# Lifetime Fitness through Female and Male Function: Influences of Genetically Effective Population Size and Density

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ABSTRACT: An individual's lifetime fitness and patterns of mating between individuals are interdependent features of sexual organisms. Mating systems (outcrossing vs. selfing or mating between close relatives) can affect the distribution of offspring fitness, which generally declines with inbreeding, which in turn is related to a population's genetically effective size (Ne). Fitness and mating patterns are also expected to vary with proximity of mates (i.e., population density). Consequently, density and Ne may influence demographic and genetic changes over generations and interact in their effects. Here, we report an experiment designed to assess the influence of these two populationlevel properties on mating system and lifetime fitness. In experimental arrays under quasi-natural conditions, we varied the density and Ne of the hermaphroditic annual legume Chamaecrista fasciculata. We recorded components of fitness for each individual and employed microsatellite markers to estimate outcrossing and assign paternity. We used aster analyses to estimate lifetime fitness for genetic families using female (seeds set) and male (seeds sired) reproduction as fitness measures. With estimates from these analyses, we assessed the evidence for a trade-off between fitness attained through female versus male function, but we found none. Lifetime fitness increased with density, especially under high Ne. Outcrossing rates increased with density under high Ne but declined modestly with density under low Ne. Our results show that density and Ne have strong direct effects on fitness and mating systems, with negative fitness effects of low  $N_e$  limiting the positive effects of increasing density. These findings highlight the importance of the interactive effects of density and  $N_{\rm e}$  on lifetime fitness.

*Keywords: Chamaecrista fasciculata*, mating system, population density, siring success, soft selection.

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### Introduction

An individual's lifetime fitness can be operationally defined as its contribution of viable offspring to its population. Accordingly, the average of individual absolute fitness relates directly to the population growth rate, a key ecological property. When variation among individuals in reproductive output reflects genetic differences, disproportionate genotypic contributions across generations result in changes in a population's genetic composition. An individual's fitness depends on the environment in which it develops, as do a population's mean and genetic variance of fitness. These two metrics represent the close interplay between ecological and evolutionary change in populations and, implicitly, their dependence on environmental context. In particular, genetic variation in fitness may depend on population-level properties, such as density and genetic composition; the consequent selection is termed "soft selection" (Wallace 1975; Reznick 2016). Such concepts are well established (reviewed in Antonovics 1992), but empirical assessment of how selection varies with these populationlevel properties under natural conditions is required to clarify the interconnections between ecological and evolutionary dynamics in the wild.

Populations in nature vary widely in both density and genetically effective size  $(N_e)$ , factors that affect both their ongoing evolution and growth. Random genetic sampling (i.e., genetic drift; Crow and Kimura 1970), which is intimately connected to inbreeding (Falconer and Mackay 1996, ch. 5), has greater evolutionary influence the smaller the population is. The random changes in allele frequencies at any locus tend to reduce genetic variation, including loss of alleles. The degree to which a population is subject to genetic drift is encapsulated by its  $N_e$  (Wright 1931). Reduced  $N_e$  can impair population persistence, as Newman and Pilson (1997) found for *Clarkia pulchella*.

Antonovics and Levin (1980) surveyed influences of density on the ecology and genetics of populations. Increased

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density tends to decrease growth, survival, and seed production of individuals (Harper 1977; Shaw and Antonovics 1986; Shaw et al. 1995; Mustajärvi et al. 2001; Gunton and Kunin 2009; Putz et al. 2015; Campbell et al. 2017), often as a result of intensified competition, but these effects are not always found (e.g., Shaw 1987). A positive relationship between density and fitness, known as an Allee effect (Stephens et al. 1999; Elam et al. 2007; Wagenius et al. 2007), indicates that sparse populations are at greater risk of extirpation. The effects of increasing density may act in a compensatory role across a range of  $N_e$  (Waples 2006) and alleviate the effects of enhanced drift and inbreeding in populations with small  $N_{\rm e}$ . Given the known effects of density and  $N_{\rm e}$ on population dynamics and that these two factors can vary independently, it is likely that density and Ne have interactive effects on fitness, although empirical studies of them (e.g., Wade 1980) are few.

In diploid, sexual organisms, population dynamics are typically considered through the number of offspring that females produce, neglecting paternity. This simplification of fitness holds whenever seed production, along with resources to provision offspring through early development, limits population growth (Gotelli 1995; but see Campbell et al. 2017). Consideration of evolutionary change, however, requires accounting for fitness through paternity as well as through maternity, since both contribute equally to the transmission of nuclear genes. For hermaphroditic organisms, which can produce offspring through both female and male functions, lifetime fitness through maternity and lifetime fitness through paternity are especially closely associated (Charlesworth and Charlesworth 1987; Barrett 1998). Most flowering plants are hermaphroditic, accumulating fitness through female (pollen import and seeds set) and male (pollen export and seeds sired) functions. Patterns of selection and resource allocation that are specific to each sex function can arise (Bateman 1948; Morgan 1994; Bonel 2018) and influence the potential for adaptation (Olito et al. 2018) and the growth, stability, or decline of populations (Eckert et al. 2010). As sex-specific selection on traits may differ or even be in conflict (Campbell 1989; Aigner 2004; Delph and Ashman 2006; but see Sahli and Conner 2011), fitness through female and male function may contribute asymmetrically to overall fitness, and these two components may be inversely related (i.e., may trade off). However, because of the difficulty of determining paternity (Conner 2006), comparisons of fitness through female and male function are scarce (but see Conner 1996; Hodgins and Barrett 2008; Kulbaba and Worley 2013; Ison et al. 2014).

