Article

GREATER NUMBER OF POLLEN DONORS IMPROVES FEMALE REPRODUCTIVE SUCCESS BUT NOT PROGENY VIGOUR IN *ALLIUM STELLATUM*

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Abstract—Plant-pollinator interactions affect the quantity and identity of pollen delivered to stigmas, influencing plant genetics and fitness. Here, we test the pollen competition hypothesis, which predicts that competition among pollen grains yields higher-quality offspring, by hand-pollinating *Allium stellatum* with pollen from one, two, or three donors while controlling pollen load size. We germinated seeds and assessed seed and seedling traits using generalised linear mixed-effects models. We found that flowers that received pollen loads with a greater number of donors had slower growing seedlings but also had a greater proportion of seeds that successfully germinated. These results provide mixed support for the pollen competition hypothesis, in that greater donor diversity leads to higher female reproductive success, but with a possible trade-off in progeny vigour. Pollen donor diversity thus affects reproductive outcomes and should be considered when examining how pollinators influence plant population dynamics.

Keywords—Pollen competition; pollen load; plant-pollinator interactions; population fitness; trade-offs; multiple mating.

INTRODUCTION

Animal pollinators are necessary for successful reproduction in 80% of angiosperm species, making plant-pollinator interactions an important ecological interaction (Ollerton et al. 2011). Variation in visit frequency, pollinator community, pollinator functional group, and pollinator behaviour can influence the amount and composition of deposited pollen (Bernasconi 2003; Pannell & Labouche 2013; Krauss et al. 2017; Minnaar et al. 2019; Torres-Vanegas et al. 2021). The genetic quality of the deposited pollen, in turn, can impact plant reproduction: large amounts of self or closely related pollen can reduce seed production (Knight et al. 2005; Aizen & Harder 2007), while greater genetic diversity can increase the number of fertilised ovules and the number of sires within a single fruit (Ellstrand 1984; Montalvo 1992; Rhodes et al. 2017).

Variation in the amount and composition of pollen received by a stigma also creates different selection pressures through pollen competition. Pollen competition occurs when the number of grains deposited and the number of pollen tubes growing toward the ovary is greater than the number of ovules. The pollen competition hypothesis (Mulcahy 1979) predicts that such pollen competition improves progeny vigour by favouring faster-growing pollen tubes. This can lead to two benefits. First, analogous to the 'good genes' hypothesis in animal research (Dhole et al. 2018), it may impart higher-fitness paternal genes to the offspring (Janzen 1977; Mulcahy 1979; Stephenson 1981; Stephenson & Bertin 1983; Lee 1984). Studies have shown a large overlap in gene expression of both microgametophytes (pollen) and sporophytes (Pedersen et al. 1987; Honys & Twell 2003; Beaudry et al. 2020), with evidence of a relationship between genes expressed in pollen and subsequent seedling vigour (Warman et al. 2020). Second, analogous to the 'sexy sons' hypothesis in animal research (Prokop et al. 2012), faster pollen may simply lead to offspring that themselves have faster pollen and thus higher chance of siring seeds. Either or both of these benefits would predict that pollen pools with a greater number of donors will lead to higher-quality seeds and they are also more likely to contain more high quality microgametophytes.

There is some support for the relationship between pollen competition and offspring quality with studies testing effects of pollen load, pollen size, and location of pollen placement on the stigma. In multiple species, larger pollen loads have been shown to decrease germination time but increase the number of seeds per fruit, fruit set, seed mass, germination success, and seedling growth rate (Winsor et al. 1987; Bertin 1990; Palmer & Zimmerman 1994; Niesenbaum 1999; Holland et al. 2009). Pollen competition between large and small pollen grains in *Ipomoea purpurea* leads to larger pollen grains having higher siring success (McCallum & Chang 2016). In *Dianthus chinensis*, which has an elongated stigmatic surface, pollen loads that were deposited farthest from the ovary led to seeds with greater mass and earlier germination times, suggesting that increases in offspring quality are more likely when the time or distance to reach the ovary is great enough for pollen competition to occur (Mckenna 1983; Mulcahy & Mulcahy 1987). However, comprehensive analyses of pollen competition are still inconclusive as to whether pollen competition improves offspring quality (Baskin & Baskin 2015; Baskin & Baskin 2019), pointing to the need for continued research on the topic.

An extension of the pollen competition hypothesis is the idea that the donor diversity of deposited pollen, in terms of the number of potential fathers represented in the pollen load, can also mediate pollen competition. In the few studies on the role of donor diversity in pollen competition, there have been mixed results, with evidence for both positive and negative effects of increased diversity on reproductive success and offspring fitness (Snow 1990; Niesenbaum 1999; Paschke et al. 2002). Snow (1990) found no effects of donor diversity on reproductive output of *Raphanus raphanistrum*, however, flowers in the study came from an "intense" competition line which was expected to already have high fitness, and treatments were repeated on the same maternal plant, which would not have controlled for effects related to that specific maternal individual. Niesenbaum (1999) showed an increase in donor number increased fruit set for *Mirabilis jalapa*, however treatments were also repeated on the same maternal individual. Paschke et al. (2002) found that a higher number of donor numbers in *Cochlearia bavarica* improved reproductive success but also led to lower offspring fitness. Pollen donors, however, were all selected 0.5 m from the recipient plant, which may have led to confounding effects of relatedness.

Although it is often overlooked, the donor diversity found in pollen loads is likely affected by the type of pollinator. For example, a comparison across taxa shows fruits from bird-pollinated plants are sired by almost twice the number of fathers as fruits from insect-pollinated plants (Krauss et al. 2017). Additionally, a meta-analysis on plants with pollinators with differing mobilities show more mobile pollinators facilitate higher proportions of half-sibs, meaning they are depositing pollen from a greater number of donors (Breed et al. 2015). Studies comparing pollinator types typically just focus on the amount of pollen transferred, thus potentially missing differences in donor diversity and any associated impacts on plant reproduction (Krauss et al. 2017).

