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RIVERSIDE

Seed Packaging and Seed Characteristics
in a *Raphanus* Hybrid-Derived Lineage

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Plant Biology

by

Silvia Margarita Heredia

December 2012

Dissertation Committee:
Dr. Norman C. Ellstrand, Chairperson
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The Dissertation of Silvia Margarita Heredia is approved:

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Committee Chairperson

University of California, Riverside

ACKNOWLEDGMENTS

To my three boys,

and to my Colombian, Italian and North American families.

ABSTRACT OF THE DISSERTATION

Seed Packaging and Seed Characteristics
in a *Raphanus* Hybrid-Derived Lineage

by

Silvia Margarita Heredia

Doctor of Philosophy, Graduate Program in Plant Biology
University of California, Riverside, December 2012
Dr. Norman C. Ellstrand, Chairperson

Hybridization is accountable for a considerable number of plant species of which some are remarkably good invaders. Interspecific hybridization can promote plant invasiveness by leading to (1) higher reproductive output, (2) higher genetic diversity, and (3) novel traits or genotypes resulting in range expansion. Through descriptive and experimental analyses, the aim of this study is to understand if an invasive hybrid-derived lineage, California wild radish, varies in novel traits such as fruit structure and within-fruit seed characteristics, relative to its two progenitors, the cultivar *Raphanus sativus* and the wild *R. raphanistrum*. I first asked if changes in fruit structural and material properties have important effects on pre-dispersal granivory. Data included fruit morphometrics, biomechanical measurements of the strength necessary to break open the fruit wall, and records of removal after experimentally exposing the fruits to granivores.

Combining these data revealed that hybrid fruits differ structurally from fruits of both progenitors, thereby lowering the damage rates by granivores. Second, I asked if pre-dispersal seed predation differs among the three *Raphanus* lineages and if there are fitness consequences resulting from this. Plants from all three lineages were sown in common gardens, either protected or unprotected from avian granivory experiments. Relative to both progenitors, the hybrid lineage suffered the highest rates of granivory at the unprotected treatments but its seed and fruit production was not depressed by granivory. Finally, I explored how within-fruit seed characteristics may be a feature explaining the competitive superiority of the hybrid-derived lineage. I addressed this topic by directly comparing patterns of within-fruit seed size, paternity, and fitness variables including fecundity and phenology. Within-fruit seed paternity revealed that all three lineages have multiple paternity and that in natural conditions the identity of the pollen donor affects seed weight and final reproductive output. The hybrid-derived lineage had larger peduncular seeds. Taken together, these results indicate that the strength of the fruit wall is a key defense mechanism underlying invasion success in the hybrid-derived lineage.

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INTRODUCTION

Damage by biotic agents is one the most important challenges to plant survival and reproduction (Coley et al. 1985). Animal influences on plants may be antagonistic (i.e., cause damage), mutualistic (pollination and seed dispersal), or some combination of the two. The responses by plants to herbivory influence their chemistry, morphology, phenology, and ecology (Edwards 1989; Herms and Mattson 1992; Edwards et al. 2000; Fine et al. 2004), and can also lead to the evolution of defense traits. The research described here was motivated by the expectation that this evolutionary process would be especially apparent by comparing an invasive hybrid-derived plant with its two progenitor lineages.

Biological defenses are defined as adaptive responses to selective forces imposed via interactions with other organisms (Herms and Mattson 1992). In plant biology, chemical defenses have received considerably more attention than structural defenses (Grubb 1992; Hanley et al. 2007). Many structural defenses also have physiological effects such as aiding in water retention and cold tolerance, and it has been suggested that the physiological role is typically the original target of selection with anti-herbivore defensive features evolving thereafter (Edward 1989; Strauss and Agrawal 1999). Structural defenses can take several forms such as spinescence, pubescence and sclerophylly (reviewed in Hanley et al. 2007).

Plants have structural defenses that protect both vegetative and reproductive components. For the latter, the investment of the plant in the propagule generally

increases with phenology and is at its highest level during the period of seed dispersal. Seeds represent one of the most vulnerable stage in a plant's life cycle, so their defense is essential to the parent plant's reproductive success (Kolb et al. 2007). A common mechanism of defense is to package seeds in fruits, which are evolved structures that mediate maturation and seed dispersal, and can also protect seeds by reducing consumption from granivores (Ferrándiz 2002).

Pre-dispersal seed predation has the potential to affect the ecology and evolution of plant populations (Kolb et al. 2007). Some plants lose a significant proportion of their seeds to seed predators and these loses have the potential to change the number of subsisting individuals, their occurrence and dissemination modifying the structure and interactions of the ecosystem. Each species responds differently based on intrinsic as well as the environmental characteristics. At the evolutionary level, seed predators may act as selective agents when feeding differentially among plants with different heritable traits (Kolb et al. 2007). This can be assessed by considering how plant traits are altered in response to predation pressure. However, it is still difficult to predict the ecological and evolutionary consequences of seed predation with considering plant-animal interactions in spatially- and temporally-explicit frameworks. For example, the amount of seeds lost is not necessarily a good estimate of the impact of the granivores (Kolb et al. 2007). Factors other than predator pressure, such as variation in seed density, seed predator density, or presence of other interacting species, can contribute to predation rate. Arguably, one of the most useful features of hybrid plant systems is that one can regenerate or work with existent hybrid lineages and compete it with its pure lines of progenitors to examine the

interactions between ecological and evolutionary processes with spatial and temporal precision.

Many invasive plants are hybrid-derived. There are multiple examples of invasive lineages derived from progenitors in which both members are introduced (non-natives), as well as from crosses between introduced and native plants (Ellstrand and Schierenbeck 2000). A recent study illustrates how hybridization between native *Rosa mollis* and invasive *R. rugosa* is reducing the genetic diversity of *R. mollis* as well as its abundance (Kellner et al. 2012). When referring to hybrid plant susceptibility, Fritz et al. (1994) have proposed four alternative hypotheses to the null hypothesis that the are no differences in susceptibility to damage when comparing F1 hybrid-derived with progenitor lineages. The four alternative hypotheses consider how resistant traits are inherited and expressed as follows: (1) additive hypothesis: when hybrid susceptibility is intermediate. The average resistance is not different from both progenitors; (2) dominance: when the mean susceptibility of the F1 resembles to one of the progenitors; (3) hybrid susceptibility: the F1 susceptibility exceeds both progenitors. Mixed stands of progenitors and hybrids will have more herbivores residing on hybrid plants (i.e. sink hypothesis by Whitham 1989); finally (4) hybrid resistance: F1 hybrids are more resistant than either progenitor. Experimentally testing hybrids produced *de novo* allows for the determination of inheritance patterns. In contrast, a comparison of inherited traits in advanced hybrids with pure lines of both progenitors is likely to be confounded by evolutionary history.

Radish plants in the genus *Raphanus* are a traditional model system for research in plant ecology (Stanton 1987a, Stanton 1987b, Conner et al. 1995, Conner 1996) and plant evolution (Snow and Campbell 2005, Campbell et al. 2006, Campbell et al. 2009a, Campbell et al. 2009b, Hegde et al. 2006, Ridley et al. 2008, Ridley and Ellstrand 2009, Ridley and Ellstrand 2010). The genus *Raphanus* belongs to the Brassicaceae family. *Raphanus* flowers are insect pollinated, self-incompatible based on a single S-allele locus (Sampson 1967) and hermaphroditic. The genus has a well-studied reproductive and sexual selection system (Marshall and Ellstrand 1986; Hill and Lord 1986; Hill and Lord 1987; Stanton 1987b; Marshall and Ellstrand 1988; Marshall and Whittaker 1989; Nason and Ellstrand 1995; Jablonski 1997; Marshall 1998; Marshall 1991; Marshall 1998; Agrawal et al.1999, Marshall et al. 2007) and high genetic polymorphism (Stanton 1987a; Conner 1996; Strauss et al. 2003; Hegde et al 2006; Sahli et al. 2008). *Raphanus* species have non-fleshy pod-like fruits that dry out attached to the plant. The fruit develops from the pistil to form an indehiscent silique pod-like fruit attached to the plant by a pedicel (Panetsos 1953). Each fruit holds from 1 to up to 12 seeds in total. Mature seeds have a globular shape weighing 2mg to 12 mg (Stanton 1985). Several features of reproductive biology of *Raphanus* suggest that this is a tractable system for assessing how the evolution of plant characters and plant-enemy interactions may have contributed to its invasive success.

The central question motivating this dissertation research is: are there enhanced seed packaging and seed characteristics in a hybrid-derived invasive lineage relative to its progenitors? This problem is considered from several perspectives that are described over

three chapters. Chapter one concerns seed packaging and addresses three topics: (1A) What are the differences in fruit packaging traits such as within-fruit seed distribution, fruit shape, and fruit wall strength? (1B) Are there differences in the type and quantity of damage inflicted by the granivore on the fruits? (1C) Is the fruit material homogenous across the length of the fruits? Chapter two concerns granivore effects on fecundity by addressing the following topics: (2A) What species of bird granivore is the main consumer of radish seeds? (2B) How much does the bird granivore affect relative fecundity and relative potential fecundity? (2C) Are the variables likely to affect the birds' selection of individual plants such as days to germination, plant final weight, total fruit production and potential reproduction, correlated with fruit damage? (2D) Are there viable seeds in the debris due to granivory resulting from the bird foraging behavior under damaged plants? Chapter three considers within-fruit seed characteristics, and with five major objectives: (3A) To compare seed weight among all three *Raphanus* lineages and their populations; (3B) To determine if within-fruit seed positioning influences seed weight, fecundity, and other morphological as well as fitness related characteristics; (3C) To compare if single and to mixed hand pollination crosses influence fitness values; (3D) To document the occurrence of multiple paternity in all three lineages; (3E) To assess maternal and paternal effect on seed weight and other fitness related variables. Collectively, these studies examine the inter-relationships among fruit mechanical defense, granivory and fitness. The results provide insights into how interspecific hybridization, pre-dispersal seed predation defenses and propagule quality determine if a hybrid lineage will become a successful invader that is able to replace its progenitors.

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CHAPTER 1

Fruit Wall Strength and Seed Packaging in the Hybrid-Derived Lineage,

California Wild Radish

ABSTRACT

Hybridization is a significant evolutionary force in plant speciation. Inter-specific hybridization may have considerable effects on plant structural defenses that contribute to the success of invasive hybrid-derived lineages. Changes in fruit structural and material properties can have key effects on pre-dispersal granivory. Here, I describe and experimentally test the fruit of an invasive hybrid-derived lineage relative to its wild and domesticated *Raphanus* progenitors. I measured the total length and weight of the fruits as well as the diameter, volume, shape, internal seed distribution and number of seeds at three different sections of the fruits. By coupling morphological measurements with the strength necessary to break open the fruit wall along with experimentally exposing the fruits to granivores, our data reveals that the hybrid fruit: (1) differs structurally from both progenitors, (2) its fruit wall is not structurally homogenous lengthways with harder peduncular sections, (3) within-fruit seed arrangement varies from highly arranged comparable to the wild progenitor to loosely arranged similar to the domesticated progenitor, at times with both arrangements present within individual fruits, and (4) suffers lower rates of damage by the granivores. Based on the variation in material and structural properties of the fruits among three lineages, I propose that the fruit wall is a key defense mechanism underlying invasion success in the hybrid-derived lineage.

INTRODUCTION

Inter and intra-specific hybridization is an important evolutionary force in plant speciation and phenotypic diversification in flowering plants (Arnold 1992; Rieseberg and Ellstrand 1993; Rieseberg 1997; Ellstrand 2003; Mallet 2007). Successful hybrids display novel combinations of phenotypes that can be selectively advantageous in novel environments compared to progenitor lineages (Anderson and Stebbins 1954; Rieseberg et al. 2003). This same evolutionary force may have significant effects during the invasion process, particularly on the establishment and propagation of invasive species (Ellstrand and Schierenbeck 2000). Relative to both progenitor species, resistance to damage by biotic agents (e.g. herbivores, granivores, pathogens) in native hybrids ranges from more susceptible to more resistant (Fritz 1999; Fritz et al. 1999). Material properties such as stiffness, extensibility and strength, and structural properties, such as size and shape, determine some of defensive traits that help plants to resist damage (Read and Stokes 2006, Hanley et al. 2007; Onoda et al. 2011). Despite their importance in plant survival and fitness (Lucas et al. 2000; Hanley et al. 2007; Zhang et al. 2011) very little is known about the biomechanical features underlying invasiveness (Grosholz 2010).