Self-compatible plants can produce seeds via selfing (autogamy) when pollen fertilizes ovules within the same flower or when pollen is transferred among flowers on the same plant (geitonogamy). This increases the potential for inbreeding compared with dioecious and self-incompatible organisms, which inbreed solely through mating with related individuals (biparental inbreeding). Inbreeding, whether through selfing or biparentally, tends to reduce the fitness of resulting offspring (inbreeding depression). The degree of outcrossing and selfing, which can be quantified with heritable molecular markers, has been extensively studied in flowering plants. Most self-compatible hermaphroditic flowering plant species engage in highly variable mixed mating (i.e., seed production through outcrossing and selfing; see Jarne and Charlesworth 1993; Goodwillie et al. 2005; Whitehead et al. 2018). Evidence for the underlying causes of mating system variation is mixed and strongly temporally variable.

Plant and floral density are likely to influence mating systems via effects on pollinator behavior. High floral density may attract and retain pollinators, promoting pollen transfer among close neighbors. Outcrossing rates have been found to increase with floral density in some cases (Karron et al. 1995; Herlihy and Eckert 2004; Brunet and Sweet 2006) but to decrease in others (Ellstrand et al. 1978; Watkins and Levin 1990; Harder and Barrett 1995), or they have no detectable relationship (Schoen 1982) with floral density. Greater spacing between populations has been predicted to promote long-distance pollen movement (Loveless and Hamrick 1984). For example, Schulke and Waser (2001) found that Delphinium nuttallii plants received substantial pollen loads even when they were isolated from large populations by 400 m. Dependence of pollinator behavior and pollen movement on plant density contributes to overall effects of density on plant fitness. Moreover, density dependence of pollen movement is likely to interact with  $N_{\rm e}$ in its effect on fitness because as  $N_{\rm e}$  declines, the greater probability of pollen transfer between closely spaced plants is more likely to result in biparental inbreeding.

We present a field experiment to investigate how genetic effects on individual fitness and, hence, selection depend on the population properties of density and  $N_e$  in the hermaphroditic annual legume Chamaecrista fasciculata (Michx.) Greene, a species shown to suffer strong inbreeding depression (Fenster 1991a, 1991b). We augmented an experimental design that Newman and Pilson (1997) devised to investigate the effect of  $N_{\rm e}$  on the probability of population extinction. They established replicate populations, manipulating the number of founding individuals to vary N<sub>e</sub> (Crow and Kimura 1970, eq. [3.13.4]). In our experiment, we established experimental plots that differed in density (three levels) as well as in the number of genetic founders (two levels of  $N_e$ ). We addressed the following questions: To what extent does individual and genotypic fitness depend on population density and Ne? How closely does genotypic fitness through maternity match genotypic fitness through paternity, and how does this relationship vary with density? Specifically, is there evidence for genetic familybased trade-offs between fitness gained through maternity and paternity? Furthermore, in this largely outcrossing species, how does inbreeding (biparentally and through selfing) vary with density and  $N_e$  of populations?

#### Methods

## Study Species

To study the effects of density and  $N_e$  on lifetime fitness and mating system variation, we established experimental arrays of *Chamaecrista fasciculata*. This species is native to the experimental region and ranges from the sand prairies of Minnesota to Central Mexico (Irwin and Barneby 1982). *Chamaecrista fasciculata* is a self-compatible annual that produces somatically enantiostylous (alternating morphs of style orientation among flowers; Fenster 1995), buzzpollinated flowers (Lee and Bazzaz 1982). The primary pollinators of *C. fasciculata* are bumblebees (*Bombus* spp.). For this study, we focused on a population from the McCarthy Lake Wildlife Management Area (44°15′16N, 91°57′05W), where we collected seeds from 50 individuals >5 m apart in the fall of 2014.

#### Greenhouse Crosses and Planting Design

Our field experiment consisted of three replications of the factorial combinations of three plant densities (with distance

between neighbors 30, 10, or 5 cm) and two levels of  $N_e$  (high and low). To achieve distinct levels of  $N_e$ , we established plots with individuals from two full-sibling families (four genetic founders, low  $N_e$ ) versus six full-sibling families (12 founders, high  $N_e$ ). Thus, there were six treatments (fig. 1) replicated three times in 18 plots altogether.

We generated full-sibling families of seeds by performing reciprocal crosses by hand in the greenhouse on plants grown from the field-collected seeds. These seeds were sterilized in diluted (8%) bleach and thoroughly rinsed with distilled water. We manually scarified seeds with a sterilized razor blade, removing 1-3 mm of the seed coat opposite the embryo. Scarified seeds were placed in sterile distilled water to imbibe for 24 h. After imbibing water, seeds were planted in 5 × 5-cm peat trays with sterilized sand and SunGro potting mix to germinate. Once seedlings produced three or four true leaves, plants were transplanted into 2-gallon (7.57-L) pots with sterilized sand and Osmocote slow-release fertilizer (NPK: 14-14-14). Juvenile plants were inoculated with a slurry produced from soil obtained from the original population site to ensure equal resource opportunity for plants to be used to generate the full-sibling families for the field experiment.

