind-pollinated plant after experimentally controlling for male allocation (similar to a  
132 series of studies in free-spawning animals; Yund and McCartney 1994, McCartney 1997, Yund  
133 1998, Johnson and Yund 2009) and to quantify mate diversity through paternal function.

134

135 **Methods**

136 *Study Species*

137 *Ambrosia artemisiifolia* L. (common ragweed) is an early successional weed that inhabits a  
138 broad range of habitats including roadsides, cultivated fields, and disturbed lands (Bazzaz 1974).  
139 The species is monoecious, producing male inflorescences at the tips of branches and  
140 inconspicuous female flowers in leaf axils (Payne 1963). Ragweed can produce between 200 and  
141 6000 seeds depending on the plant's condition (Fumanal et al. 2007), although fecundity is  
142 substantially lower when plants are disturbed or transplanted. Plants are usually weakly  
143 protandrous at the plant level (i.e. male flowers open before female flowers) and there is  
144 plasticity in the degree and order of dichogamy (Friedman and Barrett 2011a). Ragweed is self-  
145 incompatible (Friedman and Barrett 2008), so that all seed are outcrossed. In addition, it is an  
146 annual plant reproducing entirely through seed (Bassett and Crompton 1975), which allows for  
147 more easily quantifying fitness.

148

149 *Experimental Design*

150 Between June 1-5 2017, we collected *A. artemisiifolia* seedlings at the 4-leaf stage from 18  
151 natural populations around Syracuse, NY, USA. The populations were selected arbitrarily,  
152 ensuring they were approximately 4 km apart (range: 4-9 km). We collected plants from multiple  
153 sites to increase the genetic variation in microsatellite loci, which we would later use for  
154 paternity assignment. We dug up seedlings and immediately transplanted them into 2.5 cm pots  
155 filled with Sunshine Mix4 (Sun Gro Horticulture). We maintained the plants in their pots for 8  
156 days, then transplanted them into randomly predetermined locations in three blocks in a field at  
157 Syracuse University, NY, USA (43°00'47"N 76°07'07"W). The three blocks were spaced  
158 approximately 15m from each other, and each block was covered with groundcloth to inhibit



159 weed growth. Within each block, we planted 64 plants in a square grid, with individuals 50cm  
160 away from each other. We planted equal numbers of all subpopulations into each block (n=4 per  
161 subpopulation per block) and recorded the specific location of each plant. We monitored the  
162 plants' condition and replaced any dead plants up to two weeks after transplanting in the field  
163 (n=12 transplants).

164

165 We minimized pollen contamination by naturally occurring ragweed by mowing the field around  
166 the three blocks every two weeks and manually removing any ragweed surrounding the field site  
167 at least twice a week. The larger area around the experimental garden was maintained by  
168 Syracuse University and received considerable mowing and horticultural maintenance, which  
169 reduced the overall presence of ragweed plants.

170

171 To limit confounding sex allocation with overall condition or plant size (Emms 1993, Schärer  
172 2009), we artificially manipulated male allocation by randomly assigning each plant to one of  
173 four male allocation categories that differed in their maximum number of branches bearing male  
174 inflorescences per plant. We started the manipulations between August 1-5 (which coincided  
175 with when the very first plants began flowering) after allowing plants to grow for about seven  
176 weeks. We cut male inflorescences from each plant to match its pre-assigned allocation category,  
177 and maintained the number of inflorescences by removing a subset of newly emerged male  
178 inflorescences twice a week. The four categories had a maximum of 4, 8, 16, or 32 branches  
179 bearing male inflorescences per plant. Although the number of branches increased exponentially,  
180 subsequent branches have fewer and shorter inflorescences, so this approximated a linear

181 increase in male flowers (Figure S1: category 4 has greater variance because we could only  
182 remove excess branches from the randomly assigned individuals).

183

184 We measured plant height from ground level to the base of the inflorescence spike, and measured  
185 width at the widest point of the plant including branches. We recorded these size measurements  
186 three weeks after transplanting (17 July 2017) and repeated the measurements on 7 August and 7  
187 September. We also recorded the date the first female and male flower opened on each plant.

188 Subsequent to the initial difference in onset of female and male flowers, plants continue  
189 producing new flowers of both sex functions throughout the blooming period (see Figure 4 in  
190 Friedman and Barrett 2011a). Once plants started senescing (between October 2 and 15, 2017),  
191 we harvested male inflorescences from each plant and separately collected above-ground plant  
192 material. We dried the male inflorescences and above-ground plant material for 3 days at 70°C,  
193 then recorded the biomass as proxies of male allocation and plant size, respectively.

194

195 We allowed plants to pollinate naturally within the field. We randomly selected a subset of plants  
196 (n=24 per plot, total=72) from which we collected at least 60 seeds twice: once when the plants  
197 first matured seeds (between September 12 and 26, 2017), and once before the plants were  
198 harvested (between October 1 and 12, 2017). We refer to these plants as ‘maternal’ plants but  
199 recall that the species is monoecious, so these focal 24 plants are also ‘paternal’ plants. We  
200 ensured that the maternal plants were evenly represented among the four male allocation  
201 categories. We avoided collecting seeds from plants at the edges of plots as they were not  
202 surrounded by experimental plants in all directions.

203

204 *Parent and progeny genotyping*

205 We collected fresh leaf tissue from all experimental plants in the field on August 23-25 for  
206 subsequent genotyping. We stored leaf tissue at -80°C until DNA extractions could be  
207 performed. On December 21, 2017, we stratified 60 seeds (from an equal mix of early and late  
208 collection dates) from each of the 72 focal maternal plants in the dark at 4°C for 16 weeks, and  
209 then planted them in 96-well plug trays filled with Sunshine Mix4 mixed with 25% sand. We  
210 planted more seeds than we intended to genotype to ensure we had 24 seedlings per maternal  
211 plant. The average germination probability of the seeds was 68.5 percent. We maintained plants  
212 in the greenhouse under 16-hr photoperiod and 21°C/18°C day/night temperatures. After six  
213 weeks, once the majority of plants were at the 4-leaf stage or larger, we collected fresh leaf tissue  
214 from 24 progeny per maternal plant, and stored the plates at -80°C until DNA extractions could  
215 be performed.

216

217 We extracted DNA from frozen leaf tissue of the 1728 progeny and 192 field plants using a  
218 modified CTAB protocol. We then used seven polymorphic microsatellite markers that were  
219 previously developed for *A. artemisiifolia* (Genton et al. 2005; Chun et al. 2009) to identify male  
220 parentage. We performed polymerase chain reaction using Bio-Rad thermocyclers (see Table S1)  
221 and labelled PCR products were analyzed at the Institute of Biotechnology at Cornell University  
222 on an ABI 3730xl DNA Analyzer using GeneScan LIZ500 size standard (Applied Biosystems,  
223 Foster City USA). We manually scored the genotypes using GeneMarker v.2.2.0 (Soft Genetics,  
224 State College USA). We used CERVUS version 3.0.7 (Field Genetics Ltd, London UK), to  
225 perform paternity analyses using stringent parameters with a minimum of 3 typed loci and

226 accounting for a 1% genotyping error rate and assigned paternity using a strict (95%) confidence  
227 criterion.

228

### 229 *Data analysis*

230 We assessed reproductive success in three ways. First, we quantified the total number of seeds  
231 sired by each plant and refer to this as *male reproductive success*. Second, we quantified the  
232 number of unique plants on which a given individual sired seed and we refer to this as *male mate*  
233 *diversity*. Third, we quantified the number of seeds sired by different pollen donor plants on a  
234 given focal plant and we refer to this as *female mate diversity*. Note that we did not quantify total  
235 seed production by plants (female reproductive success). Our primary aim was to evaluate the  
236 association between male allocation and the measures of male reproductive success (total  
237 number of seeds sired and number of mates), including their relation with each other, and effects  
238 of size and flowering time.

239

240 All analyses were performed using R version 4.1.1 (R Core Team 2021). We used generalized  
241 linear mixed models (GLMMs) using the *glmmTMB* package (Brooks et al. 2017) and marginal  
242 means were estimated using the *emmeans* package (Lenth 2021) We used the *DHARMA*  
243 package for diagnostic tests of residuals (Hartig 2017). We had three sets of models, where the  
244 response variable was either male reproductive success, male mate diversity, or female mate  
245 diversity. In all models, we included the fixed effects of male allocation, female flowering day,  
246 male flowering day, plant width, plant height, biomass, and whether the plant was located on the  
247 edge or interior. The latter effect was excluded in models of female mate diversity since all  
248 plants were located in the interior; in this model we additionally included germination

249 probability of seed. Two random effects were included in all models: block and source  
250 population. We identified significant terms in the model using AIC scores and log-likelihood  
251 ratios of model fit, and present the simplest model of best fit (except when the term reflected  
252 experimental design, like edge effect). We tested for significant differences among categories  
253 using Dunn-Sidak adjustment for multiple testing. Recall that we quantified male allocation in  
254 two ways—the categorical manipulation the plant received, and the continuous measure of male  
255 inflorescence weight. Thus, we ran separate models including either measurement as a fixed  
256 effect. The two sets of models produced very similar results, which is not surprising because the  
257 two measures of male allocation are strongly statistically associated (Figure S1).

258

259 Models of male reproductive success used a negative binomial distribution with the canonical  
260 link function. Additionally, to specifically evaluate the shape of the male gain curve, we fit a  
261 power function ( $y=ax^b$ ; Charnov 1979; Johnson and Yund 2009) to the continuous male  
262 allocation data. The exponent of the power function ( $b$ ) describes the shape of the curve. If the  
263 exponent does not differ significantly from 1 then reproductive success approximates a linear  
264 function of male allocation; if it is significantly  $<1$  or  $>1$ , reproductive success is either a  
265 saturating or accelerating function of male allocation, respectively. Models of mate diversity  
266 required accounting for the number of seeds sired. Thus, we used a binomial model (unique  
267 mates/total seeds sired) with prior weights, which best captured the underlying biological process  
268 and accommodated the frequent occurrence of the upper bound. Models of female diversity used  
269 a poisson distribution with an offset parameter of the number of seeds genotyped and the  
270 canonical link function.

