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Mating consequences of contrasting hermaphroditic plant sexual systems

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For hermaphroditic angiosperms with multiple flowers, the sex roles can be exclusively combined in bisexual flowers (monocliny), strictly separated among different flowers (monoecy), or arrayed in mixtures of bisexual flowers with female flowers (gynomonoecy) or male flowers (andromonoecy). The hypothesized benefits favoring the evolution of these contrasting hermaphroditic sexual systems are typically examined individually, usually by assessing success through only one sex role. We tested predictions of most hypotheses experimentally with an andromonoecious species, *Anticlea occidentalis* (Melanthiaceae), based on the performance of intact plants (andromonoecy) and those with emasculated bisexual flowers (functionally monoecious) or emasculated male flowers (functionally monoclinous with sterile peripheral flowers). Andromonoecy in this species enables efficient, size-dependent resource allocation, emphasizing female function in large plants. Emasculation revealed that anthers in male flowers promote female mating quality (outcrossing rate and mate diversity), whereas anthers in bisexual flowers promote male mating quantity (pollen dispersal distance and probability of any siring success). Thus, different hermaphroditic sexual systems likely evolve to capitalize on suites of benefits, rather than just one, and provide compromises between quantitative and qualitative reproductive components. These compromises apparently maximize an individual's combined genetic contributions through female and male functions, rather than separate contributions through each sex role.

KEY WORDS: Andromonoecy, angiosperm, hermaphrodite, monocliny, monoecy, sexual systems.

Flowering plants exhibit unparalleled diversity of sexual systems, or gamete packaging within and among individuals (Barrett 2002a). This diversity mostly involves monomorphic populations with only hermaphroditic individuals, as only ~6% of angiosperm species have polymorphic sexual systems with mixtures of hermaphroditic, female and/or male individuals (Renner 2014). By definition, each hermaphrodite can contribute alleles to the next generation as both a maternal and a paternal parent. Hermaphroditism, coupled with production of multiple flowers per individual and the composite nature of individual flowers (perianth whorls, androecium, and gynoecium), allows considerable scope for plastic and evolutionary modification of the organization of female and male functions on individual plants (see Lloyd and Bawa 1984; Diggle et al. 2011; Torices et al. 2011; Diggle 2014). Importantly, this combination of characters enables division of labor among flowers, including pollinator attraction (bisexual and sterile flowers: Morales et al. 2013) and the sex roles (e.g., Harder et al. 2000; Vallejo-Marín and Rausher 2007a, b; Mamut et al. 2014), and reduces sexual interference (Barrett 2002b).

Sexual systems evolve by modification of sex-allocation patterns within and among individuals (Charlesworth and Charlesworth 1979; Charnov 1982) in response to the prevailing intrinsic and extrinsic conditions that determine reproductive success (e.g., Lloyd and Bawa 1984; Brunet and Charlesworth 1995; McCauley and Brock 1998; Ashman 2006; Zhang 2006). Most hermaphroditic angiosperms produce only bisexual flowers (Torices et al. 2011), and so have a monoclinous sexual system (Sakai and Weller 1999). To the extent that the gynoecium and androecium develop independently (see Diggle et al. 2011), internal gradients of signaling molecules can create systematic variation in floral gender among bisexual flowers within inflorescences

(Diggle 2014). In the extreme, such within-individual variation can generate mixtures within inflorescences of qualitatively different flower types, including bisexual flowers and those that lack a functional gynoecium or androecium or both (Diggle et al. 2011). These mixtures typify several minority hermaphroditic sexual systems, including gynomonoecy (female and bisexual flowers), andromonoecy (male and bisexual flowers), and monoecy (female and male flowers: Sakai and Weller 1999). In addition, abortion of both gynoecium and androecium produces sterile flowers within inflorescences, which occur in a few species that also produce bisexual fertile flowers (e.g., Jin et al. 2010; Barabé 2013) and therefore have a (variant) monoclinous sexual system. Given such capacity to generate within-plant variation, whether selection favors a hermaphroditic sexual system that includes unisexual flowers will depend on the associated advantages compared to monocliny.

The recognized benefits of individual plants producing multiple flower types include effects on mating quantity and/or quality, involve one or both sex roles, and are relevant to several or specific sexual systems (Table 1). Some of these benefits arise directly from resource allocation to the sex roles, including committing resources to female function only in flowers likely to produce fruit (resource efficiency); enabling facultative adjustment of investment on specific sex roles in response to current plant size and/or growth conditions (flexible resource allocation); and adaptively shifting within-plant sex allocation as the population floral sex ratio changes during flowering seasons (gender matching). Other benefits arise indirectly from effects on pollination, including enhanced pollinator attraction; reduced within-flower self-pollination (autogamy) and/or among-flower self-pollination (geitonogamy); reduced interference between anthers and stigmas during pollen removal and deposition and lessening of pollen and ovule discounting (sexual interference); and general promotion of pollen export. In addition to the possible benefits relative to production of only bisexual flowers, Huang (2003) proposed that inclusion of some bisexual flowers within inflorescences provides benefits over monoecy if autonomous autogamy provides reproductive assurance when seed production is pollen limited.

As Table 1 illustrates, various benefits of hermaphrodites producing unisexual flowers can be realized simultaneously (e.g., Harder et al. 2000; Dai and Galloway 2012), depending on plant characteristics, the pollination and mating environments, and abiotic conditions (e.g., compare Elle and Meagher 2000; Vallejo-Marín and Rausher 2007b). Nevertheless, most studies of the effects of different hermaphroditic sex systems in angiosperms have addressed, at most, two possible benefits (see references in Table 1), so their overall adaptive significance is poorly understood. Furthermore, most studies of these sexual systems quantified female productivity (fruit and/or seed production), but not siring success, and they did not consider qualitative aspects of mating performance, as reflected in female and male outcrossing rates and mate diversity. These limitations can lead to spurious conclusions, such as "andromonoecy by itself is not an out-breeding mechanism" (Primack and Lloyd 1980, p. 361), which was subsequently rejected when male success was considered (e.g., Elle and Meagher 2000).

In this article, we compare the reproductive consequences of three deployments of sex function that typify different, naturally occurring hermaphroditic sexual systems. Working in a natural population of the andromonoecious species *Anticlea* (syn. *Stenanthium*) *occidentalis* (A. Gray) Zomlefer & Judd (Melanthiaceae), we emasculated some flowers of subsets of plants, creating treatments representing contrasting sexual systems, which then competed simultaneously for reproductive opportunities. A third of plants were left intact to represent andromonoecy, which is a relatively rare (<2% of angiosperm species) but widespread sexual system, occurring in at least 33 families (Vallejo-Marín and Rausher 2007b). For the second third of plants, we emasculated the basal bisexual flowers (referred to as BE plants), but not the distal male flowers, creating the most common monoecious state with male flowers above female flowers. Monoecy occurs in ∼5% of angiosperm species (Torices et al. 2011) among >130 families (Renner and Ricklefs 1995). For the remaining plants, we emasculated only the male flowers (referred to as ME plants), creating plants with basal fertile bisexual flowers and distal sterile flowers. ME plants had a monoclinous sexual system with respect to their capacity for sexual interference and self-pollination within and among flowers, but differed from most monoclinous species by having sterile distal flowers. We retained the emasculated flowers to maintain the floral display size of these plants, thereby not confounding the effect of monocliny with that of reduced pollinator attraction (see Ohashi and Yahara 2001). Sterile peripheral flowers occur among occasional monoclinous angiosperm species in a few unrelated families, and enhance pollinator attraction (Morales et al. 2013) without aggravating geitonogamy and associated pollen discounting (Harder and Barrett 1996). Note that the two classes of emasculated plants although simulate the arrangements of female and male function among flowers that characterize monocliny and monoecy, the reduced maleness caused by emasculation may not represent the typical gender of plants with these sexual systems.

