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# REPRODUCTIVE ECOLOGY OF *ERIGERON SPECIOSUS*, A MONTANE PERENNIAL HERB: EVIDENCE OF SELF-INCOMPATIBILITY

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ABSTRACT—We conducted an experiment to determine the reproductive biology of *Erigeron speciosus* (Asteraceae), a perennial montane herb that is widespread throughout Western North America. Pollination of *E. speciosus* was manipulated to understand the following questions: (1) What is the mating system for *E. speciosus* (outcrossing vs. selfing)? (2) Is *E. speciosus* self-incompatible? (3) Does pollen donor distance affect reproductive success? (4) Is reproductive success limited by pollen receipt (i.e., pollen limitation)? We compared seed set and seed viability among five pollination treatments: ambient pollination (control), pollinator exclusion (bagged capitula), self-pollination only, and two outcrossing treatments (near and far pollen donors). We found that *E. speciosus* is largely self-incompatible and depends on outcrossing for its reproduction. Despite this, reproduction was not pollen-limited at our study site. We also found some evidence that *E. speciosus* reproduction is susceptible to outbreeding depression.

RESUMEN—Realizamos un experimento para determinar la biología reproductiva de *Erigeron speciosus* (Asteraceae), una hierba montano perenne que se extiende por el Oeste de Norteamérica. Polinización de *E. speciosus* fue manipulado para entender: (1) ¿cuál es el sistema de apareamiento para *E. speciosus* (cruzamiento vs. autofecundación)? (2) ¿Es *E. speciosus* autoincompatible? (3) ¿La distancia del donador de polen afecta el éxito de la reproducción? (4) ¿El éxito de la reproducción será limitada por la recepción de polen (i.e., limitación polínica)? Comparamos la producción de semillas y la viabilidad de semillas por cinco tratamientos de polinización: polinización ambiente (control), exclusión de polinización (flores embolsadas), autopolinización solamente, y dos tratamientos de cruzamiento (donadores de polen cercanos y lejanos). Encontramos que *E. speciosus* es por gran parte autoincompatible y depende en cruzamiento para su reproducción. Aun así, la reproducción no fue limitada por polen en el sitio de nuestra investigación. También encontramos evidencia que la reproducción de *E. speciosus* es susceptible a depresión por exogamia.

It is estimated that approximately 88% of flowering plant species rely on animals for pollination (Ollerton et al., 2011). Despite the ubiquity of animal pollination among angiosperms, plant species exhibit considerable variation in their reproductive strategies. These strategies can be influenced by many ecological factors that ultimately interact to influence a plant's reproductive success. Understanding the multifaceted reproductive ecology of a species is an essential component of its basic biology and is important for effective conservation and prediction of how species might respond to environmental change over the short- and long-term (Waser and Price, 1989; Barrett, 2003; Ashman et al., 2004; Biesmeijer et al., 2006; Charlesworth and Willis, 2009; Inouye, 2008, 2020).

Plants are capable of sexual reproduction, asexual reproduction, or some mixture of both (i.e., a mixed mating system). Many species are capable of self-pollination (selfing). Selfing can occur through pollen donation to a stigma within a flower (autogamy) or through pollen donation to a stigma of a different flower of the same plant (geitonogamy; Barrett and Harder, 2017). In contrast to species capable of selfing, many others have molecular mechanisms that prevent successful fertilization of genetically similar genotypes, thereby preventing self-pollination, and instead promoting reproduction among genetically dissimilar individuals (outcrossing). Beyond sexual reproduction occurring via outcrossing or selfing, asexual reproduction involves the production of genetically identical offspring, which can form via nonreproductive organs of a plant (vegetative reproduction) or from the reproductive organs of a plant (apomixis; Lei, 2010).