271

272 We investigated the fitness accrued through male function by mating with additional mates by  
273 estimating the slope of a least-square regression between standardized male mating success and  
274 standardized male reproductive success ('Bateman gradient': Arnold and Wade 1984). The  
275 model included covariates for flowering time, male allocation, edge location, and random effects  
276 accounting for block and source population. We compared the likelihood of linear and quadratic  
277 terms in the regression using AIC scores and log-likelihood ratios of model fit.

278

279 We considered the spatial dispersal of successful pollen transfer by calculating the Euclidean  
280 distance between a pollen donor and the plant on which it sired offspring. Note that because of  
281 our experimental design there was a 15m gap between any two blocks. We modeled mating  
282 distance using generalized linear mixed models (GLMMs) using the glmmTMB package, with a  
283 gamma distribution. To investigate the effect of morphological and phenological variables on  
284 dispersal distance, we included male inflorescence weight, plant height, plant width, biomass,  
285 female flowering time, male flowering time and edge as fixed effects, and candidate father  
286 identity as a repeated random effect. To investigate the relation between mean dispersal distance  
287 and reproductive success we modeled the effects of male siring success and male mate diversity  
288 as fixed effects. To visualize variation in dispersal kernels, for every pollen donor plant that sired  
289 more than 5 seeds, we fit a Weibull distribution to its dispersal kernel, and extracted the shape  
290 parameter,  $\kappa$ , and the scale parameter,  $\lambda$  for each pollen donor plant. We also used the software  
291  $NM\pi$  (Chybicki 2018) to calculate the parameters of the pollen dispersal model. Specifically, we  
292 estimated average dispersal distance ( $\delta_p$ ), the shape of the exponential-power dispersal kernel  
293 ( $b_p$ ), the intensity of directionality of dispersal ( $\kappa_p$ ), and the prevailing direction of pollen  
294 dispersal ( $\alpha_p$ ).

295 **Results**

296 *Identification of mating outcomes*

297 A total of 1257 (72.7%) offspring were assigned paternity, the remaining 471 (27.3%)  
298 individuals were excluded because either confidence was low, or no paternity matches were  
299 found using the parental genotypes (ie. the seed were sired by plants outside the experimental  
300 fields). Of the 192 experimental plants, 176 plants (91.7%) sired seed. Of the 16 plants that were  
301 not represented in the paternity of seed, three plants were removed from the analysis because  
302 they were typed at less than three loci, and the remaining 13 plants were not identified as the  
303 father for any genotyped seed.

304

305 *Pollen dispersal and siring distance*

306 The incidence of mating strongly declined with distance, with a heavy-tailed distribution. Plants  
307 sired most of their seeds locally, where siring peaked within half a meter of pollen source and  
308 declined rapidly over the 25 m distance of the field (distance between mates: mean = 2.81 m, SD  
309 = 5.03 m.; Figure 1A, solid line). Known intermate distances were vastly smaller than the  
310 distances between all pairs of plants (ie. all possible mates;  $t=52.91$ ,  $P<0.001$ ) and also smaller  
311 than the distances of all within-block pairs of plants ( $t=4.39$ ,  $P<0.001$ ) —reflecting the  
312 predominance of mating amongst near neighbours. Of the seed we successfully genotyped,  
313 90.7% were sired by fathers within the same experimental block (1127 of 1242), and only 9.3%  
314 were due to longer distance siring events between blocks. None of the morphological or  
315 phenological variables we investigated (male inflorescence weight (or allocation category), plant  
316 biomass, width, height, the onset of female or male flowering) had any effect on siring distance,  
317 and there was also no relation between siring distance and either the number of seeds sired or

318 mate diversity. The only variable that was significantly associated with dispersal distance was  
319 whether plants were located in the interior or the edge (adjusted for other effects in the model,  
320 mean dispersal distance: edge: 2.92 m, 95% CI: 2.38-3.59; interior: 2.24 m, 95% CI: 1.89-2.66;  
321  $t=1.94$ ,  $P<0.05$ ).

322

323 There was substantial variation among plants in their fitted dispersal distributions. To visualize  
324 the variation in the distribution of inter-mate distances, we plot the fitted Weibull distribution for  
325 a random subset of 10 plants (Figure 1B) and also show the trajectories of pollen dispersal for  
326 the same subset of 10 plants (Figure 1C). The Weibull distribution is described by the shape  
327 parameter,  $\kappa$ , and the scale parameter,  $\lambda$ . Among individual candidate fathers, estimated shape  $\kappa$   
328 had range=0.62-4.74 (mean=1.51) and the estimated scale  $\lambda$  had range=0.72-6.39 (mean=2.30).

329 We next used NM $\pi$  (Chybicki 2018) to calculate properties of the pollen dispersal kernel.

330 Similar to the results using other methods, the estimated average dispersal distance ( $\delta_p$ ) was 1.2m  
331 with high variance (S.E.=1.3m), the shape of the exponential-power dispersal kernel ( $b_p$ ) was  
332 0.18 (S.E.=0.14), the intensity of directionality of dispersal ( $\kappa_p$ ) was 0.48 (S.E.=0.31), and the  
333 prevailing direction of pollen dispersal ( $\alpha_p$ ) was 2.68 with high variance (S.E.=8.93).

334

### 335 *Male reproductive success*

336 Individual plants sired a mean=6.60 (SD=6.53, Range=0-39) seed, of the successfully genotyped  
337 offspring. The effect of our experimental manipulations resulted in plants with greater male  
338 allocation siring significantly more seed than plants with less male allocation (Table 1A; Figure  
339 2A). Adjusted for other effects in the model, plants in the lowest allocation category sired an  
340 average of 4.44 seeds (95% CI: 3.36-5.87) while plants in the highest allocation category sired an



341 average of 8.05 seeds (95% CI: 6.28-10.31). When we consider allocation as a continuous  
342 variable modeled as male dry inflorescence weight, we found that the number of offspring sired  
343 increases significantly with male inflorescence weight (Table 1B; Figure 2B). The exponent of  
344 the power function ( $b=0.85$ ,  $SE=0.30$ ) saturates slightly, but did not significantly differ from one  
345 ( $t_{168} = -0.45$ ,  $P=0.65$ ), indicating a significant linear relation between male allocation and siring  
346 success ( $F_{1,168}=7.68$ ;  $P<0.001$ ).

347

348 We found a strong effect of the timing of onset of male flowering on male reproductive success  
349 where earlier flowering plants sired significantly more seed (Table 1). Moreover, we found a  
350 significant interaction between allocation and male flowering time, whereby only plants in  
351 categories 1, 2, and 3 experienced greater reproductive success if they started male flowering  
352 earlier (Table 1A; Figure 2C). Plants in the highest allocation category had similar reproductive  
353 success regardless of when they flowered (Table 1A; Figure 2C).

354

355 In contrast to our expectation that larger plants would sire more offspring, we discovered that  
356 neither plant height nor biomass had a significant effect on the number of seeds sired. Instead,  
357 we found that wider plants had greater male reproductive success (Table 1;  $\beta \pm SE = 0.08 \pm$   
358  $0.005$ ; Figure S3). Plants located on the edge of the arrays sired significantly fewer seeds than  
359 plants located in the interior (edge: mean: 4.97 seeds, 95% CI: 4.06-6.08; interior: mean: 6.52  
360 seeds, 95% CI: 5.45-7.82; Table 1).

361

362 *Mate diversity*

363 There was a significant positive linear Bateman gradient indicating a strong relation between  
364 mating and reproductive success (Figure 3;  $\beta \pm SE = 0.71 \pm 0.04$ ,  $\chi^2 = 235.41$ ,  $P < 0.0001$ ). We  
365 also found a significant quadratic term ( $\gamma \pm SE = 0.12 \pm 0.03$ ,  $\chi^2 = 24.75$ ,  $P < 0.0001$ ). These  
366 relations were independent of male allocation.

367

368 We calculated male mate diversity by quantifying the number of mates to which a pollen-donor  
369 plant successfully dispersed pollen and sired seed. Of the genotyped seed, plants donated pollen  
370 to and mated with a mean=4.91 (SD=2.55, Range=1-14) different individuals. In raw numbers,  
371 males with larger male allocation had greater mate diversity (mean cat1: 3.71; cat2: 4.08; cat3:  
372 5.61; cat4: 5.98), however this was likely a consequence of siring more seeds overall. In models  
373 that accounted for the total number of seeds sired, we found no significant effect of male  
374 allocation on mate diversity (Table 2).

375

376 We previously reported that early flowering plants sired more total seeds (Figure 2C), when we  
377 investigate mate diversity it appears that many of those seeds were more likely to be full-sibs  
378 (i.e. sired on the same plant). Individuals that flowered later had a probability of greater mate  
379 diversity (Table 2; Figure 4A), that is, they donated pollen to and sired seeds on more unique  
380 mates. Amongst the morphological characteristics we measured (plant width, plant height, and  
381 biomass) none of them had a significant effect on mate diversity, after controlling for the number  
382 of seeds sired.

383

384 We quantified female mate diversity as the number of different pollen donor plants that sired  
385 seed on a given plant. We genotyped a subset of seed per plant (mean=17.25, SD=2.43,

386 Range=11-22). Of these genotyped seed, plants mated with a mean=11.06 (SD=2.64, Range=5-  
387 16) different pollen donors. Plants that initiated female flowering later produced seeds that were  
388 sired by a greater diversity of pollen donors, than those with an earlier onset to female flowering  
389 (Table 2, Figure 4B). There was no association between female mate diversity and the allocation  
390 class of the individual, nor any of the measured morphological traits (height, width, biomass),  
391 nor germination probability.