This experiment tests general and specific hypotheses concerning the advantages of different hermaphroditic sexual systems. It generally assesses the extent to which a sexual system's relative advantage depends on the diversity of its consequences for female and male performance, rather than on a single key influence. It also evaluates expected advantages associated specifically with monocliny, monoecy, and andromonoecy (see

Table 1. Possible consequences of the inclusion of multiple flower types within hermaphroditic inflorescences compared to inflorescences with only bisexual flowers (monocliny).

Consequence	Mating effect	Affected sex role(s)	Relevant sexual system
Enhanced resource efficiency (no investment in gynoecia that cannot mature into fruits)[5,12]	Quantity	Female—but only with resource limitation	Andromonoecy
More flexible resource allocation to sex roles[3,11,14,16,17]	Quantity	Either or both, depending on seed production limits	Gynomonoecy Andromonoecy Monoecy
Matching of instantaneous plant gender to population mating opportunities[7,10]	Quantity	Both	Gynomonoecy Andromonoecy Monoecy
Enhanced pollinator attraction (larger displays)[13]	Quantity Quality (greater mate diversity)	Both—female quantitative effect requires pollen limitation	Gynomonoecy Andromonoecy Monoecy
Reduced within-flower self-pollination (autogamy)[2]	Quantity (if less pollen discounting) Quality (if less inbreeding depression)	Both Female	Gynomonoecy Monoecy
Reduced self-pollination among flowers (geitonogamy)[1,6,8]	Quantity (less pollen discounting) Quality (if less inbreeding depression)	Male Both	Monoecy
Reduced sexual interference[9,15]	Quantity	Both	Gynomonoecy Andromonoecy Monoecy
Enhanced pollen export (perhaps a secondary effect of other consequences)[1,4,8,18]	Quantity	Male	Andromonoecy

Quantitative mating effects refer to the numbers of seeds produced (female) or sired (male), whereas qualitative effects refer to outcrossing rate, mate diversity, and inbreeding depression. References identified by superscripts provide supporting evidence for the identified consequence.

[1]Dai and Galloway (2012); [2]Davis and Delph (2005); [3]Diggle (1993); [4]Elle and Meagher (2000); [5]Emms (1996); [6]Harder et al. (2000); [7]Huang et al. (2002); [8]Jordan and Harder (2006); [9]Kawagoe and Suzuki (2005); [10]Méndez (1998); [11]Miller and Diggle (2003); [12]Miller and Diggle (2007); [13]Podolsky (1992); [14]Primack and Lloyd (1980); [15]Quesada-Aguilar et al. (2008); [16]Reuther and Claßen-Bockhoff (2013); [17]Sarkissian et al. (2001); [18]Schlessman et al. (2004).

Table 1). Among hermaphroditic sexual systems, the likely benefits of monocliny arise from maximal capacity to function as both a mother and father in the face of variable pollination quantity and quality and resource availability, and the joint contributions of investment in pollinator attraction and reward to both sex roles (Lloyd and Bawa 1984). Compared to monocliny, both monoecy and andromonoecy offer resource-allocation benefits of producing unisexual flowers and reduced sexual interference (Table 1). Monoecy also limits opportunities for autonomy and geitonogamy, whereas andromonoecy should enhance pollen export (Table 1). Finally, for self-compatible species, andromonoecy and monocliny can allow reproductive assurance, unlike monoecy. As this experiment was conducted in a natural *A. occidentalis* population, it compares andromonoecy and simulated monocliny and monoecy in conditions that likely favor andromonoecy.

Methods

STUDY SPECIES AND STUDY DESIGN

Anticlea occidentalis is a bulbous, herbaceous perennial. Reproductive plants produce a single, andromonoecious paniculate inflorescence with bronze, bell-shaped flowers (Fig. S1A, C, D). In addition to the main axis, inflorescences of about a third of plants produce up to three basal axillary branches with a few flowers. Most flowers are bisexual, but some distal (later) flowers lack pistils and are functionally male (Fig. S1C, D). All flowers have tubular perianths with six stamens in their mouths. Bisexual flowers have exserted stigmas and so exhibit approach herkogamy. Flowers open acropetally and are relatively long-lived, lasting a mean ± SD of 11.9 ± 2.2 days (20 plants; unpubl. data). Consequently, most of a plant's flowers are open simultaneously, so that male flowers can contribute to pollinator attraction and geitonogamy when bisexual flowers are open. The bisexual flowers are self-compatible (Ida et al. 2013) and partially protandrous, with a few-day male phase and a longer bisexual phase. Fruits are dry dehiscent capsules that disperse seeds passively (Fig. S1B). At the study site, flowering occurs during July, and seeds mature from mid- to late September. *Anticlea occidentalis* does not propagate clonally: none of the study plants had identical genotypes for seven microsatellite loci.

Despite many hours of observation during multiple years, the pollinators of *A. occidentalis* remain unknown. Anthers are largely not exserted from the perianth (Fig. S1C, D), so wind pollination is unlikely. Although flowers have tubular perianths, they produce no nectar: thus, visitation by insects for pollen and/or shelter seems most likely to cause cross-pollination. The only insects observed visiting flowers are many opportunistic flies, which mostly use the flowers as resting places, rather than probing them purposefully. Self-pollination significantly reduces both fruit set and seed set (Ida et al. 2013), indicating that this species is subject to at least predispersal inbreeding depression. Studies during 2010–2012 within 500 m of our study site indicated chronic pollen-limitation of female success by *A. occidentalis*, as only 35–42% of naturally pollinated plants produced any fruit, 46–57% of flowers on fruiting plants set fruit, and fruits contained a mean of 2.7–9.2 seeds, despite flowers producing >40 ovules (T. Y. Ida and L. D. Harder, unpubl. data). This low female success, even though individual flowers last an average of 12 days, indicates that whatever pollinates *A. occidentalis* locally visits flowers very sporadically. Furthermore, the chronic pollen limitation likely results in plants competing for mating opportunities through both female and male functions.

We studied *A. occidentalis* near the University of Calgary's Barrier Lake Field Station in the Kananaskis Valley, Alberta, Canada (51°03′ N, 115°03′ W) during 2014. The study area covered approximately 50 × 80 m (0.4 ha) in a mixed forest (*Populus tremuloides* Michx., *Picea glauca* (Moench) Voss, *Pinus contorta* Douglas ex Loudon). This site was chosen because plants were separated from conspecifics by ≥50 m. This separation was expected largely to isolate the study plants from nonstudy conspecifics, based on the generally short-distance pollen dispersal of other herbs (see Appendix S1). For example, mean intermate distances reported for two other forest Melanthiaceae were 2.0 m for *Trillium cuneatum* (Gonzales et al. 2006) and 9.5 m for *Chamaelirium luteum* (Meagher 1986). Furthermore, mating between *C. luteum* plants separated by <10 m occurred more frequently than expected given the distribution of distances between all pairs of female and male plants. We studied all 163 reproductive plants within the study area (study plants), which had an aggregated distribution (Fig. 1A). To account for possible spatial heterogeneity in reproductive conditions and quantify intermate distances we mapped the study plants by triangulation with a Leica DISTO$^{\text{TM}}$ E7400x laser distance meter.

As described above, our experiment imposed simultaneous competition for mating opportunities between andromonoecy (intact plants), simulated monoecy (BE plants), and simulated monocliny with sterile distal flowers (ME plants). This design assessed the relative merits of these sexual systems under identical conditions, which would not be possible if each system was considered alone in a different site or period of the flowering season. To eliminate possible confounding associations between the effects of a plant's local environment and its experimental treatment, we selected trios of adjacent plants with incipient inflorescences prior to flowering and randomly assigned one plant to each treatment. Flowers were emasculated by removing the undehisced anthers (but not the filaments) on the day of anthesis. During flowering, nine plants (three of each treatment) did not produce any male flowers. These plants were excluded from analyses of mating outcomes because they did not adequately represent any experimental treatment, leaving 154 "experimental plants" ($n = 51$ intact, 52 BE, 51 ME). Nevertheless, the nine nonexperimental plants still served as possible paternal parents in analyses of female success and as possible maternal parents in analyses of male success.