For the crosses, we randomly paired individuals from distinct field-sampled individuals, each of which was represented in only one crossing pair. Reciprocal pollinations were made on all days that both plants displayed open flowers. We removed pollen from anthers using a vibrating



**Figure 1:** Experimental design to assess the effects of density and genetically effective population size ( $N_e$ ). Each circle represents an individual plant, and colors represent full-sibling families. Experimental treatments consist of three different densities where plants differed in physical proximity (low: 30 cm; medium: 10 cm; high: 5 cm) and two levels of  $N_e$  (low  $N_e$ : six plants from two full-sibling families). All experimental plots consisted of 12 plants in a 1 × 1-m plot and were replicated three times. Numbers in the top left indicate individual plant positions.

electric toothbrush to trigger pollen release and collected it in a small (50-mm) petri dish. During pollen extraction, we reflexed the stigma behind the corolla to avoid self-pollination. Pollen was transferred to the stigmas of open flowers other than those used to collect pollen on the assigned mate. Altogether, seeds of 21 independent full-sibling families were collected from mature pods and stored at 4°C. These progeny were used in the field experiment described below.

As noted above, the field planting consisted of 18 plots, three for each combination of the three plant densities and two levels of  $N_e$ . They were planted at the University of Minnesota Rosemount Research and Outreach Center (44°41′17.9″N, 93°04′26.4″W) on June 2–3, 2017. Plots were positioned within fenced areas, affording protection from deer browsing, and maintained free of other vegetation. They were separated by at least 82 m to reduce the likelihood of pollen transfer between plots. Nearby flowering plants included *Dalea candida*, *Dalea purpurea*, *Monarda fistulosa*, *Anemone cylindrica*, *Bouteloua curtipendula*, and *Schizachyrium scoparium*.

To maintain consistent numbers of individuals in all plots while manipulating density, we planted 12 seeds in each plot, using three different spacings between nearest neighbors (30, 10, and 5 cm) in a hexagonal design (Antonovics and Fowler 1985). We varied  $N_{\rm e}$  through the number of independent full-sibling families included in each experimental plot. Plots consisted of either six individuals from each of two full-sibling families (low  $N_e$ ) or two individuals from each of six full-sibling families (high  $N_e$ ). These treatments represent different  $N_{\rm e}$  because they differ in the number of genetically distinct founders (4 vs. 12 parents). Twelve seeds that had been sterilized, scarified, and imbibed in water were planted in each 1-m<sup>2</sup> plot. Because the number of seeds in each full-sibling family was limited, most families were not represented in all plots. Additional seeds of each family were germinated in the greenhouse to be available at the same growth stage if needed to replace seeds that did not germinate in situ.

## Lifetime Fitness Surveys

We recorded components of fitness for each individual plant in our experiment. To maintain 12 plants in each plot, we replaced instances of germination failure with family-specific seedlings of similar size. A total of 12 replacement seedlings (<6%) were planted in eight experimental plots within the first week of the experiment. We recorded fitness data from all 216 plants at four times across the duration of the experiment: June 18–19, July 11–12, August 8–9, and August 29–30, 2017. For each plant, we recorded seedling survival, survival to flowering, and the numbers of fruits produced. Because of the large range in fruit pro-

duction (1–533; median: 14) and the fruits' tendency to explosively dehisce, we were unable to collect all fruits from each plant. However, we could determine the total number of fruits produced by counting the number of persistent pedicels on stems. To characterize the number of seeds and aborted embryos each plant produced, counts of both were obtained on a subsample of fruits produced; this subsampling was accounted for in statistical analyses (see below). We completed the collection of fruits and seeds on September 27, 2017.

Thereafter, we measured total individual plant biomass and the number of rhizobial root nodules of all experimental plants. After fruit maturation and collection, each plant was carefully exhumed. Plants were separated into aboveand belowground components (separated at level of soil), dried, and weighed to 0.1 g.

## Mating System and Siring Success

To assess mating system variation and infer paternity, we examined variation at 13 polymorphic microsatellite loci using primers generated by L. Wallace and M. Nobarinezhad (unpublished data; table S1 [tables S1, S2 are available online]) from a *C. fasciculata* transcriptome (Singer et al. 2009).

From each plant growing in the arrays (N = 216), we extracted DNA from leaf tissue and from 10 randomly selected seeds (2,160 seeds) using a modified cetyltrimethlammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1987). We genotyped DNA from seeds instead of germinated seedling tissue to ensure that we included instances of inbreeding fertilizations that produced inviable offspring (predispersal inbreeding; Harder et al. 2012). Before DNA extraction, seeds were scarified and imbibed in water to loosen the maternal seed coat and facilitate its removal. We then extracted DNA from the embryo and endosperm of seeds. Thirteen microsatellite loci were amplified in three multiplexed reactions described in table S1. All markers were manually scored with Peak Scanner 2.0 software (Applied Biosystems, Foster City, CA).

