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Effect of Inflorescence Size on Female Choice in Wild Radish, *Raphanus raphanistrum*

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ABSTRACT

Sexual selection is an important mechanism of speciation. Originally studied in animals, researchers now argue that plants also exhibit characteristics that have been altered by sexual selection. Flower size, nectar production, pollen load, flower scent, and petal color are plant traits that participate in attracting mates through an intermediate pollinator. Although female choice has been demonstrated to enhance offspring quality in animals, a thorough analysis remains to be conducted in plants. In this study, I experimentally altered female choice by manipulating flower number on the hermaphroditic plant, *Raphanus raphanistrum*. Plants with smaller floral displays had reduced female choice; thus, allowing me to determine whether female choice can play a role in improving offspring quality. The effect of this manipulation was determined by measuring seed production, seed mass, and assessing offspring quality via a growth experiment in which time of germination, time of flowering, and aboveground biomass were determined. The results indicated that female choice did not result in improved offspring quality as there were no differences in time to germination, time to flowering, and aboveground biomass. Mean seed mass was greater for individuals with fewer flowers, implying that there may have been a tradeoff due to resource limitation. I conclude that although differences in offspring quality were not detected in the present greenhouse study in which there was unlimited nutrients and lack of competition, the results may have differed if progeny were grown in the field under natural conditions.

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ACKNOWLEDGEMENTS

I acknowledge and express my gratitude to the people who provided generous amounts of support during this scholarly endeavor. First and foremost, I would like to thank my parents; Percy and Haydee Salinas, because although they did not have the opportunity to get a college degree, they made sacrifices and hard choices to allow my sister and I to obtain a higher education. Their love, care, and guidance allowed me to be part of the first generation in my family to obtain an undergraduate degree, and for that I am very grateful.

I thank my advisor, Kari Segraves, for all of her guidance, patience, and instruction in the ways of the research world. I am honored to have worked with such an intellectual, thoughtful, and strong woman. She mindfully molded my curiosity and questions to help me figure things out on my own and for that extra push, I am eternally grateful. I thank David Althoff for his helpful suggestions and patient advice over the past two years. To my beloved ‘Segoff lab’ members Becky Ruppel, Kendra Tatusko, Nathan Schwarting, Andy Johncox, and Megan Larson; thank you for making my lab experience enjoyable even when I felt hopeless that my studies would never succeed. I also hold in deep gratitude my friends Rachel Eldridge, Karen Hernandez, and Sonia Mercado for encouraging me through the long lab nights and for helping me with data collection.

This project was funded by a Ruth Meyer Scholarship Program, a Renée Crown Honors Program Award, and Louis Stokes Alliance for Minority Award to Mylenne H. Salinas, and a National Science Foundation grant to Kari Segraves and David Althoff (DEB 0743101).

ADVICE TO FUTURE HONORS STUDENTS

Upon entering the Renée Crown Honors Program I took an honors seminar which advocated that as an honors student I should be an active learner. This means that if I made an effort to be curious about all subjects that learning would not be as difficult. I found that the key to learning is not just being a receptacle; it also involves taking the initiative to learn. This fully embraces the Syracuse motto: *suos cultores scientia coronat*: “knowledge crowns those who seek her.” I have learned through completing this Capstone the value of your own personal effort. Simply put, your experience in college will be a reflection of your personal effort. So my advice is to not only get involved in your interests but also to be passionate about your interests. The dedication will reflect in the end and will leave you with a feeling of accomplishment and pride.