The role of the fruit wall as a material and structural defense against pre-dispersal granivory has seldom been explored in the context of plant invasiveness. Seeds are the most vulnerable yet most critical stage of angiosperm plants' life, and fruits have evolved not only to facilitate their fertilization, maturation and dispersal (Ferrándiz 2002; Groszmann et al. 2011) but also to protect them. For instance, it has been proposed that the initial function of the pulp in fleshy fruits was defensive against seed predators; the

pulp has subsequently evolved as a reward to seed dispersers (Mack 2000). In the case of serotinous plants, seeds are stored in the canopy inside protective cones or fruits for a year or more (Lamont et al. 1991), and the thickness and density of the fruits are correlated to the level of serotiny and protection against granivory and abiotic conditions (Groom and Lamont 1997). As a result, fruit characteristics can be viewed as a set of packaging traits with one function being to provide protection against pre-dispersal biotic conditions.

The three *Raphanus* system has previously been used to study how structural properties, i.e. fruit morphology, has evolved through hybridization. This system consists of the two parental lineages: *Raphanus sativus* and *R. raphanistrum* together with their hybrid derived lineage, the highly successful invasive plant named here as California wild radish. The striking variation in fruit structural characteristics among different *Raphanus* species has being of interest since early 1900's and it has also been suggested that the material properties, specifically fruit wall strength, may be an additional barrier against seed removal by predators (Trouard-Riolle 1914; Frost 1923; Panetsos 1953; Hegde et al.2006). If true, then the material and/or structural properties of the fruit should also vary among lineages and possibly at different parts of the fruit.

Raphanus species have fully indehiscent (Hall et al 2011) non-fleshy loments or pod-like fruits (figure 1.1) that dry out attached to the plant. The fruit develops from the pistil in the flower attached to the plant by a pedicel (Panetsos 1953). In most cases the structure of the fruit narrows into a seedless beak on the stylar end that can account for up to half the total length of the fruit. Recently, Cousens et al. 2010 found that the

segmented fruit wall around single dispersing seeds of *Raphanus raphanistrum* slows the

rate of desiccation for short periods of time, primes germination, and induces dormancy if

conditions of moisture prevail for long period of time. Because fruit structure is one of

the genetically based traits that differentiate all three *Raphanus* lineages (Panetsos and

Baker 1967; Hegde et al. 2006), it is possible to perform comparative experiments. Here I

address the following questions: (1) what are the differences in the fruit packaging traits

of within-fruit seed distribution, fruit shape, and fruit wall strength? (2) are there

differences in the type and quantity of damage inflicted by the granivore on the fruits?

and (3) are the fruits materially homogenous across the length of the fruits?

MATERIALS AND METHODS

Plant material - The plants that produced the fruits used in this experiments were reared

in a common garden during Spring 2005 and Winter 2006. The seed sources for those

maternal plants are described in table 1.1. The fruits are the result of natural open

pollination in common gardens at the Agricultural Operations (AgOps) of the University

of California-Riverside (UCR). More details on how the plants were grown can be found

in Ridley and Ellstrand (2009). Cultivated radish (Rs) and the hybrid derived wild radish

(CAwr) included 4 populations each, whereas wild radish (Rr) contained 3 populations.

For all populations, I selected at random 10 mothers, and then selected at random 10

fruits from each mother; Rs and CAwr had 400 fruits each and Rr 300 fruits for a total of

1100 fruits.

Fruit packaging traits - I studied five variables associated with fruit structure from each of the three lineages under study. All fruits were measured for total fruit length (mm), from the most stylar to the most peduncular ends of the fruits, total weight (mg), and the length (mm) of the seedless portion of the fruits or beak (b). I also estimated fruit volume (mm^3) using the fruit diameters in the middle (m) of the seeded portion of the fruit and 2/3 away from that central point towards peduncular (p) and the stylar (s) portions inside the seeded portion of the fruit. Two perpendicular measurements of the diameter were recorded and averaged at each of 3 sections (p, m, s). To estimate the volume I assumed the fruits were formed of two joined frustum shapes, one from the peduncular to the middle section and the other from the middle to the stylar section. The total volume of the fruit (V_f) was calculated as follows:

$$V_f = \left[\frac{\pi \frac{h_t}{2}}{12} \left(d_p{}^2 + d_p d_m + d_m{}^2 \right) \right] + \left[\frac{\pi \frac{h_t}{2}}{12} \left(d_m{}^2 + d_m d_s + d_s{}^2 \right) \right] ,\text{where } h_t \text{ is the total length of}$$

the seeded portion, d_p is the average of the peduncular diameter, d_m is the average of the middle diameter and d_s is the average of the stylar diameter. The fruit volume did not include the beak portion (seedless area) of the fruit.

A potentially key material trait for fruit protection is the fruit wall strength, which is defined as the stress value at which the wall breaks (Lamont et al. 1991; Groom and Lamont 1997). This was measured with a custom-designed pliers device, designed and fabricated at the Department of Biology at UCR (based on Grant 1999; figure 1.2). The device has a Dillon universal force gauge model "U" with a 50-pound force capacity. The

contact area between the pliers and fruit is the bottom of a 6/32 screw with a flat bottom and an area of 5.04 mm^2. The force measurements in Pounds were converted to Newtons and normalized by the contact area in mm^2 to give measurements of stress (N/mm^2). Therefore the strength has the same units. The strength was measured at exactly the same place where the diameters were measured: peduncular (p), middle (m), stylar (s), besides the seedless beak (b) at the stylar end of the fruits (figure 1.2). The same person pliers operated the pliers to standardize the strength applied to obtain the measurements. In some fruits the strength was measured in only one of the halves of the fruit wall (H) for each section wall when it was necessary to extract intact seeds for further analysis (see below). For the remaining fruits, the strength was recorded on whole fruits (W).

To visualize the distribution and number of the seeds for each of the three sections within the fruits, I used X-radiation (X ray). I visualized three fruits from five mothers selected at random from each of the four populations of Rs, three populations of CAwr, and three populations of Rr. The X rays were standardized at 44 kilovolts (kVp) and 3 milliampere/seconds (mAs). The distance to the high definition plate remained constant for all trials.

Experimental tests of damage - During a 2010 field season that involved all three lineages of *Raphanus* plants, I observed and filmed house finches (*Carpodacus mexicanus*, Fringillidae) feeding on *Raphanus* fruits. Based on these observations, I decided to experimentally characterize the damage and quantify the effect of the granivore by exposing fruits produced in the greenhouse to: (1) house finches in the field and (2) to zebra finches (*Taeniopygia guttata,* Estrildidae) in cages.

Five *Raphanus raphanistrum* (Rr) seeds and six California wild radish seeds (CAwr) were planted in one-gallon pots in a climate controlled greenhouse. Hand-pollinations were performed and labeled with green tie tape when all plants had reached maturity to ensure the fruits were all formed with no more than a few days difference among individuals. The plants were kept in the greenhouse, water daily and pruned to preserve only the branches with pollinated flowers, until all fruits had matured. Before moving the plants to the field, length of all fruits was measured. In the field, the pots were placed 2 meters apart in two rows on top of a mesh to avoid losing the fruits that eventually fell to the ground. The plants were left in the field for two weeks and visited daily to record damage and collect any fruits found on the ground. After two weeks, all the remaining fruits were collected and brought to the lab to measure final length and assess damage.

Two plants from each lineage were kept in the greenhouse. The fruits of these plants were exposed to a pair of caged zebra finches because house finches are not commercially available. I recognize that compared to house finches, zebra finches are almost half the size, belong to a different family within the order Passeriformes and vary in jaw structure and, probably, maximal bite force. Lower values of maximal bite force increase the time spent manipulating the seeds not necessarily limiting the opening of harder food (van der Meij and Bout 2006). Because the zebra finches had unlimited time to access the fruits in the cage, food handling (husking time) was not affected by the presence of predators. With these data I address the following questions about seed damage by granivores: (1) are immature fruits more easily damaged? (2) when most of

the bird diet contains radish seeds, are there any whole radish seeds found in the bird

droppings? and (3) is the observed pattern of the damage, from stylar end onwards, the

result from the way the fruit is presented in the plants? Following experiments, the zebra

finches were returned to the commercial supplier. The Institutional Animal Care and Use

Committee of the University of California Riverside approved the procedures.

Data analysis - The three lineages under study: *Raphanus sativus* (Rs), California wild

radish (CAwr) and *R. raphanistrum* (Rr) are closely related (Warwick and Black 1997)

and cannot be considered as independent units. However, it is not clear which model for

data relatedness is appropriate when comparing a hybrid and its two progenitor lineages. I

employed conventional parametric and non-parametric statistics to assess fruit

differences. In addition, I know that the mother plants of the fruits used in this study were

collected from distant places and grown in contrasting conditions and environments (i.e.

domesticated vs. wild or Europe vs. USA refer to table 1.1; Ridley 2008).

Data were normalized when needed with lognormal or Box Cox transformations

(R Development Core Team 2010). One-way analyses of variance (ANOVA) were used

to compare fruit traits at the lineage level. Significant results were followed by

TukeyHSD post hoc tests for multiple paired comparisons of means at the lineage and

populations levels. Linear regression models were performed to find the relationship

between log-transformed fruit weight and fruit length for every lineage. Because fruits

with same volume might have different shapes, I decided to explore in detail how the

diameters at the different portions of the fruit vary. All peduncular (p), middle (m) and

stylar (s) section diameters and within each lineage were compared with paired Wilcoxon

tests. Because the strength was obtained for both whole (W) and halved (H) segments of the fruit wall, I tested the hypothesis that the strength required to break H would be half of the strength required to break W. I employed a Welch two-sample t-test for unpaired samples, which indicated if the strength differed, and then compared the means to determine if differences matched the predicted pattern. I then tested the relationship between fruit diameter and strength with Spearman rank correlation tests. Kernel density plots per lineage for each section were plotted to visually assess the distribution of the fruit wall strength. Comparisons among lineages and within lineages at the three different portions of the fruits were assessed with goodness of fitness chi-square tests to reveal differences in (1) the number of seeds visualized by the X-rays and (2) the different types of damage. The goodness of chi-square tests were at times followed by Pearson's chi-square with 10000 permutations when the differences among row were lower than five. Significant P values were adjusted a posteriori with sequential Bonferroni tests to adjust for type I error (Rice 1989). All statistical tests were implemented using the R statistical program (R Development Core Team 2008) and extra statistical R packages were downloaded from the Comprehensive R Archive Network (CRAN 2012).

<center>RESULTS</center>

Fruit characteristics

    Lengths, diameter, volume and weight -   The three lineages differ significantly in fruit length ($F_{2,\ 1.087} = 461.35$, $P < 0.0001$) and beak length ($F_{2,\ 1.067} = 589.87$, $P < 0.0001$). For both measurements CAwr fruits have intermediate lengths, Rr

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fruits have the longest lengths, and Rs fruits have the shortest lengths. These differences among all lineages are significant (Tukey post hoc, all $P < 0.00001$) as are most of the comparisons among populations (appendix A and appendix B). One notable exception is the Rr-DK1 population, which has similar beak and length values as In1-CAwr and fruit length value as Cst-CAwr populations.

