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Can't live with them, can't live without them? Balancing mating and competition in two-sex populations

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Two-sex populations are usually studied through frequency-dependent models that describe how sex ratio affects mating, recruitment and population growth. However, in two-sex populations, mating and recruitment should also be affected by density and by its interactions with the sex ratio. Density may have positive effects on mating (Allee effects) but negative effects on other demographic processes. In this study, we quantified how positive and negative inter-sexual interactions balance in two-sex populations. Using a dioecious grass (*Poa arachnifera*), we established experimental field populations that varied in density and sex ratio. We then quantified mating success (seed fertilization) and non-mating demographic performance, and integrated these responses to project population-level recruitment. Female mating success was positively density-dependent, especially at female-biased sex ratios. Other demographic processes were negatively density-dependent and, in some cases, frequency-dependent. Integrating our experimental results showed that mate-finding Allee effects dominated other types of density-dependence, giving rise to recruitment that increased with increasing density and peaked at intermediate sex ratios, reflecting tension between seed initiation (greater with more females) and seed viability (greater with more males). Our results reveal, for the first time, the balance of positive and negative inter-sexual interactions in sex-structured populations. Models that account for both density- and sex ratio dependence, particularly in mating, may be necessary for understanding and predicting two-sex population dynamics.

1. Introduction

From foundational theory to the present, population ecologists have tended to ignore sex structure. For example, in a database of animal demography including 405 species [1], only 9% of studies separately considered females and males, and an even smaller subset (approx. 3%) explicitly considered interactions between the sexes. Ignoring sex is convenient and probably appropriate for asexual organisms or self-compatible hermaphrodites. It may also be appropriate for dioecious organisms (those with separate sexes) but only if female fertility is not limited by the availability of males. This condition may be reasonably satisfied under a realistic range of sex ratio variation [2]. In such cases, a 'female-dominant' perspective that ignores males may be a useful simplification because it provides a linear approximation to a nonlinear interaction (female fertility, a function of male availability, is treated as constant). However, there is growing recognition that assumptions of female dominance may often be violated, particularly in populations that experience selective harvesting [3,4] and in global change-related contexts, such as skewed sex ratios at invasion fronts [5,6], and the distributional limits of native species [7].

Theoretical advances for two-sex populations have generalized female-dominant approaches to explicitly accommodate both sexes and their interactions

[8–10]. This theory allows for sex-specific demographic rates to generate skew in the population sex ratio. Skewed sex ratios, in turn, can modify recruitment via a ‘mating function’ that specifies the dependence of reproduction on the relative abundance of females and males, generating feedbacks between population sex ratio and individual vital rates [11]. The canonical and widely used mating functions that define these feedbacks are frequency-dependent but density-independent [10,12], a property that makes them mathematically tractable but potentially unrealistic. There are at least two ways in which the operation of both frequency- and density dependence, and their interaction, may be important in two-sex populations.

First, mating success may be density-dependent because individuals are more likely to encounter mates at higher densities. The large literature on Allee effects suggests that mate-finding difficulties are a common mechanism of positive density dependence at low density [13]. Mate-finding Allee effects may be especially pronounced in sessile organisms that rely on wind or water for the dispersal of gametes [14]. Theoretical studies of two-sex populations have shown that strong mate-finding Allee effects are likely to arise in small populations, even at sex ratios that would promote population growth at higher densities [15]. Mate-finding Allee effects can also exacerbate the negative effects of skewed sex ratios, where frequency dependence already limits reproduction [16]. Combined, the largely independent bodies of work on frequency-dependent mating functions and density-dependent mate-finding suggest a need for a unified understanding of both axes of variability.

The second way in which density- and frequency-dependence could interact in two-sex populations is through resource competition [17,18] and other antagonistic inter-sexual interactions such as harassment [19,20] and disease transmission [21]. Competition occurs both within and between the sexes, and several studies have shown sex differences in competitive ability [18,22]. Hence, density-dependent competition may play out differently at different sex ratios. For example, if females are superior competitors [18], then, for the same density, a female-biased sex ratio represents a more challenging competitive environment than a male-biased sex ratio. The response of female and male vital rates to density- and frequency-dependent competition should affect the population sex ratio and thus modify the competitive and mating environments. These density- and frequency-dependent feedbacks in two-sex populations have been the subject of some theoretical study [8,9] but little empirical work [19].

Density- and frequency-dependence in inter-sexual mutualism (mating) and inter-sexual antagonism (competition) could have opposing effects on recruitment at the population level, evoking the old adage that neither sex can live with or without the other. For example, the sex ratio that maximizes female fertility may be different from the sex ratio that minimizes competition. Similarly, increasing density may improve mate-finding but also increase the competitive environment. In nature, the outcomes of inter-sexual interactions probably reflect a balance of these processes, but their contributions have not previously been decomposed, to our knowledge. Our goal was to understand how frequency- and density dependence interactively affect mating and competition, and how these forces combine to affect population-level recruitment.

Using a dioecious grass (*Poa arachnifera*) as a model system, we conducted a field experiment that assigned replicate populations to a range of densities and sex ratio frequencies. Such response surface experimental designs are a powerful way to

study interspecific interactions but we are aware of only two studies, both laboratory-based, that have used this approach to study inter-sexual interactions [23,24]. A sessile focal organism allowed us to establish and track replicated populations with assigned composition of females and males under natural conditions, a manipulation that would be difficult in many animal systems.