Although there are fitness tradeoffs associated with these different reproductive strategies, perhaps the most important factor among sexually reproducing species lies in the fitness costs and benefits of reproductive assurance from selfing vs. genetic diversity from outcrossing (Barrett and Harder, 2017). In scenarios of low population densities, low

pollinator visitation, or more stable environmental conditions (Barrett and Harder 2017), selfing can be a viable mode of reproduction. However, selfing also increases homozygosity, which can lead to inbreeding depression; this has been widely documented in studies of plant reproduction (Lande and Schemske, 1985; Charlesworth and Willis, 2009). The greatest fitness benefit from outcrossing comes from the culminating genetic diversity in progeny (Charlesworth and Charlesworth, 1987; Barrett and Harder, 2017). Through increased heterozygosity, populations possess greater robustness against various environmental changes (Lei, 2010).

Alongside the existence of inbreeding depression, the converse phenomenon has also been observed, which is known as outbreeding depression. With outbreeding depression, outcrossing disrupts local adaptation when offspring experience a fitness reduction as a result of too much genetic variation (or too little genetic similarity; Waser and Price, 1989). Outbreeding depression can occur when parental plants are geographically far apart because genetic similarity negatively correlates with physical distance among plants (Slatkin, 1975; Waser and Price, 1989). When inbreeding and outbreeding depression simultaneously act on a plant population, an optimal outcrossing distance can emerge (Waser and Price, 1989; Grindeland, 2008). Understanding the existence of an optimal outcrossing distance for a given plant population is important for more completely understanding the drivers of reproductive success, and how populations might fare under environmental change.

The various reproductive strategies of plants have implications for the degree to which pollinators limit reproductive success. For example, plants that predominantly reproduce by selfing may not be hindered by insufficient pollination compared with outcrossing species (Morgan et al., 2005). Pollen limitation occurs when pollen receipt becomes the limiting resource in a plant's reproductive output: a plant possesses sufficient ovules and resources such that more fruits or seeds could have been produced if more pollen were received (Knight et al., 2005). A species' mating system (selfing vs. outcrossing), variety or specificity of pollinators, and the presence or density of neighboring plants are a few factors that can influence the magnitude of pollen limitation within a population (Ashman et al., 2004; Morgan et al., 2005; Davila et al., 2012).

Here, we studied the perennial herb, *Erigeron speciosus* (Asteraceae) in a subalpine ecosystem in the Colorado Rocky Mountains, USA. Although it is clear that *E. speciosus* is visited by a wide range of potential pollinators in our study area (Kearns, 1992; Pohl et al., 2011; CaraDonna and Waser, 2020), the details of its basic reproductive biology remain unknown. We therefore ask the following questions: (1) What is the mating system for *E. speciosus* (outcrossing vs. selfing)? (2) Is *E. speciosus* self-incompatible? (3) Does pollen donor distance affect reproductive success? (4) Is reproductive success limited by pollen receipt (i.e., pollen limitation)? Other species within the genus *Erigeron* have been observed to be self-incompatible (Armbruster and McGuire, 1991; Allphin et al., 2002); therefore, we predicted that *E. speciosus* would exhibit an outcrossing mating system and self-incompatibility. Furthermore, inbreeding depression is common in outcrossing plants (Charlesworth and Charlesworth, 1987), so we predicted that reproductive success would be higher in plants receiving pollen from farther distances because of opportunities for greater genetic variation. Finally, pollen limitation is widespread in nature (Knight et al., 2005; Bennett et al., 2020), so we predicted that *E. speciosus* reproduction would be pollen-limited.

Materials and Methods—*Study Site*—This study was conducted in 2018 at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (38°57.50N, 106°59.30W). The RMBL is in the subalpine zone of the Colorado Rockies at 2,900 m above sea level. The area consists of open meadows with numerous long-lived perennial forbs and grasses, intermixed with aspen (*Populus tremuloides*) and spruce forests (*Picea engelmannii*). Our study population of *E. speciosus* occurred primarily in a dry, open meadow habitat near the edge of an aspen forest. The study site is covered by snow for much of the year (ca. October–April), with the growing and flowering season typically occurring from May to September (CaraDonna et al., 2014; Cordes et al., 2020).