The fruits of the three lineages also differ in the relative diameters at the peduncular (p), middle (m), and stylar (s) sections. Rs fruits have a swollen equatorial region as indicated by significant Wilcoxon tests (Wt) pairwise comparisons (Wt $P:_{p\ vs.\ m}< 0.0001$, $_{p\ vs.\ s}< 0.01$, $_{m\ vs.\ s}< 0.0001$). Conversely, CAwr fruits are shaped more like a parabolic cone with similar diameters at the peduncular and middle sections (Wt $P_{p\ vs.\ m}= 0.1169$), and significantly smaller diameters at the stylar region (Wt $P:_{p\ vs.\ s}< 0.0001$, $_{m\ vs.\ s}< 0.0001$). Rr fruits are long and thin with significant decreases in diameter at each section from the peduncular to stylar direction (Wt $P_{p\ vs.\ m}= 0.032$; $P_{p\ vs.\ s}< 0.0001$; $P_{m\ vs.\ s}=0.0048$). Pairwise comparisons among lineages reveal significant differences at each of the three fruit regions (appendices C and D). Rr fruits have the lowest diameters in all sections. Among populations, the same trends described for the lineages are observed but the inter-lineage variation is higher in CAwr and Rs populations. Peduncular and middle sections are larger than the stylar sections in all cases (appendix C).

The relative values in volume and weight follow a different pattern from length and diameter values. CAwr fruits have the highest volume, Rs fruits have intermediate volume and Rr fruits have the lowest volumes (table 1.2). These differences are highly significant ($F_{2,\ 336}= 37.916$, $P < 0.0001$; all Tukey post-hoc tests $P < 0.0001$; appendix E).

Inter-lineage fruit volume variation is high for both CAwr and Rs. The weights of CAwr

fruits are also 40% higher than that of both progenitors Rs and Rr (table 1.2, $F_{2, 1.087}=$

94.479, $P < 0.0001$), which does not differ between them (Tukey post-hoc, $P = 0.28$).

Greater overlap is detected at the population level (Appendix F) with Rs-BSp and Rs-

WhI having similar weights as fruits from the CAwr populations Hemet (CA-In2) and

Lindcove (CA-In3) and from the Rr fruits from Denmark (Rr-DK1) and Mexico (Rr-

MX1). I also assessed the scaling relationships between the logs of fruit weight and

length (figure 1.3). All three lineages exhibit positive allometry (expected slope for

isometry = 0.33).

Fruit wall strength -   The strength measured from halved (H) fruits was half the

strength of breaking for whole (W) fruits in many but not all cases (data not shown). The

most consistent deviation from the prediction is that the strength at the beak (~0.44

N/mm^2) of the fruit is indistinguishable between the two conditions for all three lineages.

Rs fruits have similar strength at the middle and stylar sections. The H forces at the

peduncular section are significantly lower than W strength in this lineage but their value

is 66% rather than 50%. Based on these results, I focus exclusively on the strength

measured in whole portions (W) because: 1) there are significant differences in

magnitude between halved and whole forces and 2) at both pre and post-dispersal phases

these fruits do not dehisce, and even when the fruits fragment, the fruit wall still covers

the seeds.

The fruit wall strength for the three main sections of the fruit (not including the

beak) range across all samples from a minimum of 0.04 N/mm^2 in the stylar section of an

Rr fruit to 12.01 N/mm^2 in the peduncular section of a fruit of that same lineage. Mean values for each lineage and fruit sections are listed in table 1.2. Pair-wise comparisons between the different fruit sections are all significantly different (W>113406, 0.02<p<0.00001). Within lineages, similar differences are found with a few exceptions where the fruit strengths are similar for Rs stylar and middle sections and Rr middle and peduncular sections. When each section of the fruit wall strength was compared across the three lineages, I found that: (1) the beak is equivalent in all cases, (2) only Rs and CAwr differ at the stylar section, (3) Rs middle sections differ from the two other lineages whereas CAwr and Rr are similar, and (4) all lineages differ at the peduncular sections with CAwr with the highest values and Rr with intermediate (figure 1.4, table 1.2). Overall the peduncular section is the hardest to break and Rs populations have the softest fruits. The distributions of the fruit wall strength (figure 1.5) at all four fruit sections are almost identical between Rr and CAwr wild lineages and rather different from Rs. It is important to note that the X-axes differ for all three lineages, the largest for CAwr. Rs has a larger Y-axis indicating a tighter distribution; a third of Rr and fourth of CAwr.

The strength and the corresponding diameters at each of the three sections are significantly correlated in the case of Rr fruits. The opposite is true for CAwr with negative correlation coefficients at the middle and peduncular sections. Rs fruits have a significant correlation between force and diameter at the peduncular portion.

Within-fruit seed distribution - Seed distributions inside the fruits differ among the three lineages as revealed by X-ray imaging (figure 1.5). Rr fruits have the

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most consistent arrangement with seeds neatly organized in one row with at least 2 mm spacing between them (figure 1.5, middle row). Rs and CAwr fruits have less consistent arrangements. For example, Rs seeds have four seed distribution patterns: (1) one untidy row (figure 1.5, e.g., fruit # 60), (2) alternating seeds (e.g. fruit # 24), (3) two separate rows (e.g. fruit #21), or (4) simple without clear configuration (e.g. fruit # 37). The hybrid CAwr fruits have examples of seed configuration patterns exhibited by both Rr and Rs progenitors (figure 1.6, top row). However, when CAwr fruits have two rows of seeds, they are found at the peduncular regions (top row, e.g. fruits #96 and #88). The fraction of seeds found at the different section of CAwr fruits does not deviate from Rs or Rr (three last columns table 1.2).

Experimental tests of damage - I describe the damage inflicted by the granivores on Rr and CAwr fruits, with 8 different types of damage or no damage, that can be grouped in two categories: I-no seeds removed or II- seeds removed. The first category included: 1-whole intact fruits found attached to the plant, 2-whole intact fruits that fell off and were found on the ground, 3-fruits that suffer minor attempts to damage and were only scratched, 4-fruits most likely struck with the beak (i.e. pecked) and almost reached the seeds, and 5-fruits with broken or missing sections usually the seedless beak section. The second category included: 6-fruits most likely struck with the beak (i.e. pecked) resulting in seed removal, 7-fruits with seeded sections removed, and 8-fruits fully eaten (figure 1.6). Pair-wise comparisons between both lineages per sections revealed that the damage inflicted by the birds at the peduncular (p), middle (m) and stylar sections are significantly different while no difference were found at the beak section. When each

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type of damage was examined individually and accross lineages, damages 2, 6,7 and 8 differed highly. When differences in damage were compared within lineages per sections, no differences were found in any case for Rr fruits. The opposite is true for CAwr. All pair-wise comparisons were significantly different with the exception of peduncular (p) vs. middle (m) sections.

DISCUSSION

This study provides evidence for a mechanism that allows a hybrid-derived plant to displace its progenitors and expand its range as an invasive lineage (Hegde et al. 2006). The innovative combination of structural and material properties of the hybrid California wild radish (CAwr) fruits provides superior protection to its seeds relative to its progenitors. The hybrid has some intermediate characters such as fruit length, beak length, and within fruit seed distribution. Other characters are similar to one or both progenitors, including fruit diameters at specific sections and the positive allometry of fruit length compared to fruit weight. The fruit features that are most different relative to the progenitors are larger volume, greater weight, the strength (breaking force) at the peduncular section, and the tendency to increase the diameter in this area. It is not surprising that the wild lineages (CAwr and Rr) have harder fruit walls compared to the domesticated lineages (Rs), but the extreme value for fruit wall strength in the penduncular section of the hybrid suggests that hybridization events combined with mechanisms of selection are acting on this section. Uncoupling the effects of these two processes on the structure of the fruit remains to be demonstrated.

The parental lineages express divergent values in fruit structure and seed packaging traits. The cultivated *Raphanus sativus* (Rs) has easily breakable fruits that contain a large number of seeds, and thus appear to be especially vulnerable to granivores. The wild *R. raphanistrum* (Rr) fruits are considerable harder and have uniform fruit wall protection along with internal seed compartments that provide additional shielding for broken fruits. The results presented here indicate that Rr has two additional mechanisms to protect the fruits. The first is the "fell off" mechanism (figure 1.6), which may compensate for lower values in fruit wall strength. The second is the ability to break into individual one-seeded segments offering additional benefits after dispersal from the mother plant (Cousens et al. 2010). In the case of the hybrid-derived (CAwr), fruit wall strength is greater particularly at the peduncular section of the fruits, which is also where most double row seeds as well as where the largest seeds dwell in this lineage (Marshall and Ellstrand 1988). The internal structure of the CAwr fruit maintains the seed compartments in most cases providing a similar protection as Rr fruits. Overall, the strength required to break *Raphanus* fruits is influenced by differences in material properties at the fruit wall among lineages. Our data indicates that the fruit shape alone does not explain the differences among fruit hardness.

Prior studies have reported *Raphanus* fruit characteristics, mostly based on observations and general measurements. Fruits of *Raphanus sativus* (Rs) or cultivated radish have been described as smooth, spongy and corky and at maturity easily crushed (Trouard-Riolle 1914; Panetsos 1953) whereas the fruits of *R. raphanistrum* (Rr) are harder to break and shrink in diameter around the seed compartments when ripening

eventually breaking into individual seed segments (Panetsos 1953; Cheam and Code 1995). The hybrid fruits are intermediate with hard fruit walls that do not break at maturity, smooth surfaces (Panetsos and Baker 1967), and heavier and larger volumes (Hegde et al.2006). The present study provides additional detail of the fruit structure and experimentally examines the interaction of the fruit traits and the seed predators. Finch granivory and seed predation has been previously reported in *Raphanus* plants (Trouard-Riolle 1914; Frost 1923; Hegde et al.2006; Ridley 2008; Cousens et al.2009) but never tested. To our knowledge there are no reports on European finches feeding in the wild on *R. raphanistrum*, but there are numerous granivore species in Europe (Svensson et al. 2010). In addition, seeds of *R. sativus* are typically found in commercially manufactured bird food and have been reported as part of the diet in granivore birds in northern European farms (Wilson et. el. 1999).

Post-invasion success has received considerable attention with most mechanistic studies being focused on chemical defenses. The evolution of biomechanical defense in invasive plants is largely unexplored despite being a genetically based trait but the few available studies indicate its importance. Seeds from roadside populations of introduced rose clover (*Trifolium hirtum*) in California were found to retain hairier calyx than populations from old rose clover plantations (Jain and Martins 1979). This feature is associated with enhanced seed survival. Fruit wall thickness was measured during a common garden experiment at the invaded range between European originated and the North American invasive counterpart of *Silene latifolia* plants (Blair and Wolfe 2004). The European originated fruits appeared to have better protected fruits when seed mass

was considered compared to fruits from the invasive range. The influence of invasive plant fleshy fruit characteristics on seed dispersal agents has received more attention although these studies do not focus on fruit defenses (Vilà and D'Antonio 1998; Gosper et al.2005; Buckley et al.2006). Invasive plants are not exempted of mutualistic and antagonistic interactions, even if the composition and nature of those agents changes at the novel range (Maron and Vilà 2001; Colautti et al.2004; Mitchell et al. 2006). Natural or novel enemies drive the evolution of plant characters in the same way native or introduce host plants might drive the enemies characters (Caroll et al.1998; 2001; 2005 and 2007).

Fruit morphology is a fundamental trait in plant evolution (Ferrándiz 2002; Hall et al. 2006; Hall et al. 2011). Fruit and/or seeds produced within the same plant that vary in morphology are heteromorphic (Imbert 2002). The genus *Raphanus* belongs to the Brassiceae, a tribe in the Brassicaceae family. Fewer than half of the species in the Brassiceae tribe have heteromorphic fruits, in particular heteroarthrocarpic fruits, characterized by having segments (Hall et al. 2006; Hall et al. 2011). Heteroarthrocarpic fruits, in contrast with non-heteroarthrocarpic ones, do not release their seeds from a particular fruit and/or plant at once into the environment at maturity (Hall et al. 2011). Seed dispersal variations depend on different heteroarthrocarpic fruit types, described in detail elsewhere (Hall et al. 2011). As a consequence, different proportions of seeds from the same fruits disperse without pericarp (fruit wall) and others disperse in units that include entire fruits or sections of the fruits, distal/stylar or proximal/peduncular (Hall et al. 2011). Systematic, developmental, and ecological aspects of these characteristics have

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been studied in different species of Brassiceae (Barbour 1970; Keddy 1980; Keddy 1981; Payne and Maun 1981; Zhang and Maun 1992; Donohue 1997; Donohue 1998; Hall et al. 2006; Lu et al. 2010). Understanding the genetics of fruit development, morphology and physiology has also been key to improve crop yield and agriculture in general (i.e. Ferrándiz 2002; Arnaud et al. 2011; Groszmann et al. 2011). In natural and agricultural environments fruit traits are essential to protect seeds from both biotic and abiotic factors at pre-dispersal, dispersal and post-dispersal seed stages (Herrera 1992; Groom and Lamont 1997, Kolb et al. 2007; Beckman and Muller-Landau 2011). The present study supports that fruit structure and morphology is part of plant structural defenses in a hybrid *Raphanus* lineage. It remains to study to what extend this structural defense influences pre-dispersal seed predation and plant fitness. Also, since the fruit wall hardness varies along the fruit, it would be worthwhile to correlate the fruit wall hardness with within-fruit seed characteristics.