First, we tested for density- and frequency-dependence in female mating success (seed fertilization). We hypothesized that mate-finding Allee effects would generate positive density dependence in female mating success and that Allee effects would be stronger under female-biased sex ratios, when females were most male-limited. Second, we tested for sex differences in demographic performance in response to variation in the competition determined by density and sex ratio. We hypothesized that there may be baseline differences in demographic performance of females and males expressed at low density, that density-dependent competition would have negative effects on performance, and that females and males may contribute differently to density dependence due to sex-specific competitive ability. We separated sex differences in competitive effect (ability to suppress competitors) and competitive response (the change in performance due to increasing density) [25]. Finally, we integrated our understanding of competition and mating to test how their combined effects determine population recruitment, including sexual and asexual reproduction. We hypothesized that the density and sex ratio at which *per capita* recruitment is maximized reflect the opposing forces of competition and mating.

2. Material and methods

(a) Study system

Texas bluegrass (*P. arachnifera*) is a perennial dioecious grass native to the southern Great Plains of North America. It commonly occurs in grassland and savannah habitats of central Texas and Oklahoma, usually in the dappled shade of trees and shrubs. Texas bluegrass is a cool season (C₃) grass that flowers in April and May and, like other grasses, is strictly wind-pollinated. Flowering individuals can produce multiple panicles (branched inflorescences), each panicle includes many spikelets and each spikelet includes multiple florets.

This species grows in bunches of 2–50 tillers, each of which includes multiple leaves, and is capable of rhizomatous growth by which bunches are connected underground. We treat rhizomatous growth as asexual reproduction, because bunches remain viable and become physiologically independent when rhizomes break. The rhizomatous growth habit of this species allowed us to propagate large quantities of known-sex individuals from field-collected source material (sex in Texas bluegrass is genetically based [26]).

(b) Source material and study site

The source material for our experiment came from a naturally occurring population near Groesbeck, Texas (latitude 31°33′38.9″ N, longitude 96°38′12.8″ W). In spring 2012, we collected flowering individuals (so that their sex was known) from 63 (32 male, 31 female) separate individuals (despite clonal growth, individual patches are well defined in the field). We propagated them in greenhouses at Rice University. Single tillers were separated from a clonal parent plant and grown in ProMix potting soil for 2–3 months in cylindrical containers before transplanting into the field experiment.

We carried out the field experiment at Lake Lewisville Environmental Learning Area, a 500-ha protected area near Lewisville, TX

(33°03'32.4" N, 96°55'49.4" W, altitude approx. 170 m) approximately 169 km from the source population. We established our experimental plots in a honey mesquite (*Prosopis glandulosa*) savannah with a grass layer dominated by Texas wintergrass (*Nassella leucotricha*). Soils at the site are typical of blackland prairie (40–60% clay).

(c) Experimental design

For the response surface experiment, each replicate population was assigned to one of 34 combinations of density and sex ratio, with absolute densities spanning 1 to 48 plants and sex frequencies from 0 to 100% female (electronic supplementary material, figure S1a). Surveys indicate that extreme sex ratios occur in natural populations of Texas bluegrass (mean % female = 48, minimum = 0.9, maximum = 100, $N = 15$ populations; TEX Miller, unpublished data). Experimental populations were established by transplanting individuals into 0.4 m × 0.4 m field plots. Each transplanted individual was randomly assigned from the pool of 63 clones we collected in 2012. Thus, potential systematic bias in clonal diversity across plots would be expected from sampling effects (i.e. least clonal diversity in one-plant plots). Most treatments were replicated two to four times, with high-density treatments having the lowest replication (electronic supplementary material, figure S1a) because they required the most plants to establish; unbalanced replication of response surface designs is not a problem and can even be beneficial [23]. The experiment included 124 experimental plots and 2074 individuals. Each plot was planted under the partial shade of a honey mesquite canopy, the natural microhabitat.

It was important that replicate populations be independent such that the mating environment was not contaminated by movement of pollen between plots. Plots were separated by at least 15 m to minimize potential between-plot pollen movement. This distance was based on a preliminary study of seed viability from naturally occurring females, which suggested that greater than 90% of wind pollination came from males within 13 m (TEX Miller, unpublished data). To test the adequacy of our plot spacing, we included the most replication for one-female plots (15 replicates; electronic supplementary material, figure S1a), distributed widely over our 3.5-ha study area, because these served as controls to detect between-plot pollen movement (any seeds produced must have been sired by males greater than 15 m away).

We established experimental populations in November 2013. For each plot, we plowed to a depth of 5 cm and transplanted greenhouse-raised propagules in a standardized grid, assigning individuals to plots randomly. We marked up to 10 planted individuals (five individuals per sex maximum) with a unique identifier, to allow individual-level measures of demographic performance (henceforth called 'census individuals'). In treatments that included more than five individuals per sex, census individuals were selected randomly. Each plot received one gallon of water at the time of planting to promote establishment but was subsequently unmanipulated.

(d) Data collection

We collected data at the time of plot establishment and in the spring flowering seasons (April) of 2014 and 2015. We intended to track mating success of females (we did not track male mating success because we could not assign paternity to seeds) and non-mating demographic performance of females and males. Individual-level data came from the census individuals described above. In the field, for each census individual at each sampling period, we recorded survival, size (number of leaves) and number of panicles. We collected all panicles from census individuals at maturity to estimate reproductive effort per panicle. In the laboratory, we removed seeds from the female panicles by rolling them between fingertips for 2 min, and then counted them. Because unfertilized seeds

shatter from the panicle along with fertilized seeds, this count reflects female reproductive effort (seeds initiated) and not mating success (seeds fertilized). We quantified male reproductive effort by counting the number of spikelets on each male panicle, assuming this quantity is proportional to pollen production.