PLANT AND FLORAL TRAITS

For all plants, we measured the basal diameter of the flowering scape (stem) to represent overall reproductive effort, and the positions, number, and sizes of each flower type as well as their pollen and ovule production to represent allocations to female and male functions. Corolla length (L) and width (W) were measured on the day of anthesis for each flower with digital calipers and used to estimate flower volume ($\pi L[W/2]^2$) as a general measure of flower size. Anthers removed from BE and ME plants during experimental emasculation were used to quantify pollen production by bisexual and male flowers. Undehisced anthers were collected in microcentrifuge tubes and allowed to dehisce naturally before being preserved and stored in 70% ethanol. We counted pollen from a random subsample of flowers from the top, middle, and bottom positions on the main inflorescence axis using an Elzone 5380 particle analyzer (Micromeritics Instrument Corporation, Norcross, GA) following Harder (1990). Because anthers were sampled from bisexual flowers of only BE plants and from male flowers of only ME plants, treatment and flower type were confounded. We ascribe any differences in pollen production to flower type because application of the emasculation treatments could not have affected pollen production.

We measured several components of female reproductive success. Stigmas from experimental plants were collected during early fruit development to assess pollen deposition. Stigmas from the first and last perfect flower of every plant from each treatment were collected and preserved in 70% ethanol. Pollen grains on these stigmas were stained with basic fuchsin dye (Beattie 1971)

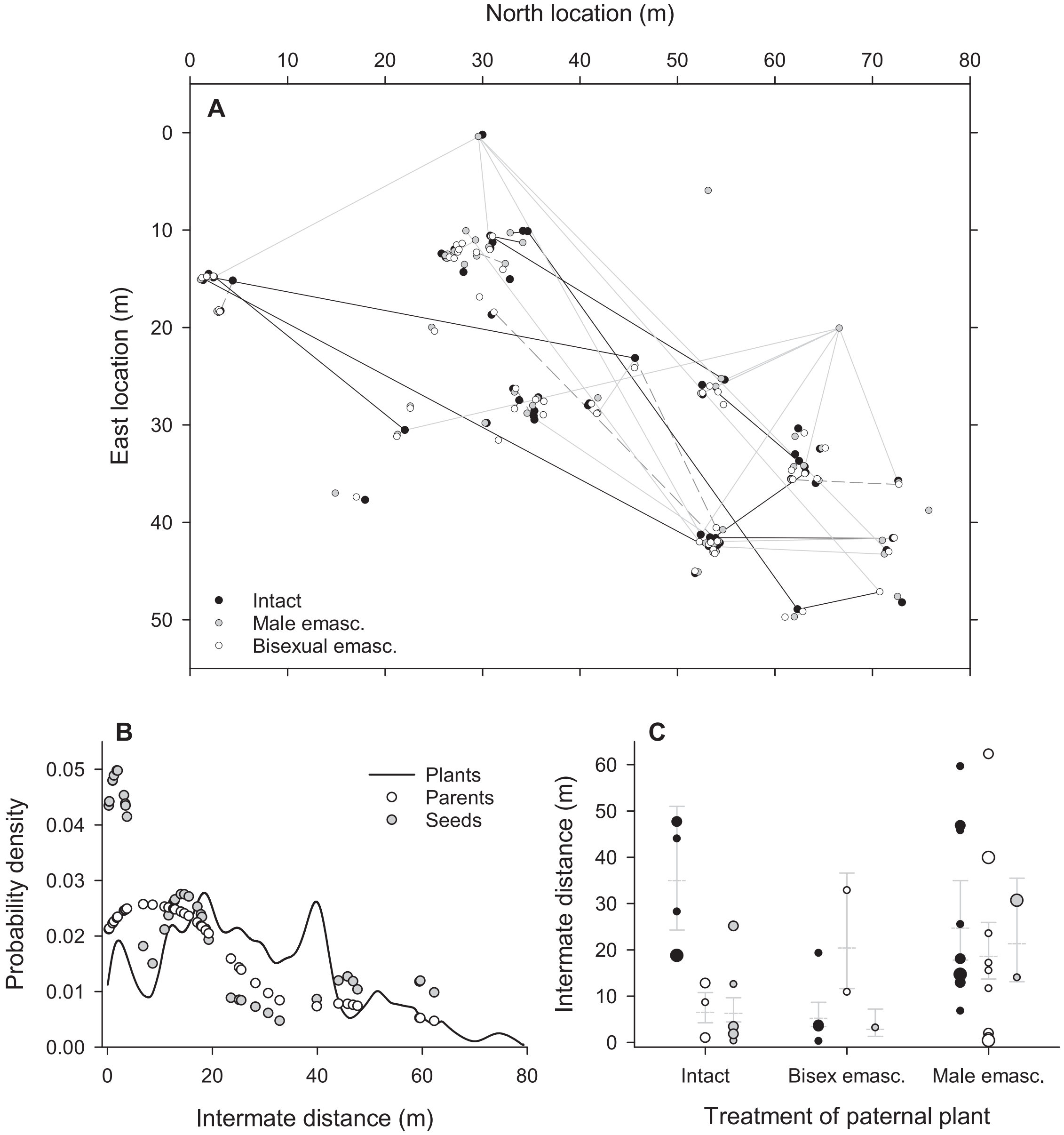

Figure 1. (A) Distribution of 163 *Anticlea occidentalis* plants within the study area, with lines linking mating between known maternal and paternal plants, (B) the frequency distributions of interplant and intermate distances, and (C) the relation of intermate distances to the experimental treatments experienced by the maternal and paternal mates. In (A), line characteristics identify the experimental treatment of the paternal plant (black solid line, intact flowers; dashed line, emasculated bisexual flowers; gray solid line, emasculated male flowers). In (B), the black curve represents the probability density distribution (PDD) of distances between all study plants, and the symbols depict PDDs for intermate distances, either unweighted (open symbols), or weighted by the number of seeds for which both plants were the parent (gray symbols). In (C), symbols represent individual matings between paternal plants that were subjected to the treatments identified by the ordinate labels and maternal plants that were subjected to treatments identified by symbol color (black, intact flowers; white, emasculated bisexual flowers; gray, emasculated male flowers). Symbol size depicts the number of seeds sired on the respective female mate (1, 2, or 3). Gray lines illustrate least-squares means (±SE) back-transformed from ln estimates, hence the asymmetrical standard errors.

and counted using a compound microscope. All mature fruits on experimental plants were collected and counted. Unfertilized ovules, aborted seeds, and seeds were also counted: their sum equals total ovule production.

MATING-SYSTEM ESTIMATION

The genotypes of all study plants and 271 seeds (18.2% of total seed production) were determined using seven polymorphic microsatellite loci (see Appendix S2) to quantify outcrossing rates and identify male parentage. One leaf was collected from each flowering plant in September, when fruit were almost mature, and dried with silica gel. From each maternal plant that produced a fruit we randomly selected six seeds, or used all seeds if fewer than six were available. Genomic DNA was extracted from dried leaf tissue and seeds using a standard cetyltrimethlammonium bromide (CTAB) protocol (Doyle and Doyle 1987). Intact seeds were assayed, as no genomic DNA and amplification products were detected for maternal seed-coat tissue removed from a sample of two seeds from each of four maternal plants. Amplified microsatellite loci were analyzed with an Applied Biosystems 3500xl Genetic Analyzer with LIZ500 size standard (Applied Biosystems, Foster City, CA). Alleles for all loci were scored manually with GeneMapper v3.2 software (Applied Biosystems).

We estimated the probability that each seed was outcrossed and inferred its most likely father with Colony 2.0.6.1 (Jones and Wang 2010; Wang et al. 2012). This program infers parentage and sibships jointly by maximizing the likelihood of the inferred pedigree of sampled individuals. The analysis incorporated estimates of genotyping error rates (Hoffman and Amos 2005) for each locus (0–1.2%, median 0.4%) based on regenotyped DNA from 95 seeds selected randomly from 943 seeds from a separate experiment (unpubl. ms.). Whether seeds were outcrossed or selfed was determined with considerable certainty, as the estimated probability of outcrossing was either 0 or 1 for all but five of the 271 assayed seeds. The inferred probability that a seed was outcrossed and the identity of its most likely father were the basic observations in statistical analyses of mating outcomes.