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Figure 1.1. *Raphanus* fruits: *R. sativus* (Rs, top), *R. raphanistrum* (Rr, bottom), and hybrid lineage California wild radish (CAwr, middle).

Table 1.1

SEED SOURCES FOR ALL MATERNAL PLANTS

Lineage	Source / location	Population	ID
Raphanus sativus Rs	Ferry-Morse Seed Co.	Cherry Belle	Rs-ChB
	Ferry-Morse Seed Co.	French Breakfast	Rs-FrB
	Botanical Interests, Inc.	Round Black Spanish	Rs-BSp
	Botanical Interests, Inc.	White Icicle	Rs-WhI
CA wild radish CAwr	USA - California	Riverside Riverside County	CA-In1
	USA - California	Hemet Riverside County	CA-In2
	USA - California	Lindcove Tulare County	CA-In3
	USA - California	Morro Bay State Park San Luis Obispo County	CA-Cst
R. raphanistrum Rr	Denmark	Roskilde University Botanic Garden	Rr-DK1
	Mexico	Mexico City	Rr-MX1
	USA	Conanicut Island Rhode Island	Rr-NE9

Note.- To eliminate maternal effects, fruits and seeds were reared in the greenhouse from pure lineages of Rr and CAwr (Ridley 2008) and Rs, from purchased seeds. Fruits from CAwr populations comprise one coastal and three inland natural populations.

Figure 1.2: Schematic representation of the pliers device showing the four different sections of the fruits where the breaking forces or strengths (lb) to break the fruit walls were measured at: peduncular (p), middle (m), stylar (s) and beak (b). The single device is depicted four times to illustrate the four different sections of the fruit studied.

Table 1.2

FRUIT STRUCTURAL AND MATERIAL PROPERTIES - MEAN VALUES

	Length (mm)		Weight (mg) fruit	Volume (mm³) fruit	Diameters (mm)			Fruit wall strength (N/mm²)			Seed number		
	fruit	beak			p	m	s	p	m	s	p	m	s
Rs (N=393)	28.4 (8.4)	6.3 (2.8)	81.1 (55.0)	473.4 (359.6)	4.8 (9.8)	5.4 (10.7)	4.5 (8.5)	0.9 (0.6)	0.6 (0.5)	0.6 (0.4)	2.0 (1.0)	2.3 (1.0)	1.7 (0.8)
CAwr (N=397)	48.3 (10.5)	14.6 (5.4)	129.4 (62.5)	676.8 (414.5)	5.3 (8.5)	5.0 (7.5)	3.9 (7.2)	3.4 (2.9)	2.6 (2.2)	2.4 (2.3)	2.1 (1.1)	1.9 (0.9)	1.6 (0.7)
Rr (N=300)	53.0 (17.1)	17.3 (6.4)	86.1 (48.0)	183.3 (155.3)	2.7 (4.8)	2.4 (4.6)	2.1 (4.2)	2.6 (3.0)	2.7 (2.8)	2.0 (2.3)	1.9 (0.7)	1.8 (0.7)	1.8 (0.6)

Note.- p=peduncular, m=middle, s=stylar; standard deviation values in parentheses.
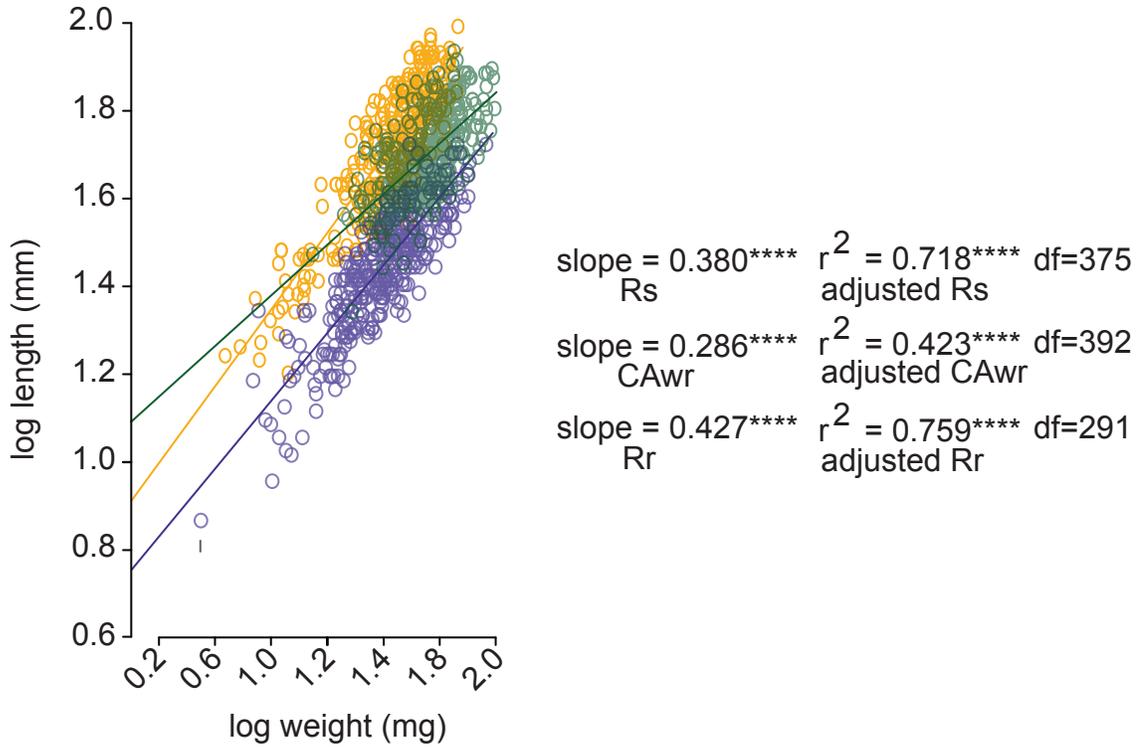
Figure 1.3. Regression results for fruit length vs. fruit weight. The variables were log transformed. Data for the three lineages are color coded as follows: *Raphanus sativus* is represented in purple, California wild radish in green, and *R. raphanistrum* in yellow. *P*<0.00001****.
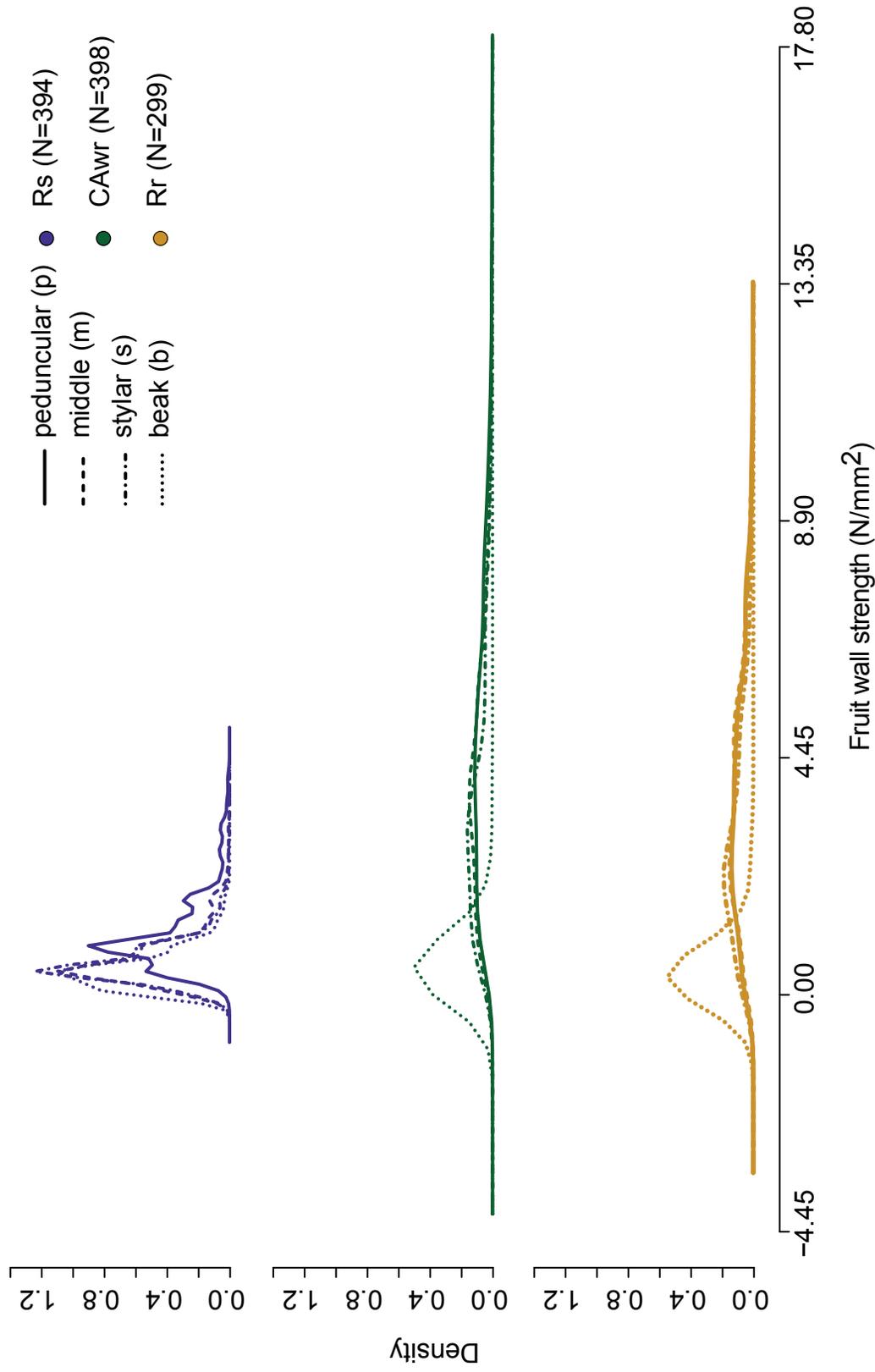
Figure 1.4. Kernel density plots of the fruit wall strength per fruit sections for all three linages. *Raphanus sativus* (Rs) is represented in purple, *R. raphanistrum* in yellow and the hybrid-derived (CAwr) in green.
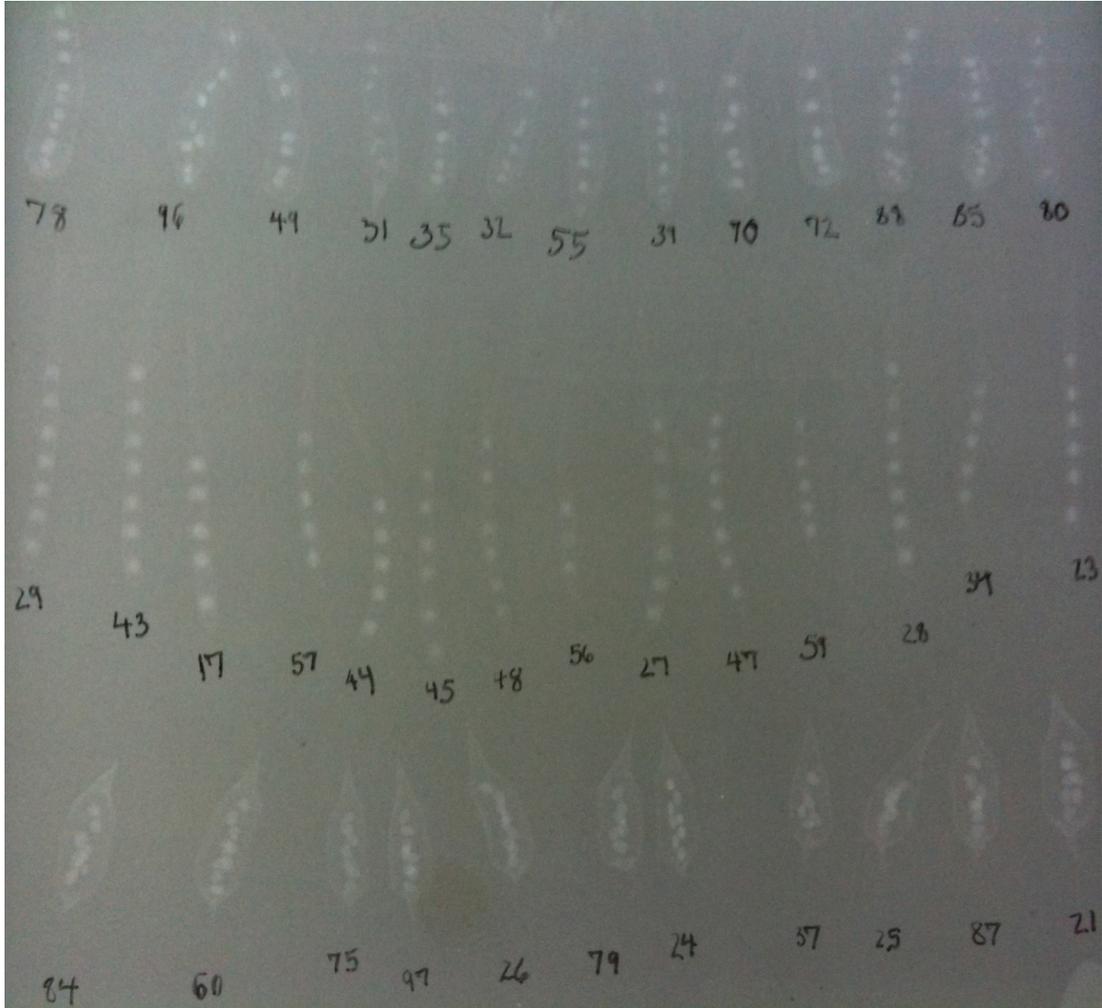
Figure 1.5. X-ray taken from a subsample of fruits of the three lineages: top row, fruits of the hybrid derived California wild radish (CAwr); middle row, fruits of *Raphanus raphanistrum* (Rr); and third row, *R. sativus* (Rs) fruits.