In this study we used hand-pollinations to test the pollen competition hypothesis in *Allium stellatum* and to determine the implications of multiple mating on offspring and reproductive fitness. We specifically tested if the number of donors deposited on a recipient flower influences the number of seeds per flower and progeny vigour. We expect a greater number of donors will increase the number of seeds per flower and progeny vigour.

MATERIALS AND METHODS

STUDY SYSTEM

Allium stellatum (prairie onion) is a perennial herb found on limestone glades in central Canada from Ontario to Saskatchewan and in central United States from Illinois to Texas. Flowers are protandrous and are arranged in umbels of 25-30 flowers, with about six ovules per flower (Molano-Flores et al. 1999; Weiherer et al. 2020). Weiherer et al. (2020) concludes that *A. stellatum* is an obligate outcrosser but show that 5% of selfed flowers set fruit.

Our study took place in September 2020 and 2021 at the Missouri Botanical Garden's Shaw Nature Reserve (SNR) in Gray Summit, Missouri (38°27'56.9"N 90°49'23.7"W) where the population has approximately 2,000 individuals (Weiherer et al. 2020). Flowers at SNR are primarily visited by beetles and bees in the families Apidae, Halictidae, and Megachilidae (Weiherer et al. 2020). In this population, 46% of *A. stellatum* flowers set fruit and stigmas receive on average 2.8 pollen grains and with each flower producing on average 3.0 seeds from open pollination (Weiherer et al. 2020).

POLLINATION TREATMENTS

To determine effects of donor diversity on progeny fitness, we implemented treatments of pollination by one, two, and three donors. We also had open-pollination and geitonogamous self-pollination treatments for additional comparisons. Geitonogamous selfing and 1-, 2-, and 3-donor treatments were performed with hand-pollinations. For N = 150 individuals, four individual flowers received the open pollination and 1-, 2-, and 3-donor treatments, with the four treatments nested within an individual. For an additional N = 150 individuals, one individual flower received the geitonogamous selfing treatment. One hundred and nineteen individuals were chosen as donors from a different location at SNR about 200-300 meters away, separated by walkways and a wooded patch. We rotated through these donors ensuring no individual was used as a donor for separate treatments within the same individual.

To prepare flowers for hand-pollinations, we cut off excess flowers from umbels and emasculated flowers receiving pollen treatments before stigma receptivity. For individuals receiving the open pollination and donor treatments, all but four flowers were cut off, one for the open pollination treatment and three for the 1-, 2-, and 3-donor treatments. For the open pollination treatment, we chose flowers that were past stigma receptivity as indicated by wilted stigmas and therefore could have received both self and outcross pollen. The open-pollinated flowers were chosen before any experimental manipulation; therefore, these flowers would have been exposed to pollinators during the entire time their stigmas were receptive. For the geitonogamous selfing treatment on separate individuals, we left one emasculated flower as well as one unmanipulated flower that was the source of self-pollen. After emasculating flowers, we covered umbels with organza bags for two days until stigma receptivity. Once stigmas were receptive on flowers for each treatment, we deposited self-pollen for geitonogamous treatments and deposited a pollen mixture from one, two, or three donors for the remaining hand-pollination treatments. No pollen donor was used in more than one mixture within the same maternal plant. Hand-pollinations were performed by touching the plastic ball tip of a sewing pin to a dehisced anther of a donor individual. To make donor mixtures, we tapped the plastic tip of the sewing pin to different anthers on different areas of the plastic tip. We then used the metal tip of another sewing pin to mix pollen together on the surface of the plastic tip. Mixtures were then deposited by tapping the plastic tip to stigmas, maintaining similar amounts of pollen transfer across all treatments.

We checked for any differences in pollen load size in our 1-, 2-, and 3-donor treatments by collecting stigmas from every fifth individual and counting fuchsin-stained pollen grains with a microscope. On average, 26.41 grains (SE = 8.04) were deposited in the 1-donor treatment, 29.23 grains (SE = 6.56) in the 2-donor treatment, and 20.96 grains (SE = 4.11) in the 3-donor treatment. An ANOVA showed no difference in pollen number between the 1-, 2-, and 3-donor treatments ($F_{2,85}$ = 0.043, P = 0.958).

Resulting seeds were collected four weeks after pollinations. Seeds were cold stratified in damp sand at 4°C for 60 days and then planted in Black Gold® seedling mix (a mixture of peat moss and perlite) and watered once a week. Planted seeds were grown in an incubator at 18°C with 12-hour day and night cycles and monitored weekly for six months. Seeds were randomly place in a tray and trays were rotated between different shelves and different locations on shelves each week.

FITNESS METRICS

As measures of maternal and offspring fitness, we measured seed and seedling characteristics. For seeds, we measured the number of seeds per flower and seed mass. For seedlings, we recorded number of weeks to germination, as well as seedling height and number of leaves each week.

We also calculated seedling growth rates as change in height (cm) per week. After 24 weeks, we dried all seedlings in an oven for 48 hours and measured their biomass. We also calculated the proportion of germinated seeds per flower as well as the number of germinated seeds per flower. Measurements for number of seeds per flower included flowers that did not set seed and all other fitness measurements excluded flowers that did not set seed.

STATISTICAL ANALYSIS

To test differences in the percentages of flowers that set seed for different treatments we performed a chi-square test.

Because the identity of the maternal individual potentially impact fitness, we performed an analysis of variance (ANOVA) to test for differences in fitness metrics between maternal individuals. If there was a significant difference in a fitness metric between maternal individuals, we included maternal individual as a random factor in the linear model for that particular measurement.