*Study Species*—*Erigeron speciosus* (Asteraceae) is an herbaceous, perennial wildflower species that is found across many habitat types of western North America. The species is present in 11 states of the western United States and western Canada, and in a small portion of Baja California (Gucker and Shaw, 2018). *Erigeron speciosus* can be found in both wet and dry areas (Inouye, 2008) as well as over the elevation range of 600–3,400 m above sea level. (Nesom, 2006). *Erigeron speciosus* is a long-lived perennial; an individual's life can span for decades (Inouye, 2008). The species possesses a robust root system including rhizomes and a caudex. From this system, a single individual can yield numerous stalks, each terminally ending in one or several radiate inflorescences (Gucker and Shaw, 2018). *Erigeron speciosus* is a mid-to-late flowering species (CaraDonna et al., 2014; CaraDonna and Bain, 2016) and is visited by a wide variety of potential pollinators, including bumble bees (Apoidea: *Bombus* spp.), several species of solitary bees (Megachilidae, Halictidae), syrphid flies (Syrphidae), bombyliid flies (Bombyliidae), and lepidopterans (Nymphalidae, Pieridae) (Kearns, 1992; Burkle and Irwin, 2009; Pohl et al., 2011; CaraDonna and Waser, 2020).

*Field Experiment*—For our experiment, we selected 12 experimental blocks of 5 individual plants each. Plants within each block had similar size, similar number of capitula, and were located in the same microhabitat. We then randomly assigned each plant per experimental block to one of five pollination treatments: (i) control (ambient pollination conditions); (ii) pollinator exclusion; (iii) self-pollination; (iv) outcross pollination from a nearby pollen donor within the focal study meadow (within ca. 25 m); and (v) outcross pollination from a faraway pollen donor collected from nearby but different meadows separated by aspen or spruce forest (most of which was from the range of ≥100 m away, but not >1,000 m away). We collected outcross pollen from the outcross-far treatment from meadows with similar elevation as that of the focal study meadow. The distances used to distinguish our outcross-near from outcross-far treatments are based on other studies that have documented evidence of outbreeding depression taking

place over distances ranging from 30 m to 1,000 m (Price and Waser, 1979; Waser and Price, 1989, 1991, 1994; Waser, 1993; Grindeland, 2008). We placed metal tags by plants to designate the experimental block and treatment.

We did not manipulate plants assigned to the "control treatment"; they received ambient pollination. Plants assigned to the "pollinator-exclusion" treatment received no animal pollination via the application of mesh pollinator-exclusion bags that were placed on all individual capitula and were not removed until after capitulum senescence; plants in this treatment were still capable of autogamous selfing. We placed all bags over capitula before any florets were open and therefore before any pollen shedding took place. We placed only one or two capitula within each bag. We similarly placed pollinator-exclusion bags over all capitula for plants assigned to the self-pollination treatment, but temporarily removed these bags for experimental self-pollination between capitula of the same individual (i.e., geitonogamous selfing). We accomplished this by gently rubbing disc florets of two entire capitula together. We gave plants assigned to the "outcross-near" treatment supplemental hand-pollination from capitula of *E. speciosus* plants within the same meadow of the focal plants (i.e., within ~25 m). We administered plants assigned to the "outcross-far" treatment supplemental hand-pollination from capitula of *E. speciosus* plants from different meadows than the study meadow (>100 m but not >1,000 m). We unbagged outcross-near and -far treatment plants, which therefore also received ambient pollination. We conducted all pollination manipulations (i.e., self-pollination, outcross-near, and outcross-far) 3 times/week on Monday, Wednesday, and Friday while plants were in flower. The study plants occurred within a larger population in which there was an ample supply of pollen, and capitula used for the hand-pollination treatments (self, outcross-near, and outcross-far) were only used if multiple florets were clearly shedding pollen. Additionally, we collected demographic data for each plant: the maximum height, the number of stems, and number of capitula per plant.