STATISTICAL METHODS

Most analyses of mating outcomes involved generalized linear (mixed) models (Stroup 2013) as implemented in the glimmix or genmod procedures of SAS/STAT 14.1 (SAS Institute Inc. 2015). These analyses considered sampling distributions and link functions appropriate for the characteristics of the dependent variables (see Table S1). All analyses considered emasculation treatment as a fixed factor (or the emasculation treatments of both maternal and paternal plants for inter-mate distance), except that for pollen production per flower, which instead included flower type as a fixed factor. Analyses of floral traits involved only flowers on the main axis of inflorescences. Some analyses also included other independent variables (see Table S1) to control for the effects of nuisance variables (e.g., number of genotyped seeds in the analysis of female-mate diversity), or to assess the effects of variables relevant to specific hypotheses (e.g., ln[stem diameter] to analyze size-dependent resource allocation).

Plant nested within treatment was included as a random effect in two situations: to account for repeated measurement for flower-level analyses; and to model covariance in plant-level responses associated with spatial proximity (Stroup 2013: see Table S1). In the latter case, the covariance among pairs of plants was modeled as a declining power function of the distance between them ($\sigma^2\rho^d$, where σ^2 is the overall variance of the dependent variable, ρ is the general correlation of the dependent variable among plants, and d is the specific interplant distance for a mate pair). When the random plant effect was included, the Laplace approximation was used to estimate the conditional fixed effects (Stroup 2013). Results of associated tests are presented in Appendix S3.

Two analyses involved underdispersed distributions, requiring alternative statistical approaches, which we implemented with the nlmixed procedure of SAS/STAT 14.1 (SAS Institute Inc. 2015). First, zero is not possible for the proportion of pistillate flowers that set fruit on plants that produced at least one fruit, so fruit set could not follow a binomial distribution. Therefore, we used the zero-truncated binomial distribution (see Appendix S4) to estimate the mean logit of fruit set for the three treatments and compared them pairwise using the delta method (Cox 1998), accounting for multiple comparisons with the Dunn–Šidák procedure (Kirk 1995). The Akaike's Information Criterion (AIC) for the zero-truncated binomial distribution was 14.3 units smaller than that for the binomial distribution, indicating that the former provided a superior fit to the data. Second, initial analysis of the outcross mate diversity of plants that sired seeds revealed considerable underdispersion compared to a Poisson distribution, precluding analysis with a standard generalized linear model. To address this problem, we fit the data to a double Poisson distribution, which can represent either an overdispersed distribution ($\theta < 1$) or an underdispersed distribution ($\theta > 1$: Efron 1986). The nonlinear models depicted variation in the number of a plant's female outcrossed mates (M) as a power function of the number of seeds that it sired, S, or $M = aS^b$. We assessed treatment effects by comparing the maximum likelihoods for models with separate or common estimates of a for the three treatments using a likelihood-ratio (G) test. For the best-fitting model, $\hat{\theta} = 11.04$ (95% confidence interval, 3.76–18.31), indicating significant underdispersion compared to a Poisson distribution.

We illustrate the spatial extent of mating relative to plant dispersion based on the probability density distributions (PDDs) of distances between all pairs of plants and between mates. PDDs were estimated using nonparametric density estimation (Silverman 1986; Sheather 2004), as implemented in the KDE procedure of SAS/STAT 14.1 (SAS Institute Inc. 2015). We estimated two PDDs for intermate distance, one based on the distance between parents and the other weighted by the number of seeds genotyped for the maternal plant that were sired by the paternal plant.

We present results from generalized linear models back-transformed from the scale of measurement of the associated link function. This generally produces asymmetric standard errors and confidence intervals. For categorical variables, notably experimental treatment, we present least-squares means, which represent effects in isolation from those of other independent variables included in a linear model.

Results

PLANT AND FLORAL CHARACTERISTICS

The characteristics of *A. occidentalis* plants and flowers set the context for the phenotypic impact of the experimental manipulations. On average (±SD), the study plants produced 9.2 (±3.91) bisexual flowers and 5.6 (±3.72) male flowers. The main inflorescence axis produced mainly bisexual flowers (median 8 vs. 3 male flowers), whereas axillary branches produced mainly male flowers (median 2 vs. 0 bisexual flowers). Of the 163 study plants, 35 produced flowers only on the main axis, whereas the others also produced flowers on up to three axillary branches (median = 1). Larger plants, as measured by ln(stem diameter), produced more flowers than smaller plants ($F_{1,160} = 26.78$, $P < 0.001$; partial regression coefficient, $b \pm \mathrm{SE} = 0.531 \pm 0.103$). However, this effect resulted solely from a positive association for bisexual flowers ($t_{160} = 6.45$, $P < 0.001$; $b \pm \mathrm{SE} = 0.883 \pm 0.137$), as male-flower number varied independently of stem diameter ($t_{160} = 1.17, P > 0.2$: overall interaction $F_{2,182} = 11.76, P < 0.001$; Fig. 2A). Consequently, large plants were phenotypically more female than small plants (see Appendix S5). For the 154 experimental plants, neither total flower number ($F_{2,151} = 0.99$, $P > 0.9$) nor the proportion of bisexual flowers ($F_{2,151} = 0.33$, $P > 0.7$) varied significantly among the three treatments.

Bisexual and male flowers differed in several aspects of reproductive investment and allocation. As axillary inflorescence branches produced few bisexual flowers, we compared the characteristics of flowers on only the main axis. Based on average volume, male flowers were smaller than bisexual flowers ($F_{1,695.2} = 9.44$, $P < 0.005$; Fig. 2B), but the size difference varied with flower position within the inflorescence (interaction, $F_{1,929.7} = 5.18$, $P < 0.025$). Male flowers had relatively similar flower volume regardless of flower position ($b \pm \mathrm{SE} = -13.81 \pm 10.93$, $t_{950.9} = 1.26$, $P > 0.2$), whereas the mean size of bisexual flowers declined steadily with position along an inflorescence ($b \pm \mathrm{SE} = -39.17 \pm 2.54$, $t_{607.6} = 15.44$, $P < 0.001$; Fig. 2B). Flower volume did not differ significantly among treatments ($F_{2,123.2} = 1.08, P > 0.3$). On average, bisexual flowers produced 43.9 ovules (LSE = 2.23, USE = 2.35). Ovule number declined with ln(flower position), like overall flower size (Fig. 2C: $b \pm \mathrm{SE} = -0.128 \pm 0.060$, $F_{1,124} = 4.61$, $P < 0.05$), and varied positively with ln(stem diameter) ($F_{1,124} = 8.00$, $P < 0.01$; 0.567 ± 0.200), but did not differ significantly among treatments ($F_{2,57} = 3.53$, $P > 0.05$). Overall, male flowers at middle and top positions (lower flowers were all bisexual) produced more pollen than bisexual flowers ($F_{1,54.5} = 12.38$, $P < 0.001$; Fig. 2D) and this difference was most evident for top flowers (flower type × position interaction, $F_{1,58.9} = 5.33$, $P < 0.025$). Pollen production per flower also varied positively with ln(stem diameter) ($F_{1,54.5} = 16.41$, $P < 0.001$: $b \pm \mathrm{SE} = 0.907 \pm 0.229$).

OVERALL MATING PATTERNS

Of the 245 seeds genotyped from the 54 fruiting experimental plants, 98 were selfed and 147 were outcrossed. Selfed seeds were identified in the fruits of 32 of these plants. Outcrossed seeds represented mating of 102 unique pairs of maternal and paternal plants. Of these pairs, 39 (61 seeds) involved paternal plants within the study area and the remaining 63 pairs (83 seeds) involved fathers beyond the study area. In general, the incidence of mating declined with distance (Fig. 1B), and a median of 14.7 m separated known maternal and paternal parents of seeds (mean = 19.1 m; Fig. 1B, open symbols). Overall, known intermate distances greatly exceeded the distances between neighboring plants (median = 0.36 m, mean = 0.70 m), reflecting the predominance of mating between, rather than within, plant clusters (Fig. 1A). Nevertheless, mating among plants within 10 m of each other occurred more often than expected given the frequency of interplant distances, whereas mating among plants 20–40 m away was less common than expected (Fig. 1B, compare open symbols and black curve), especially when weighted by the number of seeds per sire (Fig. 1B, gray symbols).

