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**UNRAVELING THE MYSTERIES OF PLANT MATING
SYSTEMS IN *RAPHANUS SATIVUS***

by

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**PREVIOUS DEGREES
B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2011**

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Abstract

Plant mating systems include a variety of mechanisms that result in non-random success of self- and outcross pollen, pollen from different compatible mates, and pollen from the same or different species. Although some of these mechanisms have received considerable attention (i.e., Charlesworth et al. 2005; Pannell and LaBouche 2013), most previous study has been of only one of these mechanisms at a time. Further in the field, a plant might simultaneously receive pollen of several types; thus, it is likely that multiple mechanisms of sorting among pollen operate simultaneously. If these mechanisms interact in ways that alter sorting among mates, we may be missing important pieces in our understanding of plant mating systems. To begin to ask whether different types of sorting among mates (e.g., sorting against self pollen, sorting among compatible mates, and sorting against heterospecific pollen) interact, I conducted two experiments that ask whether sorting among compatible mates changes when self incompatibility and/or sorting against heterospecific pollen also occur. By using two different *Raphanus sativus* types with different histories of introgression with *R. raphanistrum*, I also asked whether past history of hybridization affects ability to discriminate against heterospecific pollen. I applied different mixes of pollen to recipient plants and investigated the ability to sire seeds when different identities of pollen

were present. I found that the regardless of the identity of the pollen, sorting compatible mates did not change in either type of *R. sativus* (California wild radish; $P=0.15$ and Purple plum; $P=0.34$). This result suggests that the mechanisms involved in sorting different pollen types are independent in this species. I also found differences in ability to discriminate against the *R. raphanistrum* in the two types of *R. sativus*. The relative proportion of seeds sired by *R. raphanistrum* on California wild radish was significantly less than on the cultivar Purple plum ($P<0.0001$), but Purple plum showed seed abortion and reduced size of seeds when *R. raphanistrum* pollen was used. Thus, it appears that the mechanism for discriminating against heterospecific pollen acted earlier (prior to fertilization) in California wild radish than in Purple plum. This may give an advantage to California wild radish, since seed abortion has a negative impact on reproductive fitness.

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INTRODUCTION

Plant mating systems have long been an important area of study for evolutionary biologists because the mating system (i.e., who mates with whom and how often) alters gene transmission within a population, determines patterns of inbreeding and outbreeding, influences the quality of offspring, and affects the opportunities for speciation (Barrett et al. 1996; Bomblies and Weigel 2007). In the field, a plant may receive pollen from several sources including pollen from different species, compatible mates, close and distant relatives, and itself (Waser 1978; Schemske 1981). A number of sorting mechanisms may select among different types of pollen that reach the pollen recipient, a process that frequently starts with rejection or acceptance of self-pollen. Sorting against individuals with genes that are too closely related to the recipient plant is, in part, what drives patterns of inbreeding and outbreeding (Charlesworth et al. 2005). Avoiding self-fertilization and mating with close relatives may act to prevent inbreeding depression (Barrett et al 1996; Goodwillie et al. 2005; Hiscock and Allen 2008). Plants also sort among compatible pollen; this category of sorting leads to competition among pollen grains for access to ovules and mate choice by the pollen recipient and may lead to sexual selection (Marshall 1986; Marshall and Folsom 1991; Skogsmyr and Lankinen 1999; Marshall et al. 2010; Carlson et al. 2009; Fitz Gerald et al. 2014) as well as higher fitness of progeny (Bernasconi et al. 2004). Finally, a plant may sort against pollen from different species. This sorting process allows or prevents hybridization (Wang and Cruzan 1998; Montgomery et al. 2010) and affects the rate of introgression and speciation (Barrett et al. 1996).

The parts of the mating system that sort against self-pollination have received the most attention. Physiological incompatibility systems that prevent self-pollination are the most studied mechanisms of pollen sorting. Of the two types of genetic control over self-

incompatibility — gametophytic and sporophytic self-incompatibility—the gametophytic system terminates growth of pollen tubes in the style when there is a shared S-allele with the recipient plant (Barrett 1998; Dixit and Nasrallah 2001; Charlesworth et al. 2005).

Sporophytic incompatibility occurs at the stigmatic surface preventing germination of pollen grains from pollen donors that share an S-allele with the pollen recipient. These sorting processes have been well described at a molecular level, particularly the sporophytic incompatibility system in the Brassicaceae (e.g., De Nettancout 2001; Kachroo et al. 2002; Busche and Schoen 2008; Franklin-Tong 2008), and their consequences for mating and population structure have also been well studied (Barrett 1998; Elam et al. 2007; Koelling and Karoly 2007; Figueroa-Castro and Holtsford 2009; Kariyat et al. 2013).

Other studies, centered on sorting among compatible mates, allow researchers to understand how pollen competition for access to ovules and female mate choice influence the mating system (Marshall and Diggle 2001; also see review by Pannell and LaBouche 2013). The molecular and physiological mechanisms that underlie these mating patterns are little understood, but recent investigations in *Arabidopsis thaliana* have begun to describe the genetic mechanisms in both male- and female-mediated non-random mating (Carlson et al. 2009; Carlson et al. 2011; Fitz Gerald et al. 2014).

Studies of the sorting of pollen from different species have largely focused on heterospecific pollen competition and the effect this competition may have on population structure particularly in hybrid zones (Alarcon and Campbell 2000; Chevre et al. 2007; Chapman and Goring 2010; Arceo-Gomez and Ashman 2011; de Jong and Hesse 2012). The consequences of these patterns of sorting range from loss of male and female fitness through loss of opportunities for compatible pollen to sire seeds to the possibility of a new hybrid species (see Morales and Traveset 2008). Recent work in *Nicotiana tabacum* suggests

that specific proteins within the style inhibit the growth of pollen tubes from different species (Eberle and Anderson 2013), giving us a start in describing a physiological mechanism involved in sorting against heterospecific pollen.

Moreover, studies of the mating system have traditionally focused on one category of pollen sorting at a time (Barrett 1998; De Nettancout 2001; Marshall and Diggle 2001; Kachroo et al. 2002; Elam et al. 2007; Koelling and Karoly 2007; Busche and Schoen 2008; Franklin-Tong 2008; Kariyat et al. 2013; Pannell and LaBouche 2013) rather than examining the interactions among various mechanisms of mate sorting. Studies that have investigated multiple categories of sorting in the mating system have done so not to test whether the mechanisms interact, but rather have concentrated on competition between heterospecific pollen and conspecific pollen using species pairs that co-occur in various hybrid zones. This type of investigation has been done within several genera: *Ipomopsis* (*I. aggregate* and *I. tenuituba*), *Mimulus* (*M. guttatus* with three heterospecific donors that co-occur in a single hybrid zone, *Helianthus exilis*, *Stachys albens*, and *Mimulus nudatus*), *Brassica* (*B. campestris* and *B. napus*), *Silene* (*S. latifolia* and *S. dioica*), *Helianthus* (*H. annuus* and *H. petiolaris*), *Betula* (*B. occidentalis* and *B. papyrifera*) and *Nicotiana* (*N. longiflora* and *N. plumbaginifolia*) (Alarcon and Campbell 2000; Aldridge and Campbell 2006; Arceo-Gomes and Ashman 2011; Hauser et al. 1997; Rahme et al. 2009; Montgomery et al. 2010; Rieseberg et al. 1995; Williams et al. 1999; Figueroa-Castro and Holtsford 2009). These studies indicate that different types of pollen may influence one category of sorting among mates; that is, for example, asking whether heterospecific pollen is less likely to sire seeds when conspecific pollen from a single donor is available. What they don't address is whether mechanisms that act during one type of pollen sorting are influenced by mechanisms that operate at another. That is, for example, what happens when both sorting among multiple compatible mates and sorting against self-

or heterospecific pollen operate simultaneously? However, a recent study has begun to fill this gap by examining the differential effects of heterospecific pollen on conspecific pollen's (both self- and outcross conspecific) ability to germinate and fertilize ovules, in a mixed mating system (Arceo-Gomez and Ashman 2014).