At the plot level, we recorded the total number of female and male panicles (including census and non-census individuals) and the number of new tillers produced by rhizomatous growth as a measure of asexual reproduction. These new tillers occurred in the interspaces between transplants. While we could not assign these tillers to clonal parents or to a sex (except for one-plant plots), we used these data to estimate population-level *per capita* rhizomatous tiller production.

In the laboratory, we measured seed fertilization success as the proportion of seeds initiated that were viable. We quantified viability in two ways: germination and tetrazolium assays. We carried out germination trials for seeds from 112 panicles, belonging to 84 census females from 50 plots, spanning the range of density and sex ratio treatments (electronic supplementary material, figure S1b). Samples were uneven across treatments because of variation in the numbers of panicles per individual and individuals per plot (electronic supplementary material, figure S1c). Each trial included 25 seeds from one female panicle, which were placed on potting soil in a greenhouse during the winter following seed collection. We recorded germination twice per month between February and June, removing seedlings as they emerged to estimate cumulative germination. The fraction of seeds that emerged as seedlings was a proxy for seed viability. However, it is possible that seeds that did not germinate were also viable (e.g. in case of long-term seed dormancy, which was not known). We, therefore, additionally used a tetrazolium-based viability assay on a subset of 65 panicles (belonging to 63 separate females, all of which were included in the germination assay) to determine whether germination was a good proxy for viability (mode: 30 seeds per female; range: 17–57 seeds). To perform these assays, we first let seed batches imbibe on a moistened paper towel for 12 h. We then bisected the seeds in half and soaked them in a pH buffer solution containing 0.1% of tetrazolium for 12 h. The pH buffer solution contained 0.57% of sodium phosphate and 0.36% of potassium phosphate. A seed was scored as viable if the embryo stained pink [27].

(e) Data analysis

Our analyses focused on the mating success and demographic performance of Texas bluegrass in relation to focal plant sex, total density, population sex ratio and their interactions. We did not analyse the survival of transplants because almost all (97%) transplants survived. Our analyses of panicle production, reproductive effort per panicle and seed viability were limited to 2014 because very few plants flowered in 2015, probably owing to severe flooding at our study site.

Most of our analyses relied on generalized linear mixed models (GLMMs) in R v. 3.3.0 [28] using packages *glmmADMB* v. 0.8.3.3 [29] and *lme4* v. 3.3.1 [30]. Models for individual-level responses included random effects for plot, to account for the non-independence of observations from census individuals. We used Akaike information criterion (AIC)-based model selection to quantify support for alternative hypotheses, described below. We used AIC to rank candidate models and calculate their relative degree of support via AIC weights [31]. To account for model uncertainty, we focused on the set of ranked models comprising greater than or equal to 95% of AIC weight. Where appropriate, we used multi-model inference to compute a weighted average model on which we based our inferences. We calculated model averages as recommended by Cade [32].

(i) Female mating success

We modelled female mating success, or the proportion of viable seeds, as a binomial process using GLMMs with a logit link

function. Candidate models included as predictors total density of panicles in the plot, sex ratio of panicles, neither (null model), or both density and sex ratio as additive and interactive factors (electronic supplementary material, table S1). The density and sex ratio of panicles were closely but not perfectly correlated with those of transplants, because transplants varied in panicle number. This made panicle density and sex ratio the better indicators of the mating environment. Our data were unbalanced across treatments because we intentionally over-replicated one-female plots to serve as controls for between-plot pollen movement (electronic supplementary material, figure S1a). To account for the imbalance in sampling, we repeated analyses across 200 bootstrap samples of the complete dataset. Each bootstrap sample included a random sample of at most two replicates per density \times sex ratio treatment. We calculated 95% CI on model predictions for seed viability across these bootstrap samples.

(ii) Demographic performance

The five response variables associated with demographic performance were growth rate of individuals, the number of panicles produced per individual, female reproductive effort per panicle (number of seeds initiated), male reproductive effort per panicle (number of spikelets) and the production of new tillers through asexual recruitment. Growth rate was calculated as $\log(L_{t+1}/L_t)$, where L_t is size (number of leaves) at time t . Growth rate was estimated for two different time intervals: between autumn 2013 and spring 2014, and between spring 2014 and spring 2015. We analysed the growth data as a normally distributed response variable. Because the growth data came from different time intervals, years were not directly comparable, so we analysed 2014 and 2015 data separately. All other measures of demographic performance were counts, for which we used a negative binomial response distribution.

Growth rate and panicle production were individual-level responses shared by both females and males. Our analysis considered three ways in which these may differ between the sexes (electronic supplementary material, table S2). First, there may be intrinsic sex differences, independent of the competitive environment. We tested for this difference by including the sex of the census individual as a fixed effect. Second, the sexes may differ in their competitive response to density, which would give rise to a statistical interaction between sex of the census individual and total population density (e.g. female but not male performance is suppressed by increasing density). Third, the sexes may differ in their competitive effects on census individuals, which would give rise to a statistical interaction between density and sex ratio (e.g. increasing density has a stronger effect at female-biased sex ratios). Finally, any two or all three of these sex differences may operate simultaneously (electronic supplementary material, table S2). Unlike the analysis of female mating success, here we used the density and sex ratio of transplants (not panicles) to characterize the competitive environment.