The treatments experienced by both the maternal and paternal mates had significantly interacting effects on intermate distance ($F_{4,22} = 4.09$, $P < 0.025$). This result reflects generally greater intermate distance between plants subjected to the same treatment, as well as overall farther pollen dispersal from ME plants (Fig. 1C).

FEMALE REPRODUCTIVE SUCCESS

All but six of the 129 examined stigmas received pollen, with mean = 11.7 grains and median = 7.0 grains (maximum = 207 grains). Whether a bisexual flower received pollen did not differ among treatments (Table 2) or whether it was located on the main inflorescence axis or an axillary branch ($F_{3,35} = 0.02$, $P > 0.99$). Similarly, pollen receipt per flower did not vary significantly among treatments (Table 2), with ln(total flower number) ($F_{1,35} = 0.01, P > 0.9$) or the proportion of male flowers ($F_{1,35} = 0.75, P > 0.3$). Unsurprisingly, pollen receipt per flower varied positively with the ln(number of stigmas) that flowers contained ($F_{1,35} = 7.07, P < 0.025$).

Damage by a hailstorm during late flowering (August 3) affected fruit production by many plants. Hail broke the inflorescence pedicels of 21 experimental plants, distributed randomly among the treatments ($F_{2,151} = 1.16$, $P > 0.3$). Hail also broke some flowers from 50.4% of the remaining plants, including 32.6% (LSE = 6.5%, USE = 7.3%) of intact plants and 58.9% (LSE = 5.2%, USE = 5.0%) of plants in the two

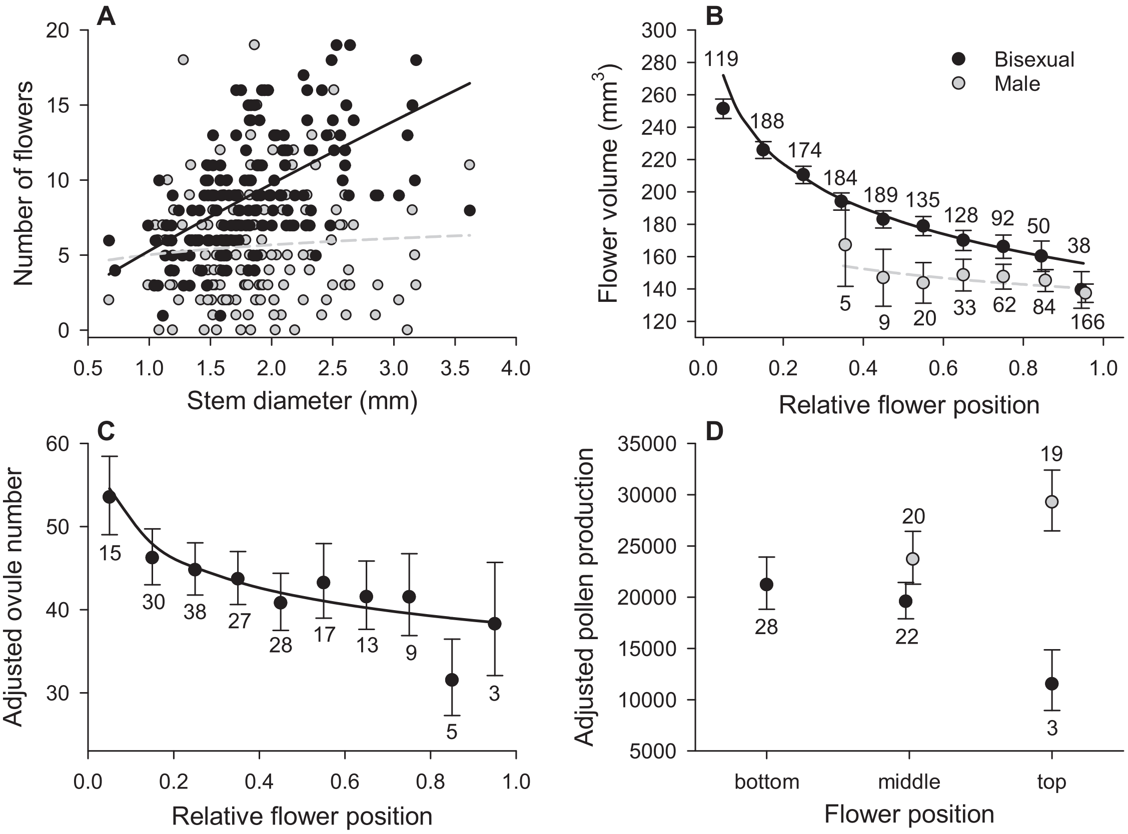

Figure 2. (A) Size-dependent variation in production of bisexual and male flowers among the 163 study plants, and within-inflorescence variation in least-squares mean (±SE), (B) flower volume (163 plants), (C) ovule production (61 plants), and (D) pollen production for *Anticlea occidentalis* (36 BE plants, 32 ME plants). In all panels, black and gray symbols represent bisexual and male flowers, respectively. Numbers in (B)–(D) indicate the numbers of sampled flowers. In (B) and (C), relative flower positions 0 and 1 refer, respectively, to bottom and top flowers within inflorescences. Values in (C) and (D) are adjusted for variation associated with plant size, as measured by the basal diameter of the inflorescence stem. Results in (C) and (D) are back-transformed from ln estimates, hence the asymmetrical standard errors.

emasculation treatments ($F_{2,130} = 3.90$, $P < 0.025$), which experienced damage with equivalent frequency ($F_{1,130} = 0.01$, $P > 0.9$). On average, partially damaged plants lost 2.6 bisexual flowers (LSE = 0.30, USE = 0.33), with no difference among treatments ($F_{2,64} = 0.25$, $P > 0.75$). None of these aspects of hail damage varied significantly with plant size, as measured by ln(stem diameter) ($P > 0.15$ in all cases).

The studied plants experienced very limited female reproductive success. Of the 133 plants with undamaged inflorescences after the hailstorm, only 63 (47.4%) produced ≥1 fruit, with no treatment difference in the incidence of fruiting plants (Table 2). Whether a plant set any fruit varied positively with ln(stem diameter) ($F_{1,129} = 4.73$, $P < 0.05$). For undamaged plants, including those that failed to produce fruit, only 7.1% (lower standard error [LSE] = 1.32%, upper standard error [USE] = 1.59%) of bisexual flowers produced fruit, with no difference among the three treatments (Table 2). For undamaged fruiting plants, 24.4% of bisexual flowers set fruit, with significant differences among the treatments (likelihood-ratio test, $G_2 = 11.54$, $P < 0.01$; Fig. 3A). Fruiting intact plants had significantly higher fruit set than fruiting BE plants ($t_{63} = 3.41$, $P < 0.005$). Fruit set by fruiting ME plants lay between these extremes and did not differ significantly from either ($P > 0.2$ in both cases). Fruit set on the main inflorescence axis of undamaged fruiting plants varied significantly with a bisexual flower's relative position ($F_{8,389.6} = 5.24$, $P < 0.001$), being highest for the basal 50% of flowers (Fig. 3B). In addition, fruit set on the main axis varied negatively with its production of male flowers ($F_{1,61.9} = 9.78$, $P < 0.005$; Fig. 3C). In particular, inflorescences in which the main axis naturally lacked male flowers produced 24.7% of all fruits.

Even though individual bisexual flowers produced an average of 44 ovules, fruits produced an average of only 5.5 seeds (LSE = 0.57, USE = 0.65: after accounting for a positive effect of ln[ovules], partial regression coefficient ± SE = 1.09 ± 0.216, $t_{98} = 5.05$, $P < 0.001$). For all surviving plants, total seed production per plant ranged from 0 (52.6% of plants) to

Table 2. Statistical comparisons of female and male reproductive outcomes for plants with intact flowers (I), emasculated bisexual flowers (BE), and emasculated male flowers (ME).