392

### 393 **Discussion**

394 Our results are consistent with the hypothesis that wind pollinated plants experience linear  
395 fitness gains with increased male allocation. Experimentally manipulating the number of male  
396 flowers elicited predicted changes in the number of offspring sired—plants with more male  
397 flowers sired significantly more offspring than plants with fewer male flowers. We found  
398 minimal direct effects of plant height on reproductive success, although plants with greater width  
399 (longer branches) had higher male fitness. We found significant, and surprising, influences of the  
400 timing of flowering, where an earlier onset to male flowering was beneficial for reproductive  
401 success, especially for plants with fewer male flowers. In contrast, individuals with later male  
402 flowering had relatively higher male mate diversity, and similarly later onset to female flowering  
403 was associated with seeds sired by a greater diversity of mates. Finally, our quantification of  
404 seed siring distance identified a strongly skewed and heavy-tailed distribution of successful  
405 pollen, with most seeds sired on nearest neighbours. Below, we consider the implications of  
406 these effects of sex allocation, intermate distance, and flowering time and their possible  
407 consequences on the evolution of reproductive strategies in wind pollinated plants.

408

409 *Incidental and unintended experimental consequences*

410 Here we address the possible implications of several features of our experiment. First, we  
411 artificially created classes of plants with different male allocation to avoid confounding our  
412 intended variable of interest with overall condition or budget effects of plant size. This treatment  
413 necessarily involved removing male flowers, and for obvious reasons we could not artificially  
414 add male flowers. One implication of this is that we cannot test whether further exaggeration of  
415 male allocation will result in a saturation of reproductive success. We followed previous workers  
416 and fit a power function to our male gain curve data (Johnson and Yund 2009), and an implicit  
417 assumption of this is that males could theoretically produce enough pollen to monopolize  
418 fertilizations, which is presumably biologically impossible under at least some range of  
419 conditions. Second, because of the inflorescence architecture of *A. artemisiifolia*, when we  
420 removed male inflorescences we necessarily interfered with the height and width of plants,  
421 because male inflorescences are borne on terminal racemes. There was a modest, but significant,  
422 reduction in height and width for the lowest allocation treatment, but none of the other treatments  
423 differed significantly from each other (Figure S2). Our analyses accounted for this statistically by  
424 initially including all effects as covariates, but nonetheless we may have reduced power to  
425 identify direct effects of size on reproductive success. Third, we used a square lattice design  
426 which meant that plants are not equidistant to their first-order neighbours (i.e. horizontal and  
427 vertical neighbours are 0.5 m, but neighbours along the diagonal are 0.7 m). This is not a  
428 problem, per se, but should be considered when interpreting intermate distance.

429 *Male reproductive success and pollen dispersal*

430 Our results are consistent with predictions from sex allocation theory that male fitness increases  
431 linearly with male allocation. To our knowledge, the only other explicit test of the male gain

432 curve in wind pollinated plants comes from a natural stand of white spruce trees, where trees that  
433 produced more male cones sired a greater proportion of seeds (Schoen and Stewart 1986). There  
434 is some evidence that in the wind-pollinated herb *Mercurialis annua* plants with greater pollen  
435 production have greater reproductive success at high density (Tonnabel et al. 2019a). In this  
436 species, males with morphological traits that facilitated farther pollen dispersal achieved greater  
437 fitness, and only marginally improved fitness with greater pollen production. A previous study in  
438 *A. artemisiifolia* demonstrated that male reproductive success increased with height and male  
439 reproductive investment (Nakahara et al. 2018), but whether this was due to budget or direct  
440 effects of plant size was unclear. Other studies have reported that male reproductive success  
441 increases linearly with male allocation in spermcasting marine invertebrates (Yund and  
442 McCartney 1994; McCartney 1997), or shown that the male gain curve becomes more linear  
443 under certain ecological conditions like intense local sperm competition or mating over larger  
444 distances (Yund 1998; Johnson and Yund 2009). Our finding of a linear gain curve means that  
445 increased allocation to pollen production should be favored under some ecological conditions.  
446 Previous work demonstrated substantial genetic variation for plasticity in male allocation in  
447 ragweed (Friedman and Barrett 2011a), so that allocation may respond adaptively to ecological  
448 conditions.

449  
450 Because we experimentally manipulated plant size, we removed budget effects (i.e. the effect  
451 where plants in better condition or larger plants invest more in reproduction because they have a  
452 larger resource budget), any remaining effects of plant size on male fitness would more likely be  
453 due to direct effects of size (Klinkhamer et al. 1997). Experimentally controlling for male  
454 investment, we found no association between plant height and siring success. Similarly, there

455 was no significant effect of plant height on male reproductive success in *M. annua* (Tonnabel et  
456 al. 2019). But our result is discordant with a different study in *A. artemisiifolia* that found a weak  
457 effect of plant height on siring success, although the effect depended on model assumptions  
458 about neighbourhood size (Nakahara *et al.* 2018). All of these studies were conducted in  
459 common garden arrays where the benefit of height might be diminished compared to natural  
460 settings, because of the reduction in intervening vegetation. Nonetheless, for herbaceous plants  
461 occurring in open environments, siring success may not be strongly influenced by plant height,  
462 but by other aspects of size that affects the ability to better disperse pollen.

463  
464 We identified a benefit of plant width on male reproductive success. Although we did not  
465 initially predict this, the architecture of the plant suggests that this effect is due to the  
466 aerodynamics of pollen release in wind pollinated plants (Niklas 1985). All objects are  
467 surrounded by a layer of still air, and the size of the boundary layer is determined by the size of  
468 the solid structure disrupting air flow. An important adaptation for wind pollinated plants is  
469 positioning male flowers away from vegetative structures to get them out of the boundary layer  
470 and enhance pollen liberation from anthers (Timerman and Barrett 2021). In many species this  
471 involves extending the anthers on long filaments that vibrate in the wind (Timerman and Barrett  
472 2018). However, in *A. artemisiifolia* dehiscent anthers extend only just below the downward-  
473 pointing floret (Payne 1963), and so the position of the staminate head on branches that extend  
474 beyond foliage may better expose them to the wind to increase vibration and facilitate the release  
475 of pollen into the airstream (Friedman and Harder 2005). Similarly, in *M. annua* selection favors  
476 males with wider diameters in combination with longer branches and greater biomass (Tonnabel

477 et al. 2019a). Thus, there is increasing evidence that branching architecture may provide direct  
478 beneficial effects for male fitness in wind-pollinated plants.  
479  
480 Wind-dispersed pollen typically has a leptokurtic distribution from point sources (Bateman 1947;  
481 Gleaves 1973; Levin and Kerster 1974), so that the seed set of recipients should decrease rapidly  
482 with distance from the pollen donor. Our results are consistent with this. Most plants sire seeds  
483 on their nearest neighbours, and the distance between mates shows a strongly fat-tailed  
484 distribution. Other studies have found similar results, for example pollination success in *Taxus*  
485 declined with plant spacing (Allison 1990); in dioecious *Thalictrum* species female plants at  
486 greater distance from males had reduced seed set (Steven and Waller 2007); and in *Festuca*  
487 *pratensis*, most pollen was deposited within 75m of donors (Rognli *et al.* 2000). In our  
488 experiment, none of the morphological variables we measured had any significant effect on the  
489 distance between mates. Similarly, Nakahara et al. (2018) found no effect of plant height on the  
490 the maximum distance between mates. These findings are in contradiction to the theoretical  
491 expectation that taller wind pollinated plants will have farther pollen dispersal (Burd and Allen  
492 1988), and indicate that plant height may have only limited consequences for pollen dispersal in  
493 herbaceous plants in open fields. Indeed, successful matings at the farthest distances in our  
494 experiment (between blocks) appear to be stochastic. Nonetheless, for plants that sired fewer  
495 seeds overall, these long-distance siring events represent a greater proportion of their total seeds  
496 sired. While most mating occurs very locally in wind pollinated plants, rare longer-distance  
497 mating events may profoundly impact genetic structure and patterns of genetic variation  
498 (Loveless and Hamrick 1984).  
499

500 *Mating portfolios and the benefits of mate diversity*

501 We found high variance in both male reproductive success and male mating success, as expected  
502 under Bateman's principle and sexual selection (Tonnabel et al. 2019b). To investigate the  
503 fitness accrued by mating with more partners, we estimated the Bateman gradient for male  
504 function, and identified significant positive linear and quadratic (accelerating) terms. This  
505 relation was not influenced by allocation category or male inflorescence weight, suggesting  
506 consistent benefits for all individuals. Similarly, while individuals with more male flowers had  
507 greater mate diversity in absolute terms, this was entirely driven by their concomitant increase in  
508 reproductive success. This finding supports the proposition that multiple mating in plants is a by-  
509 product of selection on male function to increase siring success (Pannell and Labouche 2013).  
510 Mating with multiple female partners (high mate diversity) provides a quantitative advantage  
511 through male function, and our data corroborate that mating opportunities constrain male  
512 reproductive success.

513

514 There is scant evidence that mate diversity, per se, is under selection or beneficial (Barrett and  
515 Harder 2017). However, several lines of evidence suggest that genetic diversity may be  
516 advantageous through a process of 'genetic bet-hedging'. When stochasticity is incorporated into  
517 measures of natural selection, then fitness depends on both the mean and the variance in  
518 offspring number (Gillespie 1974) and increasing the variance in offspring number of a genotype  
519 will decrease its fitness (Gillespie 1977). To the extent that mating between any two individuals  
520 leads to low fitness (e.g. due to genetic compatibility), then having a greater diversity of mates  
521 could reduce the variance in offspring number and increase mean fitness. A second related  
522 argument is that genetic diversity within families is beneficial for offspring success in the face of



523 temporal or spatial heterogeneity. When environments are heterogeneous, offspring diversity  
524 raises the chances that some offspring succeed, thus decreasing the variance in success and  
525 increasing geometric mean fitness (Slatkin 1974; Simons 2011). In animals, various lines of  
526 evidence suggest that genetic bet hedging is unlikely to be solely responsible for maintaining  
527 polyandry, unless the costs of multiple mating are very low (Yasui 1998; Jennions and Petrie  
528 2000). Indeed, in plants the costs of multiple mating are likely low, especially for wind  
529 pollinated plants that are not investing in showy flowers, raising the likelihood that genetic bet-  
530 hedging provides a selective advantage.