The per-panicle reproductive effort of females and males was estimated by different quantities (seeds initiated and spikelets, respectively). We, therefore, analysed these responses separately and did not test for baseline sex differences or sex differences in competitive response, though we did test for sex differences in competitive effect, including population density and sex ratio as candidate predictors (electronic supplementary material, table S2).

As asexual recruitment was 'anonymous' (new tillers were not assigned clonal parents), we took a different statistical approach. We fit a Beverton–Holt-like model [33] to predict asexual recruitment based on sex differences in asexual reproduction and sex-specific competitive effects and responses. The basic Beverton–Holt model predicts the total number of asexual recruits A_{t+1} as a function of total transplant density at the time of planting, summed over females and males ($N_t = F_t + M_t$):

$$A_{t+1} = \frac{\lambda N_t}{1 + bN_t}. \quad (2.1)$$

Parameter λ represents the low-density, per-individual asexual reproductive rate (new tillers per transplant) and parameter b represents the response to increasing density. For $b > 0$, asexual recruitment saturates with density due to negative density dependence. If there are baseline differences in asexual reproductive rate between the sexes ($\lambda_F \neq \lambda_M$) but no differences in contributions or responses to density dependence, then A_{t+1} is the sum of female and male contributions:

$$A_{t+1} = \frac{\lambda_F F_t + \lambda_M M_t}{1 + bN_t}. \quad (2.2)$$

Alternatively, under sex-specific competitive responses, females and males are differentially sensitive to total density:

$$A_{t+1} = \frac{\lambda F_t}{1 + b_F N_t} + \frac{\lambda M_t}{1 + b_M N_t}. \quad (2.3)$$

Finally, under sex-specific competitive effects, parameter a weights the effects of males relative to females:

$$A_{t+1} = \frac{\lambda N_t}{1 + b(F_t + aM_t)}. \quad (2.4)$$

When $a > 1$, males contribute more strongly to negative density dependence than females, and vice versa. We considered a total of eight candidate models (electronic supplementary material, table S3), including all possible combinations of sex differences in baseline asexual reproductive rate, competitive response and competitive effect. As with the individual growth data, we analysed 2014 and 2015 data separately. We fitted all candidate models by maximum-likelihood estimation using Nelder–Mead optimization (R function `optim()`).

(iii) Combined effect of mating and competition on population recruitment

Finally, we developed a simple model, integrating results of the above analyses, to estimate how mating and competition combine to determine population recruitment under variable densities and sex ratios. For a population of F females and M males, we define total recruitment $r(F, M)$ as the sum of sexual and asexual recruits:

$$r(F, M) = s(F, M) \cdot z(F, M) + a(F, M). \quad (2.5)$$

The first product represents sexual recruits, where s gives the total seeds initiated and z gives the seed fertilization probability, both of which may respond to the density and sex ratio environment defined by F and M . Total seeds initiated by F females is given by the product of panicles per female (p_f) and seeds initiated per panicle (s_p):

$$s(F, M) = F \cdot p_f(F, M) \cdot s_p(F, M). \quad (2.6)$$

The seed fertilization function $z(F, M)$ converts female and male densities into densities of female ($F \cdot p_f(F, M)$) and male ($M \cdot p_m(F, M)$) panicles, following our statistical approach defined above. The Beverton–Holt recruitment model for rhizomatous tiller production (electronic supplementary material, table S3) defines $a(F, M)$, the expected asexual propagules given densities of F females and M males.

All components of this framework were estimated from our analyses described above, excluding the analyses of growth rate (because this recruitment model is not explicitly size-dependent) and male reproductive effort (because we could not estimate the effects of variable pollen production per male). Using empirical parameter estimates, we evaluated the total recruitment function $r(F, M)$ over the range of female and male densities included in our experiment (electronic supplementary material, figure S1). For visualization, we report results *per capita* ($r(F, M)/(F + M)$) over axes defined by total density

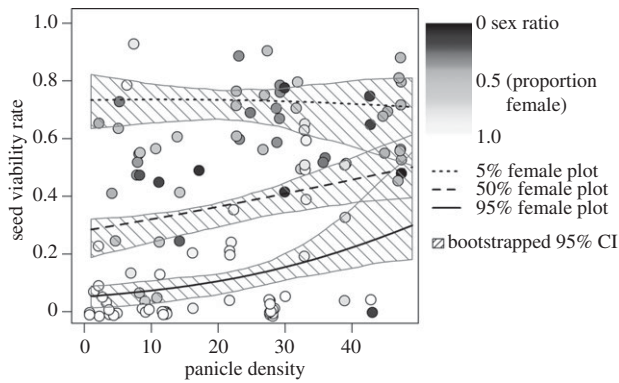


Figure 1. Seed viability as a function of the total density and sex ratio of panicles. Viability data are from the seed germination assay. Data were jittered on the x - and y -axes. The shading of circles represents the panicle sex ratio following the legend; dotted, dashed and solid lines represent model predictions for plots where 5%, 50% and 95% of panicles were female, respectively. Dashed polygons delimit the upper and lower 95% CI across 200 bootstrap samples.