INTRODUCTION

Peacock tails, the calls of the bird of paradise, the mane of a lion, and the horns of the Irish elk are all derived from a common principle of evolution: sexual selection. Sexual selection is a subset of natural selection that directly affects traits involved with increasing an organism's chance for reproduction (Darwin 1871). Charles Darwin first introduced this concept, and since then, sexual selection has been considered a major mechanism that creates new species. These new species often show exaggerated characteristics because competition for mates places pressure on populations to evolve traits that increase mating success (Krebs & Davies 1993). Although these exaggerated traits are most often displayed in males, females also experience sexual selection (Fisher 1958; Krebs & Davies 1993). Females face the problem of selecting the highest quality mate because reproduction is physically costly for females in terms of pregnancy and rearing the offspring. Males that have genetically inherited attributes that signal their quality as a mate are selected by females (Fisher 1958; Searcy 1982). As a consequence, females often prefer males that display the most costly traits such as bright plumage or large antlers (Krebs & Davies 1993). Therefore, sexual selection has two major components. The first component is competition between males for access to females (male to male competition) and the second component is female choice. Together these components create selective pressures because each sex has different mating objectives (Searcy 1982). Generally, females should be more discriminating in mate choice because they often provide a majority of the parental investment (Trivers 1972). Because males usually invest less in

reproduction, males are less choosy and may mate with any receptive female (Burley 1977). Sexual selection intensifies when the ratio of mating individuals is unequal (Krebs & Davies 1993).

Sexual selection was originally studied in animals; however, researchers have argued that plants also exhibit characteristics that have been altered by sexual selection (Bateman 1948; Willson 1994). Studying sexual selection in plants is crucial to understanding species interactions in the botanical world, and furthermore, because sexual selection has been deemed as a major contributor of speciation in animals (Lande 1981), it may likewise play a key role in plant evolution.

Plant and animal systems have been compared to determine if sexual selection occurs in plants. For instance in animals, males participate in male to male competition by fighting or displaying certain exaggerated characteristics for a female's attention. Similarly, flower size, nectar production, pollen load, flower scent, and petal color are plant traits that are involved in attracting mates through an intermediate pollinator (Stanton et al. 1989; Devlin & Ellstrand 1990; Conner & Via 1993; Campbell 1996). Male to male competition is not only demonstrated during the pre-fertilization period but also after sperm or pollen delivery. For example in animals, after the sperm have been delivered, there is direct competition between individual sperm to fertilize the egg (Krebs & Davies 1993; Alcock 2005). In plants, once pollen is transferred by pollinators onto the stigma, there is also direct competition for fertilization of ovules (Walsh & Charlesworth 1992; Willson 1994). In animals, females choose mates displaying the most

costly traits, presumably because these males are of higher quality (Searcy 1982; Krebs & Davies 1993; Alcock 2005). If the male selected as a mate benefits the female's fitness, then the advantage of female choice is apparent. In plants, female choice usually occurs after pollination but before fertilization. After pollen is placed on the flower, females may selectively choose pollen donors by responding to rates of pollen tube growth as well as pollen loads (Spira et al. 1992; Walsh & Charlesworth 1992; Snow 1994; Willson 1994; Delph et al. 1998). Thus, plants exhibit sexual selection via male to male competition and female choice.

The size of the floral display (e.g., inflorescence size, number of flowers) is a key plant trait that is often linked to the two components of sexual selection. An increase in floral display may increase the production of pollen and nectar on the plant which implies an increased attraction of pollinators (Willson 1994; Arista & Ortiz 2007). This will increase male to male competition because pollen grains will be participating in a race to sire the female ovule. Ultimately, plants with larger inflorescences will produce more seeds (Sutherland & Delph 1984). Since plants are resource limited, plants with more flowers may potentially have an enhanced ability to choose the flowers with the best sires, keeping only those flowers that will develop into fruit containing the highest quality offspring. To date, no studies have directly examined whether offspring quality is impacted by female choice mediated via the size of the inflorescence. I assume then, that plants with larger inflorescences will have greater ability to choose higher quality mates.

Although no studies have directly measured whether female choice improves offspring quality, a number of studies have indirectly measured offspring quality in terms of seed mass and have shown that larger seeds produced higher quality offspring (Campbell 1989; Campbell et al. 1991). For example, in *Raphanus raphanistrum*, seed mass variance represents variance in maternal investment. Stanton (1985) showed that seeds with greater seed mass produced plants with larger cotyledons; these plants also produced the most flowers. Therefore, seed mass was positively related to reproductive output (Stanton 1985).