531  
532 Mate diversity can also be considered from the perspective of the interactions among siblings –  
533 both during seed development and subsequent dispersal and establishment. First, genetic  
534 variation among developing embryos provides an opportunity for maternal resources to be  
535 distributed to the highest quality embryos and potential abortion of incompatible or low-quality  
536 embryos (Zeh and Zeh 1996; Haig and Westoby 1988). However, sibling competition within the  
537 developing fruit can be detrimental to both maternal and paternal parents, and several plant  
538 reproductive strategies may have evolved to reduce mate diversity within an ovary (Kress 1981;  
539 Bawa 2016). For example, generalist, indiscriminate pollinators are most likely to deliver  
540 unrelated pollen grains onto stigmas, and there is a well-established association between uni-  
541 ovulate flowers and abiotic and generalist pollinators (Charlesworth 1993; Friedman and Barrett  
542 2011b). Like most wind pollinated plants, *A. artemisiifolia* has flowers with single ovules, so  
543 differential maternal investment would occur among developing fruits on a plant. Second, greater  
544 mate diversity increases the number of half-sib families rather than full-sib families, so in species  
545 with restricted seed dispersal establishing seedlings are less related. Genetic diversity among

546 seedlings reduces sib-competition thereby increasing maternal (and paternal) fitness (Cheplick  
547 1992). Furthermore, to the extent that pollen dispersal is limited, genetic diversity of offspring  
548 will reduce biparental inbreeding (Uyenoyama 1986) and any accompanying inbreeding  
549 depression (Charlesworth and Willis 2009). Future work is necessary to partition the relative  
550 influence of these factors on selection for mate diversity.

551 *The benefits of temporal separation in flowering*

552 The temporal separation of flowering between sexes (dichogamy) generates uneven sex ratios  
553 across a flowering season (Brunet and Charlesworth 1995; Sargent and Roitberg 2000), which is  
554 paradoxical because frequency-dependent selection should act to equalize the availability of  
555 pollen and ovules at every point in time. The benefits of dichogamy (and protandry in particular)  
556 include avoiding interference between male and female sex organs (Lloyd and Webb 1986;  
557 Bertin 1993) and preventing selfing (Darwin 1876). Neither of these mechanisms are likely  
558 responsible for protandry in *A. artemisiifolia* because the species is monoecious and self-  
559 incompatible. In a simulation model of the asynchrony in timing of pollen and ovule  
560 presentation, Medan and Bartoloni (1998) demonstrated that selection favours protandrous  
561 genotypes when there is substantial overlap in male and female function; as they have the  
562 greatest access to mates (both pollen and ovules), although flowering too early can be wasteful.

563

564 Several results support this balance of influences on protandry. We found that earlier onset to  
565 male function increased male reproductive success (especially for plants that had lower overall  
566 male allocation), but later flowering increased the probability of mating with diverse partners.  
567 Although we cannot identify the mechanisms here, we speculate that the potential for male  
568 mating success depends on competition for access to ovules from other individuals—more

569 ovules will be available later in the season, but there will also be greater pollen competition.  
570 Plants benefit from early flowering by monopolizing siring opportunities on the few individuals  
571 that have female flowers, but shifting male flowering to later when more plants are blooming  
572 benefits relative mate diversity. Similarly, slightly delaying female function resulted in seed sired  
573 by a more diverse pollen pool. Other mechanisms may be at play, for example if plants with  
574 earlier flowering donated their pollen to more fecund mates, had greater pollen competitive  
575 ability, or if their mates had declining fruit set (Brunet 1996, Weis and Kossler 2004, Austen and  
576 Weis 2016b). The pattern in our results suggests that the staggered onset to male and female  
577 flowering benefits both sex functions (in “harmony” between the sexes: Delph and Ashman  
578 2006), while fecundity selection and sexual selection through male function are acting in  
579 opposing directions on the start of male flowering.

580  
581 Sex ratio selection and availability of ovules will eventually constrain the continued evolution of  
582 dichogamy, demonstrated in a theoretical model by Sargent et al. (2006). An extended blooming  
583 season alleviates some of the costs of skewed sex ratios—the earliest blooming plants sacrifice  
584 some male mating opportunities that are lost due to an absence of available ovules, but the  
585 wasted pollen represents a small fraction of overall investment. This scenario is exemplified by  
586 *A. artemisiifolia*, where flower production increases, and under some conditions accelerates,  
587 through time (Friedman and Barrett 2011a: see Figure 4), mitigating the costs of wasted mating  
588 opportunities by early blooming flowers. Further, plants flower for 6 weeks or more, and the  
589 average degree of dichogamy in our experiment was 4.2 days (range= -9 to 17 days; 9% of plants  
590 were protogynous), so plants express both sex functions for the majority of their flowering.

591

592 The duration of overlap between male and female function, or their temporal separation, may be  
593 an adaptive response to environmental stochasticity. Under conditions where resource  
594 availability is unpredictable and resource acquisition varies during the flowering season, the best  
595 allocation strategy should be in favor of the sex function with the higher return on investment  
596 (Zhang 2006). Indeed, *Ambrosia artemisiifolia* experiences substantial plasticity in sex allocation  
597 and in the degree and order of dichogamy (Paquin and Aarssen 2004; Friedman and Barrett  
598 2011a). Here we have demonstrated that the male gain curve is linear or nearly so, and if we  
599 assume that the female gain curve is mostly linear (Nakahara et al. 2018), then we would expect  
600 a sharp transition between sex functions (Zhang et al. 2006). The gradual transition that we  
601 observe may be explained because male flowers are photosynthetic and contribute to available  
602 resources (Bazzaz and Carlson 1979), and when reproductive resources are not a pool, but an  
603 income, then the constraints on staminate flowering are altered (Burd and Head 1992).  
604 Nonetheless, although the male gain curve is linear, there are likely diminishing marginal returns  
605 of male investment through time due to the saturation of ovules.

## 606 *Conclusion*

607 Experimental tests of the shape of male gain curves and factors that affect it are scarce compared  
608 to the amount of existing theoretical work. Our study is one of the few studies that empirically  
609 test theoretical expectations of male gain curve in wind pollinated plants. In agreement with  
610 predictions, we found a linear increase in fitness returns for increasing investment in male  
611 function. Fitness through male function is likely limited by the availability of mates, and  
612 increasing male investment results in proportional increases in the number of mating partners. In  
613 wind-pollinated *A. artemisiifolia* there is likely strong selection on producing more pollen, with  
614 the direct benefit increasing siring success and indirectly leading to more mating partners. While

615 early onset male flowering (particularly for lower male allocation plants) benefits male  
616 reproductive success, later onset results in greater probability of mating with diverse partners  
617 through both male and female function. Together this suggest an adaptive role for protandry to  
618 adjust the pool of competing pollen for available ovules, and a balance between fecundity  
619 selection and sexual selection through male function. Plants with lower male allocation might  
620 experience particular benefits by flowering earlier and avoiding the competitive arena during full  
621 population blooming, although a more explicit test of this prediction is necessary to rule out  
622 alternative explanations.

623 **Author Contributions:** JF conceived the study, AA and JF designed the study, AA conducted  
624 the field work and genetic assays, AA and JF analyzed the data and JF drafted the manuscript  
625 with input from AA.

626

627 **Acknowledgements:** The authors acknowledge funding from NSF EAGER 1546106 to JF, a  
628 R.C. Lewontin Early Award from the Society for the Study of Evolution to AA, and support  
629 from Syracuse University and Queen's University. Thanks to Matthew Rubin and Karine Leydet  
630 for assistance with field and laboratory work. The ideas and analyses in this manuscript were  
631 greatly improved by helpful discussions with Spencer Barrett, David Timerman, Scott Pitnick,  
632 and Crispin Jordan.

## 633 References

- 634 Ackerly, D. D., & Jasieński, M. (1990). Size-dependent variation of gender in high density  
635 stands of the monoecious annual, *Ambrosia artemisiifolia* (Asteraceae). *Oecologia*, 82(4),  
636 474–477.  
637
- 638 Allison, T. (1990). Pollen production and plant density affect pollination and seed production in  
639 *Taxus canadensis*. *Ecology*, 516–522.  
640
- 641 Antonovics, J., & Ellstrand, N. C. (1984). Experimental studies of the evolutionary significance  
642 of sexual reproduction. I. A test of the frequency-dependent selection hypothesis.  
643 *Evolution*, 38(1), 103–115.  
644
- 645 Arnold, S. J., & Wade, M. J. (1984). On the measurement of natural and sexual selection: theory.  
646 *Evolution*, 38(4), 709–719.  
647
- 648 Austen, E. J., & Weis, A. E. (2016a). The causes of selection on flowering time through male  
649 fitness in a hermaphroditic annual plant. *Evolution*, 70(1), 111–125.  
650
- 651 Austen, E. J., & Weis, A. E. (2016b). Estimating selection through male fitness: three  
652 complementary methods illuminate the nature and causes of selection on flowering time.  
653 *Proceedings of the Royal Society B: Biological Sciences*, 283(1825), 20152635.  
654
- 655 Barrett, S. C. H. (2003). Mating strategies in flowering plants: the outcrossing-selfing paradigm  
656 and beyond. *Philosophical Transactions of the Royal Society of London. Series B,*  
657 *Biological Sciences*, 358(1434), 991–1004.  
658
- 659 Barrett, S. C. H., & Harder, L. D. (2017). The ecology of mating and its evolutionary  
660 consequences in seed plants. *Annual Review of Ecology, Evolution, and Systematics*, 48,  
661 135–157.  
662
- 663 Bassett, I. J., & Crompton, C. W. (1975). The biology of Canadian weeds. ii. *Ambrosia*  
664 *artemisiifolia* L. and *A. psilostachya* DC. *Canadian Journal of Plant Science. Revue*  
665 *Canadienne de Phytotechnie*, 55(2), 463–476.  
666
- 667 Bateman, A. (1947). Contamination in seed crops III. Relation with isolation distance. *Heredity*,  
668 1(3), 303–336.  
669
- 670 Bawa, K. S. (2016). Kin selection and the evolution of plant reproductive traits. *Proceedings of*  
671 *the Royal Society B: Biological Sciences*, 283(1842), 20160789.  
672
- 673 Bazzaz, F. A. (1974). Ecophysiology of *Ambrosia artemisiifolia*: A successional dominant.  
674 *Ecology*, 55(1), 112–119.  
675
- 676 Bazzaz, F. A., & Carlson, R. W. (1979). Photosynthetic contribution of flowers and seeds to  
677 reproductive effort of an annual colonizer. *The New Phytologist*, 82(1), 223–232.