In the field it is likely that the mechanisms that sort among the different types of pollen work at the same time; thus understanding any interactions between the sorting mechanisms is critical to understanding operation of mating systems. Although there is considerable information on how different types of sorting work independently, and investigation of interactions between the different mechanisms involved in pollen sorting have begun, there is still very little known about whether and how these mechanisms interact. Therefore, I chose to ask whether the simultaneous operation of multiple categories of sorting affects the outcome of mating. To help answer this question, I chose to study a plant in the Brassicaceae because several parts of the mating system are already well described. For example, the mechanism for self-recognition and incompatibility has been studied extensively in this family (Brugiere et al. 2000; De Nattancourt 2001; Dixit and Nasrallah 2001; Katchroo et al. 2002; Chapman and Goring 2010). Sorting among compatible mates is known to occur frequently in the genus *Raphanus* (Marshall and Ellstrand 1985; Marshall and Ellstrand 1986; Marshall 1986; Marshall and Ellstrand 1988; Marshall and Ellstrand 1989). Finally, heterospecific pollination sometimes leads to hybridization (Panetsos and Baker 1967; Hauser et al. 1997; Chevre et al. 2007; Ridley et al. 2008; Campbell and Snow 2009; Hovick et al. 2012).

Research Questions:

In order to test whether the mechanisms that sort among mates interact, I focused on the outcome of one type of sorting, sorting among compatible mates, because this is well

studied in *Raphanus sativus*. I then asked whether the amount of sorting among compatible mates changed when other types of sorting in the mating system were also invoked. I posed three questions (1) Does adding self-pollen (pollen from the same plant) affect sorting among compatible mates? (2) Does adding heterospecific pollen (pollen from a second related species) affect sorting among compatible mates? (3) Does adding both self-pollen and heterospecific pollen affect sorting among compatible mates?

Populations of wild radish found in southern California are known to be introgressed with *Raphanus raphanistrum* due to hybridization between escaped crop plants and wild *R. raphanistrum* more than 100 years ago (Panetsos and Baker 1967; Ridley and Ellstrand 2008). This history of hybridization might affect the ability to sort against heterospecific pollen. This information led me to ask two additional questions: (4) Do the patterns of pollen sorting, specifically sorting against heterospecific pollen, differ in *R. sativus*: California wild radish and *R. sativus*: Purple plum (a cultivar that is not introgressed with *R. raphanistrum*)? And (5) is California wild radish less able to sort against heterospecific pollen than Purple plum?

If the mechanisms responsible for sorting mates in the different categories are working independently of one another, then no significant change in sorting among compatible mates will occur when other types of sorting are invoked. In this case, competing compatible donors would sire the same proportion of seeds, regardless of the action of other categories of sorting. If the mechanisms of sorting mates do interact, one of two outcomes is possible. Either (1) the other mechanisms involved in sorting “saturate” the system, causing the stringency of sorting to falter. This saturation would make the proportion of seeds sired by competing compatible donors more equal to one another. Or (2) the other mechanisms act to “tighten” the sorting process, resulting in an increased difference in the proportion of

seeds sired by competing compatible donors. If the introgressed *R. sativus*: California wild radish is less able to discriminate against the heterospecific pollen, a higher proportion of seeds may be sired by the *R. raphanistrum*, which would suggest that the mechanism involved in sorting against heterospecific pollen has broken down.

METHODS AND MATERIALS

Study Species

Easily grown in a greenhouse, the annual *R. sativus* quickly produces numerous flowers per plant, which allows for multiple types of crosses and replications (Marshall and Diggle 2001). Additionally, *R. sativus* has well-developed isozyme markers, allowing for analysis of seed paternity (Ellstrand 1984; Marshall and Ellstrand 1986). Finally, *R. sativus* has sporophytic self-incompatibility; hence, self-fertilization does not confound analysis of seed paternity (Hinata et al. 1980; Nasrallah et al. 1991).

Two types of *R. sativus* were used in this investigation, California wild radish and a cultivar named Purple plum. The California wild radish is the result of introgression between *R. raphanistrum* into an unknown cultivar of *R. sativus* (Panetsos and Baker 1967). California wild radish behaves as a wild species, is found in disturbed areas, and has persisted for more than 100 years (Panetsos and Baker 1967, Ridley and Ellstrand 2008). Plants used in this investigation were grown from seeds that were collected from a population near Riverside, California.

R. sativus: Purple plum (Baker Creek Heirloom Seed Co., Mansfield, MO, 65704) is an heirloom cultivar believed not to have an introgression history with *R. raphanistrum*, since Baker Creek uses tent enclosures to house the plants, and controls pollination by releasing bumblebees within the tents. This method helps prevent cross-pollination from other species, as well as preventing cross-pollination between strains. (Baker Creek Heirloom Seed Co). In fact, the Purple plum cultivar did not display the floral colors associated with the *R. raphanistrum* (yellow), which is a signal that introgression has occurred (personal observation).

Using two types of *R. sativus* allowed me to test for differences in the ability to sort among compatible mates in both an introgressed and non-introgressed variety. Both types:

Purple plum and California wild radish underwent the same treatments to determine the ability of the radishes to sort among compatible mates when multiple types of sorting within the mating system are working in concert.

To test for sorting against heterospecific pollen, I used a related species, *R. raphanistrum*, which is known as an agricultural weed found near fields and in disturbed areas (Conner and Via 1993). The *R. raphanistrum* seeds used in this study were collected from a population in New York state, obtained from Dr. Jeffery Conner (Michigan State University).

Breeding for seeds

Purple plum was chosen for this project out of 16 heirloom cultivars tested because it was the only cultivar that contained enough variation in Phosphoglucose Isomerase (PGI) phenotypes for paternity analysis. Prior to the start of the experiment, I conducted one generation of breeding of Purple plum to produce seeds of the needed homozygous PGI phenotypes which were named 22, 33, and 44 based on their position on starch gels after electrophoresis. I planted 600 Purple plum seeds in flats containing a 4:1:1:1 ratio of masonry sand, Metromix 360 (Sun Gro Horticulture Canada Ltd. Seba Beach, AB), peat moss, and perlite; the flats were placed under automated sprayers in the University of New Mexico (UNM) research greenhouse. All seedlings were tested for their PGI phenotype using leaf tissue; a total of 52 were selected for breeding (Table 1). I performed crosses among all plants with the 33 phenotypes, among those with the 22 phenotypes, and among those with the 44 and 24 phenotypes. Both types of parents were used to generate 44 because only a small number of 44 plants were available in the original sample. In total I performed 281 crosses in four weeks. Fruits with more than one seed were collected 4-6 weeks after crosses were completed.