In this study, seed mass and additional measures of seedling growth were used to determine if female choice impacted offspring quality in the wild radish, *Raphanus raphanistrum*. By eliminating flowers from a plant, I experimentally manipulated female choice so that plants with smaller floral displays would have less female choice. My overall hypothesis was that plants with larger inflorescences would have more choice, and hence, would produce higher quality offspring than plants with less choice.

METHODS

Raphanus raphanistrum L., is a self-incompatible (Reynolds 1964), hermaphroditic, annual plant that occurs in the United States. These plants grow in old fields and disturbed habitats such as roadsides and are pollinated by a variety of bees, butterflies, and flies (Stanton et al. 1989; Carlson 2007). Because wild radish reaches maturity quickly, it is particularly amenable for studies

assessing offspring quality. Under greenhouse conditions, seedlings emerge after three to five days of regular watering and rapidly grow from a taproot. Wild radish flowers in 30-40 d, and produces an average of 300 flowers on a single plant (Conner & Via 1993). These flowers are yellow or white and bloom acropetally into a cruciform shape; they range from 5 to 20mm in diameter. Wild radish has an indeterminate life cycle meaning that adult plants produce 10 to 25 flowers per day for weeks if resources are not limited (Mazer 1992). Flowers that do not give rise to a fruit usually abort and are left as pedicels. Plants typically produce large numbers of dry dehiscent siliques with seeds that range from less than 2 mg to more than 12 mg in mass.

Female choice manipulation

Maternal plants were collected as seeds in Binghamton, N.Y. by J. Conner. I used 16 maternal families from the Binghamton population. This was done to minimize variance among individuals receiving the treatments. Eighteen seeds were planted from each family to obtain a large sample size. Seeds were sown in the greenhouse in 4-inch diameter pots on June 3, 2009. Sterilized Metromix 360 potting medium was mixed with 0.3 g of Osmocote microfertilizer (17N-9P-13K) and placed in pots. Plants were grown under 12:12 (L:D) light cycle with a temperature of 27°C during the day and 17°C at night. Using a flood bench, plants were bottom watered to saturation every other day. The position of plants in the greenhouse was randomized once per week. Each maternal family was divided into blocks of three individuals, and some families had sufficient

sample size for replicate blocks. There were a total of sixteen maternal lines and forty-eight blocks. Individuals began to flower asynchronously on June 30, 2009. Once they began flowering, the plants were transferred to an experimental garden on the Syracuse University campus.

To experimentally manipulate female choice, one of three treatments was randomly assigned to each member of an experimental block. Daily flower treatments consisted of counting the newly opened flowers and applying a standard flower removal treatment to each of the three plants per block. Within each block there was a low (10%), medium (50%), and high (90%) flower removal treatment. Percentages were standardized to the total number of flowers on the low treatment. Additional damage was applied to the individuals receiving 10% and 50% flower removal treatments so damage being done to the individuals receiving the 90% flower removal treatment could be standardized. Flower pedicels that were remaining from previous days were removed in accordance to the damage done that same day (Lehtilä & Strauss 1999; Strauss et al. 2001). Plants were separated far enough from each other to avoid brush pollination. Native pollinators were allowed to forage on the plants, and each day I re-applied the treatments on the newly opened flowers to continuously control inflorescence size. After three weeks of treatment application, plants were returned to the greenhouse where the fruit were allowed to develop.

Offspring Quality

Seeds from each plant were manually removed from the fruit and counted to determine the total number of viable and inviable seeds per plant. For each plant, all seeds were pooled and then ten seeds were randomly drawn for the offspring growth experiment. A total of 1,440 seeds were planted. Seeds were individually weighed to the nearest tenth of a milligram and then sown under the same growth conditions described above. Offspring were grown for 40 d. All plants were inspected daily to determine the dates of germination and flowering. Plants were harvested after 40 d. Aboveground biomass was harvested, placed in labeled paper bags, and dried in a drying oven at 45 °C for 48 h. Aboveground biomass was measured to the nearest tenth of a milligram using an analytical balance.