- 678  
679 Bertin, R. I. (1993). Incidence of monoecy and dichogamy in relation to self-fertilization in  
680 angiosperms. *American Journal of Botany*, 80(5), 557–560.  
681
- 682 Bertin, R. I., & Newman, C. M. (1993). Dichogamy in angiosperms. *The Botanical Review*,  
683 59(2), 112–152.  
684
- 685 Brookes, R. H., & Jesson, L. K. (2010). Do pollen and ovule number match the mating  
686 environment? An examination of temporal change in a population of *Stylidium armeria*.  
687 *International Journal of Plant Sciences*, 171(8), 818–827.  
688
- 689 Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A.,  
690 Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB Balances Speed and  
691 Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. In *The*  
692 *R Journal* (Vol. 9, Issue 2, pp. 378–400). [https://journal.r-project.org/archive/2017/RJ-](https://journal.r-project.org/archive/2017/RJ-2017-066/index.html)  
693 [2017-066/index.html](https://journal.r-project.org/archive/2017/RJ-2017-066/index.html)  
694
- 695 Brunet, J. (1992). Sex allocation in hermaphroditic plants. *Trends in Ecology & Evolution*, 7(3),  
696 79–84.  
697
- 698 Brunet, J. (1996). Male reproductive success and variation in fruit and seed set in *Aquilegia*  
699 *caerulea* (Ranunculaceae). *Ecology*, 77(8), 2458–2471.  
700
- 701 Brunet, J., & Charlesworth, D. (1995). Floral sex allocation in sequentially blooming plants.  
702 *Evolution*, 49(1), 70–79.  
703
- 704 Burd, M., & Allen, T. F. H. (1988). Sexual allocation strategy in wind-pollinated plants.  
705 *Evolution*, 42(2), 403–407.  
706
- 707 Burd, M., & Head, G. (1992). Phenological aspects of male and female function in  
708 hermaphroditic plants. *American Naturalist*, 140(2), 305–324.  
709
- 710 Charlesworth, D. (1991). Allocation of resources to sex functions in flowering plants.  
711 *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*,  
712 332(1262), 91–102.  
713
- 714 Charlesworth, D. (1993). Why are unisexual flowers associated with wind pollination and  
715 unspecialized pollinators? *American Naturalist*, 141(3), 481–490.  
716
- 717 Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews*  
718 *Genetics*, 10(11), 783–796.  
719
- 720 Charnov, E. L. (1979). Simultaneous hermaphroditism and sexual selection. *Proceedings of the*  
721 *National Academy of Sciences of the United States of America*, 76(5), 2480–2484.  
722



- 723 Charnov, E. L. (1982). *The theory of sex allocation* (Vol. 18, pp. 1–355). Princeton University  
724 Press.
- 725
- 726 Chen, J., Niu, Y., Li, Z., Yang, Y., & Sun, H. (2017). Sex allocation in gynodioecious  
727 *Cyananthus delavayi* differs between gender morphs and soil quality. *Plant Reproduction*,  
728 30(2), 107–117.
- 729
- 730 Cheplick, G. P. (1992). Sibling competition in plants. *The Journal of Ecology*, 80(3), 567–575.  
731
- 732 Christopher, D. A., Mitchell, R. J., Trapnell, D. W., Smallwood, P. A., Semski, W. R., & Karron,  
733 J. D. (2019). Hermaphroditism promotes mate diversity in flowering plants. *American*  
734 *Journal of Botany*, 106(8), 1131–1136.
- 735
- 736 Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in  
737 flowering plants. *Annals of Botany*, 125(1), 1–9.
- 738
- 739 Chun, Y. J., Fumanal, B., Laitung, B., Caullet, C., & Bretagnolle, F. (2009). Eight microsatellite  
740 markers isolated from common ragweed (*Ambrosia artemisiifolia* L.) and cross-  
741 amplification with herbarium specimens. *Molecular Ecology Resources*, 9(5), 1375–1379.  
742
- 743 Chybicki, I. J. (2018). NM $\pi$ -improved re-implementation of NM+, a software for estimating  
744 gene dispersal and mating patterns. *Molecular Ecology Resources*, 18(1), 159–168.  
745
- 746 Darwin, C. (1876). *The effects of cross and self fertilization in the vegetable kingdom*. John  
747 Murray.
- 748
- 749 Delph, L. F., & Ashman, T.-L. (2006). Trait selection in flowering plants: how does sexual  
750 selection contribute? *Integrative and Comparative Biology*, 46(4), 465–472.  
751
- 752 Emms, S. K. (1993). On measuring fitness gain curves in plants. *Ecology*, 74(6), 1750–1756.  
753
- 754 Friedman, J., & Barrett, S. C. H. (2008). High outcrossing in the annual colonizing species  
755 *Ambrosia artemisiifolia* (Asteraceae). *Annals of Botany*, 101(9), 1303–1309.  
756
- 757 Friedman, J., & Barrett, S. C. H. (2011a). The evolution of ovule number and flower size in  
758 wind-pollinated plants. *American Naturalist*, 177(2), 246–257.  
759
- 760 Friedman, J., & Barrett, S. C. H. (2011b). Genetic and environmental control of temporal and  
761 size-dependent sex allocation in a wind-pollinated plant. *Evolution*, 65(7), 2061–2074.  
762
- 763 Friedman, J., & Harder, L. D. (2005). Functional associations of floret and inflorescence traits  
764 among grass species. *American Journal of Botany*, 92(11), 1862–1870.  
765
- 766 Fumanal, B., Chauvel, B., & Bretagnolle, F. (2007). Estimation of the pollen and seed  
767 production of common ragweed in Europe. *Annals of Agricultural and Environmental*  
768 *Medicine: AAEM*, 14(2), 233–236.

- 769  
770 Genton, B. J., Jonot, O., Thevenet, D., Fournier, E., Blatrix, R., Vautrin, D., Solignac, M., &  
771 Giraud, T. (2005). Isolation of five polymorphic microsatellite loci in the invasive weed  
772 *Ambrosia artemisiifolia* (Asteraceae) using an enrichment protocol. *Molecular Ecology*  
773 *Notes*, 5(2), 381–383.  
774  
775 Gillespie, J. H. (1974). Natural selection for within-generation variance in offspring number.  
776 *Genetics*, 76(3), 601–606.  
777  
778 Gillespie, J. H. (1977). Natural selection for variances in offspring numbers: a new evolutionary  
779 principle. *American Naturalist*, 111(981), 1010–1014.  
780  
781 Gleaves, J. T. (1973). Gene flow mediated by wind-borne pollen. *Heredity*, 31(3), 355–366.  
782  
783 Haig, D., & Westoby, M. (1988). On limits to seed production. *American Naturalist*, 131(5),  
784 757–759.  
785  
786 Harder, L. D. (1990). Behavioral responses by bumble bees to variation in pollen availability.  
787 *Oecologia*, 85(1), 41–47.  
788  
789 Harder, L. D., & Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite  
790 plants. *Nature*, 373(6514), 512–515.  
791  
792 Harder, L. D., & Thomson, J. D. (1989). Evolutionary options for maximizing pollen dispersal of  
793 animal-pollinated plants. *The American Naturalist*, 133(3), 323–344.  
794  
795 Hartig, F. (2021). *DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed)*  
796 *Regression Models. R package version 0.4.4.* <http://florianhartig.github.io/DHARMA/>  
797  
798 Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic  
799 benefits. *Biological Reviews*, 75(1), 21–64.  
800  
801 Johnson, S. L., & Yund, P. O. (2009). Effects of fertilization distance on male gain curves in a  
802 free-spawning marine invertebrate: a combined empirical and theoretical approach.  
803 *Evolution*, 63(12), 3114–3123.  
804  
805 Karron, J. D., & Marshall, D. L. (1990). Fitness consequences of multiple paternity in wild  
806 radish, *Raphanus sativus*. *Evolution*, 44(2), 260–268.  
807  
808 Klinkhamer, P. G., de Jong, T. J., & Metz, H. (1997). Sex and size in cosexual plants. *Trends in*  
809 *Ecology & Evolution*, 12(7), 260–265.  
810  
811 Korpelainen, H. (1998). Labile sex expression in plants. *Biological Reviews*, 73(2), 157–180.  
812  
813 Kress, W. J. (1981). Sibling competition and evolution of pollen unit, ovule number, and pollen  
814 vector in angiosperms. *Systematic Botany*, 6(2), 101–112.

- 815  
816 Lenth, R. V. (2021). *emmeans: Estimated Marginal Means, aka Least-Squares Means*.  
817 <https://CRAN.R-project.org/package=emmeans>  
818
- 819 Levin, D. A., & Kerster, H. W. (1974). Gene Flow in Seed Plants. In T. Dobzhansky, M. K.  
820 Hecht, & W. C. Steere (Eds.), *Evolutionary Biology* (pp. 139–220). Springer.  
821
- 822 Lloyd, D. (1980). Sexual strategies in plants I. An hypothesis of serial adjustment of maternal  
823 investment during one reproductive session. *The New Phytologist*, *86*(1), 69–79.  
824
- 825 Lloyd, D. G. (1984). Modification of the gender of seed plants in varying conditions.  
826 *Evolutionary Biology*, *17*, 255–338.  
827
- 828 Lloyd, D., & Webb, C. J. (1986). The avoidance of interference between the presentation of  
829 pollen and stigmas in angiosperms I. Dichogamy. *New Zealand Journal of Botany*, *24*(1),  
830 135–162.  
831
- 832 Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant  
833 populations. *Annual Review of Ecology and Systematics*, *15*, 65–95.  
834
- 835 Lüdtke, D. (2018). Ggeffects: Tidy data frames of marginal effects from regression models.  
836 *Journal of Open Source Software*, *3*(26), 772.  
837
- 838 Maynard Smith, J. (1976). A short-term advantage for sex and recombination through sib-  
839 competition. *Journal of Theoretical Biology*, *63*(2), 245–258.  
840
- 841 McCartney, M. A. (1997). Sex allocation and male fitness gain in a colonial, hermaphroditic  
842 marine invertebrate. *Evolution*, *51*(1), 127–140.  
843
- 844 McKone, M., & Tonkyn, D. W. (1986). Intrapopulation gender variation in common ragweed  
845 (*Asteraceae*, *Ambrosia artemisiifolia* L.), a monoecious, annual herb. *Oecologia*, *70*(1),  
846 63–67.  
847
- 848 Medan, D., & Bartoloni, N. (1998). Fecundity effects of dichogamy in an asynchronously  
849 flowering population: a genetic model. *Annals of Botany*, *81*(3), 373–383.  
850
- 851 Nakahara, T., Fukano, Y., Hirota, S. K., & Yahara, T. (2018). Size advantage for male function  
852 and size-dependent sex allocation in *Ambrosia artemisiifolia*, a wind-pollinated plant.  
853 *Ecology and Evolution*, *8*(2), 1159–1170.  
854
- 855 Nakamura, R. R., Stanton, M. L., & Mazer, S. J. (1989). Effects of mate size and mate number  
856 on male reproductive success in plants. *Ecology*, *70*(1), 71–76.  
857
- 858 Niklas, K. (1985). The aerodynamics of wind pollination. *The Botanical Review*, *51*(3), 328–386.  
859