We collected all capitula from all study plants once they reached a similar developmental stage, when all of the achenes were mature (expanded and brown) but prior to dispersal. We placed mature achenes from each focal plant into separate coin envelopes, which were marked to denote the identity of parental plant and the fruit collection date. By definition, each achene represents a single seed (from one ovule). We then counted seeds in the laboratory using a dissecting microscope (Motic SMZ-168 Series 7.5X-50X Zoom Stereo Microscope; Motic, Hong Kong). We scored ovules as either containing a viable seed or not (binary response). We determined whether seeds were viable by visual inspection: we considered seeds to be viable if the achene appeared to be dark and swollen. Ovules that did not develop into a viable seed were either empty or may have been fertilized but did not fully develop (i.e., nonviable 'seeds'). Although ovules may be delineated into those producing nonviable seeds and those that were never-fertilized in some plant species (e.g., Husband and Schemske, 1997), we were unable to make this distinction. We confirmed that our visual assessment of viability was accurate via X-ray image analysis of a subsample of achenes at the Chicago Botanic Garden (Model MX-W; Faxitron, Tucson, Arizona [Kamra, 1976; Al-Turki and Baskin, 2017]). The X-ray images confirmed that viable seeds were filled with an opaque embryo. Although the presence of an embryo does not guarantee that the seed will germinate, for our purposes, we define a viable seed as containing an embryo. *Erigeron speciosus* plants produce numerous ovules per capitulum with each capitulum yielding numerous seeds (on average 33 seeds/capitulum but up to 148 seeds/capitulum); therefore, we counted seeds in a haphazard subset of capitula on each individual plant, making sure that each treatment was sampled relatively equally. On average, we counted the seeds from 12 capitula/plant. The mean number of capitula per plant was 13.7 (maximum 37), so we counted all seeds from several plants.

*Data Analysis*—We investigated our study questions using two response variables: (i) the number of viable seeds per capitulum and (ii) the proportion of ovules that developed into viable seeds. We first summed the total number of viable seeds and total capitula for each plant in each treatment (from our sampled capitula). We then divided the number of viable seeds by the number of capitula to get a value of seeds per capitulum for each individual plant (from our sampled capitula). We divided the totals for each plant, as opposed to calculating mean seeds per capitulum for each plant, because each envelope contained a variable number of capitula, depending on how many were at the developmental stage for collection on that day. Achenes detach in the envelopes; thus, we did not have a specific count for each capitulum. Thus, taking a sum of seeds for each plant and then dividing the sum by total capitula gives equal weight to each capitulum, thereby standardizing across plants with variable numbers of capitula. We calculated the proportion of viable seeds per ovule in a similar way, by dividing the number of viable seeds by the number of ovules for each plant.

We first conducted analysis of variance tests to determine whether seeds per capitulum and seeds per ovule differed across our pollination treatments. We then used Tukey's Honestly Significant Difference Post Hoc test to examine pairwise differences in responses between each treatment. We log transformed he number of seeds per capitulum to meet assumptions of normality and analyzed these data with a Gaussian error distribution. We analyzed the proportion of viable seeds (seeds per ovule) with a quasi-binomial error distribution to account for the response variable being overdispersed, proportion data.

Results—The number of viable seeds per capitulum differed significantly across treatments ($F_{4,55} = 23.07$, $P < 0.0001$; Fig. 1). Control plants produced on average 61.2 seeds/capitulum. Plants in the pollinator-exclusion and self-pollination treatments produced very few viable seeds (<5 seeds/capitulum), significantly less than plants in the control and both outcross treatments (outcross-near: mean 54.2 seeds/capitulum; outcross-far: mean 41.1 seeds/capitulum; Table 1; Fig. 1). There was no difference in seed production between the outcross-near and outcross-far treatments (Table 1; Fig. 1). Finally, there was no evidence of pollen limitation because we observed no difference in seed production between either of the outcross treatments and control plants (Table 1; Fig. 1).

The proportion of viable seeds (seeds per ovule) also differed significantly across treatments ($\chi^2_{4,55} = 121.4$, $P < 0.0001$). On average, 49.1% of ovules produced viable seeds in control plants. Plants in the bagged and self-pollination treatments had a low proportion of viable seeds per ovule, on average <7% (Fig. 2). Plants in both outcross treatments (near and far) had a higher proportion of viable seeds per ovule than did plants in the self and control treatments (outcross-near: 51.8%;outcross-far: 36.7%; Table 2; Fig. 2).