($F + M$) and sex ratio ($F/F + M$). We show total *per capita* recruitment as well as its decomposition into seeds initiated ($s(F, M)$), seed fertilization ($z(F, M)$) and asexual propagation ($a(F, M)$)—the three components that collectively determine recruitment but which may respond to density and sex ratio variation in different ways.

This approach equates rhizomatous tiller production (a) to total sexual recruits (sz), assuming that all germinated seeds survive at the same rate as rhizomatous tillers. To quantify the sensitivity of our results to seedling survival, which may be lower than that of asexual tillers, we evaluated $r(F, M)$ under realistic variation in seedling survival rates (electronic supplementary material, figure S5).

3. Results

(a) Effects of density- and frequency-dependence on female mating success

Plots consisting of single females and plots containing multiple females but no males produced very few viable seeds (mean: 5% and 5.6% viability, respectively) and had significantly reduced seed viability compared with plots with one or more males (48% viability, $z = -8.2$, $p < 0.0001$ and 46% viability, $z = -15.3$, $p < 0.0001$, respectively). This indicates that mating occurred primarily at the intended, within-plot scale.

We found an interactive effect of sex ratio and density of panicles on seed fertilization (model selection results in electronic supplementary material, table S1). There was a strong effect of sex ratio, with maximum seed viability in male-biased populations (figure 1 shows viability based on seed germination). Additionally, there was positive density dependence in seed viability (a mate-finding Allee effect), especially at female-biased sex ratios. In female-biased populations, the mean viability approximately tripled from the minimum to maximum densities, while at male-biased sex ratios, viability was consistently high, with essentially no density response (figure 1). Even under male-biased sex ratios, seed viability rarely exceeded 80%, suggesting limits on fertilization that are not related to mate availability. Seed viability estimates based on the tetrazolium assay were strongly correlated with those based on germination ($r = 0.739$, $p < 0.0001$)

and gave qualitatively identical results (electronic supplementary material, figure S2), but showed even stronger positive density dependence at female-biased sex ratios and greater maximum seed viability (approx. 90%). This suggests some potential for seed dormancy, making our germination assay (figure 1) a conservative estimate of seed viability.

(b) Effects of density- and frequency-dependence on demographic performance

For the two measures of demographic performance shared by females and males (panicle production and growth), there was little evidence for sex differences in baseline rates, competitive effects or competitive responses. First, the growth rate of individuals did not strongly differ between the sexes in both 2014 (figure 2a) and 2015 (electronic supplementary material, figure S3). Moreover, we found evidence for negative density dependence in 2015 (electronic supplementary material, table S4 and figure S3) but not 2014 (figure 2a). Second, we found weak positive density dependence in panicle production (figure 2b), and the density-only model received the most support from the data (electronic supplementary material, table S2).

Female and male reproductive effort per panicle showed negative density dependence and negative frequency dependence, meaning stronger competitive effects of same-sex individuals, though this pattern was stronger in females (figure 2c,d). For females, the number of seeds initiated per panicle declined with increasing density and was more negatively affected by density at female-biased sex ratios (figure 2c). On the other hand, for male reproductive effort (spikelets per panicle), the interaction of density and sex ratio had opposite sign (figure 2d). Yet, in male reproductive effort models, the support for density- and frequency-dependence was weaker, and the null model received a slight majority of support (electronic supplementary material, table S2). The production of new tillers showed negative density dependence but weak sex differences. The null model received the majority of support, and three other models with sex-specific parameters were closely ranked (electronic supplementary material, table S3). After accounting for model uncertainty, there was clear evidence for negative density dependence, but little difference in asexual recruitment in female- versus male-biased plots (figure 2e). Results from the second year of the study were very similar (electronic supplementary material, table S5 and figure S4).

(c) The combined effect of mating and competition on *per capita* population recruitment

Total *per capita* recruitment, including sexual and asexual reproduction, was dominated by sex ratio variation and was less responsive to density (figure 3). There was maximum recruitment at intermediate sex ratios (figure 3d), reflecting the opposing forces of greater seed production with increasing female bias (figure 3a) but greater likelihood of seed viability under male bias (figure 3b). Population density modified the sex ratio at which recruitment was maximized, from 40% female at low density to 55% female at high density (figure 3d). This result reflects the interaction of density- and frequency-dependence in seed fertilization (figure 1), whereby greater female bias can be tolerated at higher densities to achieve the same level of seed viability.