Experimental conditions

All plants were grown in the UNM research greenhouse under environmental conditions set on a monitoring system that kept the temperature between 18° and 25° C and the relative humidity near 25% (Wadsworth Control System INC. Arvada, CO 80002). All seeds were soaked in gibberellic acid (40mg/100ml) for one hour prior to planting. All seeds were planted in flats and placed under sprayers, controlled by an automated watering system, and watered three times per day for two minutes. Seedlings were transplanted into 2 L plastic pots filled with the 4:1:1:1 mix of masonry sand, peat moss, perlite, and MetroMix 360 (Sun Gro Horticulture Canada Ltd. Seba Beach, AB) and placed on individual drippers (3.79 L/hour). To avoid wilting, all plants were watered three times per day for five minutes until the end of the experiment. Plants were fertilized with Jack's 20:20:20 (JR Peters, INC. Allentown, PA 18106) twice per week (3.6 g/L) and Chelated liquid iron with other micronutrients (Voluntary Purchasing Groups, Inc. 230 FM 87 Bonham, TX. 75418) every other week (3.6g/L) beginning four weeks after planting and continuing through the duration of the experiment.

Selection of experimental plants

R. sativus: Purple plum, and *R. raphanistrum* were planted in January 2013; *R. sativus*: California wild radish, was planted in February 2013. The two *R. sativus* varieties were planted four weeks apart to allow all pollinations of one experiment to be completed before plants for the second experiment had begun to flower. The difference in the planting times means that changes of day length may confound the differences seen in these two types of *R. sativus*. However, previous studies of California wild radish have shown that plant age is a more important factor in sorting pollen than flowering date (Marshall and Oliveras 2001; Marshall et al. 2010). Though day length may have increased, the age of the plants when

undergoing the experimental crosses was the same and the environmental conditions (i.e., temperature, humidity, and watering) were kept constant for both experiments.

To find the PGI phenotypes that would facilitate paternity analysis, starch gel electrophoresis was performed using leaf tissue from all the available plants. Genotyping occurred approximately two weeks after planting. I screened Purple plum for the phenotypes: 22, 33, and 44. I selected twenty-five potential pollen recipients of PGI phenotype 22. This group was the source of the plants on which experimental crosses were performed. Thirty-three potential pollen donors of PGI phenotype 33 were screened for use as the pollen donors whose ability to sire seeds in competition was tested (hereinafter known as target pollen donors) and was compared among the treatments. Sixteen potential pollen donors of PGI phenotype 44 were evaluated for use as standard competitors (hereinafter known as test pollen donors) to be used in mixed pollinations the target pollen donors. The California wild radishes were screened for the phenotypes: 11, 22, and 33. Twenty-four plants were used to find pollen recipients of PGI phenotype 22. I selected fourteen plants of PGI phenotype 33 to be potential target donors, and six plants were available to evaluate as potential test donors of PGI phenotype 11 (Table 2). Nine *R. raphanistrum* plants with a 22 phenotype were evaluated for use as heterospecific pollen donors. Each plant type was screened for a different phenotype to make paternity testing more manageable. Using the same PGI phenotype for the *R. raphanistrum* and the pollen recipients was acceptable in this study since both are sporophytically self-incompatible species; self-fertilization was not an issue.

Once genotyping was complete and all plants had at least three open flowers, I started crosses to find pollen donors that were compatible with the plants that would become pollen recipients. Each potential donor (target donors, test donors and

heterospecific donors) was reciprocally crossed with the potential pollen recipient by rubbing an anther directly onto the stigma of each flower. All plants were pollinated in this manner. In total, 900 crosses were performed, and all plants were re-examined 7-10 days after pollination for the presence or absence of fruit. A pair was considered compatible if a fruit was produced with multiple seeds. If a fruit had a single seed or if no fruit was produced, the cross was repeated to verify incompatibility. If again no fruit was produced, the plant was excluded from the group of potential pollen donors or recipients. If a fruit was produced, the cross was performed one more time for verification and was then included in the group of potential pollen donors or recipients. After compatibility testing was complete and appropriate mates were selected, I began the experimental pollinations.

Experimental design

The two parallel experiments used *R. sativus*, Purple plum for one experiment and *R. sativus*, California wild radish for the second. The same protocol was used for each experiment.

Eight types of crosses (four single and four mixed) were performed on all pollen recipients with five replicates each. Single pollinations were done with each type of plant described in Table 2. Self-pollen was used to test the reliability of the self-incompatibility (SI) system. The target and test donors were used in single pollinations to compare ability to sire seeds without competition. Finally, *R. raphanistrum* plants were tested to ensure ability to sire seeds on *R. sativus*, and to ask whether *R. raphanistrum* sired as many seeds in single pollinations as conspecific pollen. Mixed pollen crosses were performed to answer the questions posed by this study (Table 3). The mix of pollen from target and test donors served as a baseline of the ability for the target donor to sire seeds in competition.

Single donor pollinations were performed by rubbing a single anther from the pollen donor directly on the stigma of the recipient. Each mixed pollination contained pollen from one of the target donors, a test donor that was chosen haphazardly, self-pollen, and *R. raphanistrum* as described in Table 3 above. To perform the mixed pollinations, I collected a flower from each plant type and tapped its pollen into a petri dish. The pollen was then mixed with forceps and, subsequently, applied to the stigma of the recipient plant using tissue wrapped forceps. Since the pollen was applied differently in single and mixed pollinations, any differences in the resulting numbers of pollen grains per stigma have the potential to affect results. However, Marshall et al. (2007) showed that pollen number per stigma in a wide range does not affect seed paternity in this species.

A total of 2,400 hand pollinations were performed. This number was determined as follows: 5 pollen recipients X 3 Target donors X 2 *R. raphanistrum* X 8 cross types X 5 replicates X 2 experiments = 2,400 crosses or 240 crosses per plant.

All experimental crosses took 11 days per experiment to complete. Plants were checked for the presence of fruit 7-10 days after pollination. The fruits ripened within 4-6 weeks after pollination. Fruits were collected from the plants when fully ripened. All collected fruit were opened and the number of seeds per fruit, and individual seed mass were recorded. Seeds from single crosses were stored in individual coin envelopes labeled with lineage. Seeds from single crosses were not tested for paternity as it was assumed that the sire of the seed is the donor used in the cross. The seeds from the mixed pollinations were placed into 96-well plates after mass was recorded and were stored until I performed starch gel electrophoresis to determine paternity (Ellstrand 1984). All seeds produced from mixed pollinations were analyzed for paternity.

Data Analysis

The results of single pollinations were compared using mixed model ANOVAs followed by Tukey's Studentized Range tests when significant differences were observed. Seed number per fruit, good seeds per fruit, aborted seeds per fruit, and seed mass were used as the dependent variables. Pollen recipient, cross type, and their interactions were independent variables. Pollen recipient and the interaction of pollen recipient and cross type were treated as random effects.

All data for mixed pollen crosses were analyzed using mixed model ANOVAs followed by Tukey's Studentized Range tests. To compare the proportion of seeds per fruit sired by target donors, I used the arcsine square root transformation of the proportion of seeds sired per fruit as the dependent variable. Pollen recipient, target pollen donor, replicate, cross type, and all two-way interactions were the independent variables. Pollen recipient and target pollen donor and their interactions were treated as random effects. Replicate and cross type were treated as fixed effects.