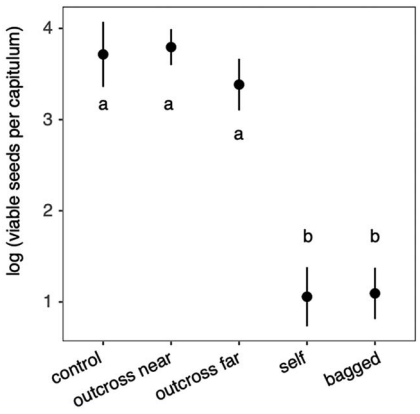


FIG. 1—The number of viable seeds per capitulum (log transformed) in each pollination treatment for *Erigeron speciosus* in the Rocky Mountains of Colorado, USA. Controls were open pollinated. Outcross-near and -far received additional pollination from other individuals, on top of what was naturally received. Plants in the self-treatment were bagged and received pollen from another capitulum on the same individual, and bagged capitula were enclosed in bags for the entire experiment. Treatments were applied at the plant level, and data were analyzed for a subsample of capitula on each plant ($N$ = 60 plants; 12/treatment). Letters indicate significant differences between treatments from Tukey's multiple comparisons.

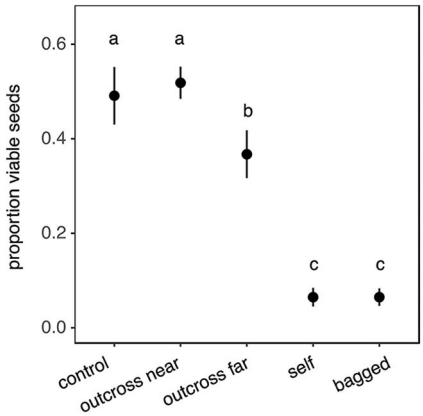


FIG. 2—The proportion of viable seeds (viable seeds per number of ovules) in each pollination treatment for *Erigeron speciosus* in the Rocky Mountains of Colorado, USA. Pollination treatments were applied at the plant level, and data were analyzed for a subsample of capitula on each plant ($N$ = 60 plants; 12/treatment). Letters indicate significant differences between treatments from Tukey's multiple comparisons.

Additionally, plants in the self-pollination treatment had a lower proportion of viable seeds than did plants in the controls (Table 2; Fig. 2). In contrast to seeds per capitulum, plants in the outcross-near treatment had a higher proportion of viable seeds per ovule than did plants in the outcross-far treatment (Table 2; Fig. 2).

DISCUSSION—We conducted a series of pollination experiments to determine the reproductive ecology of the perennial plant, *E. speciosus,* within a subalpine ecosystem in Western Colorado, USA. We found evidence that *E. speciosus* is predominantly outcrossing and self-incompatible, relying on animal pollination for its reproduction. Our experiments also revealed evidence that our study population of *E. speciosus* might experience moderate outbreeding depression and is not pollen-limited (at least in the year of study).

Our results suggest that *E. speciosus* is predominantly outcrossing and self-incompatible. First, plants in the ambient pollination (control) and outcrossing treatments yielded significantly more seeds per capitulum and a higher proportion of viable seeds per ovule than did plants in the selfing or pollinator-exclusion treatments. This finding is consistent with reports of self-incompatibility among other members of the genus *Erigeron* (Armbruster and McGuire, 1991; Allphin et al., 2002; Edwards et al., 2014; Zhang et al., 2019). That being said, ovules that did not produce viable seeds might have produced nonviable seeds that failed to

TABLE 1—Tukey's multiple comparisons for the number of *Erigeron speciosus* seeds per capitulum (per plant) across all pollination treatments, from a model in which treatment was a categorical predictor and log-transformed seeds per capitulum was a continuous response. 95% CI is 95% confidence interval.