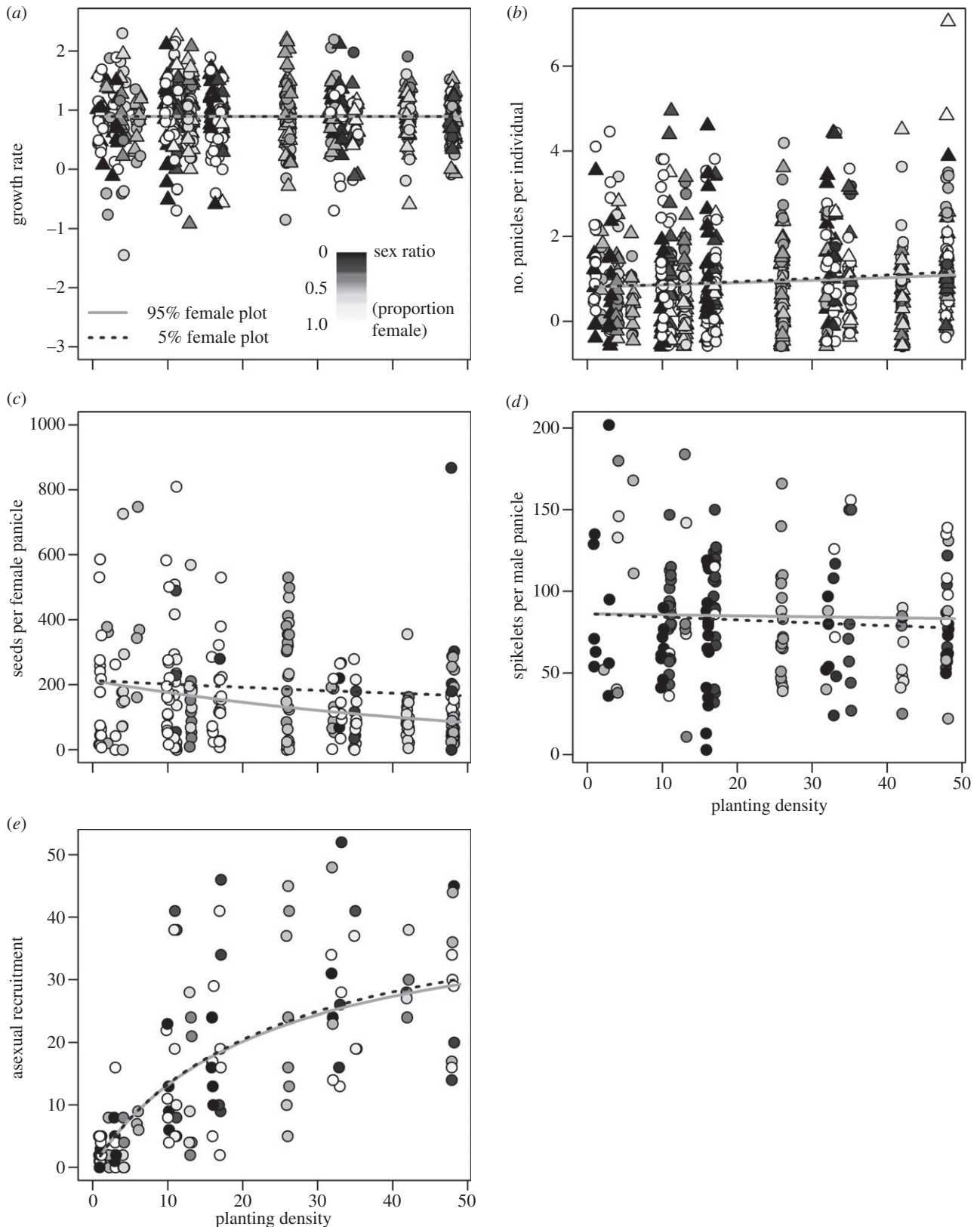


Figure 2. Vital rates associated with non-mating demographic performance in relation to plant density and sex ratio: (a) growth rate, (b) number of panicles per individual, (c) female reproductive effort (seeds initiated per panicle), (d) male reproductive effort (spikelets per panicle), (e) asexual recruitment (total new tillers via rhizomatous growth). Data were jittered on the x-axis. The meaning of symbol shading and line types is the same as in figure 1.

The overall effect of density on recruitment ranged from neutral at male-biased sex ratios to weakly positive at female-biased sex ratios. Seed initiation was negatively density-dependent, especially under female-biased sex ratios (figure 3a), reflecting the stronger competitive effect of females (figure 2c). However, positive density dependence prevailed because the strong Allee effect in mating overwhelmed the

negative effects of competition on female reproductive effort. While asexual tiller production showed strong negative density dependence (figure 3c), the number of asexual recruits was 1–2 orders of magnitude lower than the number of seed recruits (compare scale bars in figure 3). Therefore, strong negative density dependence in asexual recruitment was not sufficient to counterbalance the Allee effect in sexual reproduction.