We estimated the frequency of seeds that resulted from outcrossing events between relatives (i.e., biparental inbreeding) using the software MLTR (Ritland 2002). For each density- $N_e$  treatment combination, we determined the fraction of seeds produced by mating between full-sibling relatives (i.e., biparental inbreeding) as the difference between the multilocus ( $t_m$ ) and single-locus ( $t_s$ ) outcrossing rate with MLTR. This represents a minimal estimate of biparental inbreeding (Shaw et al. 1981). On the basis of the number of founders (4 vs. 12), we predicted the proportion of seeds produced by outcrossing to a sibling under a null hypothesis of random transfer of pollen among plants within each plot. We compared the empirical estimate of this proportion with the predicted value to aid the interpretation of

empirical patterns of biparental inbreeding as departures from these null predictions. Under null expectations, the proportion of seeds resulting from biparental inbreeding is 1 of 12 (0.083) for high- $N_e$  plots (1 of 12 plants is a full sibling) and 5 of 12 (0.417) for low- $N_e$  plots (5 of 12 plants are full siblings).

To examine the relationship among families between seed production through pollen and ovules, we assigned paternity to seeds produced in high-Ne plots. We focused on high- $N_{\rm e}$  plots because of the uncertainty in assigning paternity to a particular individual in a full-sibling group (six in the case of low- $N_e$  plots). Of the 1,080 genotyped seeds from the high- $N_{\rm e}$  plots, we assigned paternity to the most likely full-sibling pair of potential sires using COLONY 2.0.6.4 (Jones and Wang 2010; Wang et al. 2012). These analyses incorporated estimates of locus-specific genotyping error rates (Hoffman and Amos 2005) based on regenotyped DNA from 125 seeds selected randomly from the primary sample of 2,160 seeds genotyped (0%-1.4%; median: 0.4%; table S1). Using a confidence threshold of 0.85 to determine the most likely siring fullsibling pair, we successfully assigned paternity to 1,018 seeds (of 1,080 seeds; 94%) from the high- $N_e$  plots (329 low density, 340 medium density, 349 high density). Failure to assign paternity to these seeds may have resulted from genotyping errors, the movement of pollen between experimental plots, or germ line mutations. However, given the small number of seeds (62; 6%) that could not be assigned paternity at our conservative confidence threshold, we infer that pollen movement between plots in our study was rare; we excluded these seeds from subsequent analyses of male fitness. The total number of seeds that a full-sibling pair sired was used as the basic observation in aster analyses of fitness realized through male reproductive output.

#### Statistical Methods

Total lifetime fitness for each individual was analyzed using aster modeling (Geyer et al. 2007; Shaw et al. 2008) to jointly analyze fitness components because the multimodal distributions of lifetime fitness that arise from consecutive components of fitness expressed over life histories severely violate the assumptions of traditional parametric analyses (Geyer et al. 2007; Shaw et al. 2008). We employed graphical models reflecting the dependence of later-expressed fitness components on those expressed earlier. The graphical model included survival to flowering and fruit number and culminated in either total seeds set (female fitness) or seeds sired (male fitness; fig. 2). We followed Stanton-Geddes et al. (2012), incorporating an additional node in our graphical models to account for subsampling of fruits and thus scale up to the total seed production through maternity or paternity. We modeled survival to flowering and subsampled fruits with a Bernoulli distribution and total fruit number and seeds set or seeds sired with a Poisson distribution. We implemented unconditional aster models with the package aster (Geyer 2018) to assess the relationship between expected values of lifetime fitness (seeds set or seeds sired) and the explanatory variables, treatments (density, Ne, and their interaction), and plot ID as fixed effects (Shaw and Gever 2010). To test the effects of these factors, we used likelihood ratio tests comparing hierarchical models. From these analyses, we also obtained conditional estimates of the number of seeds produced per flower.

For the high- $N_e$  treatments, we qualitatively compared estimates of full-sibling families' fitness through maternity and paternity obtained through aster analyses culminating in seeds set and seeds sired, respectively. Because we assigned paternity to full-sibling pairs for a subsample (1,018 seeds)



A. Graphical Model for Female Reproductive Fitness – High and Low Ne

**Figure 2:** Graphical models for aster analyses of lifetime female (A) and lifetime male (B) fitness. Each node represents a fitness component and, therefore, response variable, and arrows represent conditional distributions. A Bernoulli distribution was used to model probability of flowering (0 or 1) and subsampled number of fruits, and Poisson distributions were used to model total number of fruits and seeds produced by subsampled fruits in A and total flowers produced and seeds sired in B.

of all seeds (i.e., fixed total number of seeds), siring success is a relative measure referring to the proportion of these sampled seeds sired by a given pair of full siblings. To enable straightforward comparison of genetically based fitness via maternity to fitness via paternity, we also scaled the estimates of female fitness (absolute number of seeds produced) for each full-sibling pair within a plot by dividing the number of seeds it produced (via ovules) by the total number of seeds produced by all plants in that plot.

Mating system outcomes of assayed seeds in high- and low- $N_e$  treatments across all three densities were analyzed with generalized linear models. Individual seeds determined as outcrossed or selfed were modeled as a binomially distributed (logit link function) response variable, with density treatment,  $N_e$  treatment, and plot ID (fixed effects) included as predictors.