- 860 Pannell, J. R., & Labouche, A. M. (2013). The incidence and selection of multiple mating in  
861 plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological*  
862 *Sciences*, 368(1613), 20120051–20120051.
- 863
- 864 Paquin, V., & Aarssen, L. W. (2004). Allometric gender allocation in *Ambrosia artemisiifolia*  
865 (Asteraceae) has adaptive plasticity. *American Journal of Botany*, 91(3), 430–438.
- 866
- 867 Paw U, K., & Hotton, C. (1989). Optimum pollen and female receptor size for anemophily.  
868 *American Journal of Botany*, 76(3), 445–453.
- 869
- 870 Payne, W. W. (1963). The morphology of the inflorescence of ragweeds (*Ambrosia-Franseria*:  
871 *Compositae*). *American Journal of Botany*, 50(9), 872–880.
- 872
- 873 R Core Team. (2021). R: A language and environment for statistical computing. In *Vienna,*  
874 *Austria*. R Foundation for Statistical Computing.
- 875
- 876 Ritland, K. (2002). Extensions of models for the estimation of mating systems using n  
877 independent loci. *Heredity*, 88(4), 221–228.
- 878
- 879 Rognli, O. A., Nilsson, N. O., & Nurminiemi, M. (2000). Effects of distance and pollen  
880 competition on gene flow in the wind-pollinated grass *Festuca pratensis* Huds. *Heredity*,  
881 85(6), 550–560.
- 882
- 883 Sakai, A., & Sakai, S. (2003). Size-dependent ESS sex allocation in wind-pollinated cosexual  
884 plants: fecundity vs. stature effects. *Journal of Theoretical Biology*, 222(3), 283–295.
- 885
- 886 Santos Del Blanco, L., Tudor, E., & Pannell, J. R. (2019). Low siring success of females with an  
887 acquired male function illustrates the legacy of sexual dimorphism in constraining the  
888 breakdown of dioecy. *Ecology Letters*, 22(3), 486–497.
- 889
- 890 Sargent, R. D., Mandegar, M. A., & Otto, S. P. (2006). A model of the evolution of dichogamy  
891 incorporating sex-ratio selection, anther-stigma interference, and inbreeding depression.  
892 *Evolution*, 60(5), 934–944.
- 893
- 894 Sargent, R. D., & Otto, S. P. (2004). A phylogenetic analysis of pollination mode and the  
895 evolution of dichogamy in angiosperms. *Evolutionary Ecology Research*, 6(8), 1183–1199.
- 896
- 897 Sargent, R. D., & Roitberg, B. D. (2000). Seasonal decline in male-phase duration in a  
898 protandrous plant: a response to increased mating opportunities? *Functional Ecology*,  
899 14(4), 484–489.
- 900
- 901 Schärer, L. (2009). Tests of sex allocation theory in simultaneously hermaphroditic animals.  
902 *Evolution*, 63(6), 1377–1405.
- 903
- 904 Schoen, D. J., & Stewart, S. C. (1986). Variation in male reproductive investment and male  
905 reproductive success in white spruce. *Evolution*, 40(6), 1109–1120.

- 906  
907 Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical  
908 evidence for bet hedging. *Proceedings of the Royal Society of London, Series B: Biological*  
909 *Sciences*, 278(1712), 1601–1609.
- 910  
911 Slatkin, M. (1974). Hedging one’s evolutionary bets. *Nature*, 250(5469), 704–705.
- 912  
913 Steven, J. C., & Waller, D. M. (2007). Isolation affects reproductive success in low-density but  
914 not high-density populations of two wind-pollinated *Thalictrum* species. *Plant Ecology*,  
915 190(1), 131–141.
- 916  
917 Timerman, D., & Barrett, S. C. H. (2018). Divergent selection on the biomechanical properties of  
918 stamens under wind and insect pollination. *Proceedings of the Royal Society of London,*  
919 *Series B: Biological Sciences*, 285(1893), 20182251.
- 920  
921 Timerman, D., & Barrett, S. C. H. (2021). The biomechanics of pollen release: new perspectives  
922 on the evolution of wind pollination in angiosperms. *Biological Reviews*, 96(5), 2146–  
923 2163.
- 924  
925 Tomaszewski, C. E., Kulbaba, M. W., & Harder, L. D. (2018). Mating consequences of  
926 contrasting hermaphroditic plant sexual systems. *Evolution; International Journal of*  
927 *Organic Evolution*, 72(10), 2114–2128.
- 928  
929 Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019). Sex-specific selection on plant  
930 architecture through “budget” and “direct” effects in experimental populations of the wind-  
931 pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897–912.
- 932  
933 Tonnabel, J., David, P., & Pannell, J. R. (2019). Do metrics of sexual selection conform to  
934 Bateman’s principles in a wind-pollinated plant? *Proceedings of the Royal Society of*  
935 *London, Series B: Biological Sciences*, 286(1905), 20190532.
- 936  
937 Traveset, A. (1992). Sex expression in a natural population of the monoecious annual, *Ambrosia*  
938 *artemisiifolia* (Asteraceae). *American Midland Naturalist*, 127(2), 309–315.
- 939  
940 Uyenoyama, M. K. (1986). Inbreeding and the cost of meiosis: the evolution of selfing in  
941 populations practicing biparental inbreeding. *Evolution*, 40(2), 388–404.
- 942  
943 Weis, A. E., & Kossler, T. M. (2004). Genetic variation in flowering time induces phenological  
944 assortative mating: quantitative genetic methods applied to *Brassica rapa*. *American*  
945 *Journal of Botany*, 91(6), 825–836.
- 946  
947 Williams, G. C. (1975). *Sex and Evolution*. Princeton University Press.
- 948  
949 Yasui, Y. (1998). The ‘genetic benefits’ of female multiple mating reconsidered. *Trends in*  
950 *Ecology & Evolution*, 13(6), 246–250.
- 951

- 952 Young, K. A., & Schmitt, J. (1995). Genetic variation and phenotypic plasticity of pollen release  
953 and capture height in *Plantago lanceolata*. *Functional Ecology*, 9(5), 725–733.  
954
- 955 Yund, P. O. (1998). The effect of sperm competition on male gain curves in a colonial marine  
956 invertebrate. *Ecology*, 79(1), 328–339.  
957
- 958 Yund, P. O., & McCartney, M. A. (1994). Male reproductive success in sessile invertebrates:  
959 Competition for fertilizations. *Ecology*, 75(8), 2151.  
960
- 961
- 962 Zeh, J. A., & Zeh, D. W. (1996). The evolution of polyandry I: Intragenomic conflict and genetic  
963 incompatibility. *Proceedings of the Royal Society of London, Series B: Biological  
964 Sciences*, 263(1377), 1711–1717.  
965
- 966 Zhang, D.-Y. (2006). Evolutionarily stable reproductive investment and sex allocation in plants.  
967 In L. D. Harder & S. C. H. Barrett (Eds.), *Ecology and Evolution of Flowers* (pp. 41–60).  
968 Oxford University Press.  
969

**Table 1.** Models of effects on male reproductive success in *Ambrosia artemisiifolia*. Results for A) model with factorial measure of male allocation (manipulation category), and B) model with continuous measure of male allocation (male inflorescence weight), using negative binomial GLMMs. Random effects (not shown) included the effects of source population and block. Male flowering time is abbreviated MFT, and Male inflorescence weight is abbreviated MIW. Bold *P*-values indicate significant effects.

	<b>Term Condition</b>	<b>df</b>	<b><math>\chi^2</math></b>	<b><i>P</i>-value</b>
A)	Allocation category	3	9.03	<b>&lt;0.05</b>
	Male flowering time	1	9.34	<b>&lt;0.001</b>
	Plant width	1	4.44	<b>&lt;0.05</b>
	Edge	1	5.52	<b>&lt;0.05</b>
	Allocation x MFT	3	9.46	<b>&lt;0.05</b>
B)	Male inflorescence weight	1	6.67	<b>&lt;0.01</b>
	Male flowering time	1	13.04	<b>&lt;0.001</b>
	Plant width	1	5.99	<b>&lt;0.05</b>
	Edge	1	5.62	<b>&lt;0.05</b>
	MIW x MFT	1	7.23	<b>&lt;0.01</b>

**Table 2.** Models of effects on proportional mate diversity in *Ambrosia artemisiifolia*. Results for (A) male mate diversity model with factorial measure of male allocation, (B) male mate diversity model with continuous measure of male allocation (male inflorescence weight), and (C) female mate diversity. Models in A) and B) used a binomial distribution weighted by the number of seeds sired per individual, model C) used a poisson distribution and an offset parameter of the number of seeds genotyped per individual. Random effects (not shown) in all models included the effects of source population and block. Bold *P*-values indicate significant effects.