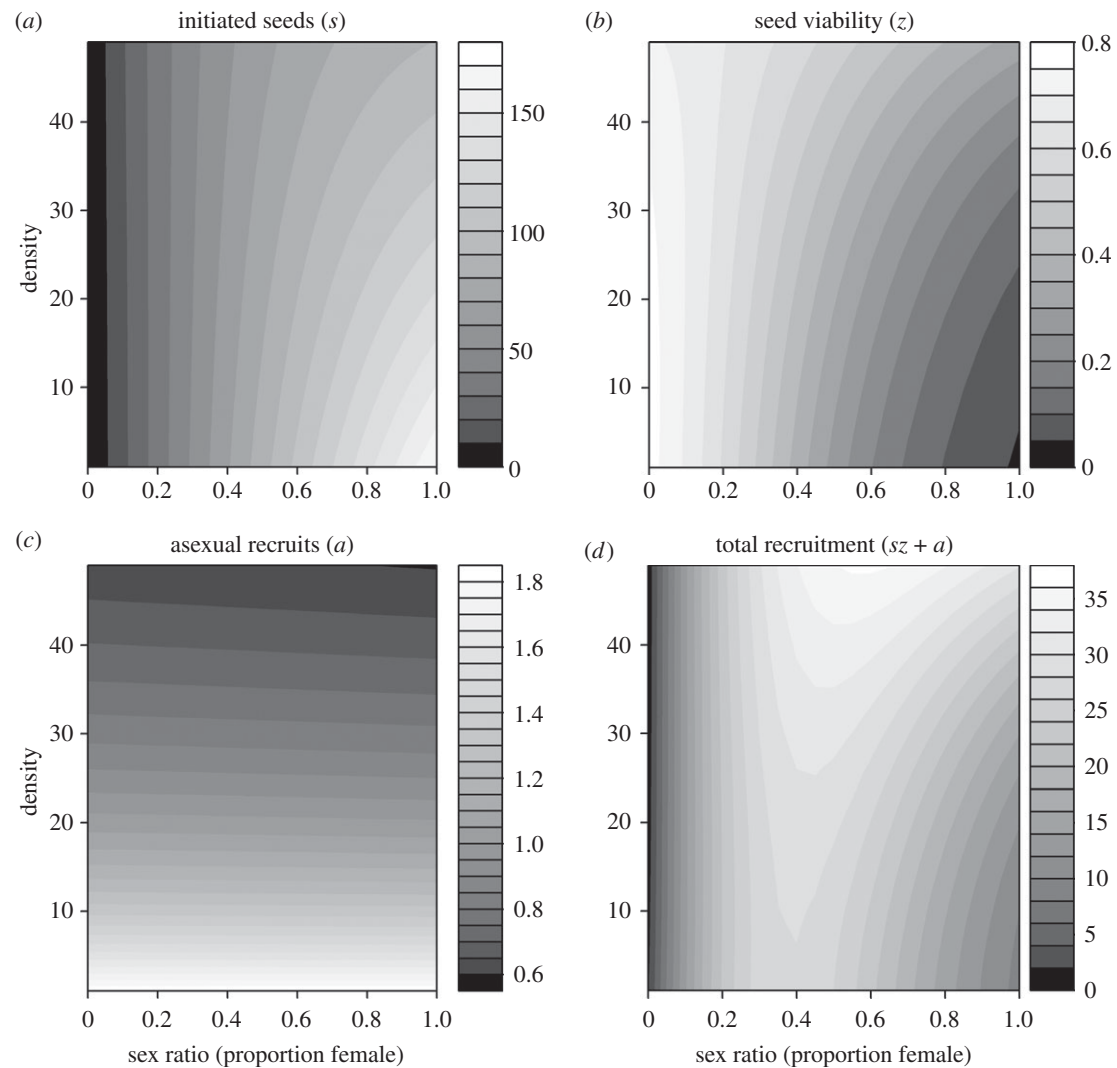


Figure 3. Surfaces showing the effect of density and sex ratio on *per capita* recruitment and its three components. Panels show number of seeds initiated, s (a), seed fertilization probability, z (b), asexual recruitment (number of new tillers), a (c) and total recruitment, $r = sz + a$ (d), all shown *per capita*. Surfaces are based on model-averaged parameter values.

Exploring the sensitivity of our results to realistic variability in seedling survival yielded qualitatively identical results (electronic supplementary material, figure S5).

4. Discussion

Sex is common in nature, but understanding the contributions of intersexual interactions to recruitment and population dynamics is challenging. Past studies of two-sex populations have emphasized the roles of frequency- or density-dependence in mating, but rarely both. Furthermore, mating dynamics do not play out in isolation from antagonistic interactions like competition, yet positive and negative inter-sexual interactions are rarely integrated in ecological studies. Consequently, population ecologists have an incomplete understanding of how the environments that define mating success align with those that define competition. In this study, we estimated the effects of sex ratio, density and their interactions, using a dioecious plant as a convenient model system, to understand how mating and inter-sexual competition combine to determine individual performance and population recruitment. We report three main results. First, mate-finding Allee effects interacted with sex ratio variation, causing female mating success to

increase with density, particularly under female-biased sex ratios (figure 1). Second, we found negative density dependence in other aspects of demographic performance, including evidence for sex-specific density dependence whereby reproductive effort was limited by competition with neighbours of the same sex more so than of the opposite sex (figure 2c,d). Finally, despite evidence for sex-structured competition, mating overwhelmed other demographic processes in determining how density and sex ratio variation affected population recruitment (figure 3). As a result, recruitment was positively density-dependent and the sex ratio (relative abundance of females) that maximized recruitment increased with increasing density, driven by the weakening of mate-finding Allee effects. Collectively, our results provide new insight into the important interaction between density- and frequency-dependence in sex-structured populations, and into tensions that may (or may not) arise between positive and negative components of inter-sexual interactions.

In our experiment, the interaction between density and sex ratio in mating occurred probably because, as density increased, the mean distance that pollen travelled to reach female stigmas decreased. Therefore, the male frequency required to maximize female fertility decreased with density. This pattern could hold for many two-sex populations, because mate-finding Allee

effects are common in nature. For example, mate-finding Allee effects occur in sessile organisms, such as plants [34] and marine invertebrates [35], as well as mobile organisms [36] albeit mediated through different mechanisms, such as reproductive asynchrony [16]. While the large literature on mate-finding Allee effects generally focuses on total density, our results suggest that sex ratio variation is a potentially important consideration. The interaction between mate-finding Allee effects and sex ratio fluctuations may be relevant in many ecological contexts, including low-density invasion fronts of species with sex-biased dispersal [5], and small populations subject to stochastic variation in sex ratio [15].