Damage type	California wild radish CAwr (N=79)				Raphanus raphanistrum Rr (N=71)			
	p	m	s	b	p	m	s	b
[icon] 8	1	1	1	1	0	0	0	0
[icon] 7	3	3	6	29	23	27	29	28
[icon] 6	3	5	16	3	8	9	7	6
[icon] 5	0	0	3	5	0	1	0	6
[icon] 4	5	4	9	9	8	10	12	7
[icon] 3	16	10	9	10	14	12	7	7
[icon] fell off 2	0	0	0	0	10	7	7	10
[icon] attached 1	72	77	56	43	37	33	38	37

Figure 1.6. Damage types in percentages. Chi-square tests for: (1) comparisons between lineages per fruit sections were significant between ([****]$P<0.00001$; [*]$P<0.01$): $X^2_{CAwrp\ vs.\ Rrp}=49.7$, df=7, [****]; $X^2_{CAwrm\ vs.\ Rrm}=49.7$, df=7, [****]; $X^2_{CAwrs\ vs.\ Rrs}=33.8$, df=7, [****]; (2) comparisons within CAwr: $X^2_{CAwrp\ vs.\ CAwrs}=18.0$, df=6, [*]; $X^2_{CAwrp\ vs.\ CAwrb}=36.5$, df=6, [****]; $X^2_{CAwrm\ vs.\ CAwrs}=15.1$, df=6, [*]; $X^2_{CAwrm\ vs.\ CAwrb}=38.2$, df=6, [****]; $X^2_{CAwrs\ vs.\ CAwrb}=26.3$, df=6, [****]; (3) comparisons within Rr were all non-significant; and (4) comparisons between lineages per damage: $X^2_{damage6}=7.8$, df=3, $P<0.05$ and $X^2_{damage7}=25.3$, df=3, [****].

CHAPTER 2

The Relative Fecundity of an Invasive Plant Lineage with and without

Protection from Avian Granivory

ABSTRACT

How invasive plants compete or compare with natives has received considerable attention but other biotic interactions, especially seed predation, have been relatively understudied. Because predation is a key component of survival and reproduction, this also represents a gap in our understanding of the evolution of invasiveness. Invasive hybrid lineages offer a powerful model for studying this process because their traits and biotic interactions can be examined experimentally relative to their progenitor lineages. Here, I take this approach with the hybrid-derived California wild radish and it progenitor lineages, the wild jointed charlock (*Raphanus raphanistrum*) and cultivated radish (*Raphanus sativus*). Plants from all three lineages were sown in common garden experiments and either exposed to avian granivory or protected from using garden mesh. Following senescence, all plants were weighed and some fitness components were measured and total and potential maternal reproduction was calculated. Fruit damage and amount of debris due to granivory were also calculated. Video recorded observations demonstrated that the house finch is the granivore that removes the seeds from attached fruits on the plant. The hybrid lineage suffered the highest rates of granivory in the unprotected treatments but its seed and fruit production were not depressed by granivory. The relative fecundity of the cultivar was the lowest at both unprotected and protected

treatments compared to both wild lineages. The wild progenitor Rr had an increase in relative fecundity in the unprotected compared to the protected treatment. Thus, the evidence that granivory has a negative effect on the fitness of these three related lineages is not definite. Characteristics such as multi-seeded fruits and well-protected seeds in combination with the bird's forging behavior (i.e. density-dependent choices) afford several layers of protection to *Raphanus* plants. Our data also suggest that avian consumption of *Raphanus* seeds solely by male house finches at the end of the breeding season may be an important feature of the bird's mating behavior.

INTRODUCTION

Interactions with local animal species influence the invasion success of plants (Richardson et al. 2000; Maron and Vilá 2001; Colautti et al. 2004; Richardson and Pyšek 2006; Parker and Gilbert 2007; Mitchell et al. 2006). These biotic interactions can range from mutualistic (review Richardson et al. 2000) to antagonistic and frequently span a broad range of this spectrum (Holmes 1990a and 1990b; Kollmann and Schill 1996; Buckley et al. 2006). It is therefore plausible that biotic interactions can help to determine the success of an invasive plant with respect to another invasive plant lacking those components. Work in applied biocontrol suggests an important role for pre-dispersal seed predation in invasive plants (McFayden 1998; Crawley 1992; Sheppard et al. 2002; Garren and Strauss 2009). Differential pre-dispersal seed predation has also been shown to influence the relative production of viable seeds in closely related species (Green and Palmbald 1975). Thus, how plants respond to predation of seeds may be an

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evolutionary response that increases invasiveness. One key challenge to testing this hypothesis is that successfully invasive plants are only defined as such retroactively.

Pre-dispersal seed predators consume seeds from the mother plant (Hulme 2002; Kolb et al. 2007) and can influence native and invasive plants through: 1) plant community interactions and demography (Hammond et al. 1999), 2) other animal-plant interactions such as frugivory (Sallabanks and Courtney 1992; Combs et al. 2011) and 3) the evolution of plant traits (Jordano 2000; Hulme and Benkman 2002). Plants employ a large array of strategies, both direct and indirect, to minimize the effect of seed predators. These include alternative strategies of regeneration, seed banks, abundant seed crops, altering phenology, spatial and temporal variation in seed productions and increasing plant defenses (Janzen 1971; Crawley 1992; Louda et al. 1990; Maron and Gardner 2000; Hulme 2002; Russell and Louda 2004; Raghu et al. 2005). It has also been demonstrated that pre-dispersal predation can facilitate the stratification of the seeds facilitating germination (Karban and Lowenberg 1992; Takakura 2002).

A hybrid-derived lineage that has already successfully invaded a new habitat represents a useful model because, when combined with its progenitors, it allows for a replicated study of the invasion process. Here, I focus on the hybrid-derived California wild radish, which is an invasive lineage in western North America (Hegde et al. 2006). Its progenitors, the cultivated radish *Raphanus sativus* and the wild radish *R. raphanistrum*, were introduced in western United States around mid 1800 (Panetsos and Baker 1967). These two lineages naturally hybridize, and there is evidence for the hybrid-derived lineage to have originated from interspecific bidirectional hybridization between

them (Ridley et al. 2008). Genetically based differences between both progenitor lineages (Hegde et al. 2006) and a partial and temporary reproductive isolation during the first generations of hybrids results from a single reciprocal translocation (Panetsos and Baker 1967; Snow and Campbell 2005). The polymorphism in fruit, flower color and shape, root morphology, chemical and structural defenses found in *Raphanus* lineages has been the focus of numerous studies in ecology, evolution, genetics and agricultural and food chemistry (e.g. Trouard-Riolle 1914; Frost 1923; Panetsos 1953; Conner and Rush 1996; Agrawal et al. 1999; Agrawal et al. 2004; Irwin and Strauss 2005; Campbell et al. 2006; Hegde et al. 2006, Blažević and Mastelić 2008; Ridley et al. 2008; Ridley and Ellstrand 2009).

Anecdotal observations have reported that bird pre-dispersal predation of seeds can be extensive in all three *Raphanus* lineages and birds are arguably the primary consumers of pre-dispersed seeds over invertebrate granivory (Frost 1923; Holm et al. 1997; Panetsos and Baker 1967; Ridley 2008, K. Hladun pers. comm.). When granivory is excluded, the hybrid-derived California wild radish (CAwr) exhibits superior fitness compared to its parental lineages in common garden experiments across its Californian distribution (Ridley and Ellstrand 2009). However, the hybrid's relative fitness and that of one of the progenitors in the presence and absence of granivores, is unknown. Our aim was to answer the following questions: (1) what species of bird granivore is the main consumer of radish seeds? (2) how much does the bird granivore affect relative fitness and relative potential fitness? (3) are the variables likely to affect the birds' selection of individual plants such as days to germination, plant final weight, total fruit production

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and potential reproduction, correlated with fruit damage? and (4) are there viable seeds in the debris due to granivory resulting from the bird foraging behavior under damaged plants? In addition, the comparison among lineages allowed us to better understand novel biotic interactions in a successful invasive hybrid-derived lineage and to propose a mechanism that led to the replacement of both progenitors (Hegde et al. 2006).

MATERIALS AND METHODS

Seed sources - The seeds used to breed the mother plants in the present study came from plants reared in a common garden during Spring 2005 and Winter 2006. The seed sources for the first generation of maternal plants are described in table 2.1. The second generation seeds are the result of natural open pollination in common gardens at the Agricultural Operations fields (AgOps) at the University of California-Riverside (UCR). More details on how the first generation plants were grown can be found in Ridley and Ellstrand (2009).

Common garden and experiment design - The common garden experiment took place during Spring and Summer seasons of 2010 at AgOps-UCR. Three replicate sites, each one consisting of two plots of 7 m by 7 m, were planted with 36 plants placed in a 6 x 6 grid with 1 m spacing in rows and columns. One of the plots at each of the three sites was covered with 3/4" x 3/4" orchard mesh (Ornex) to exclude above ground vertebrate damage while the other plot remained unprotected. These two conditions created two different treatments for the plants to grow in: (a) protected from vertebrate seed predators and (b) unprotected to vertebrate seed predators. In both cases the plants were exposed to

open pollination, invertebrates and potentially underground vertebrates. All plots were oriented in the same North-South direction.