Statistics

To determine whether offspring quality differed among treatments, analyses were performed using the mean values of each maternal plant (e.g. mean offspring biomass, mean offspring germination time). Three-way ANOVAs were used to assess whether the measures of offspring quality differed among treatments. The factors included treatment, maternal line, and block. Preliminary analyses indicated that the interaction terms were non-significant; therefore, they were removed from the final analysis. Statistical analyses were implemented using JMP version 5.0.1.2 (SAS Institute Inc. 2003. Cary, NC).

RESULTS

Flower treatment had an overall effect on mean seed weight, number of fruit, and number of seeds. The number of fruit and seeds increased with the number of flowers on each plant; thus, plants in the 10% flower removal treatment had the most fruit and seeds (Figs. 1-2). Proportion of individuals setting fruit was high. The high choice treatment (10% flower removal) plants had 71.1% fruit set, the intermediate choice treatment (50% flower removal) plants had 89.7%, and the low choice treatment (90% flower removal) plants had 100% production of fruit.

Mean seed weight was significantly different among flower treatments ($F=10.05$, $df= 2$, $P<0.0001$; Fig. 3). Plants in the 10% flower removal treatment had the lowest seed weight ($5.1 \text{ mg} \pm 0.00014$; mean \pm standard error), plants in the 50% removal treatment had an intermediate seed weight ($5.5 \text{ mg} \pm 0.00014$), and the 90% removal treatment had the highest seed weight ($6.1 \text{ mg} \pm 0.00014$). In contrast, there was no significant effect of flower treatment on the other measures of offspring quality (Table 1).

DISCUSSION

Sexual selection may elevate the rate at which populations diversify and generate new species (Lande 1981). Particularly for animals, sexual selection has been demonstrated to be integral in creating secondary sexual traits and behaviors. In plants, sexual selection is known to occur (Willson 1979; Willson 1994; Skogsmyr & Lankinen 2002); yet, we know little about the fitness consequences

of female choice (Skogsmyr & Lankinen 2002). Here I manipulated inflorescence size to assess whether female choice enhances offspring quality.

The results indicated that offspring quality was unaffected by female choice in wild radish. In contrast to the predictions, the analysis of offspring quality measurements showed that for germination time, proportion of offspring germinating, proportion of offspring flowering, flowering date, and biomass there was no significant effect due to flower removal treatment. I did observe a family effect for mean days to germination and flowering of offspring suggesting a genetic effect for these traits. In addition, there was an effect due to seed weight, but the trend was the opposite of the predictions. I had expected that plants at the highest level of female choice would have the largest seed mass; however, the results indicated that the plants with the higher level of female choice had the smallest seed mass. Although there were no differences among treatments, the opportunity for female choice was clearly present. Plants in the high choice treatment (10% flower removal) had 71.1 % fruit set, and the intermediate treatment (50% flower removal) had 89.7% fruit set relative to the number of flowers on the plant. This demonstrates that at both the high and intermediate treatments, the opportunity for female choice was present. In the low choice treatment (90% flower removal) plants achieved 100% fruit set, indicating that the individuals in this treatment exercised no female choice since all flowers were retained as fruit. Clearly, the experimental design presented an opportunity for plants to select among offspring, but the results indicate that female choice did not enhance offspring quality.

There are a number of possible explanations for these negative results. First, since progeny were planted under ideal growing conditions, subtle differences in offspring quality may have been obscured. Results may differ if seeds were grown under more competitive conditions and/or under limiting resources. Previous studies that have assessed female fitness by measuring offspring quality via seed size have provided results that are supportive of this idea. In parallel greenhouse and field studies, Stanton (1984; 1985) demonstrated that competition caused progeny in the field to have a greater variance than those grown under ideal greenhouse conditions. Blais (1989) observed a dissimilarity between plants grown under field conditions and those grown under greenhouse conditions. The plants in field environment produced larger fruit size. When plants were grown under greenhouse conditions to see if there was a correlation between fruit size and seedling growth rate, none was found (Blais & Lechowicz 1989). These studies support the idea that competition and/or limiting resources are crucial factors in quantifying offspring quality.