Demographers often model fertility using frequency-dependent 'mating functions' [10,12,37], assuming that the sex ratio at which fertility peaks is density-independent. We know of only two theoretical studies that proposed mating functions which are simultaneously density- and frequency-dependent [8,15]. On the other hand, a recent theoretical model that studied mating using an individual-based approach, rather than a formal mating function, concurs with our inference that fertility peaks depend on the interaction between frequency and density [6]. Our results suggest that studying the interaction between sex ratio and density in mating warrants more theoretical and empirical work. Additionally, it would be valuable to know whether interactions between density and frequency are more likely in sessile versus mobile organisms, the latter of which may be more efficient in locating mates.

By decomposing total recruitment into multiple demographic components, we showed that positive density dependence in mating outweighed other negative density-dependent processes. The literature emphasizes the interplay of positive and negative density-dependent processes in determining Allee effects at the population level [38]. This literature distinguishes positive density dependence in single fitness components and in total fitness as, respectively, 'component' and 'demographic' Allee effects. Density will increase total fitness only if the component Allee effects outweigh other negative density-dependent demographic processes. We found that positive density dependence prevailed across our observed range of densities, and was especially strong at high female frequencies. However, these patterns do not guarantee that a 'demographic' Allee effect operates in natural populations of our focal species. Recruitment values do not reveal the population growth rate, which would include life-cycle transitions not contemplated in our study. Because our experiments were initiated with greenhouse-raised plants, we know little about early survival and growth, particularly for sexual recruits. We partly addressed this by accounting for a range of realistic seedling establishment rates (electronic supplementary material, figure S5). Yet, negative density dependence in early seedling survival and growth could partly or entirely offset the Allee effects in viable seed production. Finally, despite what is suggested by figure 3d, recruitment would not increase indefinitely with density. Because positive density dependence in mating asymptotes at maximum seed viability, negative density dependence will eventually overwhelm mate-finding Allee effects as density increases. Extrapolating the results of our model, negative density dependence in *per capita* recruitment should occur at a density of 90 individuals per plot, more than double our maximum experimental density treatment.

Sex differences in baseline demographic rates are common in both animals [12] and plants [39], and can generate skew in the adult sex ratio relative to the birth sex ratio [40]. We were

therefore surprised to find that the vital rates we considered showed no sexual dimorphism (but see [41]). Additionally, theory suggests that sex differences in competitive ability can also affect population dynamics and sex ratios [9]. Previous studies of inter-sexual antagonism include cases where sex differences in competitive effects can lead to the disappearance of one sex [19,42]. Conversely, competition between the sexes could stabilize sex ratios [43]. In our study, there was little evidence for sex-structured competition in most aspects of demographic performance. The exception was female reproductive effort (seeds initiated per panicle), which was lower in high-density, female-biased plots, because females were more strongly affected by competition with same-sex neighbours; male reproductive effort showed a similar but weaker pattern of self-limitation. This result is consistent with previous studies of dioecious plants that report greater competitive effects of females [17,18]. We do not know what mechanisms might underlie this sex difference in competitive effect. In principle, greater self-limitation in females could lead to male-biased sex ratio optima, where females could escape female competition and maximize seed fertilization. However, female self-limitation was not strong enough in this system to counteract the overall greater seed production in female-biased populations, which maximized *per capita* regeneration. Furthermore, the competitive interactions we detected are unlikely to generate feedbacks between sex ratio and competition, as have been explored theoretically [9], because self-limitation of female seed production would not affect sex ratio. This observation, combined with the lack of baseline demographic differences between the sexes, suggests that, under the conditions of this study, the primary sex ratio of seeds is probably the main determinant of the adult sex ratio. This contrasts with recent studies of plants [44] and animals [45] that demonstrate deviations of the adult sex ratio from the primary sex ratio due to sex-specific demography. The primary sex ratio of seeds is 1:1 in Texas bluegrass (J Goldman [USDA-ARS] 2016, personal communication), yet our surveys reveal substantial variation in the operational (mating) sex ratio of natural populations (mean % female = 48, minimum = 0.9, maximum = 100). We do not yet know whether this reflects stochastic variation or sex-specific demography that is expressed under particular environmental conditions.

The evidence for sex-structured competition, whereby females produce more seeds at low female densities, raises new questions. First, such sex-structured competition may reflect niche partitioning, which occurs when the sexes compete more strongly with individuals of the same sex. However, few studies addressed niche partitioning between the sexes (e.g. [17,45]), so it is not known how common these patterns are in nature. Second, higher seed production at low density could be an adaptive, compensatory mechanism in species that experience Allee effects. In these species, selection might favour higher reproductive potential at low densities to compensate for lower reproductive success, but no study, to our knowledge, has tested this idea.

In conclusion, our work with a dioecious plant demonstrates, for the first time to our knowledge, how multiple demographic processes, sensitive to both density and sex ratio, combine in two-sex populations. We show that mate-finding Allee effects give rise to positive density dependence, exacerbated under female-biased sex ratios. In principle, Allee effects in mating could be offset by demographic rates linked to somatic (non-mating) demographic function,

which should respond to density negatively. However, we found that mate-finding Allee effects overwhelmed other sources of negative density dependence. Extending joint consideration of positive and negative inter-sexual interactions to other taxa that vary in mobility, behaviour and mating system would advance understanding in the growing field of two-sex population biology.

Data accessibility. Data and R code for the analyses are available on dryad at <http://dx.doi.org/10.5061/dryad.h3b41> [46].

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