We used generalised linear models (GLMs) or generalised linear mixed-effects models (GLMMs) to test the effect of pollination treatment on the number of seeds per flower, seed mass, number of weeks to germination, seedling height 12 and 24 weeks after planting, number of leaves 12 and 24 weeks after planting, seedling biomass 24 weeks after planting, number of germinated seeds per flower, and the proportion of germinated seeds per flower (i.e., the proportion of the seeds the flower produced that subsequently germinated). If the ANOVA showed covariance between any particular fitness metric and seed mass or showed differences in fitness metrics between maternal individuals, seed mass or maternal individual was used as a random factor in the linear model analysing that particular fitness metric.

In the linear models we used a Poisson distribution to analyse the number of seeds per flower and used a Gamma distribution to analyse seed mass, number of weeks to germination, seedling height 12 and 24 weeks after planting, number of leaves 12 and 24 weeks after planting, seedling biomass, and the number of seeds germinated per flower. We used a binomial distribution to analyse the proportion of germinated seeds per flower.

To compare seedling growth rate between treatments with different donor diversities, we performed an ANOVA analysing the effect of pollination treatment and week after planting on seedling height. Pollination treatment and week after planting were included as interaction terms to determine if the relationship between week after planting and height (i.e. growth rate) differed by treatment. Maternal individual was included as a random factor. Because seed mass can influence seedling growth rate, we also repeated the same analysis with seed mass added as another random factor.

To observe the relationships between different fitness metrics, we tested for correlations in different fitness metrics of offspring that resulted from pollen loads with different number of donors. We performed a Pearson's correlation test to determine correlations between the number of seeds per flower and seed mass along with correlations between seed mass and seedling height at 12 weeks, height at 24 weeks, number of leaves at 12 weeks, and number of leaves at 24 weeks.

All statistical analyses were performed in RStudio version 2023.06.1+524.

RESULTS

Eighty-seven out of 150 flowers (58%) from the open-pollination treatment set seed and 13 out of 150 flowers (9%) from the self-pollinated flowers set seed. Twenty-nine out of 150 flowers (19%) from the 1-donor treatment, 26 out of 150 flowers (17%) from 2-donor treatment, and 28 out of 150 flowers (19%) from the 3-donor treatment set seed. A chi-square test of all treatments showed significant differences in the percentage of flowers that set seed ($\chi^2 = 120.75$, $DF = 4$, $P < 0.001$). A chi-square test of only the 1-, 2-, and 3-donor treatments showed no significant differences in the percentages of flowers that set seed ($\chi^2 = 0.207$, $DF = 2$, $P = 0.902$).

On average, 1-donor treatments had 0.42 seeds per flower ($SE = 0.082$), 2-donor treatments had 0.40 seeds per flower ($SE = 0.087$), 3-donor treatments had 0.52 seeds per flower ($SE = 0.100$), open-pollination treatments had 1.99 seeds per flower ($SE = 0.161$), and self-pollination treatments had 0.20 seeds per flower ($SE = 0.059$). When only considering those flowers that successfully set

Table 1. Results of the generalised linear model testing the effect of pollination treatment on the proportion of germinated seeds per flower. We ran the model using a binomial distribution with maternal individual set as a random factor. Values show differences in comparison to the intercept (1-donor treatment). β shows the coefficient estimate, *SE* is the standard error associated with the coefficient estimate, the *T*-value is the t-statistic, and the *P*-value indicates the associated p-value. $^{\bullet}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	β	*SE*	*T*-value	*P*-value
Intercept (1-donor)	-0.493	0.383	-1.287	0.198
2-donor	0.493	0.548	0.899	0.369
3-donor	1.240	0.668	2.226	0.026*
open	0.400	0.440	0.909	0.363
self	0.647	0.675	0.958	0.338

seed, 1-donor treatments had 2.17 seeds per flower (*SE* = 0.222), 2-donor treatments had 2.31 seeds per flower (*SE* = 0.292), 3-donor treatments had 2.79 seeds per flower (*SE* = 0.254), open-pollinated treatments had 3.44 seeds per flower (*SE* = 0.140), and self-pollinated treatments had 2.31 seeds per flower (*SE* = 0.308).

Maternal individual was a significant covariate of all fitness metrics except for the number of seeds and number of germinated seeds per flower and therefore added as a random factor in all corresponding linear models (Appendix I).

There was no effect of donor number on number of seeds per flower, seed mass, number of weeks to germination, seedling height at 12 and 24 weeks, number of leaves at 12 and 24 weeks, seedling biomass, or number of germinated seeds per flower (Appendix II). There was a significant effect of donor number on the proportion of germinated seeds per flower, with flowers from 3-donor treatments more likely to have germinated seeds than those from 1-donor treatments (Table 1; Fig. 1).

We found that there was a significant interaction between the number of donors and week after planting (Table 2; Fig. 2). Seedlings from 1-donor treatments had an estimated growth rate of 0.825 cm/week, those from 2-donor treatments had an estimated growth rate of 0.690 cm/week, and those from 3-donor treatments had an estimated growth rate of 0.668 cm/week. Post-hoc pairwise comparisons showed that 1-donor treatments grew significantly faster than 3-donor treatments which grew (*P* = 0.051), 1-donor