	<b>Term Condition</b>	<b>df</b>	<b><math>\chi^2</math></b>	<b><i>P</i>-value</b>
A)	Allocation category	3	0.78	0.85
	Male flowering time	1	9.37	<b>&lt;0.01</b>
	Edge	1	0.03	0.86
B)	Male inflorescence weight	1	0.01	0.91
	Male flowering time	1	8.96	<b>&lt;0.01</b>
	Edge	1	0.09	0.76
C)	Allocation category	1	1.08	0.78
	Female flowering time	1	8.93	<b>&lt;0.01</b>
	Germination probability	1	1.59	0.21



**Figure 1.** (A) The frequency distributions of interplant and intermate distances of *Ambrosia artemisiifolia* plants in experimental arrays. The solid line represents the probability density distribution of distance between mates in our progeny assays, while the dotted and dashed lines represent the probability density distributions of the distance between all plants in the experiment (dotted line) and distances between plants within a block (dashed line). (B) Modelled Weibull distribution of the dispersal kernel for a random subset of 10 experimental plants. (C) Distribution of 192 plants in experimental arrays (black dots) with the curves showing the trajectory of pollen to reach the known mates for the same random subset of 10 experimental plants. Note that the physical distance between the three blocks has been compressed for ease of figure presentation.

**Figure 2.** Influences on the number of seeds sired (male reproductive success) in *Ambrosia artemisiifolia* plants, including (A) treatment effects of four experimentally manipulated categories of male allocation, (B) male inflorescence weight, and (C) interaction between male allocation category and the julian day of the start of male flowering. Panel B shows the relation described by the power function regression,  $y=ax^b$ , with  $b=0.85$ . All results represent model-adjusted and back-transformed values from ln estimates. See Table 1 for statistical details.

**Figure 3.** Male-specific Bateman gradient in *Ambrosia artemisiifolia* in experimental array conditions. The equation of best fit line is:  $y=0.71x + 0.12x^2 - 0.1$ , see text for statistical details.

**Figure 4.** Effect of the Julian day of the start of (A) male flowering time and (B) female flowering time on male and female mate diversity (number of unique mating partners through female function), respectively, in *Ambrosia artemisiifolia* plants. Results represent model-adjusted and back-transformed values from ln estimates. See Table 2 for statistical details.

Figure 1.

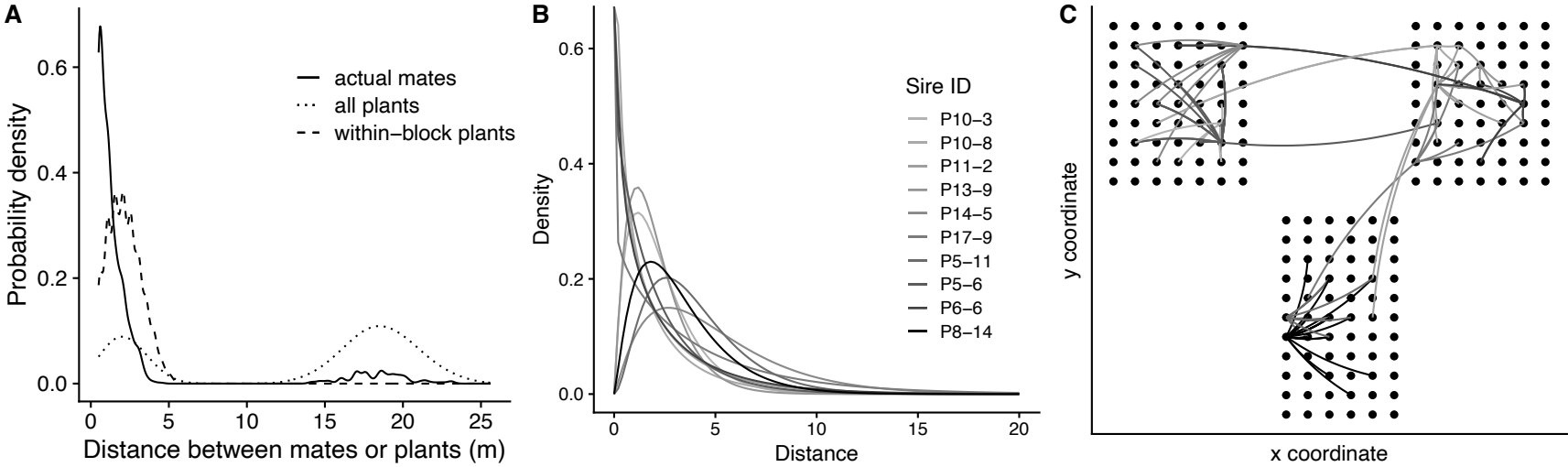


Figure 2.

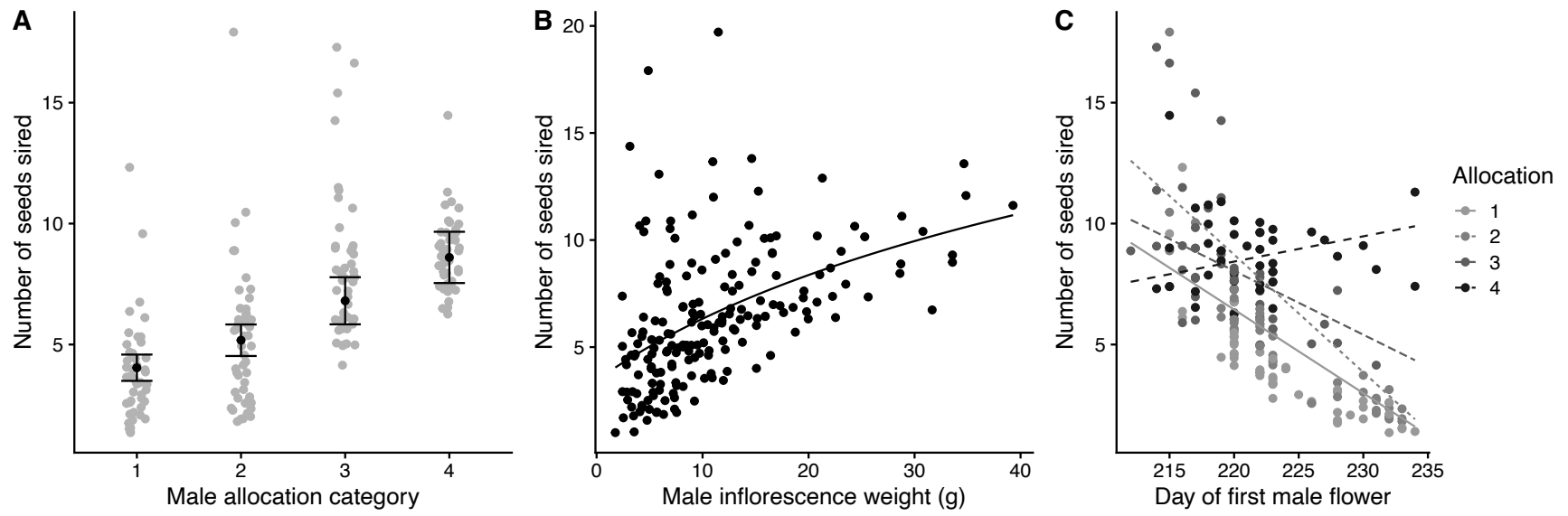


Figure 3.

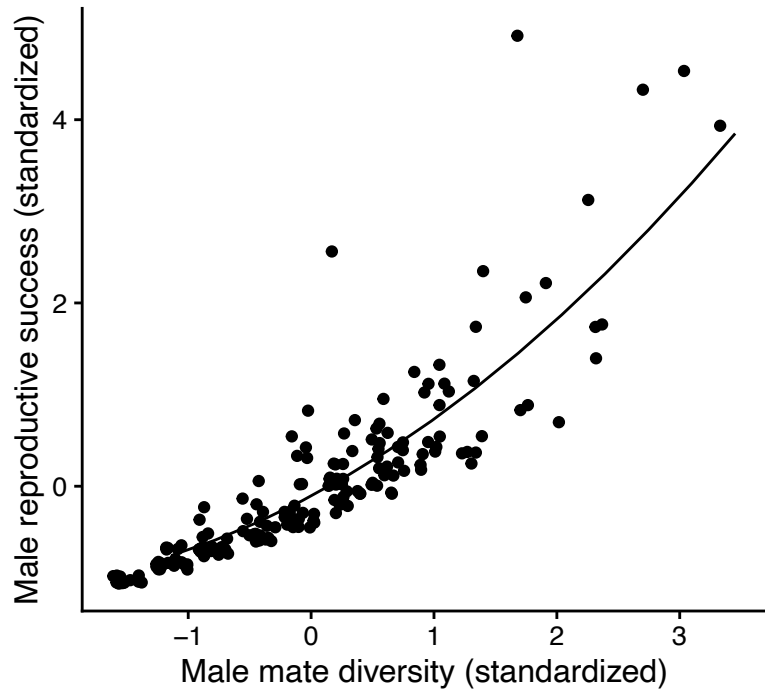
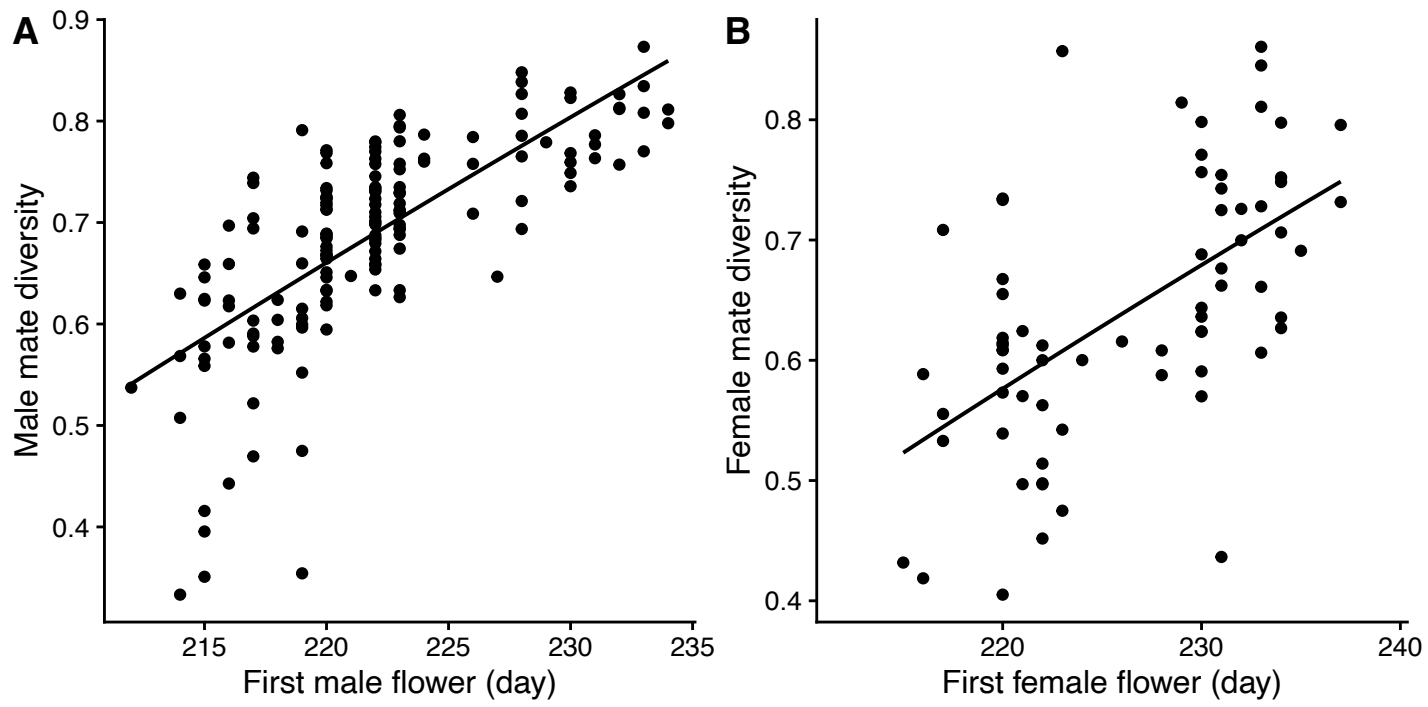