Initial analysis showed differences in the proportions of seeds per fruit sired by the target donors across replicates in the California wild radish (Table 4). A Tukey's HSD range test showed the differences to be between replicate 3 and replicate 5. There are a few possible explanations for the differences. 1) A change in the quality of either the pollen recipient or pollen donor over time that would result in a linear change in the proportion of seeds sired by the target donors. This hypothesis may in part explain the pattern of change in performance across replicates for target donor 2 in the California wild radish experiment (Figure 1). Or 2) a difference in the proportion of seeds sired per fruit by the target donor is due to the test donors being used. If the difference is a result of the different test donors, a non-linear pattern in the distribution of seeds sired by the target donors over time could be observed. This hypothesis may in part explain the pattern seen for target donor 2 in the

California wild radish experiment (Figure 2). Finally, 3) a combination of test donor identity and quality of pollen donor or pollen recipient could be responsible for different patterns seen in the pollen donors. Because the test donors were chosen haphazardly for their use in crosses and the identity of the test donor used in each cross was not recorded (two test donors were used in each experiment), differences in the abilities of test donors to sire seeds in competition may affect the results in ways that are difficult to tease out. However, since the same test donor was used for all of the crosses in one replicate on each pollen recipient, examination of the effects of replicate within plant on proportion of seeds sired by target donors can provide some indication of the magnitude of any effects of test donor identity. Since the initial analyses showed potential for confounding effects of test donor identity, the effect of replicate within pollen recipient was included in all analyses of mixed pollinations.

An analysis of variance was also performed to determine the proportion of seeds sired by *R. raphanistrum* across experiments. The arcsine square root transformation of the proportion of seeds per fruit sired by *R. raphanistrum* was the dependent variable while lineage (*R. sativus*: California wild radish or *R. sativus*: Purple plum), cross type (those that included *R. raphanistrum*), and their interaction were the independent variables. The data analysis for this paper was generated using SAS software. Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

The effects of pollen recipients were included in the analysis so that any variation due to pollen recipient would be accounted for and not lumped into the error variation. However, these effects are not discussed here as they are not relevant to the questions posed by this study. Effects of the pollen recipient on seed siring ability, seeds per fruit and seed mass

have been address in other studies of wild radish (Marshall and Fuller 1994; Marshall et al, 1996; Marshall and Diggle 2001; Marshall et al 2007 Marshall et al, 2010).

RESULTS

Single Crosses:

Although total seeds per fruit did not differ among cross types in *R. sativus*: Purple plum, both good seeds per fruit and aborted seeds per fruit were significantly different among cross types (Table 5a). *Raphanus raphanistrum* pollen donors produced the fewest good seeds per fruit and the most aborted seeds per fruit (Table 5c).

Neither, total seeds per fruit, good seeds per fruit, nor aborted seeds per fruit differed significantly among fruits sired by the various pollen donors on California wild radish (Table 5b).

The *R. raphanistrum* pollen donors sired seeds with lower mean mass than both the target donors and the test donors in the Purple plum experiment. These differences were statistically significant (Tables 5a, 5b, and 5c). Although one of the *R. raphanistrum* donors, *R. raphanistrum* donor 2, showed a lower mean seed mass than the other donors tested on California wild radish, the difference was not statistically significant (Tables 5b, and 5c).

Mixed Crosses:

For *R. sativus*: Purple plum, the mixed pollinations that contained *R. raphanistrum* pollen had the fewest good seeds per fruit and the most aborted seeds per fruit (Tables 6a and 6b). These differences were statistically significant.

For *R. sativus*: California wild radish, fruits showed no significant effect of mixed cross type on number of seeds per fruit, good seeds per fruit, or aborted seeds per fruit (Tables 6a and 6b).

In Purple Plum, mean mass of seeds sired by the two *R. raphanistrum* donors in mixed pollinations was significantly less than that of most of the other donors (Tables 7a and 7b), with the exception that *R. raphanistrum* donor 3 had similar mean seed mass to target donor

6. In the California wild radish, only one of the *R. raphanistrum* donors had a mean seed mass lower than that of the other donors (Tables 7a and 7b).

Paternity analysis: Is sorting among compatible mates affected when pollen from self (pollen from the same plant), a heterospecific donor (a second related species), or both are included in a pollen mix?

Although the proportion of seeds per fruit sired by the target donor differed slightly among the three target donors and across cross types when in competition with the test donor in *R. sativus*: Purple Plum (Figure 3), these differences were not statistically significant across either target donors or cross types (Table 8). However, the proportion of seeds sired by the three target donors did differ significantly in *R. sativus*: California wild radish. These differences were not affected by, cross type (Table 8, Figure 4).

The mean proportions of seeds sired by target donors did not differ significantly by cross types in the Purple plum (Figure 3), though the relative performance of the pollen donors appears to become more similar with the addition of each pollen type (Figure 3). However, a more careful look at variation in target donor performance reveals a different pattern. Similarity in performance of the donors was compared by calculating the variance in the mean proportion of seeds sired by the target donors in each pollen recipient. Interestingly, the mean variance in pollen donor performance increased significantly between the cross type which included self-pollen and other cross types and was generally higher when *R. raphanistrum* pollen was included (Table 9b). The variance in pollen donor performance was not significantly different by cross type in the California wild radish (Tables 9a and 9b).

***R. raphanistrum* across experiments:** is California wild radish less able to sort against heterospecific pollen than Purple plum?

The proportion of seeds sired by *R. raphanistrum* donors differed significantly between the two experiments (Table 10a). *R. raphanistrum* sired more seeds on Purple plum than on California wild radish (Table 10b). No significant differences in the proportion of seeds sired by *R. raphanistrum* among the cross types were noted within experiments (Tables 10a and 10b).

DISCUSSION

Plant mating systems include a variety of mechanisms that result in non-random success of self- and outcross pollen, pollen from different compatible mates, and pollen from the same or different species. Although some of these mechanisms have received considerable attention (i.e., Charlesworth et al. 2005; Pannell and LaBouche 2013), most previous study has been of only one of these mechanisms at a time. Further, in the field a plant might simultaneously receive pollen of several types; thus, it is likely that multiple mechanisms of sorting among pollen operate simultaneously. If these mechanisms interact in ways that alter sorting among mates, we may be missing important pieces in our understanding of plant mating systems. To begin to ask whether different types of sorting among mates (e.g., sorting against self pollen, sorting among compatible mates, and sorting against heterospecific pollen) interact, I conducted two experiments that ask whether sorting among compatible mates changes when self incompatibility and/or sorting against heterospecific pollen also occur. By using two different *Raphanus sativus* types with different histories of introgression with *R. raphanistrum*, I also asked whether past history of hybridization affects ability to discriminate against heterospecific pollen.

Does adding self-pollen (pollen from the same plant) affect sorting among compatible mates?

The addition of self-pollen did not affect the ability to sort among compatible mates since the proportion of seeds sired by the target donors was unaffected by the presence of self-pollen. This result suggests that the operation of sorting among compatible mates is independent of sorting against self-pollen in this system. A previous experiment with wild

radish showed that even when the self-incompatibility system was circumvented, the ability of compatible mates to sire seeds was not compromised suggesting that the mechanisms are distinct (Marshall 1998). Similarly, a study with *R. raphanistrum* also demonstrated that self-pollen does not interfere with ability of compatible mates to sire seeds when self-incompatible pollen and compatible pollen was applied to the stigma simultaneously (Koelling and Karoly 2007).

The mechanisms and genes that function to produce sporophytic incompatibility in related Brassicaceae species are well known (Kachroo et al. 2002; Busche and Schoen 2008; Franklin-Tong 2008; Chapman and Goring 2010), and recent work has begun to document the molecular genetic mechanism for non-random mating (or sorting compatible mates) for both male and female mediated non-random mating in the self-compatible species *Arabidopsis thaliana*. The genes thus far do not appear to be the same as those involved in the self-incompatibility system (Carlson et al. 2011; Fitz Gerald et al. 2014). These data provide support for the suggestion that my results show the presence of independent sorting mechanisms.

Nonetheless, my study has some limits that may affect both my results and my interpretations. I applied self-pollen at the same time as compatible pollen, showing that simultaneous activation of both sorting mechanisms does not alter sorting among compatible mates. However, in the field, if pollinator visits are not immediate, self-pollen might fall onto the stigma well before any outcross pollen arrives. Thus, further study may be warranted to ask whether the timing in the receipt of self-pollen affects the ability to sort compatible mates. Applying self-pollen well before outcross pollen could produce a different result.