We used linear models to evaluate effects of density and  $N_e$  on above- and belowground biomass. We log transformed above- and belowground biomass to render residuals more nearly normal. Levene's test revealed no significant departure from equal variances (all F < 1.224, all P > .420). Least squares means were contrasted on the log scale, and statistical significance was evaluated with Tukey's comparison. To assess the degree of potential nonindependence among individuals, we repeated the above linear models of biomass with the addition of plant position within a plot as a predictor. Three position categories were classified as inner (positions 1 and 12 in fig. 1), adjoining (positions 6 and 7 in fig. 1), and outer (the remaining eight positions). We analyzed the probability of a fertilized embryo being aborted with a generalized linear model. Embryo abortion was modeled as a binomial distribution with the number of aborted embryos and number of fertilized ovules as the number of "successes" and "failures," respectively. We computed back-transformed least squares means with the R package emmeans (Russel et al. 2018). All linear and generalized linear models contained plot ID, density treatment,  $N_{\rm e}$  treatment, and the interaction between density and Ne. All analyses were performed in the R 3.4.4 environment (R Development Core Team 2018).

Table 1: Summary of likelihood ratio tests comparing aster models with sequential addition of plot ID, density, family ID, genetic diversity ( $N_e$ ), density  $\times N_e$  interaction, and linear models to assess above- and belowground biomass, individual-level fitness (seeds set or relative seeds sired), and the number of aborted embryos per plant

	Analysis of individual-level fitness (seeds set and relative seeds sired)	
	Test deviance	Р
Density $\times N_{\rm e}$ + plot ID	935.37	<.001
High $N_{\rm e}$ (seeds set):		
Plot ID	1,550.80	<.001
Plot ID + density	614.18	<.001
Plot ID + density + family ID	270.53	<.001
Low $N_{\rm e}$ (seeds set):		
Plot ID	120.90	<.001
Plot ID + density	23.64	<.001
Plot ID + density + family ID	36.10	<.001
High $N_{\rm e}$ (relative number of seeds sired):		
Plot ID	.306	>.900
Plot ID + density	.103	>.900
Plot ID + density + family ID	10.12	>.080
	Analysis of biomass accumulation	
	F	Р
Plot ID	Above: $F_{1,214} = 16.22$ ; below: $F_{1,214} = 20.62$	Above: <.001; below: <.001
Plot ID + density	Above: $F_{3,212} = 7.55$ ; below: $F_{3,212} = 20.62$	Above: <.001; below: <.001
Plot ID + density + $N_e$	Above: $F_{4,211} = 7.47$ ; below: $F_{4,211} = 11.06$	Above: <.001; below: <.001
Plot ID + density + $N_{\rm e}$ + (density × $N_{\rm e}$ )	Above: $F_{6, 209} = 5.45$ ; below: $F_{6, 209} = 7.90$	Above: <.001; below: <.001
	Probability of embryo abortion	
	Test deviance	Р
Plot ID + density	4.85	<.01
Plot ID + density + $N_e$	56.69	<.001
Plot ID + density + $N_{\rm e}$ + (density × $N_{\rm e}$ )	386.82	<.001

### Results

#### Lifetime Fitness and Biomass

*Lifetime Female Fitness.* Lifetime fitness of individuals based on seeds set differed among  $N_e$  and density treatments, and these factors interacted significantly in their effects on fitness (table 1). Fitness also differed significantly among full-sibling families, implying genetic selection. In further separate analyses of the  $N_e$  treatments, density was a significant predictor of fitness in both. Counter to expectation, absolute fitness via the number of seeds set increased with density regardless of  $N_e$ . However, this fitness increase was especially prominent in high- $N_e$  treatments (fig. 3*A*). Interestingly, plants in low-density (30-cm spacing) treatments exhibited similar fitness estimates under both high and low  $N_e$ .

Biomass Accumulation. For both above- and belowground biomass, the interaction between density and  $N_e$  was significant (table 1), indicating that the effect of  $N_e$  on biomass depended on density (and vice versa). The direction of effects was consistent; high- $N_e$  plots had plants higher in biomass, but the difference between the  $N_e$  treatments was greater at higher density. Under high- $N_e$  conditions, plants in medium- and high-density conditions produced significantly more aboveground (all t > 2.32, P < .01) and belowground (all t > 5.36, P < .01) biomass compared with low-density treatments (fig. 4). Similarly, belowground biomass in low density was significantly lower than in medium and high density. Within plots, biomass did not significantly depend on plant position (all t < 0.695, P > .192), and we did not detect an interaction between position and density treatment (all t < 1.278, P > .203). Thus, our models including the factors plot ID, density, and  $N_e$  largely account for the interactions between plants. We note that, despite the inoculation treatment, not a single root nodule was found across all 216 plants.

Lifetime Male Fitness. The relative number of seeds sired (male reproductive fitness) in high- $N_e$  treatments did not significantly differ among the density levels (table 1). Moreover, variation among families in siring success was substantial (fig. 5) but was not statistically significant at P < .05(table 1). The total number of seeds produced via pollen must perforce equal the total number of seeds produced via ovules, but at the level of individual families, we found positive relationships between family-specific fitness based on contributions through maternity (seeds set) and paternity (seeds sired) in each density treatment (fig. 5). Most families produced somewhat more seeds through pollen than ovules, with a few families producing many more seeds via ovules than pollen in particular density treatments (e.g., family 7). We also found a positive relationship between seed production through female and male function at the level of individual flowers, as determined by conditional estimates of the number of seeds produced per flower from the aster analysis (fig. S1; figs. S1, S2 are available online).