Figure 4.



## Supplementary Materials

**Figure S1.** Effects of the categorical experimental treatment of male flower manipulation on mean ( $\pm$ S.E.) male inflorescence weight in experimental *Ambrosia artemisiifolia* plants. Raw data are plotted, and means associated with different lowercase letters differed significantly.

**Figure S2.** Effects of the categorical experimental treatment of male flower manipulation on mean ( $\pm$ S.E.) A) plant height, and B) plant width, in experimental *Ambrosia artemisiifolia* plants. Raw data are plotted, and means associated with different lowercase letters differed significantly.

**Figure S3.** Influence of plant width (cm) on the number of seeds sired (male reproductive success) in *Ambrosia artemisiifolia* plants. Results represent model-adjusted and back-transformed values from ln estimates. See Table 1 for statistical details.

Figure S1

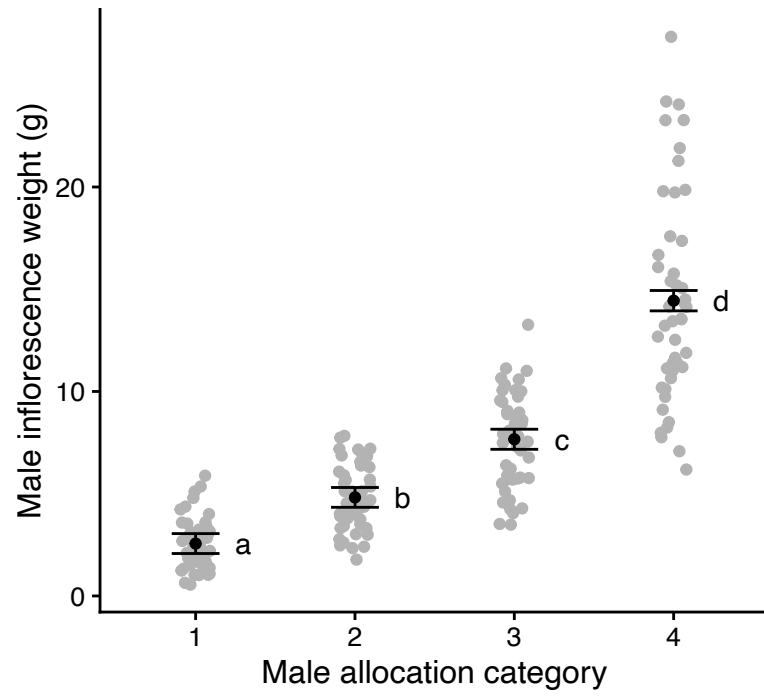


Figure S2

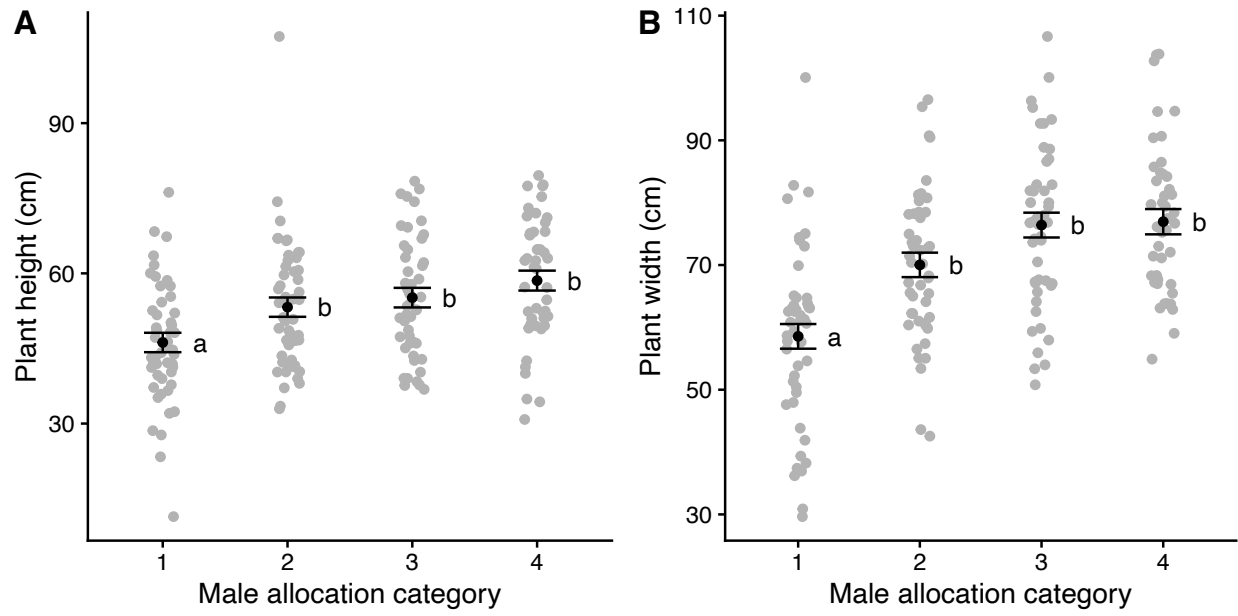
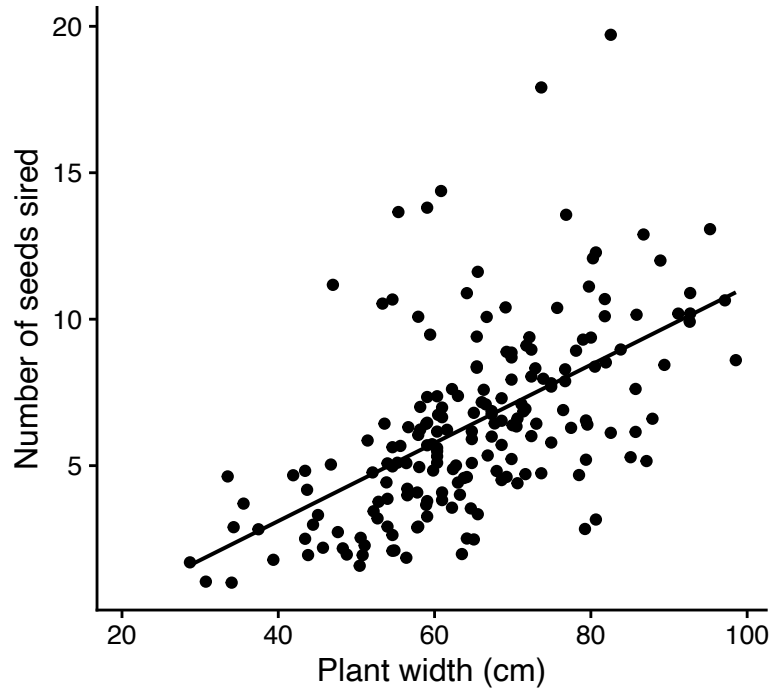




Figure S3



**Table S1.** Characteristics of the seven microsatellite loci used for paternity analysis. GenBank Accession, number of alleles, expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), and PCR conditions for amplification are provided. All PCR reactions included a final volume of 10 $\mu$ l containing approximately 10-20ng of genomic DNA, 2 $\mu$ l of 5X Taq buffer, 0.5 $\mu$ l of 25mM MgCl<sub>2</sub>, 1 $\mu$ l of 0.25mM of each dNTP, 0.1 $\mu$ l of 10 $\mu$ M of each forward and reverse primers and 0.5U of Taq Polymerase.

<b>Locus</b>	<b>GenBank Accession</b>	<b>Number of alleles</b>	<b><math>H_E</math></b>	<b><math>H_O</math></b>	<b>PCR Profile</b>
Amb15	AY849309	16	0.90	0.64	95°C for 5m; 40 cycles of: 95°C for 30s, 50°C for 30s; 65 °C for 4m; 72°C for 10m
Amb82	AY849312	28	0.92	0.73	
Ambart09	FJ595151	12	0.88	0.67	95°C for 5m; 40 cycles of: 95°C for 30s, 52°C for 30s; 65 °C for 4m; 72°C for 10m
Ambart18	FJ595153	28	0.91	0.51	
Ambart21	FJ595154	21	0.90	0.62	
Ambart27	FJ595156	32	0.91	0.87	
Amb30	AY849311	16	0.84	0.67	95°C for 5m; 40 cycles of: 95°C for 30s, 51°C for 30s; 72 °C for 30s; 72°C for 10m