Does adding heterospecific pollen (pollen from a second related species) affect sorting among compatible mates?

Since adding heterospecific pollen to mixes did not affect sorting among compatible mates, any mechanisms that sort against heterospecific pollen do not interfere with or increase sorting among compatible mates, at least in this system under these conditions. Thus, separate mechanisms may be involved. However, more information regarding the molecular genetic underpinnings of heterospecific pollen sorting is required to test this conclusion. Recent work using *Nicotiana* found a protein (class III Pistil-specific Extensin-Like Protein (PELP III)) that suppressed the growth of heterospecific pollen tubes within the stylar tissue (Eberle and Anderson 2013). If this (PELP III) or similar proteins were found to suppress heterospecific pollen tubes, but not affect relative performance of same species pollen tubes in the Brassicaceae, it would further support my hypothesis that there are independent mechanisms responsible for sorting different pollen types. My results cannot distinguish between operation of independent mechanisms and operation of one mechanism that can simultaneously produce two kinds of sorting.

Does adding both self-pollen and heterospecific pollen affect sorting among compatible mates?

Interestingly, in the Purple plum, it appeared that the mean proportion of seeds sired by the three target donors was becoming more similar (Figure 3), but the variation in the proportion of seeds sired between the three target donors increased between the cross types that contained self-pollen and cross types that contained both self- and heterospecific pollen

(Table 9b). This indicates that the relative performance of pollen donors differed more on a single pollen recipient, but the direction of the difference in donor performance varied among the pollen recipients. Perhaps the operation of mechanisms that sort self- and heterospecific pollen allowed for more selection between the two compatible donors. If operation of multiple mechanisms of sorting causes a type of stress in the pollen recipient, this might result in some decrease in selectivity among the pollen donors. This pattern has been observed in other experiments in *R. sativus* (Marshall and Ellstrand 1988; Marshall and Fuller 1994; Marshall and Diggle 2001). Alternatively, the amount of sorting among donors may simply be affected by the number of donors in a cross and not by the number of sorting mechanisms operating (Marshall 1991).

The patterns of seed paternity and the variation in pollen donor performance may be misleading, but the proportion of seeds sired per fruit by each of the target donors did not differ significantly among cross types. Since there were no significant differences in the proportion of seeds sired among cross types, this suggests that sorting against self-pollen and heterospecific pollen are independent mechanisms that do not alter sorting among compatible mates under these conditions in this species. However, in the self-compatible species *Mimulus guttatus* the presence of heterospecific pollen altered sorting among self- and outcross pollen such that the outcrossing rate increased (Arceo-Gomez and Ashman 2014). This result differs from mine and suggests that sorting mechanisms can interact. This difference may be due to the focus on a different part of the mating system or to differences in species that do and do not have sporophytic self-incompatibility

My study had a relatively small sample size and, therefore, may not be able to detect subtle interactions between the sorting mechanisms. However, a pilot study examining interactions of multiple types of pollen sorting in California wild radish had identical results

(personal communication, D.L. Marshall). The combined results of the two studies help confirm my findings since both show that the mechanisms involved in pollen sorting (against self- and heterospecific pollen and among compatible mates) did not interact.

Do the patterns of pollen sorting, specifically sorting against heterospecific pollen, differ in *R. sativus*: California wild radish and *R. sativus*: Purple plum (a cultivar that is not introgressed with *R. raphanistrum*)?

Both the California wild radish and the Purple plum responded similarly to the pollination treatments. Neither showed significant changes in the proportion of seeds sired by the target donors when self-pollen, heterospecific pollen, or both were included in the pollen mix. The patterns of sorting did not differ between California wild radish and Purple plum suggesting that the sorting mechanisms are responding in the same way in both types of *R. sativus*. However, the overall discrimination against heterospecific pollen and the timing of the discrimination was different between the two *R. sativus* types as discussed in the next section.

Is California wild radish less able to sort against heterospecific pollen than Purple plum?

Initially, it appeared that Purple plum discriminated more against heterospecific pollen, since there were fewer good seeds per fruit, more aborted seeds per fruit and a lower mean seed mass for both the single pollinations with *R. raphanistrum* and the mixed pollinations that included *R. raphanistrum* in the pollen mix (Tables 5b, 6b and 7b), whereas in

the California wild radish, there were no significant differences in the means of these characters among *R. raphanistrum* and the conspecific target donors. However, when the overall proportion of seeds sired by *R. raphanistrum* was examined in each of the experiments (Tables 10a and 10b) I found that the *R. raphanistrum* donors sired a significantly lower proportion of seeds per fruit in mixed pollinations on California wild radish than on Purple plum (Table 10b). Thus, there was a striking difference in the pattern of sorting between the two types of *R. sativus* in that the Purple plum aborted significantly more seeds in both the single pollinations with *R. raphanistrum* and in the mixed pollinations with *R. raphanistrum* included while *R. raphanistrum* sired fewer seeds in competition with conspecific pollen on *R. sativus*.

It appears that the mechanism for discriminating heterospecific pollen acted earlier (prior to fertilization) in California wild radish than in Purple plum. This may give an advantage to California wild radish, since seed abortion has a negative impact on reproductive fitness. Finding earlier sorting against heterospecific pollen in the California wild radish was a surprise since it has already introgressed with *R. raphanistrum*. However, there may be selection for this sorting since the introgressed California wild radish has been found to outperform both cultivars of *R. sativus* and wild *R. raphanistrum* in southern California (Heredia and Ellstrand 2014). Further study could examine the performance of both *R. raphanistrum* and cultivar pollen on California wild radish. Both types might be discriminated against given the fitness advantage of the California wild radish.

Conclusion:

From my data, I concluded that sorting against self-pollen, sorting among compatible mates and sorting against heterospecific pollen are independent mechanisms. More detailed molecular genetic work in the Brassicaceae agrees with this conclusion, at least

for two of the three mechanisms involved in sorting (Hiscock and McInnis, 2003; Carlson et al. 2009; Carlson et al. 2011; Fitz Gerald et al. 2014). When the genes involved in heterospecific pollen sorting are identified we can ask whether those genes are independent from the others involved in pollen sorting. When all of the relevant genes are identified it will be possible to address the origin of the sorting mechanisms. That is, are modifications of the same genes involved in all types of sorting among pollen?

Just as the mechanisms of incompatibility differ among taxa, mechanisms that sort among compatible mates and against heterospecific pollen may differ among species. Finding that these processes are independent in *Raphanus*, coupled with reports of the genes involved in various aspects of the mating system in *Arabidopsis* suggest that independence of these mechanisms could be common in the Brassicaceae. However, this does not predict the results in other species, especially those with gametophytic incompatibility.