Second, another possible explanation is that flower reduction treatments may not have been substantial enough to detect differences among treatments. For example, the proportion of flower/fruit abscission represents a female's ability to select among offspring and in the present study, approximately 29% of the flowers/fruit were abscised from the plants in the high choice treatment, 10% in the intermediate treatment, and zero in the low treatment. Although the difference between the low and intermediate treatments is considerably less than for the high and intermediate treatments, it seems unlikely that these differences

are too subtle to detect the benefits of female choice. Consequently, the negative results observed in this study may simply imply that these plants do not employ female choice when producing offspring. Female choice might not be vital in the life history of ruderal plants like *Raphanus raphanistrum*. Considering that *R. raphanistrum* is a weedy annual which grows in disturbed areas, selection for quantity of seeds produced may be a higher priority than selection for quality of the seeds produced (Stanton 1984; Mazer 1987).

Although there was no significant effect of female choice on offspring quality, the results did provide evidence for resource tradeoffs. The analysis of seed mass demonstrated that plants with the most flowers removed yielded offspring with the largest seed mass and that plants in the high and intermediate treatments were resource limited as they abscised a portion of their flowers/fruit. Since females contribute to seed development, female reproductive success is often resource limited (Arista & Ortiz 2007); therefore, plants that produce fewer seeds often have more resources to invest in those seeds. A common measure of seed quality is seed mass and studies have shown positive correlations between seed mass and seedling quality (Gross & Soule 1981; Stanton 1984; Mazer 1987). In contrast, the present results showed that seedling performance did not reflect the pattern observed with seed mass. The growth experiment results showed no effect of seed mass on offspring quality. For the reasons discussed above, the benefits of enhanced seed mass may only be realized under field conditions.

CONCLUSIONS

The results do not support the prevailing view that enhanced female choice allows plants to produce higher quality offspring. Since this study was conducted under ideal growing conditions in a greenhouse, the results are limited and may differ in the field where resources are limiting and plants experience competition. Consequently, the degree to which female choice plays a role in producing offspring of higher quality remains to be established. The present study does demonstrate evidence for tradeoffs due to resource limitation; however, seeds with larger mass did not produce higher quality offspring. In principle, female plants may choose males before, during, and after mating to maximize the quantity and quality of their offspring, especially as females contribute more resources to produce seeds. However, the present study finds no evidence for the benefits of female choice, suggesting that the choice of growth conditions and/or the life history strategy of the plant may determine the importance of choice in offspring quality.

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TABLES AND FIGURES

Table 1: ANOVA results for offspring quality measurements. Three-way ANOVAs were used to measure offspring quality variance among treatments. The factors included treatment, maternal line (family), and block.

Source	df	Sum of Squares	F
Mean days to germination			
Treatment	2	0.576	0.297
Block	1	3.459	3.568
Family	1	15.807	16.304*
Mean days to flowering			
Treatment	2	47.138	0.379
Block	1	1.482	0.023
Family	1	538.849	8.678*
Proportion offspring germinating			
Treatment	2	84.722	0.402
Block	1	26.692	0.253
Family	1	1460.297	13.867*
Proportion offspring flowering			
Treatment	2	288.888	0.311
Block	1	14.142	0.030
Family	1	3896.439	8.410*
Mean offspring biomass			
Treatment	2	0.138	0.029
Block	1	0.080	2.039
Family	1	0.001	1.756