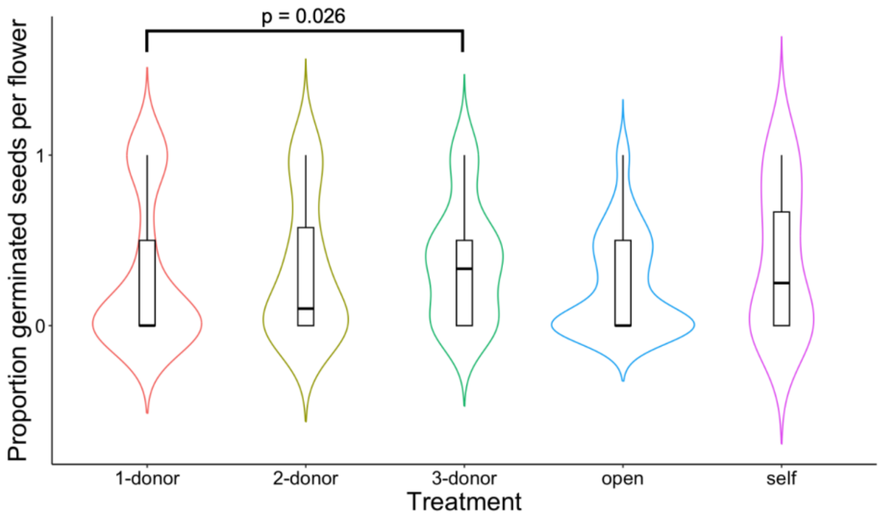

Figure 1. Violin and box plot of the proportion of germinated seeds per flower from each pollination treatment in *A. stellatum*. 1-, 2-, and 3-donor treatments consisted of hand pollinations with the number of deposited donors manipulated. Box plots show quartiles, minimums, maximums, and outliers. Violin plots show density estimations with the width showing the probability of any given value. Open treatments were unmanipulated flowers and self treatments were geitonogamous hand pollinations. Significant *P*-values indicate differences relative to the 1-donor treatment.

Table 2. Analysis of variance (ANOVA) results showing the effect of week after planting, treatment, and their interaction on seedling growth rate (cm/week) with maternal individual set as a random factor. The numerator *DF* is the degrees of freedom based on the number of groups and the denominator *DF* is the degrees of freedom based on the number of observations within a group. •*P* < 0.1, **P* < 0.05, ** *P* <0.01, * *P* < 0.001.**

	Numerator *DF*	Denominator *DF*	*F*-value	*P*-value
Intercept	1	1987	623.046	<0.001***
Week after planting	1	1987	623.047	<0.001***
Treatment	4	1987	1842.537	0.046*
Week after planting*Treatment	4	1987	4.5127	0.001**

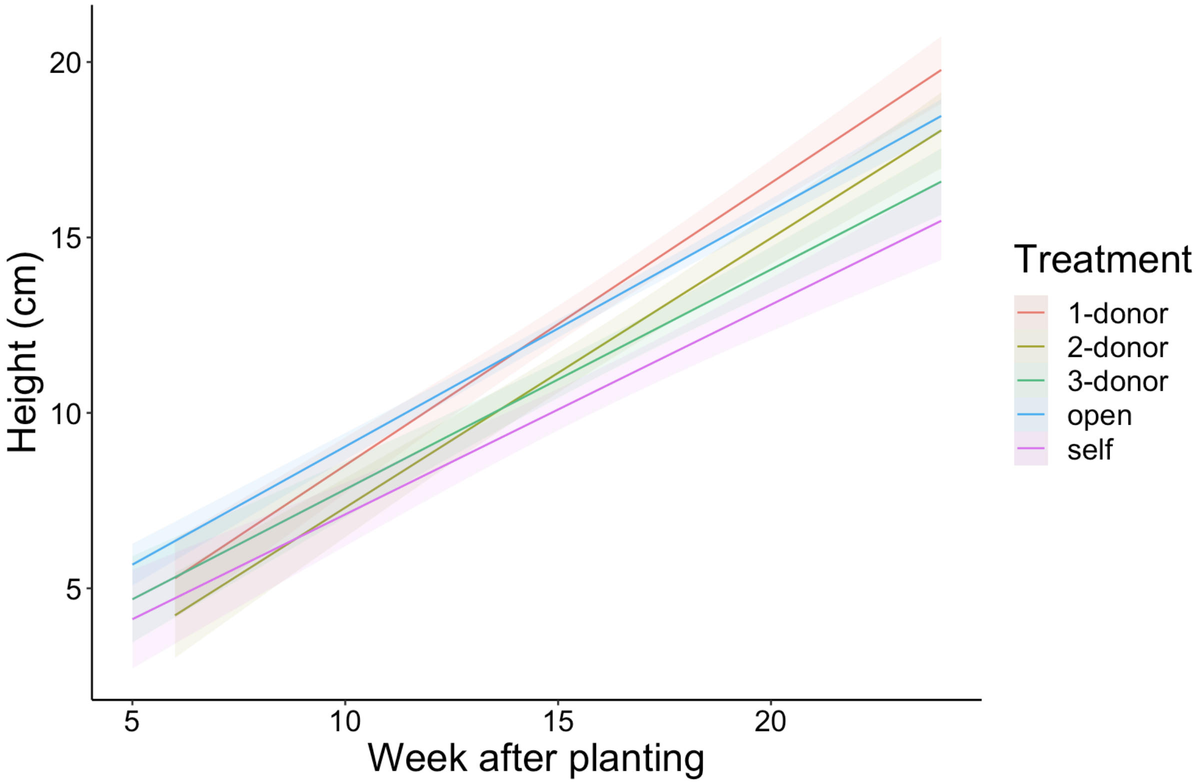

Figure 2. Growth rate, measured as the height (cm) of seedlings each week, of *A. stellatum* seedlings from pollination treatments. Slopes were calculated with a linear regression. Shaded areas show the 95% confidence interval. 1-, 2-, and 3-donor treatments consisted of hand pollinations with the number of deposited donors manipulated. Open treatments were unmanipulated flowers and self treatments were geitonogamous hand pollinations.

treatments did not grow significantly faster than 2-donor treatments (*P* = 0.151), and 2-donor treatments did not grow faster than 3-donor treatments (*P* = 0.997).