Figures

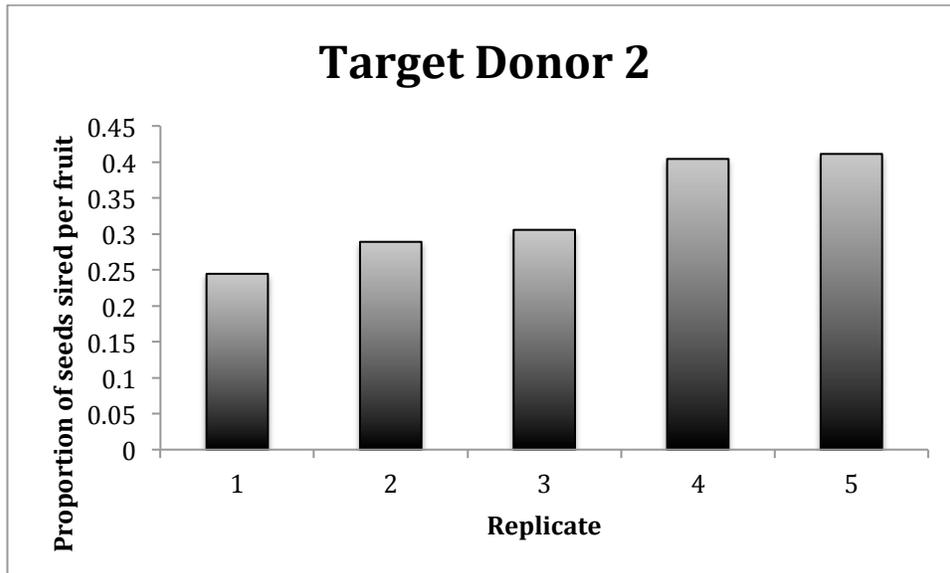


Figure 1: Charts the change in proportion of seeds per fruit sired by target donor 2 over the five replicates in a single pollen recipient (pollen recipient 'C' in California wild radish).

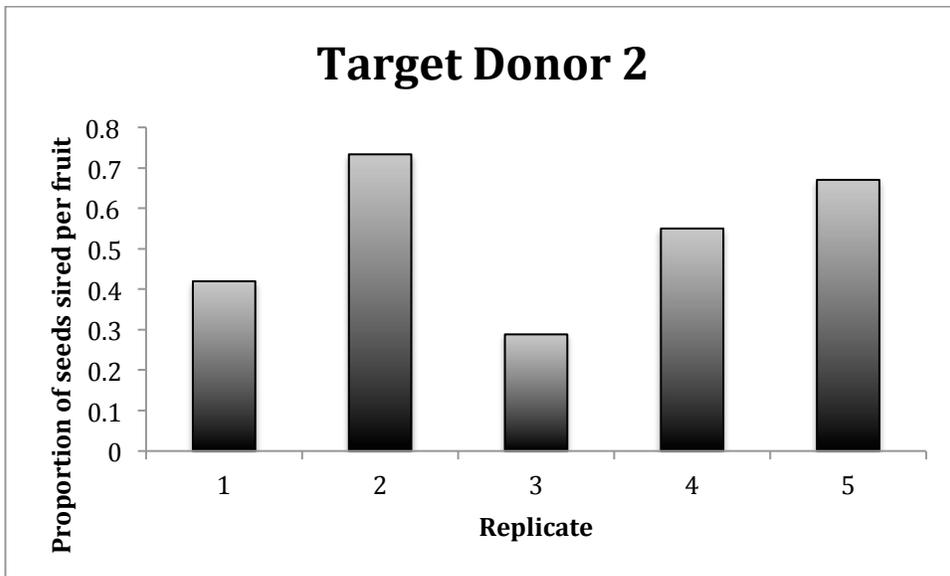


Figure 2: Charts the change in the proportion of seeds sired per fruit sired by target donor 2 over the five replicates in a single pollen recipient (pollen recipient 'E' in California wild radish).

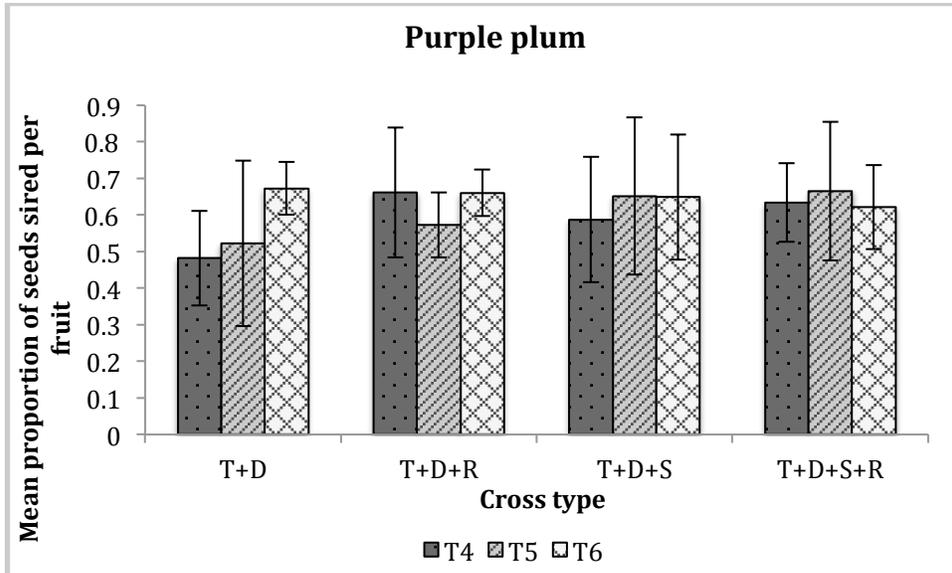


Figure 3: Relative mean proportion of seeds sired by the target donors (target donor 4 (T4), target donor 5 (T5), and target donor 6 (T6)) when compared to the test donor. Proportion of seeds sired, is shown by cross type and target donor in Purple plum. Cross type T+D is the combination of target donor and test donor. T+D+S is the target donor, test donor, and self-pollen. T+D+R is target donor, test donor and *R. raphanistrum*. T+D+S+R is the combination of all donors: target donor, test donor, self and *R. raphanistrum*.

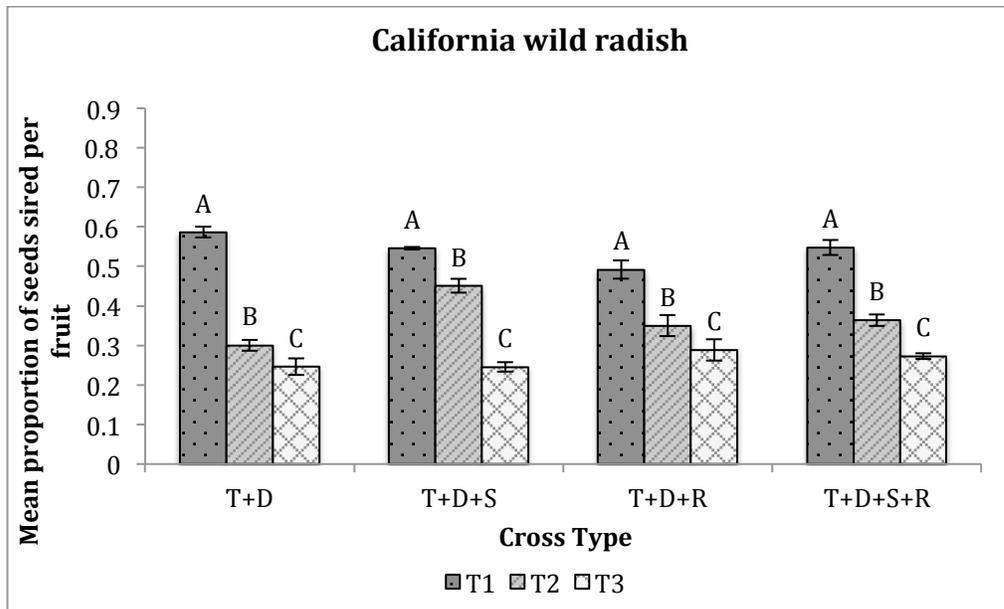


Figure 4: Relative mean proportion of seeds sired by the target donors (target donor 1 (T1), target donor 2 (T2), and target donor 3(T3)) when compared to the test donor. Proportion of seeds sired, is shown by cross type and target donor in California wild radish. Within cross types, bars with different letters were significantly different in Tukey's Studentized Range Tests. Cross type T+D is the combination of target donor and test donor. T+D+S is the target donor, test donor, and self-pollen. T+D+R is target donor, test donor and *R. raphanistrum*. T+D+S+R is the combination of all donors: target donor, test donor, self and *R. raphanistrum*.