*P<0.05

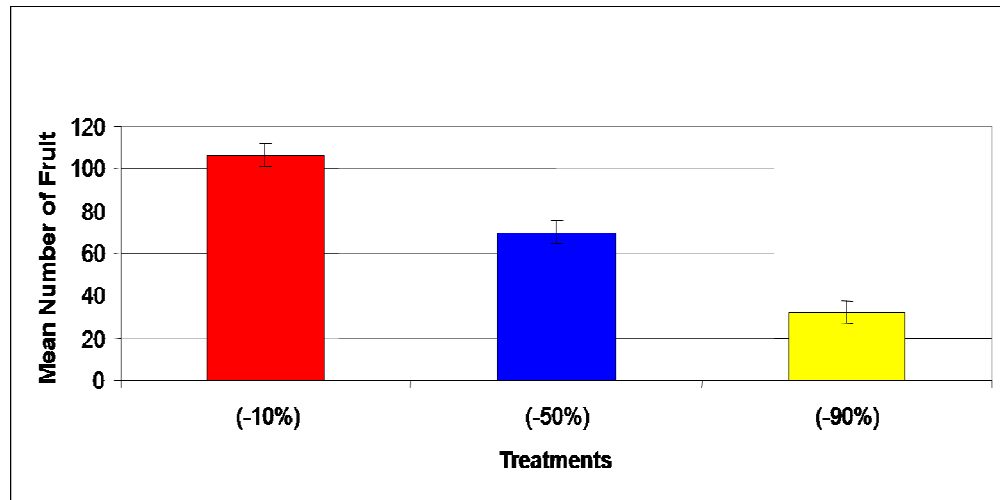


Figure 1: Comparison of the mean number of fruit (\pm standard error) across flower removal treatments in *Raphanus raphanistrum*. The 10% removal treatment represents plants with high choice whereas the 90% removal treatment represents plants with low choice.

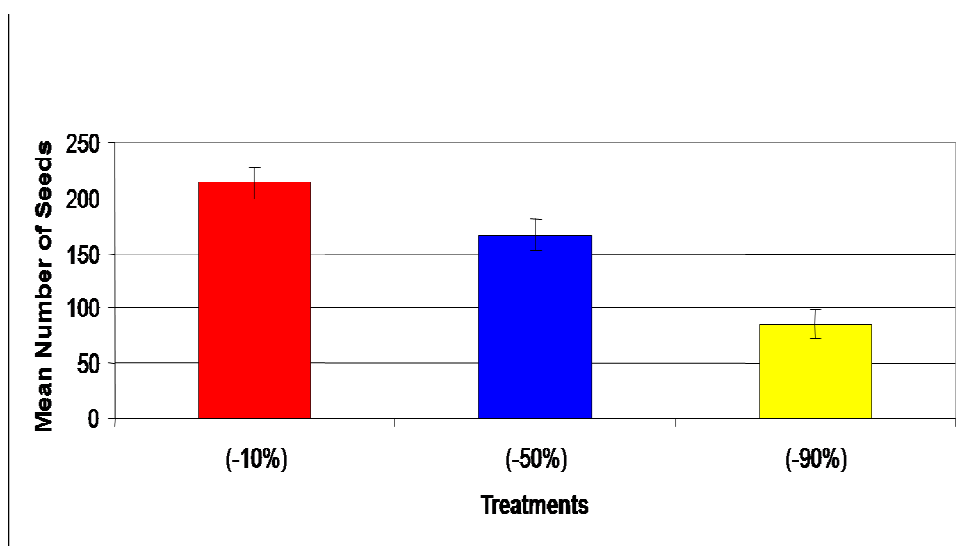


Figure 2: Comparison of the mean number of seeds (\pm standard error) across flower removal treatments in *Raphanus raphanistrum*. The 10% removal treatment represents plants with high choice whereas the 90% removal treatment represents plants with low choice.