We found significant correlations between some of the fitness metrics (Appendix III). Flowers that had a greater number of seeds per flower had seeds that weighed less (*R* = -0.270, *DF* = 81, *P* = 0.014). Twelve weeks after planting, seeds that weighed more were taller as seedlings (*R* = 0.355, *DF* = 48, *P* = 0.012) and had a greater number of leaves as seedlings (*R* = 0.325, *DF* = 48, *P* = 0.021). Twenty-four weeks after planting there was no correlation between seed mass and seedling height (*R* = 0.097, *DF* = 50, *P* = 0.500) nor number of leaves (*R* = 0.600, *DF* = 50, *P* = 0.819). There was no correlation between seedling height 12 weeks after planting and seedling biomass (*R* = 0.115, *DF* = 45, *P* = 0.442), but seedlings that were taller at 24 weeks had a greater biomass (*R* = 0.515, *DF* = 50, *P* = 0.028). There was no correlation between biomass and number of leaves 12 weeks after planting (*R* = 0.148, *DF* = 45, *P* = 9.289) and number of leaves 24 weeks after planting (*R* = -0.071, *DF* = 50, *P* = 0.618).

Seedlings that were taller at 12 weeks after planting were also taller at 24 weeks after planting ($R = 0.515$, $DF = 45$, $P < 0.001$). Number of leaves 12 and 24 weeks after planting were not correlated ($R = 0.223$, $DF = 45$, $P = 0.132$). Twelve weeks after planting, seedlings that were taller also had a greater number of leaves ($R = 0.517$, $DF = 48$, $P < 0.001$) and 24 weeks after planting ($R = 0.600$, $DF = 50$, $P < 0.001$).

DISCUSSION

Our study shows that the donor diversity of deposited pollen, in terms of the number of pollen donors, differentially affected separate metrics of fitness in *A. stellatum*. Pollination by a greater number of donors led to increased reproductive success through a higher likelihood of having germinating offspring. Unexpectedly, there was an apparent decrease in seedling growth rate with an increase in number of donors, however, this is likely due to the overall pattern across all treatments of a greater number of seeds per flower leading to smaller and therefore lower quality seeds, as the maternal plant needs to spread resources across more seeds. The lack of effects in other fitness metrics may be because the representation of each pollen donor decreases with donor diversity due to pollen mixtures being constant in size, minimising the degree of competition among donors. However, the effect of donor number on female reproductive success still shows that donor diversity of pollen loads plays an important role in plant mating dynamics.

We expected increased pollen competition to improve progeny vigour because evidence suggests pollen tube growth rate is positively correlated with offspring quality, with faster-growing pollen tubes fertilising ovules first and producing faster-growing seedlings (Mulcahy & Mulcahy 1975; Winsor et al. 1987; Bertin 1990; Richardson and Stephenson 1992). However, these studies do not provide direct evidence that pollen tube growth rate is connected genetically to offspring quality (Walsh & Charlesworth 1992). Studies looking at the effect of pollen diversity on pollen competition have found mixed results with no consistent patterns. In *Cochlearia bavarica*, Paschke et al. (2002) found an increase in donor diversity from three to nine donors increased reproductive success but found that offspring vigour was highest at intermediate levels of donor diversity. Donor diversity increased fruit set in *Mirabilis jalapa* but had no effect on seedling growth (Niesenbaum 1999). Our study shows that increased donor diversity (more fathers) actually leads to decreased seedling growth rate, thus similarly failing to support the idea that greater pollen competition will lead to increased offspring quality.

However, growth rate of seedlings is not solely determined by the quality of the pollen fathering them – it is also determined by maternal investment. A trade-off between the number of seeds per flower and seed size can occur if plants have limited resources to invest in seeds (Smith & Fretwell 1974; Shipley & Dion 1992; Leishman et al. 2000), and seed size is considered an indicator of nutrient availability and therefore seedling vigour (Westoby et al. 1992; Milberg & Lamont 1997; Leishman et al. 2000). On average, we found that having a greater number of pollen donors led to a greater number of seeds per flower, but there was no significant difference in these values (Appendix II). However, we found that seeds from larger seed sets (in terms of the number of seeds per flower) in our study were more likely to be smaller. In turn, these smaller seeds produced slower growing seedlings. We note that seed mass and seedling height were positively correlated 12 weeks but not 24 weeks after planting, which is expected as the relationship between seedling growth and seed size disappears with time as seedling growth becomes less dependent on embryo size (Westoby et al. 1992). The lack of correlation between seed mass and seedling height at 24 weeks after planting also likely explains why there was no difference in seedling biomass between treatments.

In perennial species such as *A. stellatum*, a trade-off between seed number and quality may be important for survival of the mother plant and future reproduction. Evidence suggests that high investment in reproduction has a cost to reproduction in future years (Ehrlén 1992; Obeso 2002). In fact, pollen competition studies show larger pollen loads on stigmas leads to decreased seed germination and growth rates for perennial species, but increased germination and growth rates for annual species (Winsor et al. 1987; Richardson & Stephenson 1992; Quesada et al.

1996; Niesenbaum 1999; Paschke et al. 2002), but further research is necessary to test this pattern. Because perennials must invest in resources for future reproduction and annuals do not, effects of pollen competition may differ based on maternal investment. The need for perennials to invest in future reproduction therefore could explain a potential trade-off between the number of seeds per flower and seed quality in *A. stellatum*.

Although an increased number of pollen donors leads to decreased seedling vigour in *A. stellatum*, it still benefits female reproductive success in terms of an increased likelihood of having seeds that successfully germinate. The fact that there was no significant difference in the number of deposited pollen grains across our treatments suggests that competition between the multiple fathers leads to fertilisation by pollen grains that are more likely to lead to seed germination, adding another aspect besides offspring fitness to the pollen competition hypothesis. In plants, the benefits of multiple mating are often discussed in the context of genetic benefits; here, we show reproductive success could be an important consideration.