Tables

Table 1: Number of Purple plum plants used to produce seeds.

| | | | | | |
|---------------------|----|----|----|----|-------|
| PGI Phenotype | 22 | 33 | 44 | 24 | Total |
| Number transplanted | 20 | 15 | 12 | 5 | 52 |

Table 2: Plant material used.

| Type of Plant | Number used | Purpose | PGI Phenotype |
|--|--------------------|--|---|
| Pollen recipients (all unrelated) | 5 | All crosses were performed on these plants and fruits collected for analysis. | All had a 22 PGI phenotype. |
| Target Donors-These are compatible with and unrelated to the pollen recipients. | 3 | Pollen donors whose ability to sire seeds in competition with a standard competitor was tested. | All have a 33 PGI phenotype |
| Test Donors- These plants are compatible with and unrelated to the pollen recipients | 2 | Pollen donors used as a standard competitor to test the 'target donors' ability to sire seeds in competition | Purple plum plants of this type had a 44 PGI phenotype. California wild radish of this type had a 11 PGI phenotype. |
| <i>R. raphanistrum</i> | 2 | A related species, used to test the effect of pollen from a different species (heterospecific pollen donor) | Plants of this type had a 22 PGI phenotype. |

Table 3: Mixed Pollination Crosses.

| Cross Type | Pollen Donors (repeated for each of the donors) | Questions to be answered by cross type. |
|-------------------|--|--|
| T+D | Test + Target donors | What proportion of seeds per fruit do the target donors sire when pollen competition and mate choice are possible? |
| T+D+S | Test + Target donors + self | Does adding self-pollen affect sorting among compatible mates? |
| T+D+R | Test + Target donors + R. <i>raphanistrum</i> (repeated for each target donor with each of the R. <i>raphanistrum</i>) | Does adding heterospecific pollen affect sorting among compatible mates? |
| T+D+S+R | Test + Target donors + Self + R. <i>raphanistrum</i> (repeated for each target donor with each of the <i>raphanistrum</i>) | Does adding both self- and heterospecific pollen affect sorting among compatible mates? |

Table 4: Analysis of variance of mean proportion of seeds sired by the target donors. The Arcsine square root transformation on the number of seeds sired by the target donor when in competition with the test donor is the dependent variable.

| Independent variables | California wild radish | | Purple plum | |
|--|------------------------|-----------|-------------|---------|
| | df | F-value | df | F-value |
| Pollen recipient | 4 | 2.59* | 4 | 1.34 |
| Replicate | 4 | 2.49* | 4 | 1.96 |
| Target pollen donor | 2 | 26.54**** | 2 | 0.87 |
| Cross type | 3 | 1.77 | 3 | 1.06 |
| Pollen recipient X Target pollen donor | 8 | 1.23 | 8 | 3.25** |
| Pollen recipient X Replicate | 16 | 0.64 | 16 | 1.33 |
| Pollen recipient X Cross type | 12 | 0.70 | 12 | 0.25 |
| Replicate X Target pollen donor | 8 | 1.81 | 8 | 1.13 |
| Target pollen donor X Cross type | 6 | 0.84 | 6 | 0.80 |
| Target pollen donor X Cross type | 12 | 1.51 | 12 | 0.98 |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 5a: Mixed model analyses of variance of seed and fruit measures in single donor pollinations. Seed number, good seeds, aborted seeds per fruit, and seed mass were used as the dependent variables. Pollen recipient, cross type, and their interactions were independent variables. Pollen recipient and the interaction of pollen recipient and cross type were treated as random effects.

| | Purple plum | | | | |
|-------------------------------|-------------|-------------|------------|---------------|-----------|
| Independent Variables | df | Total seeds | Good seeds | Aborted seeds | Seed mass |
| Pollen recipient | 4 | 32.21**** | 35.52**** | 2.21 | 21.64**** |
| Cross type | 5 | 0.9 | 8.96*** | 5.44** | 4.56** |
| Pollen recipient X Cross type | 20 | 0.97 | 1.18 | 1.68* | 5.26**** |
| R ² | | 0.56 | 0.67 | 0.44 | 0.47 |
| N | | 150 | 150 | 150 | 794 |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 5b: Mixed model analyses of variance of seed and fruit measures in single donor pollinations. Seed number, good seeds, aborted seeds per fruit, and seed mass were used as the dependent variables. Pollen recipient, cross type, and their interactions were independent variables. Pollen recipient and the interaction of pollen recipient and cross type were treated as random effects.

| | California wild radish | | | | |
|-------------------------------|------------------------|-------------|------------|---------------|-----------|
| Independent Variables | df | Total seeds | Good seeds | Aborted seeds | Seed mass |
| Pollen recipient | 4 | 21.79**** | 11.93**** | 4.88** | 35.2**** |
| Cross type | 5 | 0.49 | 0.38 | 0.87 | 2.33 |
| Pollen recipient X Cross type | 20 | 1.06 | 1.1 | 1.02 | 3.55**** |
| R ² | | 0.49 | 0.39 | 0.27 | 0.41 |
| N | | 149 | 149 | 149 | 921 |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 5c: Mean seed number, number of good seeds per fruit, mean of aborted seeds per fruit, and mean seed mass by pollen donor in *R. sativus*: Purple Plum and California wild radish. T1, T2, and T3 represent the three target donors, test is the test donors and R1, R2, R3 and R4 are the *R. raphanistrum* pollen donors. Values within rows that do not share a superscript were significantly different in a Tukey's Studentized Range Test ($P < 0.05$).

| | California wild radish | | | | | | Purple plum | | | | | |
|---------------------|------------------------|-----|-----|------|-----|-----|-------------------|-------------------|------------------|------------------|------------------|------------------|
| | T1 | T2 | T3 | Test | R1 | R2 | T4 | T5 | T6 | Test | R3 | R4 |
| Total seeds/fruit | 6.8 | 6.6 | 6.3 | 6.6 | 6.4 | 6.7 | 6.8 | 6.7 | 6.4 | 6.1 | 6.1 | 6.6 |
| Good seeds/fruit | 6.5 | 6.1 | 6.0 | 6.1 | 6.1 | 6.2 | 6.2 ^a | 5.8 ^a | 5.8 ^a | 5.6 ^a | 4.2 ^b | 4.1 ^b |
| Aborted seeds/fruit | 0.3 | 0.5 | 0.2 | 0.5 | 0.4 | 0.5 | 0.6 ^b | 0.9 ^{ab} | 0.6 ^b | 0.6 ^b | 1.8 ^a | 2.5 ^a |
| Seed mass (mg) | 8.2 | 8.1 | 8.6 | 8.3 | 8.4 | 7.5 | 8.0 ^{ab} | 8.0 ^{ab} | 7.7 ^b | 8.4 ^a | 6.7 ^c | 6.3 ^c |

Table 6a: Mixed model analysis of variance for mean seed number per fruit, mean good seeds per fruit, and mean aborted seeds per fruit in mixed cross types. Seed number, good seeds and aborted seeds served as the dependent variables, and pollen recipient, cross type and their interactions were the independent variables. Pollen recipient and the interaction between pollen recipient and cross type were treated as random variables.