We selected at random 8 seeds from 4 different mothers within each of the 9 above-mentioned populations for a total of 288 seeds. These seeds were divided in groups of 36, such that all mothers were represented in those 8 groups by 2 seeds. That is, each population had 4 seeds, for a total of 12 seeds per lineage. These 8 groups of seeds were germinated in Petri dishes at the beginning of March and transplanted into seed starting trays filled with sterilized UC Soil Mix III (http://agops.ucr.edu/services/) at a climate-controlled greenhouse. Once the seedlings had attained a three-leaf stage, 6 of the 36-grouped seeds were transplanted to the pre-water and plowed field plots. The two additional groups of seeds were used to replace any seed that did not germinate or any seedlings that did not survive the transplanting process. The plants were watered once daily for 10 min with a sprinkler system until most of the plants had started to flower. To maintain favorable abiotic conditions for the plants that flowered later, the watering persisted only every-other-day for 5 min.

Granivores - We visited the sites at AgOps at least every two days to ensure that the experimental conditions were kept consistent during the entire length of the study. During those visits I also spent time observing the foraging behavior of the birds that began when fruits had attained a fully formed size. Once I became familiar with the birds foraging patterns, I spend an afternoon filming their behavior. Videos were captured with a digital video camera (JVC model GR-DV800U) on a tripod. Videos are available as supplemental information.

Fruit damage, fecundity, fitness related values and debris due to granivory - Variables related to morphology, damage and fitness were recorded before planting, during the experiment, and after the surviving plants were collected. All seeds were weighed to within 0.01 mg with an analytical balance (Mettler-Toledo AB135-S/FACT). The germination and growth of seeds in the dish was recorded daily. At the end of the experiment when the plants were dry and had senesced, I recorded the final plant weight to within 0.001 g. To calculate fecundity and fruit damage, I counted total number of: (1) damaged fruits that included all fruits with clear signs of missing or damaged sections, (2) whole dropped fruits that were found detached from the dry plant, and (3) whole attached fruits. With these variables I calculated fruit damage and fecundity. We counted total numbers of: (1) flower buds, (2) flowers, (3) whole empty pedicels (and, to avoid overvaluing this variable, I subtracted the dropped fruit), and (4) broken or pedicel scars on the stems. We also collected the fruit material or debris accumulated under heavily damaged plants, herein referred to as "debris due to granivory", to discern what was discarded during the birds foraging behavior. Potential seed viability was determined by visually inspecting the seed coat and by putting pressure on each seed between the thumb and the index fingers; when unviable, seeds had black and/or wrinkled seed coats and crumbled easily.

All the previously described values and those in table 2 allow us to calculate relative fecundity and relative potential fecundity of plants in unprotected and protected plots. Because I did not count number of seed per fruits, I calculated the number of seeds based on the average number of seeds per fruit per populations. These average values,

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listed in table 2, were obtained from a previous study where I counted total number of seeds from 884 fruits that belonged to the same populations represented here (table 2). We consider these values appropriate to extrapolate the number of seed in our study because: (1) the plants that produced them developed from pure lineage seeds from the same populations represented in our study listed in table 2.1, (2) the plants were grown under similar conditions to the present study, and (3) the plants were exposed to open pollination (Ridley 2008). Total number of seeds were extrapolated for a given plant by: (1) multiplying total number of whole fruits per plant by the average value of seeds in table 2.2 according to the population of origin, followed by (2) multiplying total number of damaged fruits by the 2/3 of the average number of seeds according to the population of origin in table 2.2, and finally by (3) adding the numbers obtained for whole and damaged fruits.

Fecundity and female fitness values were calculated as follows. The average number of extrapolated seeds per population was calculated by dividing the total number of extrapolated seeds divided by the total number of fruits per population. Relative fecundity is the average number of extrapolated seeds divided by the highest average number of extrapolated seeds. Potential reproduction was calculated by adding flower buds, flowers, whole empty pedicels broken to whole, damaged and dropped fruits for a given plant. The average potential reproduction was calculated by adding the potential reproduction for a given population or lineage and dividing by the total number of plants and multiplied by 100. Finally, the percentage of the relative potential fecundity was calculated by dividing a given average potential reproduction to the highest average one

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among for populations and lineages separately and then multiplying by 100. Our fitness values did not explicitly include male fitness. Nevertheless I know based on prior studies in plants of the *Raphanus* lineage that male fertility is highly influenced by environmental factors and weakly correlated with female fertility values (Devlin and Ellstrand 1990; Devlin et al. 1992).

Data analysis - Data were normalized as needed either with log-normal or Box Cox transformations using functions in R (R Development Core Team 2008). Significant P values were adjusted a posteriori with sequential Bonferroni test to adjust for type I error (Rice 1989). We used one-way analysis of variance (ANOVA) to tests the effects of treatments and lineages on total fruit production. Variables related with fruit damage and with fitness were compared in pairs among lineages and between treatments with Wilcoxon tests. The effect of the treatments on relative fecundity and relative potential fecundity as well as average number of fruits and seeds were tested for significance with Fisher exact tests. These tests were performed to individually compare CAwr values to its progenitors. We also compared fecundity values to the highest ones with chi-square tests. Total number of fruit damaged was correlated using Spearman correlation coefficients and covariance to variables possibly related to final fruit production and general performance. Those variables included: days to germination, final plant weight (g) as well as total number of fruits and total potential reproduction. In this case each lineage was tested independently.

RESULTS

Flower buds, flowers and pedicels - No differences were found between lineages and treatments in average number of flower buds and open flowers (figure 2.1.a). With respect to the average number of pedicels, values for CAwr from protected and unprotected plots are significantly different and higher than both progenitors under protected treatment as well as for the cultivar under unprotected treatment, respectively (figure 2.1.b).

Fruits with and with no damage - We only found damaged fruits in plants that were collected in unprotected plots (figure 2.2.a). Consistent with our previous results, average numbers of fruits with damage are significantly different among lineages and treatments as revealed by Wilcoxon tests (figure 2.2.a). Whole undamaged fruits were categorized as either attached to the dry plant or detached and on the ground. The cultivar Rs differs significantly from CAwr and Rr on lower average number of whole dropped fruits, whereas both wild lineages, CAwr and Rr, are comparable (figure 2.2.b). No differences are found in the average number of whole attached fruits among lineages with the exception of CAwr and Rs from protected plots (figure 2.2.c).

The average proportions of damage, calculated as total number of damaged fruits over the total fruits produced for each lineage and population, are listed in Table 2.3. When the damage is estimated based on seeds removed, calculations of damage per population are reduced by at least 33 % and at most by 60% relative to the damage calculated based on fruit damaged. Damage, based on seeds removed, was calculated as the total number of seed removed divided by total number of seeds produced. As

mentioned earlier, I did not count the total number of seeds per fruits during this experiment. We are assuming that all damaged fruits had a third of the average number of seeds per fruit (table 2.2) removed. Fruits from Cst-CAwr suffer higher damage than interior populations (In1 and In2, table 2.3).

Fruit production - Total fruit production does not differ under protected (P) or unprotected (U) treatments but does differ among lineages (figure 2.3 a and b). The cultivated Rs lineage produced fewer fruits in protected treatments (Rs-P) relative to both wild lineages, significantly differing from both CAwr and Rr (figure 2.3.b). However, under unprotected conditions, Rs (Rs-U) only substantially differs from unprotected and protected CAwr (CAwr-U and CAwr-P) fruit production (figure 2.3.b).

Fecundity and fitness related values – CAwr has significantly higher fecundity in protected plots than in unprotected ones, as shown in table 2.4. The opposite is true for Rr and no effect is found for Rs. Overall CAwr has the highest relative fecundity with the highest fruit production (table 2.4). Average number of fruit damaged for each lineage correlates: (1) strongly with Rs and Rr fruit production, Rs and Rr potential reproduction and Rs and CAwr final plant weight, and (2) moderately with CAwr fruit production, potential reproduction and Rr final plant weight. In all the cases the covariance values are positive. No correlation was found with days to germination (table 2.5).

Debris due to granivory - Debris material due to granivory was only found under plants in unprotected plots. In general the debris due to granivory was a mix of viable and unviable seeds as well as fruit wall segments. Some seeds were found bared, with no fruit material around them, while others were either fully or partially covered by fruit wall

segments. Fruit wall segments were found fragmented and empty or whole with a seed inside. The largest amount of debris due to granivory was found under CAwr plants in unprotected plots as well as the largest number of potentially viable seeds. Most of the fruit segments were found broken and empty. The average values are listed in table 2.6. Although the debris due to granivory data was based on a small sample size, I calculated the average proportion of seeds removed for each plant by granivory in two different ways. One of the calculations was done as described in the methods. The other calculation included the subtraction of the potential "viable" seeds found in the debris due to granivory and averaged for each lineage (table 2.7). Subsequently, I compared both averages. In the case of both progenitors there are minimal differences. However, for the hybrid derived-lineage there is a 10% reduction of seeds removed.

Granivore behavior - We observed and filmed house finches (*Carpodacus mexicanus*, Fringillidae) actively manipulating and feeding on fully formed *Raphanus* fruits. No other avian species were observed on *Raphanus* plants although there are at least five other granivores regularly occurring in this location (http://gardens.ucr.edu/). At the different plots, the birds visited the plants daily in an almost predictable fashion. In all cases I observed males, easily recognizable for their characteristic red coloration around the face and head. The peak of house finch foraging activity corresponded to the period in which the fruits were fully formed but still green. We never observed the house finches feeding out of the debris on the ground; they appear to only choose fruits attached to the plants.

DISCUSSION

The house finch *Carpodacus mexicanus*, an avian granivore, affected the reproductive patterns of three *Raphanus* progenitors and hybrid lineages in dissimilar ways. When exposed to the granivore, the hybrid-derived CAwr had proportionally more fruits damaged relative to its progenitors (table 2.3). Overall relative fecundity of CAwr was significantly reduced compared to protected plants of the same lineage (table 2.4). When exposed to the granivore, CAwr had lower fecundity compared to the wild progenitor Rr. However, due to its high overall number of fruits and seed production relative to both progenitors, CAwr was not negatively affected by granivory. Under conditions where granivory is excluded, CAwr has the highest relative fecundity compared to both progenitors (table 2.4), concurring with results from a prior study (Ridley and Ellstrand 2009). The fecundity of protected Rr fruits was comparable to the high fecundity of protected CAwr fruits. A potential explanation for this result might be that Rr fruits detach easily from the mother plant. I found a large number of dropped Rr whole fruits (figure 2.2.b) relative to whole attached fruits (figure 2.2.c). Finches perch when attempting to feed, with the result that many Rr fruits fall to the ground before they can be damaged on the branch. Because the birds did not forage on the ground, the seeds in fallen fruits remained viable. Easily detachable fruits were also observed occasionally on CAwr plants but not as frequently as in the wild Rr progenitor (Panetsos 1953).

Fecundity values of the cultivated Rs lineage did not differ between unprotected and protected treatments. Rs exhibited low fecundity values overall, which is in agreement with other studies (Campbell and Snow 2009, Ridley and Ellstrand 2009).

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However, the reproductive output of Rs may be even lower than reported because our measurement protocol likely led to an underestimate of the negative impact of the granivore for this lineage. Observations by us and other researchers indicated that birds open entire fruits of the cultivar Rs and consume all the seeds inside (K. Hladun pers. comm.). Fully eaten fruits often appear similar to an empty pedicel and thus may not be counted as damaged at all. An additional caveat is that by planting the three lineages close together I may have influenced the granivore's choice to feed more often on Rr and CAwr, which are bigger and more productive compared to Rs. Density-dependence has been observed in other studies of granivory (Christensen et al 1991; Mattson 1986) and it is expected that birds make foraging choices to maximize their own fitness (Hulme and Benkman 2002).