	Female success		Male success	
Outcome	Test statistic	Differences	Test statistic	Differences
Probability of pollen receipt per flower	$F_{2,88} = 0.46$			
Pollen receipt per flower	$F_{2,87} = 0.33$			
Probability of plant setting/siring fruit	$F_{2,129} = 0.57$		$F_{2,151} = 3.39^{*}$	BE^{a} I^{ab} ME^{b}
Proportion of flowers setting fruit	$F_{2,130} = 0.70$			
Seeds set/sired[1]	$F_{2,51} = 0.63$ $F_{2,122} = 0.04$		$G_2 = 11.62^{**}$ $G_2 = 5.82$	BE^{a} I^{ab} ME^{b}
Outcrossing rate	$F_{2,51} = 6.40^{**}$	ME^{a} I^{ab} BE^{b}	$F_{2,46} = 0.22$	
Number of outcross mates	$G_2 = 8.96^{*}$	ME^{a} I^{a} BE^{b}	$G_2 = 2.80$	

For tests that detected significant differences, the treatments are ordered from smallest to largest least-squares means and contrasting superscript letters identify the means that differ significantly based on Tukey's tests ($P < 0.05$). Figure 4 presents the specific details of significant mean differences, except those for seed siring for which differences arose largely because of treatment effects on the probability of a plant siring any fruit. See Appendix S3 for statistical results for among-plant variation and spatial covariation.

$^{*}P < 0.05$; $^{**}P < 0.01$

[1] The first analysis for female success considered all seeds produced per flower, whereas the second considered total seeds per plant. The analyses for male success assessed a plant's genetic contributions to genotyped seeds: the first test involved all plants, whereas the second test involved only plants that sired some seeds.

204 seeds, with an overall mean of 10.3 seeds (LSE = 2.23, USE = 2.85). Neither seed production per fruit nor total seed production differed significantly among treatments (Table 2).

In contrast to the equivalent seed production, the female outcrossing rate differed significantly among treatments (Table 2, Fig. 4A). BE plants had a significantly higher female outcrossing rate ($t = 0.841$) than ME plants ($t = 0.426$: $t_{51} = 3.57$, $P < 0.001$; Tukey's test), with plants with intact flowers intermediate ($t = 0.628$) and not significantly different from either extreme. Of the average 4.5 seeds genotyped per seed-producing plant, 2.7 were outcrossed, being sired by a mean of 1.8 males (Fig. 4B).

After controlling for the number of genotyped seeds ($G_1 = 12.66$, $P < 0.001$), the number of pollen donors with which a plant outcrossed (male-mate diversity) differed significantly among treatments (Table 2). Based on the genotyped seeds, emasculation of male flowers reduced the number of pollen donors with which a plant mated by 40% compared to intact plants ($Z = 2.53$, $P < 0.05$; Tukey's test), whereas emasculation of bisexual flowers did not affect male-mate diversity ($Z = 0.12$, $P > 0.99$; Fig. 4B). These effects are consequences of the overall effect of the emasculation treatments on total female outcrossing, as a treatment effect was not detected ($G_2 = 2.35$, $P > 0.3$) in an analysis that accounted for variation in the number of outcrossed seeds, rather than the total number of genotyped seeds.

MALE REPRODUCTIVE SUCCESS

Of the 154 experimental plants, only 49 plants (31.8%) sired 161 of the 250 genotyped seeds (selfing and/or outcrossing). An additional 47 plants not included in the study population were also inferred as fathers of the genotyped seeds. The incidence of experimental plants siring seeds differed significantly among treatments (Table 2, Fig. 4C): ME plants were twice as likely to sire genotyped seeds as BE plants ($t_{151} = 2.54$, $P < 0.05$; Tukey's test). The incidence of plants with intact flowers siring genotyped seeds was intermediate between these extremes but did not differ significantly from either ($P > 0.2$ in both cases). The number of genotyped seeds that plants sired differed significantly among treatments for all experimental plants, but not for the subset of plants that sired some seeds (Table 2). Thus, variation in siring success depended primarily on whether a plant was a father or not, rather than on the number of seeds sired per father. Successful fathers sired an average of 2.3 genotyped seeds (LSE = 0.24, USE = 0.27).

The estimated male outcrossing rate (outcrossed seeds/total seeds sired, based on the genotyped seeds sired by individual plants), 0.393 (LSE = 0.059, USE = 0.063), was lower than the female outcrossing rate. In contrast to both overall siring success and the female outcrossing rate, the (detected) male outcrossing rate did not differ significantly among treatments (Table 2).

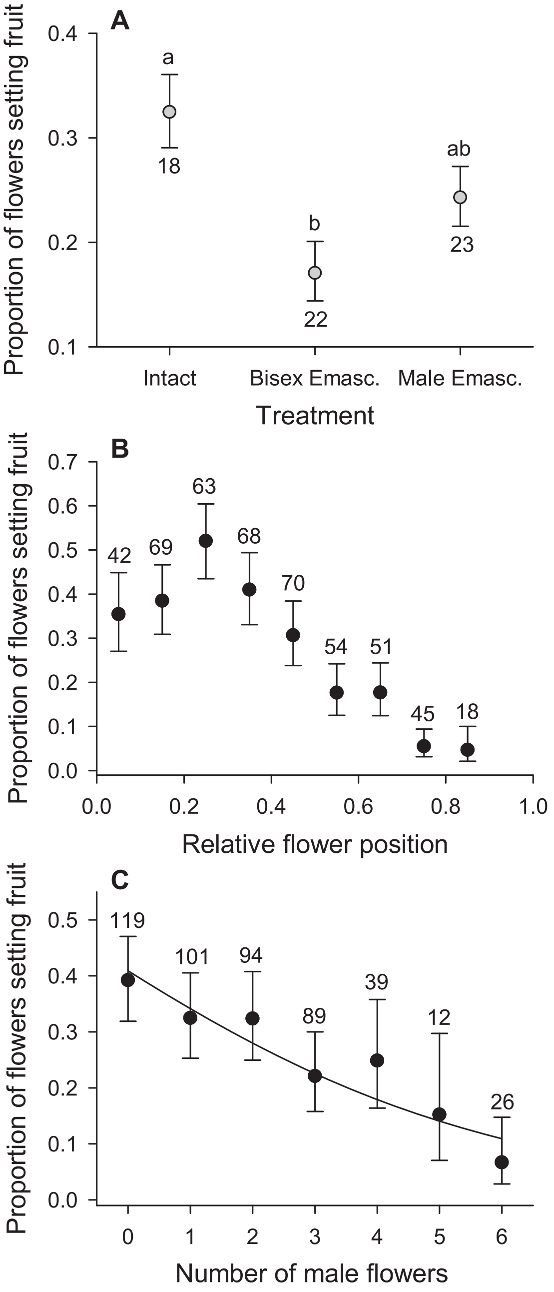

Figure 3. Influences on least-squares mean (±SE) fruit set by the 63 undamaged *Anticlea occidentalis* plants that produced at least one fruit, including (A) treatment effects on fruit set per plant, and the relations of fruit set per flower to (B) relative flower position within inflorescences, and (C) a plant's production of male flowers. In (A), means associated with different lowercase letters differed significantly and sample sizes indicate the number of plants, whereas in (B) and (C) the number of flowers is indicated. Results in (B) and (C) accounted for spatial variation in performance within the study area. All results are back-transformed from logit estimates, hence the asymmetrical standard errors.