| Independent Variables | California wild radish | | | Purple plum | | | |
|----------------------------------|------------------------|-----------------|-------------------------|----------------------------|-----------------|-------------------------|----------------------------|
| | df | Seeds/ fruit | Good seeds/ fruit | Aborted seeds/ fruit | Seeds/ fruit | Good seeds/ fruit | Aborted seeds/ fruit |
| Pollen recipient | 4 | 35.97*** | 22.48*** | 16.74*** | 87.55*** | 22*** | 5.12** |
| Cross type | 3 | 0.56 | 0.45 | 1.73 | 0.84 | 3.54* | 8.72** |
| Pollen recipient X Cross type | 12 | 1.18 | 1.1 | 0.65 | 0.71 | 2.62** | 2.51** |
| R ² | | 0.33 | 0.23 | 0.12 | 0.4 | 0.41 | 0.29 |
| N | | 449 | 449 | 449 | 452 | 452 | 452 |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 6b: Mean number of seeds per fruit, mean good seeds per fruit, and mean number of aborted seeds per fruit for mixed pollinations in *R. sativus*: Purple Plum and California wild radish. Values within rows that do not share a superscript were significantly different in a Tukey's Studentized Range Test (P<0.05). Cross type T+D is the combination of target donor and test donor. T+D+R is target donor, test donor and *R. raphanistrum*. T+D+S is the target donor, test donor, and self-pollen. T+D+S+R is the combination of all donors: target donor, test donor, self and *R. raphanistrum*.

| Cross types | California wild radish | | | Purple plum | | |
|-------------|------------------------|---------------------|------------------------|----------------------|---------------------|------------------------|
| | Total seeds/fruit | Good seeds/fruit | Aborted seeds/fruit | Total seeds/fruit | Good seeds/fruit | Aborted seeds/fruit |
| T+D | 6.7 | 6.3 | 0.5 | 6.3 ^a | 5.8 ^a | 0.6 ^b |
| T+D+R | 6.6 | 6.2 | 0.4 | 6.1 ^a | 4.8 ^b | 1.3 ^a |
| T+D+S | 6.5 | 6.1 | 0.4 | 6.1 ^a | 5.5 ^a | 0.6 ^b |
| T+D+R+S | 6.5 | 6.3 | 0.4 | 6.3 ^a | 4.7 ^b | 1.6 ^a |

Table 7a: Mixed model analysis of variance of mean seed mass in mixed pollinations. Seed mass was the dependent variable while pollen recipient pollen donor and their interactions were the independent variables. Pollen recipient and the interaction of pollen recipient and pollen donor were treated as random variables.

| Independent Variables | California Wild Radish | | Purple Plum | |
|---------------------------------|------------------------|------------|-------------|-----------|
| | df | F-Value | df | F-Value |
| Pollen recipient | 4 | 152.14**** | 4 | 68.81**** |
| Pollen Donor | 5 | 14.03**** | 5 | 6.62*** |
| Pollen recipient X Pollen Donor | 20 | 1.92** | 20 | 4.55**** |
| R ² | 0.42 | | 0.44 | |
| N | 2781 | | 2205 | |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 7b: Mean seed mass by pollen donor in mixed pollen crosses for *R. sativus*: Purple Plum and California Wild Radish. Values within rows that do not share a superscript were significantly different in a Tukey's Studentized Range Test (P<0.05). T1, T2 and T3 represent the target donors, test is the test donor and R1, R2, R3 and R4 represent the *R. raphanistrum* donors.

| | California wild radish | | | | | | Purple plum | | | | | |
|---------------------|------------------------|-------------------|------------------|------------------|-------------------|------------------|------------------|------------------|-------------------|------------------|-------------------|------------------|
| | T1 | T2 | T3 | Test | R1 | R2 | T4 | T5 | T6 | Test | R3 | R4 |
| Mean seed mass (mg) | 8.0 ^c | 8.5 ^{ab} | 8.1 ^c | 8.6 ^a | 8.2 ^{bc} | 7.3 ^d | 7.8 ^b | 7.9 ^a | 7.7 ^{bc} | 8.3 ^a | 6.7 ^{cd} | 7.0 ^d |

Table 8: Mixed model analysis of variance of the proportion of seeds sired by the target pollen donor(s) across four cross types. The arcsine square root transformation of proportion of seeds sired by the target donors when compared to the test donor is the dependent variable. Target pollen donor, pollen recipient, and their interactions were treated as random effects.

| Independent Variables | California wild radish | | Purple plum | |
|--|------------------------|---------|-------------|---------|
| | df | F-Value | df | F-Value |
| Target pollen donor | 2 | 24.98** | 2 | 0.3 |
| Cross type | 3 | 2.13 | 3 | 1.32 |
| Replicate within pollen recipient | 20 | 0.82 | 20 | 0.99 |
| Target pollen donor X pollen recipient | 8 | 0.88 | 8 | 2.43* |
| Target donor X cross type | 6 | 0.84 | 6 | 0.85 |
| Target donor X replicate within pollen recipient | 40 | 1.42 | 40 | 1.43 |
| Target donor X cross type X pollen recipient | 36 | 0.86 | 36 | 0.68 |
| R ² | 0.31 | | 0.26 | |
| N | 449 | | 450 | |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 9a: ANOVA for the mean variance in donor performance between the 4 cross-types. Pollen donor variance was the dependent variable, and cross type was the independent variable.

| California wild radish | | | Purple plum | |
|-----------------------------|-----------|----------------|-------------|----------------|
| Independent variable | df | F-value | df | F-value |
| Cross type | 3 | 0.71 | 3 | 3.73* |
| R ² | 0.12 | | 0.41 | |
| N | 20 | | 29 | |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 9b: Variance in mean proportion of seeds sired by the three target donors across the 5 pollen recipients when increasing numbers of sorting mechanisms are active in California wild radish and Purple plum. Cross type T+D is the combination of target donor and test donor. T+D+S is the target donor, test donor, and self-pollen. T+D+R is target donor, test donor and *R. raphanistrum*. T+D+S+R is the combination of all donors: target donor, test donor, self and *R. raphanistrum*. Values within rows that do not share a superscript were significantly different in a Tukey's Studentized Range Test (P<0.05).

| California wild radish | | Purple plum |
|------------------------|-----------------|---------------------|
| Cross type | Variance | Variance |
| T+D | 0.096 | 0.121 ^{ab} |
| T+D+R | 0.089 | 0.145 ^{ab} |
| T+D+S | 0.106 | 0.085 ^a |
| T+D+S+R | 0.109 | 0.163 ^b |

Table 10a: Analysis of variance of the proportion of seeds sired by *R. raphanistrum* across experiments. Lineage represents the two experiments one with *R. sativus*: California wild radish and the other with *R. sativus*: Purple plum. The cross types were combined across experiments and include only those that contain *R. raphanistrum*. The arcsine square root transformation of the proportion of seeds sired by *R. raphanistrum* is the dependent variable while lineage is the independent variables.

| Independent Variables | df | F-Value |
|-----------------------|-------|-----------|
| Lineage | 1 | 43.51**** |
| R ² | 0.007 | |
| N | 600 | |

**** P <0.0001 *** P <0.001 **P <0.01 *P <0.05

Table 10b: Table of means showing the proportion of seeds per fruit sired by *R. raphanistrum* in a combined analysis across experiments using California wild radish and Purple plum. Values within rows that do not share a superscript were significantly different in a Tukey's Studentized Range Test (P<0.05).

| | California Wild Radish | Purple Plum |
|---------------------------|------------------------|--------------------|
| Proportion of seeds sired | 0.177 ^b | 0.331 ^a |

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