One component missing in this study is the ability to pinpoint the cause of differences in female reproductive success. While the likelihood of high-quality gametophytes increases with the number of pollen donors, we are unable to determine if, in fact, the observed increase in reproductive success was due to higher quality pollen. Other potential causes for our observed effects are differences in pollen-pistil interactions or in the genetic compatibility of the donors and each recipient (Stephenson et al. 1981; McClure et al. 2000; Wheeler et al. 2001). Future studies would benefit from a genetic component to identify whether these fitness effects are truly caused by higher quality donors. Regardless of the mechanism, our experiments support the idea that increased donor diversity can increase female reproductive success.

Here we test the pollen competition hypothesis, however, female choice may be an important factor. With female choice, females can recognise high-quality pollen at the stigma (Valdivia et al. 2009; Chae & Lord 2011; Rejón et al. 2016; Goring 2018), style (Lind et al. 1996; Wu et al. 2000; Chae & Lord 2011), or after ovule fertilisation and selectively abort seeds and fruit or stop pollen tube growth from low-quality donors (Stephenson & Bertin 1983). It is also possible that females preferentially invest resources in multiply sired ovules. Female selection would then also lead to a greater number of higher quality seeds. While we are unable to test which mechanism is at play here, it is likely that a combination of both male-male competition and female choice influence the fertilisation success and quality of offspring.

From our open-pollination treatments we were able to see if our donor treatments had comparable outcomes to unmanipulated flowers. Interestingly, only 17-19% of the hand-pollinated flowers set seed while 58% of open-pollinated flower set seed. Previous work found that open-pollinated *A. stellatum* flowers receive 2.8 pollen grains on average (Weiherer et al. 2020), thus we do not expect this difference to be due to the amount of pollen received. Perhaps manipulating the hand-pollinated flowers (emasculating them and bagging them before the treatments) decreased the numbers of seeds, or perhaps our hand-pollination methods somehow negatively impacted stigmas in ways that insect pollinators do not. However, apart from the number of seeds per flower, seeds and seedlings from open-pollination treatments had similar characteristics to 1-, 2-, and 3-donor treatments in terms of viability and growth rates. Although there were no differences between self-pollination treatments and donor treatments, we feel confident that our focal individuals did not receive closely related pollen in our 1-, 2-, and 3-donor treatments. This is due to their similarities to the open-pollination treatments and that the lack of differences between donor treatments and self-pollination treatments is likely due to a low sample size from self-pollination treatments since many of those flowers did not set seed. Because open-pollinated flowers were pollinated before the 1-, 2-, and 3-donor hand pollinations, there could be a potential for a priority effect, however, in a natural setting, an *A. stellatum* individual has an umbel of 25-30 flowers of which half set fruit Weiherer et al. (2020) and therefore we do not expect there to be a priority effect among the four treatment flowers within an individual in our experiment.

The self-pollination treatments show that *A. stellatum* is self-compatible, with 5% of self-pollinated flowers setting seed, which is a similar result to Weiherer et al. (2020). Weiherer et al. (2020) performed geitonogamous self-pollination experiments on a single flower in a full umbel in *A. stellatum* and found that about 9% of selfed flowers set seed. In our experiment, we manipulated umbels so that there was only one flower in individuals that received the self-pollination treatment, meaning that a lack of competition with other flowers for resources could have influenced the results. However, because Weiherer et al. (2020) found that 9% of selfed flowers set seed, we do not feel that a lack of competition for resources influenced our results.

This study provides mixed evidence with respect to the pollen competition hypothesis but also suggests that multiple mating may be beneficial for female reproductive success in *A. stellatum*. Some of our results are consistent with the pollen competition hypothesis but they still leave us with a complex picture of the role of pollen competition in population dynamics. The pollen competition hypothesis often discusses the benefits of competition on offspring fitness but not maternal fitness, while our results show that maternal fitness could also be an important component. We unexpectedly found that progeny vigour declined with the number of pollen donors, however this may be explained by a trade-off between seed number and quality, an idea which warrants further investigation. Whether or not negative effects on offspring vigour are mediated by a trade-off, the donor diversity of deposited pollen likely plays a significant role in offspring fitness. Hand pollination experiments from this study should be repeated on other species to investigate how life history trade-offs interact with the effects of pollen competition for a more robust test of the pollen competition hypothesis. Future work should also add a genetic component to separate effects of pollen competition versus female choice and to determine the exact drivers of female reproductive fitness. Ultimately, the result that increased donor diversity while holding pollen load size constant leads to a greater likelihood of having germinating seedlings stresses the importance of understanding how pollinators differ in the number of donors represented in the pollen loads they carry.

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AUTHOR CONTRIBUTION

R.Y.: Conceptualisation, methodology, data collection, data analysis, investigation, project administration, writing. N.M.: Conceptualisation, methodology, supervision, validation, review and editing.

DISCLOSURE STATEMENT

The authors do not have any conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Data are publicly available in the Figshare data repository: 10.6084/m9.figshare.21340131

APPENDICES

Additional supporting information may be found in the online version of this article:

Appendix I. Analysis of variance (ANOVA) results comparing maternal individual to fitness metrics.

Appendix II. Results of the generalised linear mixed-effects models for the effect of pollination treatments on different offspring fitness metrics.

Appendix III. Correlation matrix for seedling characteristics and seed mass.

REFERENCES

Aizen MA, Harder LD (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology 88:271–281. https://doi.org/10.1890/06-1017.

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, D.R. Campbell, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421. https://doi.org/10.1890/03-8024.

Baskin JM, Baskin CC (2015) Pollen (microgametophyte) competition: an assessment of its significance in the evolution of flowering plant diversity, with particular reference to seed germination. Seed Science Research 25:1-11. https://doi.org/10.1017/S0960258515000033.

Baskin JM, Baskin CC (2019) How much influence does the paternal parent have on seed germination? Seed Science Research 29:1-11. https://www.doi.org/10.1017/S0960258518000417.