| Treatment comparison | Predicted difference | 95% CI | $P$ value |
|---|---|---|---|
| Control vs. self | −2.66 | −3.83 to −1.49 | <0.0001 |
| Control vs. outcross-near | 0.080 | −1.09 to 1.25 | >0.99 |
| Control vs. outcross-far | −0.33 | −1.50 to 0.84 | 0.93 |
| Self vs. outcross-near | −2.74 | −3.91 to −1.57 | <0.0001 |
| Self vs. outcross-far | −2.33 | −3.50 to −1.16 | <0.0001 |
| Outcross-near vs. outcross-far | 0.41 | −0.76 to 1.58 | 0.86 |
| Control vs. bagged | 2.62 | 1.45 to 3.79 | <0.0001 |
| Self vs. bagged | −0.037 | −1.21 to 1.13 | >0.99 |
| Outcross-near vs. bagged | 2.70 | 1.53 to 3.87 | <0.0001 |
| Outcross-far vs. bagged | 2.29 | 1.12 to 3.46 | <0.0001 |

TABLE 2—Tukey's multiple comparisons for viable *Erigeron speciosus* seeds per ovule (per plant) across all treatments, from a model in which treatment was a categorical predictor and the proportion of viable seeds per ovule was a continuous response.

| Comparison | Predicted difference | $Z$ statistic | $P$ value |
|---|---|---|---|
| Control vs. self | −3.22 | −6.26 | <0.0001 |
| Control vs. outcross-near | 0.0057 | 0.020 | >0.99 |
| Control vs. outcross-far | −0.78 | −3.07 | 0.016 |
| Self vs. outcross-near | −3.23 | −6.16 | <0.0001 |
| Self vs. outcross-far | −2.45 | −4.83 | <0.0001 |
| Outcross-near vs. outcross-far | 0.78 | 2.89 | 0.028 |
| Control vs. bagged | 3.19 | 5.18 | <0.0001 |
| Self vs. bagged | −0.034 | −0.045 | >0.99 |
| Outcross-near vs. bagged | 3.20 | 5.12 | <0.0001 |
| Outcross-far vs. bagged | 2.41 | 3.96 | <0.0001 |

develop as a result of inbreeding depression, instead of self-incompatibility. However, many of the ovules that failed to produce viable seeds appeared to be empty, consistent with self-incompatibility and lack of fertilization. We also observed that plants in the selfing and pollinator-exclusion treatments did produce at least some, albeit very few, viable seeds (<5 seeds/capitulum), suggesting that the focal population might be weakly self-compatible via both autogamous and geitonogamous self-pollination. It is possible that our bagged capitula could have reproduced via apomixis. Although apomixis seems unlikely, we cannot exclude this possibility because we did not include a treatment of bagged, emasculated capitula. It is also possible that bagged capitula could have been inadvertently bumped to transfer self-pollen when we applied other pollination treatments, or that wind or other animals might have bumped the bagged capitula to cause some transfer of self-pollen. We think these scenarios are unlikely because we applied bags before anthers were shedding pollen and were careful not to bump into them. Additionally, our targeted delivery of self-pollen 3 times/week to capitula in the selfing treatment should have transferred considerably more self-pollen than we would expect from any passive jostling of the bags.

We also found some evidence of outbreeding depression. Plants that received pollen from donor plants outside of our study meadow (i.e., outcross-far treatment, 100–1,000 m away) produced 15.1% less viable seeds per ovule compared with plants that received pollen from nearby donors within the same meadow (<25 m) and 12.4% less viable seeds per ovule compared with control plants. Although the same plants receiving pollen from donors outside our study meadow produced on average 13 fewer total viable seeds per capitulum compared with those receiving pollen from donors within our study meadow (ca. 24% fewer seeds), this difference was not statistically significant. Thus, we have not shown evidence that fitness could potentially decline as a result of receipt of pollen from far away (e.g., Price and Waser, 1979). This pattern is nonetheless intriguing because it suggests that there might be only a very weak cost (if any) to outbreeding in terms of the number of viable seeds produced per capitulum, but a more significant cost in terms of the proportion of viable seeds produced per ovule. It is possible that the distance of our outcross-far treatment was still within a range in which standing genetic diversity confers both reproductive costs and benefits: Our treatment might have included a mixture of pollen donors that conferred some beneficial and deleterious alleles alike with regards to viable seed formation. Our results also suggest that plants receiving outcross pollen from farther distances might have the maternal resources to mature more viable seeds, but perhaps pollen quality is too low to do so. Outbreeding depression, if present in our population, might occur because of local adaptation. Other studies have documented evidence of outbreeding depression taking place at various distances, from 30 m to 1,000 m (Price and Waser, 1979; Waser and Price, 1989, 1991, 1994; Waser, 1993; Grindeland, 2008). Optimality of pollen donor distance might therefore be closer than expected as a result of localized, beneficial genes.