Plant invasion often involves multiple introductions followed by a time lag during which invader populations overcome small population effects such as genetic drift and inbreeding causing Allee effect, Founder Effect, and bottlenecks (Novak 2007, Sakai et al. 2001, Ellstrand and Elam 1993). In addition, other mechanisms favor invasiveness like gene flow, potentially leading to interspecific hybridization, (Ellstrand and Schierenbeck 2000). Some invasive plants are a result of successful interspecific hybridizations, presenting defense traits that may be identical, different or additive relative to one or both progenitors, defining their susceptibility or resistance (Fritz et al. 1994). Hybrids can be more susceptible: a comparison of the densities of herbivore and pathogen species on two progenitors species, *Salix sericea* and *S. eriocephala* and hybrid willow shrubs, revealed significantly higher herbivore densities on the hybrids (*sensu* Fritz et al. 1994). Although

the trend in the Fritz et al. (1994) study and others that he and collaborators (1999) reviewed do not support higher resistance in hybrids, other studies have found evidence for this. For example, the hybrid derived *Helianthus annuus texanus* acquired resistance to two types of herbivores from both ancestors *H debilis* and *H annuus annuus* increasing its relative fitness (Whitney et al. 2006).

In the case of invasive plants, hybridization can contribute to the evolution and success of invasive lineages (Ellstrand and Schierenbeck 2000). In addition, after establishment of long-standing populations in the community in a novel environment, invasive plants evolve and with them their potential enemies (Stamp 2003). And as it is the case for all ecological interactions, invasive species exist in a complex network, where new abiotic and biotic conditions imposing a new selective pressure on the plants that may cause an adaptive response from the plants (Mooney and Cleland 2001). Considerable effort has been given to the idea that plant colonists have the advantage to be unknown by their new biotic enemies, i.e. herbivores, frugivores and pathogens. This idea, initially known as the "enemy release hypothesis", was proposed by Elton in 1958 but can be traced back to Charles Darwin in 1859 and subsequently with an added evolutionary context became the Evolution of Increased Competitive Ability hypothesis (EICA) by Blossey and Nötzold (1995). The EICA predicts that invaders have the opportunity to evolve a competitive advantage compared to native taxa by re-allocating on plant growth instead of on plant defenses. Aspects of this hypothesis have been tested empirically multiple times at many levels, at both single sites and continent-wide scales,

but the results have been equivocal (Hinz and Schwarzlaender 2004, Bossdorf et al. 2005, Hierro et al. 2005).

With respect to pre-dispersal seed predation, previous studies have found that other factors are fundamental to maintain the demographics. For example, higher predation does not translate in lower fitness rates that will affect the population size or the following year's adult density in invasive *Centaurea solstitialis* (Garren and Strauss 2009). Similarly, in spite of higher susceptibility to the effect of invertebrate pre-dispersal seed predation by the fly *Hylemya* sp. in natural and artificial hybrids crosses relative to progenitor species, *Ipomopsis aggregata* and *I. tenuituba,* flower traits and higher pollination rates compensate for seed losses (Campbell et al. 2002). One of the fruit characteristics that has been found to reduce the negative effects of seed-eaters on fitness is multi-seeded fruits (Buckley et al. 2006). In our study, estimated percentages of damage decreased when calculated based on estimated seeds removed compared to values based on fruit damage, diminishing the level of impact by the granivore. Thus, multi-seeded fruits reduce the effect of the granivore pressure on seed survival. Prior studies in non-invasive plants also support this idea; the impact of seed predation is reduced when plants produce multi-seeded fruits (Bradford and Smith 1977; Garrison and Augspurger 1983; Camilo-Alves and Mourão 2010; but Janzen 1971). To our knowledge, this study is the first to find evidence of the advantage of multi-seeded fruit in an invasive plant in the context of granivory.

Aside from the density-dependent effect that may have distracted the birds from eating fruits of Rs, I propose that fruits of each lineage provide a different level of

defense to the seeds inside. Currently it is known that fruit morphology does vary among the *Raphanus* lineages (Trouard-Riolle 1914; Frost 1923; Panetsos 1953; Hegde et al. 2006) as does fruit mechanical defense (Heredia Chapter 1). Invasive plants have been demonstrated to exhibit rapid evolutionary change (reviewed Bossdorf et al. 2005, Campbell et al 2009) suggesting that ~100 years of granivore interactions may have imposed substantial evolutionary pressure on the hybrid-derived fruits. This possibility coupled with the pre-existing hardened fruit wall in *Raphanus raphanistrum* (Panetsos 1953) leads to the hypothesis that fruit hardness is a genetically based trait that is favorably recombined as hybrids are formed and diversify in time (Ellstrand and Schierenbeck 2000). We never observed fully eaten fruits of California wild radish, but at most ~1/3 of the seeded portion. Previous work on CAwr mating system has found that the seeds inside an individual fruit are usually sired by various pollen sources (Ellstrand 1984,) and that the siring of the pollen occurs in a non-random manner (Marshall and Ellstrand 1986, Hill and Lord 1986; Hill and Lord 1987), which has been related to a within-fruit seed size variation (Stanton 1984). The combination of these traits and the impact of the granivore provide a unique situation to study the combined effects of natural and sexual selection.

To include the granivore's perspective was beyond the scope of our study. Nevertheless, our observations are in agreement with other features of the physiological ecology of house finches. We only observed male house finches feeding on our experimental radish plants. According to Hill et al. (1994) house finches have a 6-month breeding season, which in their western distribution starts in January (Hill 2002). The

fruits in the experimental plants described here were fully formed in June, which is near the end of house finch breeding season and just prior to the onset of molting. Carotenoid-based plumage pigmentation such as red, orange and yellow can only be acquired through diet (Goodwin 1984, Latscha 1990, Hill 2002, Olson and Owens 2005, Namitha and Negi 2010) and are known to have a significant function in sexual selection, immune system and senescence (Lozano 1994, Namitha and Negi 2010). To acquire the necessary pigments to color their plumage, male house finches need to include carotenoid rich food in their diet during the time the feathers are replaced and growing (Hill et al. 1994, Hill 2002). *Raphanus* plants have carotenoid contents and are particularly rich in β-carotenoids (Nithia et al. 2005, Kim et al. 2007), which are the specific class of pigments that house finches most likely require to color their feathers (Hill 1994). Female house finches prefer males with bright red plumage (Hill 1991) and the intensity of the red coloration in the males significantly correlations with: 1) an early beginning of the breeding season (Toomey and McGraw 2012), 2) increase in number of brood for a given pair (McGraw et al. 2001), as well as 3) the concentration and type of carotenoids in the food ingested (Hill et al. 2002). To our knowledge there is no study that has reported carotenoids extracted from radish seed endosperm or even fruit wall; most studies focus on cotyledons and leaves (Suzuki and Shioi 2004; El-Beltagi and Mohamed 2010). However, carotenoid pigments in canola *Brassica napus* seeds, another Brassicaceae species have been reported (e.g. Shewmaker et al 2002; Farré et al. 2010). The pattern of fruit consumption described here represents a positive effect for house finches and,

surprisingly, a positive effect for *Raphanus*. We propose a novel interpretation that granivore-fruit interactions in *C. mexicanus- Raphanus* are mutualistic.

The results of our study provide information of an invasive hybrid derived lineage performance relative to both progenitor lineages in the context of a novel biotic interaction. The hybrid as well as the wild progenitor have a combination of traits that increases their ability to survive and persist in novel environments. High fecundity and multi-seeded fruits with mechanical defenses protect and compensate for the effect of the granivore. The wild progenitor has some advantages over the hybrid lineage at the pre-dispersal seed predation phase. Although this result might be accurate I also suspect that our experimental design might have added some unwanted level of competition among the plants at the early growing stages affecting the hybrid performance (Campbell and Snow 2007). We anticipate that the amount of potentially viable seeds found in CAwr debris due to granivory with various degrees of fruit wall coverage affect: (1) seed dormancy (Cousens et al 2010) and thus widen the range of germination in the seed bank from the cohort of seed that will fall all at once with the senesced plant and, (2) availability to post-dispersal seed predation with opportunities for dispersal. Our results, combine with all previous evidence of the hybrids high genetic polymorphism, suitable life-history traits and higher fecundity both inside and outside its distribution range, provide a compelling argument for the hybrid to have displaced its progenitor lineages and for its capacity to be a successful invader (Panetsos and Baker 1967; Snow et al. 2001; Campbell et al. 2006; Hegde et al. 2006; Ridley and Ellstrand 2009; Hovick et al. 2012).

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Table 2.1

SEED SOURCES FOR ALL MATERNAL PLANTS

Lineage	Source / location	Population	ID
Raphanus sativus Rs	Ferry-Morse Seed Co.	Cherry Belle	Rs-ChB
	Ferry-Morse Seed Co.	French Breakfast	Rs-FrB
	Botanical Interests, Inc.	Round Black Spanish	Rs-BSp
	Botanical Interests, Inc.	White Icicle	Rs-WhI
CA wild radish CAwr	USA - California	Riverside Riverside County	CA-In1
	USA - California	Hemet Riverside County	CA-In2
	USA - California	Lindcove Tulare County	CA-In3
	USA - California	Morro Bay State Park San Luis Obispo County	CA-Cst
R. raphanistrum Rr	Denmark	Roskilde University Botanic Garden	Rr-DK1
	Mexico	Mexico City	Rr-MX1
	USA	Conanicut Island Rhode Island	Rr-NE9

Note.- To eliminate maternal effects, fruits and seeds were reared in the greenhouse from pure lineages of Rr and CAwr (Ridley 2008) and Rs, from purchased seeds. Fruits from CAwr populations comprise one coastal and three inland natural populations.

Table 2.2

AVERAGE NUMBER OF SEEDS PER FRUIT PER POPULATION

Lineage	Population	N	Average seed set	SD
Raphanus sativus	BSp	100	3.52	1.62
Rs	ChB	100	3.29	1.29
	FrB	94	3.20	1.40
California wild radish	Cst	99	4.86	1.64
CAwr	In1	99	4.05	1.64
	In2	99	4.85	1.09
R. raphanistrum	DK1	97	5.41	1.48
Rr	MX1	100	6.53	2.31
	NE9	96	3.88	1.47
All	9 populations	884	4.42	1.85