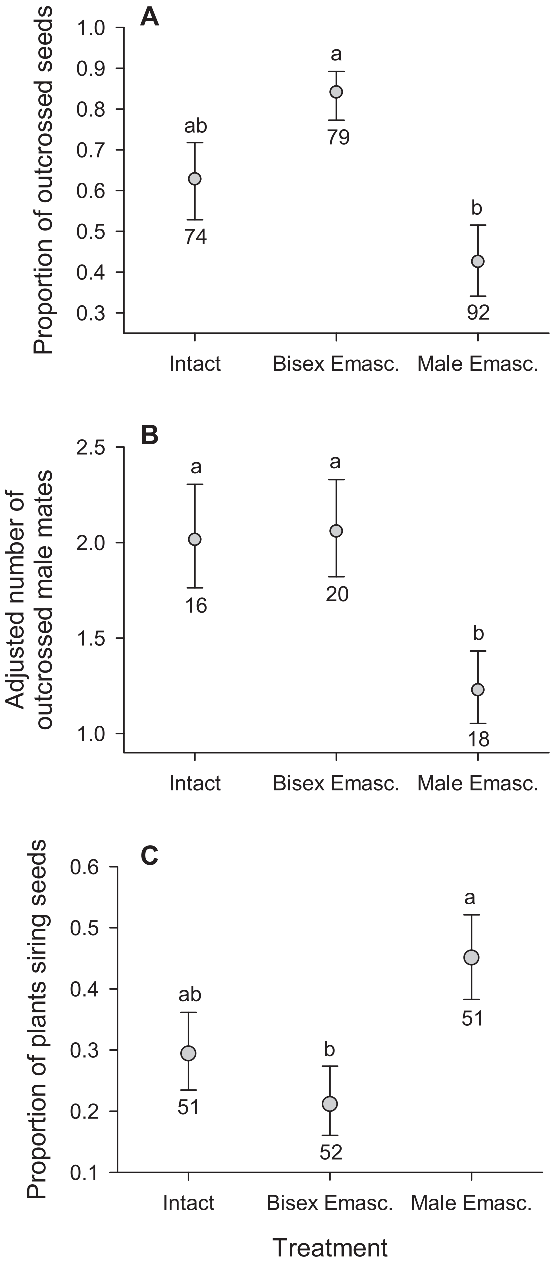

Figure 4. Effects of experimental treatment on least-squares mean (±SE) mating outcomes of *Anticlea occidentalis* plants, including (A) female selfing rate, (B) the number of males siring seeds on maternal plants, and (C) the proportion of plants siring seeds on experimental plants. Within a panel, means associated with different lowercase letters differed significantly. The results in (B) are adjusted for the effects of variation in total seed number. Sample sizes indicate the number of flowers for (A) and the number of plants for (B) and (C). The number of plants sampled per treatment for (A) is the same as indicated in (B). Results are back-transformed from logit (A and C) or ln estimates (B), hence the asymmetrical standard errors.

On average, the 26 experimental plants identified as siring genotyped outcrossed seeds mated with an average of 1.4 maternal plants after accounting for a positive effect of the number of sired seeds genotyped per male ($t_{26} = 17.11$, $P < 0.001$). Female-mate diversity of the known sires did not additionally differ significantly among treatments (Table 2).

Discussion

The preceding results illustrate diverse consequences of a plant's sexual system for the quantity and quality of its female and male success. Emasculation of either bisexual or male flowers generally reduced the fruit set of fruiting plants (Fig. 3A). In addition, emasculation of bisexual flowers enhanced female mating quality (outcrossing rate and mate diversity; Fig. 4A, B), whereas emasculation of male flowers paradoxically increased male mating quantity (primarily the probability of any siring success; Fig. 4C). This variety of responses demonstrates that the relative benefits of contrasting sexual systems arise from the mixture of benefits that each provides in a particular environment, rather than from any specific benefit. Relevant environmental characteristics include the density and characteristics of potential mates, pollinator type and abundance, and resource availability for flower and fruit production (Barrett and Harder 2017). In the specific environment in which this experiment was conducted, intact plants usually performed at least as well as plants subject to the most beneficial emasculation treatment and better than the least beneficial treatment. These results reveal that andromonoecy provides an adaptive compromise for *A. occidentalis* in its natural environment. This compromise includes the qualitative benefits of male flowers for female function and the quantitative advantages of bisexual flowers for male function. Other environments favor different sexual systems, promoting the sexual diversity evident among angiosperms (see Barrett 2002a). We now consider the relevance of our experiment for evaluating the relative merits of different sexual systems and then discuss the likely mechanisms responsible for the experimental effects and elaborate the adaptive compromise hypothesis for the different hermaphroditic sexual systems.

INCIDENTAL AND UNEXPECTED EXPERIMENTAL CONSEQUENCES

Before interpreting the results of this study, we address the possible implications of three features of our experiment for feasible conclusions.

That this study is based on an experiment conducted during a single year should have limited implications for the associated conclusions. The primary objective of the experiment was to compare the reproductive performance of three competing sexual systems, rather than to characterize variation in reproductive success for the study species. All treatments experienced similar conditions, including the impact of the hailstorm, so dissimilar performance among the treatments specifically represents effects of their contrasting arrangements of female and male function. Details of these responses might vary if this experiment was repeated, but the central conclusion that the merit of a particular sexual system in a given environment depends on the spectrum of its reproductive consequences is likely robust.

Creation of simulated monoecious (BE) and monoclinous (ME) plants from andromonoecious plants necessarily involved emasculation of some flowers. In addition to intended changes associated with the simulated sexual systems (e.g., reduced opportunity for self-pollination), manipulation of two-thirds of the study plants unavoidably reduced the outcross siring capacity of BE and ME plants and the pool of pollen for dispersal to stigmas of all plants. The direct consequences for male function are addressed below: here, we consider effects on female performance, which were informatively heterogeneous. Compared with naturally pollinated plants in the same forest during previous years (T. Y. Ida and L. D. Harder, unpubl. data), emasculation had limited effect on the proportion of intact plants that produced at least one fruit (this study 47%, previous studies 35–42%) and the mean number of seeds per fruit (this study 4.5, previous studies 2.7–9.2). Furthermore, neither pollen receipt by stigmas, the proportion of fruiting plants, nor seed number per fruit differed significantly among treatments. These results indicate that emasculation did not alter general pollen dispersal during our study. Nevertheless, fruit set by fruiting intact plants in our experiment (24%) was about half that observed during previous years (46–57%), with even lower fruit set by fruiting emasculated plants. This contrast could have arisen if the overall smaller pollen pool caused by emasculation of many plants reduced average pollen loads on individual pollinators and, in turn, the number of visited flowers per plant that received sufficient pollen to initiate fruit development.

Contrary to our expectation that limited pollen dispersal would isolate the study plants from nonstudy conspecifics, *A. occidentalis* pollen dispersed extensively (Fig. 1C). For the mating events involving experimental sires, the overall mean pollen-dispersal distance was 19.1 m. This mean is clearly an underestimate, as the sires of the remaining matings were located outside the study area. Such extensive dispersal is unusual for herbaceous species; especially those with granular pollen, like *A. occidentalis* (see Appendix S1, nonorchid species). For example, for two other forest species in the same family as *A. occidentalis* (Melanthiaceae), the estimated mean dispersal distances were 2.0 and 9.5 m (Appendix S1). Nevertheless, most dispersal of *A. occidentalis* pollen occurred relatively locally compared to the size to the study area (50 × 80 m), as 50% of matings between known mates involved plants separated by <15 m (median = 14.7 m). Furthermore, plants mated more commonly within the median distance than expected given the distribution of interplant distances, whereas matings between 20 and 40 m occurred less often than expected (Fig. 1B). This declining pattern suggests that most mating by the experimental plants occurred locally, as is the case for other herbaceous plants (Appendix S1), although some pollen likely dispersed beyond the study area.

That our study did not involve a closed population likely has limited qualitative consequences for interpretation of the results. The spatial details of pollen dispersal do not affect assessment of female performance, as all female mating events by the study plants, including those involving male mates outside of the study area, are represented by their seeds and the seed genotypes. In contrast, any pollen dispersal beyond the study area would have caused incomplete representation of outcross siring success by the study plants. In general, this incomplete sampling would reduce the statistical power to detect treatment effects. Nevertheless, statistically significant differences were evident between treatments for mating distance and the incidence of a plant siring seeds, which in turn affected the number of seeds sired. Importantly, the treatment with consistently longer mating distances (ME plants: Fig. 1C) also sired seeds on more study plants (Fig. 4C). This positive association suggests that measurements of pollen dispersal beyond the study area would, if anything, accentuate the observed differences between treatments. We therefore interpret the results of our experiment based on the quantitative differences that it revealed for female success and the qualitative differences evident for male success.