Our study population of *E. speciosus* does not appear to be pollen-limited. Plant species that are obligate outcrossers, have many ovules per flower (or in the case of *E. speciosus*, many ovules per capitulum; Knight et al., 2005), and are polycarpic (Calvo and Horvitz, 1990; Primack and Hall, 1990) are likely to experience a greater degree of pollen limitation compared with species that are self-compatible, possess few ovules per flower, or are monocarpic. *Erigeron speciosus* has a dominantly outcrossing mating system, has many ovules per capitulum, and is polycarpic; therefore, we expected its reproduction to be pollen-limited. Nevertheless, several factors might explain why we did not observe pollen limitation within our focal population. *Erigeron speciosus* is pollinated by many insect species (Kearns, 1992; Pohl et al., 2011; CaraDonna and Waser, 2020). Plants with such generalized pollination systems have been documented to be less susceptible to pollen limitation than are species with more specialized pollination systems (Horvitz and Schemske, 1990; Waser et al., 1996; Knight et al., 2005). Additionally, pollinator communities can fluctuate considerably from one season to the next (CaraDonna et al., 2021); this is a major reason why overall pollen receipt among plant species can vary considerably from year to year (Burd, 1995; Ashman et al., 2004; Knight et al., 2005), so our assessment that did not detect pollen limitation in a single season cannot definitively discern whether this population of *E. speciosus* is chronically subject to pollen limitation. This species' floral trait of possessing many ovules per capitulum does suggest the species (or at least a recent ancestor's) adaptation to stochastic environments of pollen receipt (Burd, 1995). Finally, the year in which our study took place was a drought year in our study region (2018; Faust and Iler, 2022). Resource shortages can affect reproductive success of plants (Phillips et al., 2018; Turc and Tardieu, 2018), so it is possible that reproduction was limited by these abiotic conditions instead of pollination.

It is important to mention a few additional caveats to our experiment. First, we only measured production of viable seeds (those containing an embryo). Ideally, fitness would be determined by assessing many variables throughout the entire life of offspring produced from these crosses. For example, germination rate, survival rate to maturity, rate or probability of flowering, and timing of flowering would be additionally informative (Waser and Price, 1989), but these responses take several years to manifest in a long-lived perennial like *E. speciosus*. Second, and related to the previous point, whereas our study did suggest the presence of outbreeding depression within our focal population, we do not know whether the effect size is biologically meaningful. Third, outcrossing treatments experienced ambient pollination as well as manipulated pollination, so treatment effects are not as accurate as they would have been if ambient pollination were controlled. To this point, the experimental treatments likely underestimate the effects

of manipulated outcrossing distances to some degree because ambient pollination likely included some self-pollination, which we show yields very few viable seeds. Bagging the outcross-near and -far treatments would allow for the isolation of the effects of outcross-only pollen and pollen donor distance. Fourth, our outcrossing-near treatment, although within the same meadow, might have been too distant from the parent plant to detect evidence of inbreeding in this treatment. For these reasons, we caution that although our study reveals some evidence of outbreeding depression, further research is required to address this possibility.

In summary, our experiment reveals evidence that *E. speciosus* exhibits a primarily self-incompatible mating system with very weak self-compatibility, might be susceptible to outbreeding depression, and, at least in the year of study, is not pollen-limited. Such information is beneficial for understanding *E. speciosus*'s reproductive biology and how its reproduction might be affected if faced with environmental change.

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