**Figure 3:** Total lifetime fitness via female function and standard errors (*A*) and proportion of outcrossed (left axis) and selfed (right axis) seeds and standard errors (*B*) across two treatments of genetically effective population size ( $N_e$ ) and three density treatments. Values denoted with different lowercase letters within a  $N_e$  treatment are significantly different (P < .05). Bars (with respect to left axis) represent the empirical fraction of seeds resulting from biparental inbreeding ( $t_m - t_s$ ) across density treatments for high (filled bars) and low (open bars)  $N_e$ . The solid horizontal line represents the predicted proportion of seeds produced from mating between related individuals in high- $N_e$  plots (1/12 = 0.083), and the dashed horizontal line represents the predicted proportion of seeds produced from mating between related individuals in low- $N_e$  plots (5/12 = 0.417). Back-transformed least squares means and standard errors for the probability of fertilized embryo abortion are shown in *C*.



**Figure 4:** Accumulation of aboveground (*A*) and belowground (*B*) biomass across high and low genetically effective population size ( $N_c$ ) and three population density treatments. Values represent back-transformed least squares means and standard errors. Values denoted with different lowercase letters within a  $N_c$  treatment are significantly different (P < .05).

## Mating System Variation

Under high and low  $N_{\rm e}$ , the proportion of outcrossed seeds set exhibited differing trends with density. Whereas under high- $N_e$  conditions the outcrossing rate increased with density, under low-Ne conditions, outcrossing declined slightly, although not significantly, with density (fig. 3B). In high- $N_e$ plots, plants in high-density treatments set over twice the proportion of seeds by outcrossing (multilocus outcrossing rate:  $t_{\rm m} = 0.843$ ) as plants in low-density treatments  $(t_{\rm m} = 0.396, t = 3.64, P < .001;$  Tukey's test; fig. 3B). We note that the crowding at high density juxtaposed the flowers of different plants, which could contribute to this strong trend in outcrossing with density. Furthermore, outcrossing rates differed between low- and high-Ne treatments in medium-density (t = 4.55, P < .001; Tukey's test) and high-density (t = 6.89, P < .0001) treatments (fig. 3B). Of the seeds produced via outcrossing, estimates of mating between full siblings (biparental inbreeding) in the low- $N_e$ treatment (~10%-17%) tended to exceed those at high  $N_{\rm e}$  (~6%–10%; fig. 3B). Generally, in high- $N_{\rm e}$  conditions, biparental inbreeding matched the predictions, whereas in low-Ne plots, estimates of biparental inbreeding tended to be lower than expected (fig. 3B). Our estimates of biparental inbreeding represent a lower bound due to the difficulty in differentiating between selfed and biparentally inbred genotypes (Shaw et al. 1981).

The number of aborted embryos differed significantly among density treatments but in a similar pattern across  $N_e$  treatments (table 1). In low- $N_e$  treatments, the probability of a fertilized ovule aborting increased with density but was consistently lower in the high- $N_e$  treatment (fig. 3*C*). This matched the pattern of the proportion of aborted embryos. In the high- $N_e$  treatment, the proportion of aborted embryos decreased across low (0.114), medium (0.079), and high (0.065) densities. In the low- $N_e$  treatment, the proportion of aborted embryos increased across low (0.109), medium (0.145), and high (0.209) densities.

## Discussion

In this study, we characterized the effects of population density and  $N_e$  on lifetime fitness and mating systems in experimental arrays of Chamaecrista fasciculata. We detected strong interactions in the effects of these factors on lifetime fitness through female function, a finding that was also reflected in our analyses of plant biomass. Interestingly, lifetime fitness increased with density. In high- $N_e$  arrays, this density response was considerable, whereas at low  $N_e$ , lifetime fitness was reduced regardless of density. At both levels of Ne, families differed significantly in lifetime absolute fitness via seeds set, but we did not detect significant differences among families in lifetime relative fitness via seeds sired. Family-specific estimates of lifetime fitness through female and male function showed a positive relationship, with no indication of trade-offs between fitness attained through female and male function at either the level of individuals or flowers. We found that mating system patterns differed between high- and low-Ne treatments; under high



**Figure 5:** Plant-level relative female (seeds set) and male (seeds sired) lifetime fitness for high genetically effective population size (12 plants, as two plants from six full-sibling families) across low-density (A), medium-density (B), and high-density (C) treatments. Dashed lines represent a 1:1 relationship between female and male fitness. Individual symbols represent distinct full-sibling families. Pearson's product-moment correlations between female and male fitness are 0.279 for low-density, 0.802 for medium-density, and 0.580 for high-

 $N_{\rm e}$ , rates of outcrossing were generally greater and increased with increasing density, whereas there was no clear trend with density under low  $N_{\rm e}$ . The probability of embryo abortion did, however, increase with density in low- $N_{\rm e}$  plots, indicating that the greater incidence of inbreeding when close neighbors are closely related imposed greater cost to fitness. To our knowledge, this is the first study to explicitly and empirically reveal the mating system consequences of an interaction between genetically effective population size and population density. Below, we interpret these findings, considering them in relation to other available evidence.