MECHANISMS FOR MATING OUTCOMES

Sexual system strongly influences the mating system, as illustrated by the extensive differences between the two emasculation treatments for female outcrossing rate, the diversity of male partners, and the proportion of plants siring outcrossed seeds (Fig. 4). *Anticlea occidentalis* has a mixed mating system (Fig. 4A), indicating both frequent self-pollination and considerable scope for mating-system variation. This variation facilitates assessment of consequences of contrasting hermaphroditic sexual systems for mating patterns.

Several results suggest that self-pollination in *A. occidentalis* occurs primarily within bisexual flowers with pollinator assistance (facilitated autogamy). Compared to intact plants, emasculation of bisexual flowers significantly reduced fruit production by fruiting plants (Fig. 3A), as expected for autogamy. Furthermore, emasculation of male flowers reduced the female outcrossing rate (i.e., increased selfing: Fig. 4A), contrary to the expectation of extensive geitonogamy. That autogamy specifically involved pollinators, rather than occurring autonomously, is evident from only 7% of all flowers producing fruit. Thus, compared to monoecy (BE treatment), production of bisexual *A. occidentalis* flowers primarily promotes female mating quantity (Fig. 3A) owing to the opportunity for facilitated autogamy. This quantitative benefit suggests that female function of bisexual *A. occidentalis* flowers is not subject to greater sexual interference than if this species was monoecious or gynomonoecious (unlike the species studied by Kawagoe and Suzuki 2005; Duffy and Johnson 2014). Limited interference is probably a consequence of the extreme pollen limitation of the study plants. The conclusion that the quantitative benefit of bisexual flowers arises from facilitated autogamy also differs from Huang's (2003) hypothesis of an advantage for bisexual flowers over monoecy arising from reproductive assurance against limited pollinator visitation, because facilitated autogamy requires visitation and so cannot provide assurance (Lloyd 1992). In contrast to their quantitative advantage, bisexual *A. occidentalis* flowers seem not to provide qualitative female benefits compared to monoecy, as neither the female outcrossing rate nor the diversity of male mates differed significantly between the intact and BE treatments.

The presence of male flowers distinguishes andromonoecy from the much more common monocliny. Andromonoecy has been proposed to provide three possible benefits compared to monocliny: increased pollinator attraction compared to production of fewer bisexual flowers; increased siring capacity associated with greater production of (comparatively inexpensive) pollen; and reduced sexual interference with pollen export (Table 1). Our experiment could not assess the benefits of producing some unisexual flowers for pollinator attraction, as all treatments retained male flowers—they differed solely in whether those flowers contained pollen. The experiment did provide evidence relevant to siring capacity and sexual interference; however, it contradicts both hypotheses. Although emasculation reduced siring capacity, intact plants did not realize a siring advantage, but instead had intermediate success between BE and ME plants (Fig. 4C). Furthermore, proportionally more ME plants sired seeds than BE plants, even though ME plants had only bisexual flowers and thus the greatest chance for sexual inference. We propose that these contrary outcomes arose from the effects of emasculation on pollinator behavior. The significantly lower fruit set by emasculated fruiting plants compared to intact plants ($t_{63} = 2.91$, $P < 0.005$) could have arisen if the absence of anthers/pollen in some flowers prompted pollinators to depart emasculated plants sooner than when they visited intact plants. Such premature departure would also explain the paradoxical elevated siring success of plants with emasculated male flowers, which resulted primarily from an increased probability that a plant sired any seeds on other plants (Fig. 4C).

The key to understanding this result seems to lie in the spatial extent of outcross mating by *A. occidentalis*. Intermate distances were long compared to those reported for other animal-pollinated species with granular pollen (see Incidental and Unexpected Experimental Consequences). Their median also exceeded the median nearest-neighbor distance 50-fold. Furthermore, emasculation of male flowers of paternal plants extended intermate distance more consistently than any other treatment (Fig. 1C). Three processes determine intermate distance as inferred from genotyped seeds: pollinator movement between successive plant visits (e.g., Thomson and Thomson 1989; Jersáková et al. 2006; Hobbhahn et al. 2017); the tendency of pollen to remain on pollinators' bodies, rather than being deposited on stigmas (pollen carryover: Waser and Price 1983; Thomson and Thomson 1989); and postpollination processes that bias ovule fertilization and seed development against neighbors being parents of mature seeds (e.g., Harder et al. 1985; Souto et al. 2002). Of these processes, emasculation of male flowers seems most likely to affect pollinator movement. If pollen-feeding pollinators assess plant quality based on pollen availability of male flowers, then they might move more readily and farther between plants than if they encountered intact male flowers, in keeping with area-restricted search (e.g., Heinrich 1979; Morse 1980).

HERMAPHRODITIC SEXUAL SYSTEMS AS HETEROGENEOUS ADAPTIVE COMPROMISES

That hermaphroditic sexual systems with multiple flower types can have diverse advantages over monocliny (Table 1) suggests several conclusions concerning their evolution. First, the diverse advantages are not mutually exclusive, so a sexual system can confer multiple benefits, as *A. occidentalis* illustrates clearly. In this species, male flowers are smaller, but produce more pollen than bisexual flowers, indicating resource efficiency compared to monocliny (Fig. 2B, D). This species also engages in flexible sex allocation, as large plants produce more bisexual flowers, but not male flowers, than small plants, and so invested proportionally more in female capacity (Fig. 2A, Appendix S5). Production of male flowers also reduces the effects of sexual interference and/or geitonogamy on female success, as BE plants had the highest female outcrossing rate (Fig. 4A). Finally, as discussed above, production of male flowers apparently limits otherwise extensive pollen-dispersal distances, although at the expense of overall siring success (Fig. 4C). Given this variety of consequences, an alternative sexual system could evolve from a monoclinous ancestor as long as the aggregate benefits outweigh the costs, even if each specific benefit is relatively small. This conclusion implies that analysis of contrasting hermaphroditic sexual systems should ideally assess as many feasible benefits as possible, rather than testing individual consequences in isolation.

The second conclusion concerning sexual system evolution arises from the observation that none of the identified advantages of different hermaphroditic sexual systems uniquely distinguishes any system from the others (Table 1). Thus, the evolution of a particular sexual system likely depends on the particular combination of advantages that can be realized in a particular reproductive environment and the functional compromises that are required to enhance annual reproductive success and its life-history consequences. Because of this context dependence and the multiple contributing advantages, each qualitative sexual system, such as andromonoecy, is likely heterogeneous with respect to the evolutionary opportunities exploited by different clades and the realized adaptive benefits (also see Mitchell and Diggle 2005). This heterogeneity is further manifest in the diversity of developmental mechanisms that generate the same sexual system (Diggle et al. 2011). Together, this variety of evolutionary options probably explains the fact that, despite being relatively uncommon, hermaphroditic sexual systems other than monocliny tend to be distributed widely among angiosperm lineages (the concentration of gynomonoecy in the Asteraceae [Torices et al. 2011] being an obvious exception).

AUTHOR CONTRIBUTIONS

CT, MK, and LH conceived and designed the study, CT and MK conducted the field work, MK and CT implemented the genetic assays, LH analyzed the data and drafted the manuscript, which all authors then finalized.

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DATA ARCHIVING

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Photographs of an *Anticlea occidentalis* inflorescence and infructescence, and bisexual and male flowers.
Appendix S1. Mean pollen dispersal distances estimated in natural conditions for 16 species of herbaceous perennials.
Appendix S2. Identification and characterization of seven polymorphic microsatellite markers for *Anticlea occidentalis* (A. Gray) Zomlefer & Judd (Melanthiaceae).
Table S1. Characteristics of statistical analyses.
Appendix S3. Detected among-plant variation and spatial covariation.
Appendix S4. Truncated binomial distribution.
Appendix S5. Size-dependence of phenotypic gender.