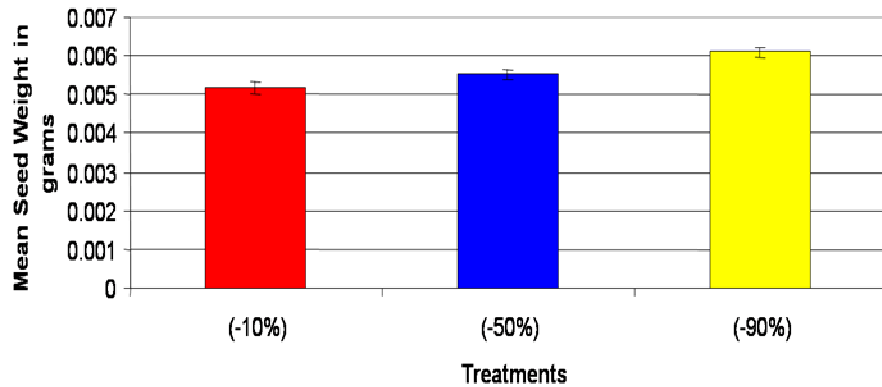


Figure 3: Comparison of mean offspring seed weight (\pm standard error) across flower removal treatments in *Raphanus raphanistrum*. The 10% removal treatment represents plants with high choice whereas the 90% removal treatment represents plants with low choice.

Written Capstone Summary

Mylene H. Salinas

Sexual selection is natural selection that arises from differences in mating success (Arnold 1994); therefore, both males and females face the challenge of propagating their genes by copulating with high quality mates. For females, selecting a high quality mate may ensure that she produces high quality offspring that have an enhanced ability to survive and reproduce. In contrast, males generally maximize their reproductive success by mating with as many females as possible, and they often compete with other males of their species for access to females. As a consequence, sexual selection contains two components: female choice and male to male competition (Darwin 1871).

Sexual selection was originally studied in animals; however, researchers have argued that plants also exhibit characteristics that have been altered by sexual selection (Bateman 1948; Willson 1994). Like animals, plants also experience the selective pressures that arise from differences in mating success. For instance, plants display male to male competition directly and indirectly. Before fertilization, males can increase pollen transfer by using inflorescence size, nectar production, flower size, scent, and number of male parts to attract pollinators. In this case, plants are competing against one another indirectly for pollinators. Male to male competition also occurs after pollen transfer when the pollen grains compete directly with one another to sire the egg. In addition to male to male competition, female choice also takes place within the flower. Females selectively choose sires by responding to rates of pollen tube growth as

well as pollen loads (Charlesworth et al. 1987; Walsh & Charlesworth 1992; Willson 1994).

The size of the floral display (e.g., inflorescence size, number of flowers) is a key plant trait that is often linked to the two components of sexual selection. For the male component, an increase in floral display may increase the production of pollen and nectar on the plant which in turn can lead to an increased attraction of pollinators (Willson 1994; Arista & Ortiz 2007). For the female component, plants with larger inflorescences will produce more seeds (Sutherland & Delph 1984). Because plants are resource limited, plants with more flowers may potentially have enhanced ability to choose among the flowers with the best sires, keeping only those flowers that will develop into fruit containing the highest quality offspring. To date, no studies have directly examined whether female choice mediated via the size of the inflorescence affects offspring quality.

Previous work, however, has provided limited measurements of offspring quality in terms of seed mass and shown that larger seeds produced higher quality offspring. For example, in *Raphanus raphanistrum*, differences among seeds in weight represents variation in maternal investment. Stanton (1985) showed that heavier seeds produced offspring with more flowers. Therefore, seed mass was positively related to reproductive output (Stanton 1985).

In this study, seed mass and other measures of offspring quality were used to determine if female choice impacted offspring quality. By eliminating flowers from a plant, I experimentally manipulated female choice. Plants with smaller floral displays had lower female choice and those with larger floral displays had

higher choice. As a result, I hypothesized that plants with larger inflorescences would produce higher quality offspring.

To answer these questions, wild radish, *Raphanus raphanistrum*, was used for this study. For the adult generation, plants from 16 maternal lines (half-sib families that have the same mother but may have different fathers) were grown in the greenhouse. Maternal lines were used to minimize the variability among individuals receiving the treatments. Plants were kept in the greenhouse until they began flowering, and were then transferred to an experimental garden on the Syracuse University campus.