Beaudry FEG, Rifkin JL, Barret SCH, Wright SI (2020) Evolutionary Genomics of Plant Gametophytic Selection. Plant Communications 1:100115. https://doi.org/10.1016/j.xplc.2020.100115.

Bernasconi G (2003) Seed paternity in flowering plants: An evolutionary perspective. Perspectives in Plant Ecology, Evolution and Systematics 6:149–158. https://doi.org/10.1078/1433-8319-00075.

Bertin RI (1990) Effects of Pollination Intensity in *Campsis radicans*. American Journal of Botany 77:178–187. https://doi.org/10.1002/j.1537-2197.1990.tb13544.x.

Breed MF, Ottewell KM, Gardner MG, Marklund MHK, Dormontt EE, Lowe AJ (2015) Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity 115:108–114. https://doi.org/10.1038/hdy.2013.48.

Chae K, Lord EM (2011) Pollen tube growth and guidance: roles of small, secreted proteins. Annals of Botany 108:627–636. https://doi.org/10.1093/aob/mcr015.

Dhole, S, Stern CA, Servedio MR (2018) Direct detection of male quality can facilitate the evolution of female choosiness and indicators of good genes: Evolution across a continuum of indicator mechanisms. Evolution 72:770-784. https://doi.org/10.1111/evo.13466.

Ehrlén J (1992) Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. Ecology 73:1820–1831. https://doi.org/10.2307/1940033.

Ellstrand NC (1984) Multiple Paternity within the Fruits of the Wild Radish, *Raphanus sativus*. The American Naturalist 123:819–828. https://doi.org/10.1086/284241.

Goring DR (2018) Exocyst, exosomes, and autophagy in the regulation of Brassicaceae pollen-stigma interactions. Journal of Experimental Botany 69:69–78. https://doi.org/10.1093/jxb/erx340.

Holland JN, Chamberlain SA, Waguespack AM, Kinyo AS (2009) Effects of pollen load and donor diversity on seed and fruit mass in the columnar cactus, *Pachycereus schottii* (Cactaceae). International Journal of Plant Sciences 170:467–475. https://doi.org/10.1086/597266.

Honys D, Twell D (2003) Comparative Analysis of the Arabidopsis Pollen Transcriptome. Plant Physiology 132:640–652. https://doi.org/10.1104/pp.103.020925.

Janzen DH (1977) A Note on Optimal Mate Selection by Plants. The American Naturalist 111:365–371. https://doi.org/10.1086/283166.

Knight TM, Steets JA, Vamosi JC Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman TL (2005) Pollen limitation of plant reproduction: Pattern and process. Annual Review of Ecology, Evolution, and Systematics 36:467–497. https://www.doi.org/10.1146/annurev.ecolsys.36.102403.115320.

Krauss SL, Phillips RD, Karron JD, Johnson SD, Robert DG, Hopper SD (2017) Novel Consequences of Bird Pollination for Plant Mating. Trends in Plant Science 22:395–410. https://doi.org/10.1016/j.tplants.2017.03.005.

Lee TD (1984) Patterns of Fruit Maturation: A Gametophyte Competition Hypothesis. The American Naturalist 123:427–432. https://doi.org/10.1086/284213.

Leishman MR, Wright IJ, Moles AT, Westoby M (2000) The evolutionary ecology of seed size. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities. Oxford University Press, Oxford, pp 31–57.

Lind JL, Bönig I, Clarke AE, Anderson MA (1996) A style-specific 120-kDa glycoprotein enters pollen tubes of *Nicotiana alata* in vivo. Sexual Plant Reproduction 9:75–86. https://doi.org/10.1007/BF02153054.

McCallum B, Chang SM (2016) Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. American Journal of Botany 103:460–470 https://doi.org/10.3732/ajb.1500211.

McClure BA, Cruz-Garcia F, Beecher B, Sulaman W (2000) Factors affecting inter- and intra-specific pollen rejection in *Nicotiana*. Annals of Botany 85:113-123. https://www.doi.org/10.1006/anbo.1999.1061.

Mckenna M (1983) Ecological aspects of gametophytic competition in *Dianthus chinensis*. In: Mulcahy D, Ottaviano E (eds) Pollen: Biology and Implications in Plant Breeding, Elsevier, New York, pp 419-424.

Milberg P, Lamont BB (1997) Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. New Phytologist 137:665–672. https://doi.org/10.1046/j.1469-8137.1997.00870.x.

Minaar C, Anderson B, De Jager ML, Karron JD (2019) Plant-pollinator interactions along the pathway to paternity. Annals of Botany 123:225–245. https://doi.org/10.1093/aob/mcy167.

Molano-Flores B, Hendrix SD, Heard SB (1999). The Effect of Population Size on Stigma Pollen Load, Fruit Set, and Seed Set in *Allium*. International Journal of Plant Sciences 160:753–757. https://doi.org/10.1086/314160.

Montalvo AM (1992) Relative Success of Self and Outcross Pollen Comparing Mixed- and Single-Donor Pollinations in *Aquilegia caerulea*. Evolution 46:1181–1198. https://doi.org/10.1111/j.1558-5646.1992.tb00627.x.

Mulcahy DL (1979) The Rise of the Angiosperms: A Genecological Factor. Science 206: 20–23.

Mulcahy DL, Mulcahy GB (1975) The influence of gametophytic competition on sporophytic quality in *Dianthus chinensis*. *Theoretical and Applied Genetics* 46:277–280. https://doi.org/ https://doi.org/10.1007/bf00269416.

Mulcahy DL, Mulcahy GB (1987) The effects of pollen competition. American Scientist 75:44–50. https://www.jstor.org/stable/27854449.

Niesenbaum RA (1999) The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). American Journal of Botany 86: 261–268. https://doi.org/10.2307/2656941.