## Lifetime Fitness, Biomass, and Mating System Variation

Lifetime Fitness and Biomass Accumulation. Estimates of lifetime fitness through seeds set and biomass responded strongly to both density and  $N_e$  treatments. Whereas fitness increased rapidly with increased density in the high- $N_e$  treatment, only modest increases in fitness with density were observed in the low-Ne treatment. Our finding of enhanced absolute fitness with density complements those of Stanton-Geddes et al. (2012), who found that survival and fitness of C. fasciculata were greater in experimental plots when heterospecific neighbors were present compared with plots lacking neighbors. These authors identified hydraulic lift as one mechanism that could contribute to this finding, as it may in our finding of fitness enhancement with closer spacing of individuals. Regardless of the mechanism, this positive relationship between individual fitness and density represents a demographic Allee effect, as has been shown previously (Berec et al. 2007; Le Cadre et al. 2008). In contrast to the expectation of reduced biomass due to increased competition (Harper 1977; Antonovics and Levin 1980), we found that individual above- and belowground biomass accumulation increased substantially with density (fig. 3A, 3B), mirroring the trend with fitness. This pattern was observed under both high and low  $N_{\rm e}$  but was especially strong in high- $N_{\rm e}$  treatments; at this level, plants in the highest density grew large enough to became entangled (fig. S2), likely enhancing interplant pollination and outcrossing (fig. 4). Whereas in some previous studies closely related neighbors (i.e., low N<sub>e</sub>) enhanced performance measures such as biomass, resulting in kin selection (Donohue 2003; File et al. 2012), increased  $N_{\rm e}$  can, conversely, enhance productivity through annidation (i.e., niche partitioning), as different

density treatments. Correlations do not account for sampling variance of estimates of female and male fitness and are therefore presented as a coarse estimate of the relationship between female and male fitness among families. Legends provide symbols that indicate each fullsibling family and, in parentheses, the number of plots in which each family was used.

genotypes are favored by different ecological aspects of the local environment (Antonovics 1978; Loreau et al. 2001; Vellend and Geber 2005).

Our finding that increasing  $N_{\rm e}$  enhanced female fitness and biomass represents a genetic Allee effect (Luque et al. 2016). Such an effect was shown by Newman and Pilson (1997) as lower rates of extirpation over 4 years for populations of Clarkia pulchella established in the wild at higher  $N_{\rm e}$ . Likewise, Wagenius et al. (2007) demonstrated that an increase in the population size of Echinacea angustifolia increased the proportion of potential matings that were genetically reproductively compatible. Our results show a strong role of a genetic Allee effect, limiting fitness increases in low-Ne plots with increasing density compared with fitness at higher Ne. Therefore, our results suggest that genetic Allee effects that limit fitness can override otherwise powerful demographic Allee effects. Such strong interaction effects have been predicted but have remained difficult to empirically demonstrate (Wittman et al. 2018; but see Johnson et al. 2010).

Mating System Variation. In a study of a natural population of C. fasciculata in situ with population densities lower than any in our study, Fenster (1991a) and Fenster et al. (2003) estimated an outcrossing rate of ~80%. This closely resembles the outcrossing rates in our medium- and high-density treatments in high  $N_{\rm e}$ , whereas our estimates are considerably lower for remaining treatments. Between the two  $N_e$ treatments, density had differing effects on the proportion of seeds resulting from outcrossing. In high-Ne treatments, the outcrossing rate consistently increased across the range of densities; under low Ne however, outcrossing slightly declined (increased selfing, nonsignificant) as density increased, leveling off just below approximately 30% outcrossing rate (fig. 3B). In addition, among the seeds produced by outcrossing, a considerable fraction resulted from mating between full siblings (fig. 3B). This biparental inbreeding increased modestly with density in both high- and low-Ne treatments but was more apparent in low- $N_{\rm e}$  plots, reaching ~17% at high density. Similar to Yin et al. (2016), in high densities, greater outcrossing in high  $N_{\rm e}$  was associated with reduced biparental inbreeding. In populations with low  $N_e$ , biparental inbreeding tended to increase with density (fig. 3B) as a result of the increasing likelihood of mating between neighbors, many of which are close relatives in this treatment. Interplant mating increases under high-density conditions because of the decrease in energetic cost to pollinators of travel between neighbors (sensu optimal foraging theory; Pyke 1984). Interestingly, rates of biparental inbreeding in low-Ne treatments were lower than predicted under random pollen transfer among individuals (including self), but the biparental inbreeding rate in high- $N_e$  treatments closely matched our prediction under random pollen transfer (fig. 3B).

We note that our estimates of biparental inbreeding are conservative because some seeds produced by biparental mating may be assigned as selfed offspring (Shaw et al. 1981). Thus, biparental inbreeding in the low- $N_e$  treatment may also have matched the prediction.