Plants were organized into blocks of three individuals from the same maternal line. To experimentally manipulate female choice, one of three treatments was randomly assigned to each member of an experimental block. Flower treatments consisted of counting the newly opened flowers on a plant and applying a standard flower removal treatment to each of the three plants per block. Within each block there was a low (10%), medium (50%), and high (90%) flower removal treatment standardized to the total number of flowers on the high treatment. Because plants in the high removal treatment were receiving more damage than the low removal treatment, I standardized damage by removing additional non-flower tissue from the plants in the low and medium treatments. Native pollinators were allowed to forage on the plants, and each day I re-applied the treatments on the newly opened flowers to control flower number. After three weeks of treatment application, the plants were returned to the greenhouse where the fruit and seeds were allowed to develop.

The unique part of my study was that I assessed offspring quality more thoroughly than previous researchers. In addition to determining seed weight, I conducted an offspring growth experiment by growing ten seeds from each experimental plant. To assess offspring quality, a total of 1,440 seeds (ten per plant) were grown from the 144 original plants manipulated in the above experiment. Seeds were individually weighed to the nearest tenth of a milligram and then sown in the greenhouse. The dates of germination and of flowering were recorded daily. Aboveground biomass after 40 d from planting was measured to the nearest tenth of a milligram using an analytical balance.

Flower treatments had an overall effect on number of fruit, number of seeds and mean seed weight. Naturally, as the number of flowers that were allowed to grow on a plant increased, so did the number of fruit and seeds produced by that plant; thus the high choice treatment (10% removal) had the most fruit. Mean seed weight was also significant among flower treatments; plants with the lowest choice treatment (90% flower removal) had the heaviest seeds. In contrast, the offspring growth experiment showed no significant effect of flower treatment.

The results indicate that offspring quality was unaffected by female choice. In contrast to the predictions, the analysis of offspring quality measurements showed that for germination time, flowering date, biomass, proportion of offspring germinating, and proportion of offspring flowering there was no significant effect due to flower removal treatment. There was a significant effect due to seed weight, but the trend was the opposite of the prediction. I had

predicted that plants at the highest level of female choice would have the largest seed mass; however, the results indicated that the plants with the most choice had the lowest seed mass (i.e., were of lower quality). Although there were no differences among treatments, the opportunity for female choice was clearly present. Plants in the high choice treatment (10% flower removal) and the intermediate treatment (50% flower removal) had lower fruit set relative to the number of flowers on the plant. This demonstrates that at both the high and intermediate treatments, the opportunity for female choice was present because plants could choose which flowers to retain. Clearly, the experimental design presented the opportunity for female choice, but the results indicated that female choice did not enhance offspring quality.

There are a number of possible reasons for these results. First, progeny were grown under ideal conditions in a greenhouse; if progeny had been grown under more realistic conditions, the results may have shown the prediction of enhanced offspring quality. Second, another possible explanation is that flower reduction treatments may not have been substantial enough to detect differences among treatments. Lastly, perhaps these specific plants have no need to employ female choice for higher offspring quality. Considering that *R. raphanistrum* is a weedy annual which grows in disturbed areas, quantity of seeds may be prioritized over seed quality.

In conclusion, the results do not support the prevailing view that enhanced female choice produces higher quality offspring. Since this study was conducted under ideal conditions in a greenhouse, the results are limited and may differ in

the field where resources are limiting and plants experience competition. Consequently, the degree to which female choice plays a role in producing offspring of higher quality remains to be established. The present study does demonstrate evidence for tradeoffs due to resource limitation; however, seeds with larger mass did not produce higher quality offspring. In principle, female plants may choose males before, during, and after mating to maximize the quantity and quality of their offspring, especially as females contribute more resources to produce seeds. However, the present study finds no evidence for the benefits of female choice, suggesting that the choice of growth conditions and/or the life history strategy of the plant may determine the importance of choice in offspring quality.

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