Obeso JR (2002) The costs of reproduction in plants. New Phytologist 155:321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x.

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x.

Palmer TM, Zimmerman M (1994) Pollen Competition and Sporophyte Fitness in Brassica campestris: Does Intense Pollen Competition Result in Individuals with Better Pollen? Oikos 69:80-86. https://doi.org/10.2307/3545286.

Pannell JR, Labouche AM (2013) The incidence and selection of multiple mating in plants. Philosophical Transactions of the Royal Society B: Biological Sciences 368. https://doi.org/10.1098/rstb.2012.0051.

Paschke M, Abs C, Schmid B (2002) Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). American Journal of Botany 89:1250–1259. https://doi.org/10.3732/ajb.89.8.1250.

Pedersen S, Simonsen V, Loeschcke V (1987) Overlap of gametophytic and sporophytic gene expression in barley. Theoretical and Applied Genetics 75:200–206. https://doi.org/10.1007/BF00249164.

Prokop ZM, L Michalczyk SM, Drobniak SM, Herdegen M, Radwan J (2012) Meta-analysis suggests choosy females get sexy sons more than “good genes”. Evolution 66:2665-2673 https://doi.org/10.1111/j.1558-5646.2012.01654.x.

Quesada M, Stephenson AG, Winsor JA (1996) Effects of pollen competition on the reproductive performance in cucurbit hybrids (Cucurbitaceae): F_1 and backcross generations. Canadian Journal of Botany 74:1113–1118. https://doi.org/10.1139/b96-136.

Rejón, J, Delalande F, Schaeffer-Reiss C, Alché J, Rodríguez-García M, Van Dorsselaer A, Castro AJ (2016) The Pollen Coat Proteome: At the Cutting Edge of Plant Reproduction. Proteomes 4:5. https://doi.org/10.3390/proteomes4010005.

Rhodes MK, Fant JB, Skogen KA (2017) Pollinator identity and spatial isolation influence multiple paternity in an annual plant. Molecular Ecology 26:4296–4308. https://doi.org/10.1111/mec.14115.

Richardson TE, Stephenson AG (1992) Effects of parentage and size of the pollen load on progeny performance in *Campanula americana*. Evolution 46:1731–1739. https://doi.org/10.1111/j.1558-5646.1992.tb01165.x.

Shipley B, Dion J (1992) The allometry of seed production in herbaceous angiosperms. The American Naturalist 139:467–483. https://doi.org/10.1086/285339.

Smith CC, Fretwell SD (1974) The Optimal Balance between Size and Number of Offspring. The American Naturalist 108:499–506. https://doi.org/10.1086/282929.

Snow AA (1990) Effects of Pollen-Load Size and Number of Donors on Sporophyte Fitness in Wild Radish (*Raphanus raphanistrum*). The American Naturalist 136:742–758. https://doi.org/10.1086/285129.

Stephenson AG (1981) Flower and Fruit Abortion: Proximate Causes and Ultimate Functions. Annual Review of Ecology and Systematics 1:253–279. https://www.annualreviews.org/content/journals/10.1146/annurev.es.12.110181.001345.

Stephenson AG, Bertin RI (1983) Male competition, female choice, and sexual selection in plants. In: Real L (ed) Pollination Biology. Academic Press Inc., New York, pp 110-149.

Torres-Vanegas F, Hadley AS, Kormann UG, Jones FA, Betts MG, Wagner HH (2021) Tropical deforestation reduces plant mating quality by shifting the functional composition of pollinator communities. Journal of Ecology 109:1730–1746. https://doi.org/10.1111/1365-2745.13594.

Valdivia ER, Stepheson AG, Durachko DM, Cosgrove D (2009) Class B β-expansins are needed for pollen separation and stigma penetration. Sexual Plant Reproduction 22:141–152. https://doi.org/10.1007/s00497-009-0099-y.

Walsh NE, Charlesworth D (1992) Evolutionary Interpretations of Differences in Pollen Tube Growth Rates. The Quarterly Review of Biology 67:19–37. https://doi.org/10.1086/417446.

Warman C, Panda K, Vejlupkova Z, Hokin S, Unger-Wallace E, Cole RA, Chettoor AM, Jiang D, Vollbrecht E, Evans MMS, Slotkin RKK, Fowler JE (2020) High expression in maize pollen correlates with genetic contributions to pollen fitness as well as with coordinated transcription from neighboring transposable elements. PLoS Genetics 16:e1008462. https://doi.org/10.1371/journal.pgen.1008462.

Weiherer D, Eckardt K, Bernhardt P (2020) Comparative floral ecology and breeding systems between sympatric populations of *Nothoscordum bivalve* and *Allium stellatum* (Amaryllidaceae). Journal of Pollination Ecology 26:16–31. https://doi.org/10.26786/1920-7603(2020)585.

Westoby M, Jurado E, Leishman M (1992) Comparative evolutionary ecology of seed size. Trends in Ecology and Evolution 7:368–372. https://doi.org/10.1016/0169-5347(92)90006-W.

Wheeler MJ, Franklin-Tong VE, Franklin FCH (2001) The molecular and genetic basis of pollen-pistil interactions. New Phytologist 151:565-584. https://doi.org/10.1046/j.0028-646x.2001.00229.x.

Winsor JA, Davis LE, Stephenson AG (1987) The Relationship between Pollen Load and Fruit Maturation and the Effect of Pollen Load on Offspring Vigor in *Cucurbita pepo*. The American *Naturalist* 129:643–656. https://doi.org/10.1086/284664.

Wu H, Wong E, Ogdahl J, Cheung AY (2000) A pollen tube growth-promoting arabinogalactan protein from *Nicotiana alata* is similar to the tobacco TTS protein. Plant Journal 22: 165–176. https://doi.org/10.1046/j.1365-313x.2000.00731.x.

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