The negative fitness consequences of inbreeding due to increased homozygosity and expression of deleterious recessive alleles have long been known (Darwin 1876; Charlesworth and Charlesworth 1987; Charlesworth and Willis 2009; Barrett and Harder 2017). Along with the strong inbreeding depression previously found for C. fasciculata (Fenster 1991b), our results imply that the levels of selfing and biparental inbreeding we have documented would reduce fitness in the next generation. Thus, considering a longer time horizon for fitness by including an individual's fitness in the fitness of its offspring, we expect that the low fitness of plants in the low-density and low-Ne treatments would be further impaired by their enhanced inbreeding relative to high density and high Ne.. Moreover, even though lifetime fitness of individuals in low  $N_{\rm e}$  increased slightly across densities, a larger fraction of their seeds was produced through inbreeding (both biparentally and by selfing). Inbreeding depression expected in the next generation would check and potentially reverse the slight increase in fitness with density that we observed in the experimental plants.

The observed effect of inbreeding on lifetime fitness in our experiment may be partially attributed to aborted embryos (as in Rigney 1995). Whereas inbreeding depression of offspring would be realized in the next generation, parental plants suffered immediate fitness costs in the form of inbred embryo abortion. In the low- $N_e$  treatment, the probability of a fertilized embryo being aborted increased with density (fig. 3*C*). In contrast, at high  $N_e$  the probability of embryo abortion remained consistent across densities. Our experiment did not explicitly account for the potential role of resource limitation in embryo abortion; however, the complete lack of rhizobial nodules suggests that belowground symbiotic relationships did not contribute to variation in individual-level resource budgets.

### Sex-Specific Fitness Estimates

Our results yielded no evidence of a genetic family-based trade-off between seed production via maternity and paternity; rather, these aspects of reproduction were similar. Some genetic families achieved slightly higher fitness through paternity than through maternity. This disparity was greatest under low density (fig. 5*A*), whereas more families had nearly equal seed production through maternity and paternity in the medium- and high-density treatments (fig. 5*B*, 5*C*). Nevertheless, at each density, families that produced the greatest

total number of progeny did so through maternity. However, the degree of fitness advantage of these families varied with density (e.g., families 2, 7, and 11; fig. 5).

The relative contribution of female and male reproductive performance to total lifetime fitness in our study may be related to variation in plant size across densities. Larger plants (greater biomass) in high-density plots exhibited higher fitness. Large flower displays increase pollinator visitation, which in turn increases pollen transfer. However, within individuals with larger floral displays, pollinators typically visit more flowers on the same plant in succession, thus effecting selfing between flowers within an individual (geitonogamy) and limiting pollen export (Harder and Barrett 1995; Barrett 2003; Lau et al. 2008). For example, Karron and Mitchell (2012) found seed production to increase with display size in *Mimulus ringens*, but because of increased inbreeding, marginal fitness gains decreased.

Fitness trade-offs between sexes have been previously reported with respect to floral traits-for example, enhanced allocation to primary (androecium) and secondary (corolla mass) male function in large-flowered Collinsia parviflora (Parachnowitsch and Elle 2004) and enhanced female function in larger-flowered tristylous Narcissus triandrus (Hodgins and Barrett 2008). Rather than focusing on a limited number of traits, we examined how variation in population density may more generally affect female and male function of genetic families. We found positive, albeit weak, relationships among families between fitness realized through female and male sex functions (figs. 5 and S2, respectively). Asymmetry toward reproduction via male or female function varied somewhat idiosyncratically among families and densities (fig. 5). This further highlights the importance of population-level ecological influences on the reproductive environment, resulting in deviations from symmetry in sex function, as described by Lloyd (1980).

Estimates of lifetime fitness culminating in both female and male reproductive output offer a more complete view of fitness compared with analyses focused exclusively on reproduction through ovules. Of the few studies that do incorporate estimates of siring success, even fewer examine fitness over the full life span (but see Conner et al. 1996), for example, focusing on how reproductive performance varies in relation to plant traits (e.g., Hodgins and Barrett 2008; Kulbaba and Worley 2012). Whereas such studies have revealed how selection through variation in reproduction can shape traits, they neglect the dependency of reproductive output on earlier components of life history. Our lifetime fitness approach to estimating sex-specific fitness, as implemented by aster analyses (Geyer et al. 2007; Shaw et al. 2008) represents a powerful means for a complete assessment of fitness. Finally, we advocate the use of aster models to further elucidate effects of population density and genetic composition on lifetime fitness to more completely describe demographic-dependent fitness and selection (i.e., soft selection; Wallace 1975; Reznick 2016).

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## Statement of Authorship

R.G.S. acquired funding. M.W.K. and R.G.S. conceived and designed the study, and M.W.K. conducted the field and laboratory work. M.W.K. and R.G.S. analyzed the data, and M.W.K. wrote the manuscript. R.G.S. and M.W.K. contributed to revising the manuscript.

## Data and Code Availability

Data and R code are available at Zenodo (https://doi.org /10.5281/zenodo.4323093; Kulbaba 2020).

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"The Great Auk or Gare-fowl . . . was about the size of a goose, with a large head, a curved, grooved and laterally flattened bill; wings rudimental, adapted to swimming only, approaching in this respect the penguins of the southern hemisphere." From "The Great Auk" by James Orton (*The American Naturalist*, 1869, 3:539–542).