Note.- Used to extrapolate fruit, flower buds and flower counts to seed numbers; SD=standard deviation; N=number of fruits.
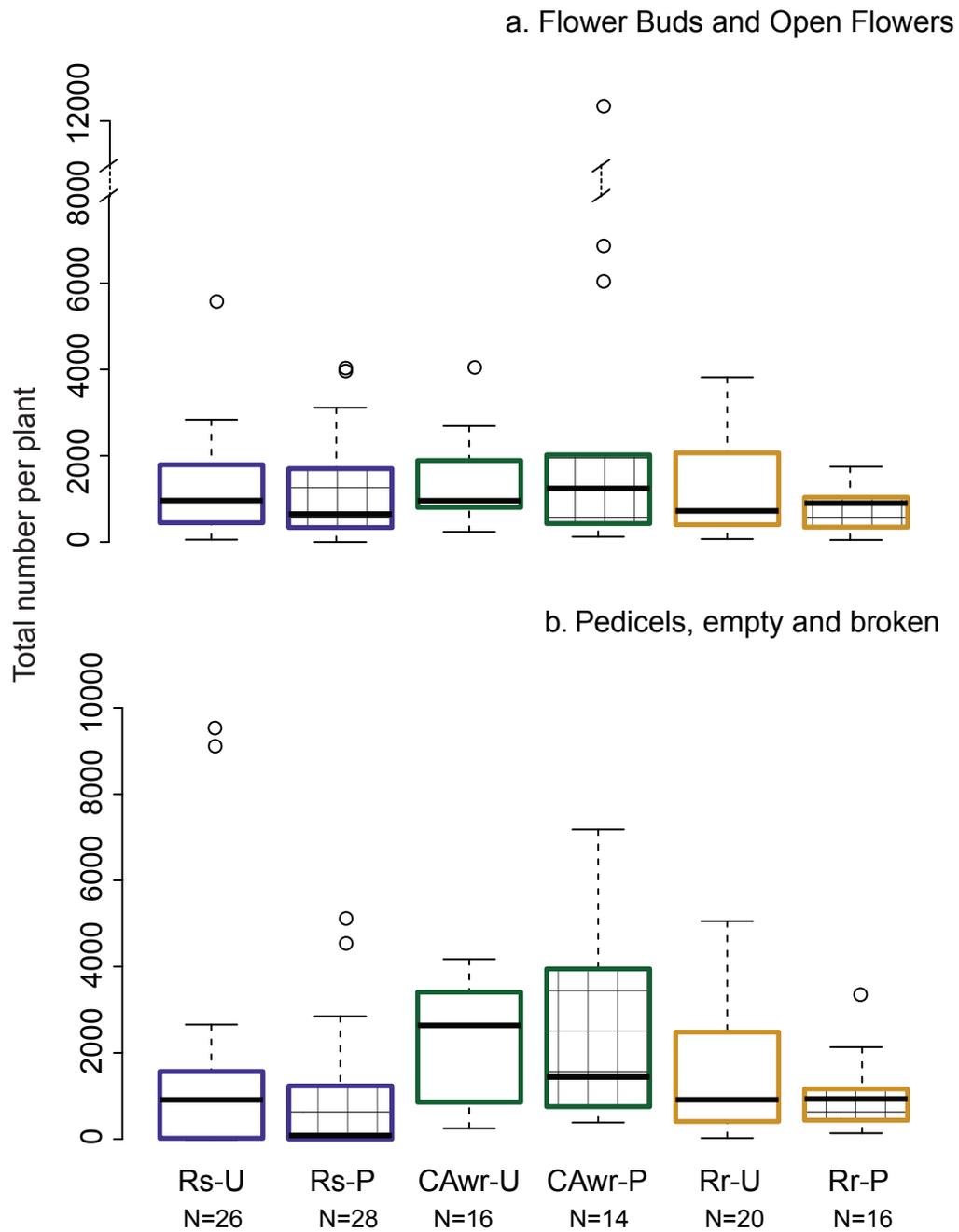
Figure 2.1. Box-plots of total number of flower buds and open flowers, and pedicels per lineage in unprotected (U, open boxes) and protected (P, gridded boxes) treatments. The lineages are represented as follows: Rs in purple, CAwr in green and Rr in yellow. Significant differences comparing average values with Wilcoxon paired tests were found between: b. CAwr-E vs. Rs-E (W=311, *), Rs-M vs. CAwr-M (W=309, *), and CAwr-M vs. Rr-M (W=161, p=0.04). *P<0.01.
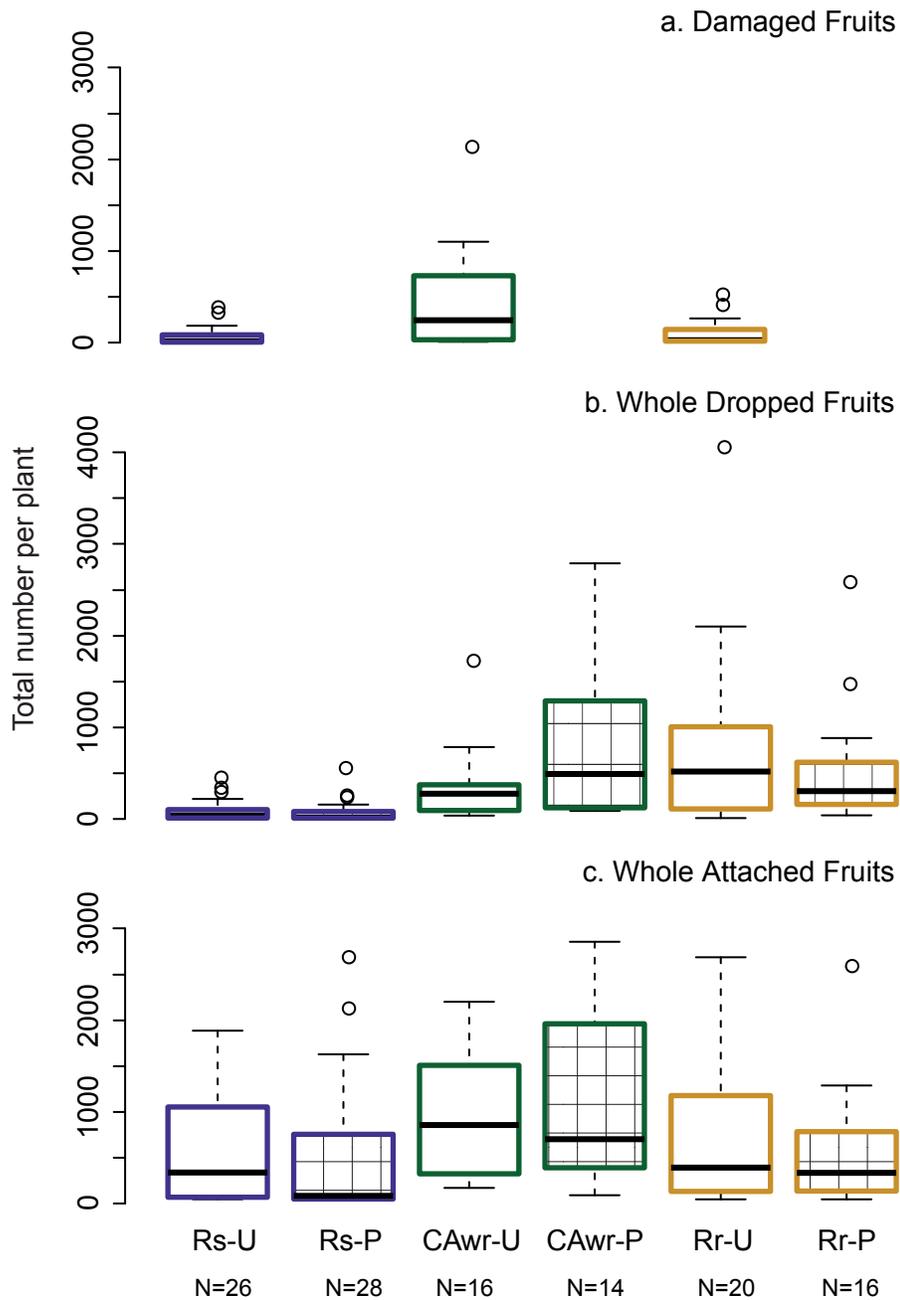
Figure 2.2. Box-plots of total number of fruits with and without damage per lineage in unprotected (U, open boxes) and protected (P, gridded boxes) treatments. The lineages are represented as follows: Rs in purple, CAwr in green and Rr in yellow. Significant differences between average values tested with Wilcoxon paired tests were found between: a. CAwr-E vs. Rs-E (W=341, **); CAwr-E vs. Rr-E (W=229, p=0.03); Rs-E vs. Rr-E (W=354, p=0.04); CAwr-M vs. CAwr-E (W=224, ****); Rs-M vs. Rs-E (W=602, ****) and Rr-M vs. Rr-E (W=320, ****); b. Rs-E vs. CAwr-E (W=350, **); Rs-E vs. Rr-E (W=430, ****); Rs-M vs. CAwr-M (W=361, ****) and Rs-M vs. Rr-M (W=410, ****); c. CAwr-M vs. Rs-M (W=304, *). ****$P<0.00001$, **$P<0.001$, *$P<0.01$.

Table 2.3

AVERAGE DAMAGE BASED ON FRUITS OR SEEDS

Lineages Populations	Fruit damaged %		Seeds removed %		Reduction %	
Rs	5.7		2.5		43.9	
BSp		9.7		4.5		46.4
ChB		4.2		1.6		38.1
FrB		1.8		0.6		33.3
CAwr	22.0		11.4		51.8	
Cst		33.2		20.0		60.2
In1		13.0		5.2		40.0
In2		14.0		5.0		35.7
Rr	8.1		3.2		39.5	
DK1		12.1		4.9		40.5
MX1		7.1		2.8		39.4
NE9		6.3		2.2		34.9

Note.- Seeds removed were estimated. Rs=*Raphanus sativus*; CAwr=California wild radish; Rr=*R. raphanistrum.*

a. ANOVA

Effect	Df	F-value
Treatment	1	2.4
Lineages	2	21.1***
Treatment x Lineages	2	0.3

b. Tukey HSD

	Rs-U				
Rs-P	0.8	Rs-P			
CAwr-U	*	***	CAwr-U		
CAwr-P	*	***	1.0	CAwr-P	
Rr-U	0.6	**	0.9	0.9	Rr-U
Rr-P	0.3	*	0.6	0.7	1.0

	CAwr	Rr
Rs	****	***
Rr	0.2	

Figure 2.3. Effect of treatments, protected (P) and unprotected (U), and lineages on the total number of fruits produced; ****$P<0.00001$; ***$P<0.0001$; *$P<0.01$.

Table 2.4

RELATIVE FECUNDITY OF PLANTS IN UNPROTECTED AND PROTECTED TREATMENTS

Lineage Population	N		Number of fruits (average)		Number of seeds (average)		Relative fecundity (%)		Relative potential fecundity (%)	
	U	P	U	P	U	P	U	P	U	P
Rs	36	36	651.2	496.9	2143.7	1699.1	22.7****	18.0****	48.6****	35.3****
BSp	12	12	775.4	811.8	2637.8	2857.6	18.3	19.8	47.3	36.9
ChB	12	12	540.2	274.8	1727.4	903.1	12.0	6.3	22.8	11.9
FrB	12	12	573.3	309.6	1817.2	990.6	12.6	6.9	24.1	20.3
CAwr	36	35	1691.0	1981.1	7374.0	9464.0	77.9^{**P}	100.0	75.8$^{0.03P}$	100.0
Cst	12	11	2121.3	2968.3	9110.4	14425.7	63.2	100.0	68.1	100.0
In1	12	12	931.0	892.7	3543.3	3615.3	24.6	25.1	32.4	27.7
In2	12	12	1569.0	1883.6	7263.4	9135.3	50.4	63.3	44.2	69.3
Rr	36	37	1469.2	1049.7	9082.7	6753.0	96.0	71.4$^{**U, 0.03}$	60.7*	38.5$^{****, *U}$
DK1	12	13	155.8	362.3	829.5	1959.8	5.8	13.6	18.5	14.0
MX1	16	13	1778.3	1278.8	11360.7	8350.8	78.8	57.9	44.6	30.8
NE9	8	11	2086.0	0.0	7924.3	0.0	54.9	0.0	96.1	0.0

Note.- U=unprotected; P=protected. Significant values were calculated relative to the highest value for each variable with goodness of fitness chi-square tests at the lineage level. To compare treatment values we used fisher exact tests;
$^{****}P<0.00001$, $^{***}P<0.0001$, $^{**}P<0.001$, $^{*}P<0.01$, $0.02<P<0.05$.

Table 2.5

FRUIT DAMAGED CORRELATIONS WITH FITNESS RELATED
VARIABLES FOR EACH LINEAGE - AVERAGE NUMBERS

Lineage	Days to G (days)		Plant weight (g)		Fruits		Potential reproduction	
	r_s	P	r_s	P	r_s	P	r_s	P
Rs N=26	0.35	>0.5	0.75	****	0.82	****	0.89	****
CAwr N=16	-0.1	>0.5	0.83	****	0.55	0.02	0.63	*
Rr N=20	0.1	>0.5	0.68	**	0.70	****	0.75	****

Note-. Rs=*Raphanus sativu*s; CAwr=California wild radish; Rr=*R. raphanistrum*; G=germination. **** *P*=0.00001; ***P*=0.001; **P*=0.01; *0.05<*P*<0.02.

Table 2.6

GRANIVORY DEBRIS CONTENT COLLECTED AT UNPROTECTED PLOTS
AVERAGE VALUES

Lineage	Weight (g)	Seeds		Fruit segments			Broken fruit segments		
		viable	non-viable	viable	non-viable	empty	viable	non-viable	empty
Rs N=2	0.5 (0.4)	1.4 (1.4)	0.0 -	0.0 -	0.5 (0.7)	0.0 -	4.0 (5.7)	1.5 (0.7)	23.5 (29.0)
CAwr N=6	28.1 (40.0)	96.0 (161.1)	11.3 (12.8)	49.7 (70.6)	48.0 (61.9)	4.7 (6.3)	132.8 (220.5)	33.2 (42.5)	458.7 (618.7)
Rr N=4	2.1 (1.7)	5.3 (6.4)	0.3 (0.5)	21.5 (27.4)	12.5 (13.8)	0.8 (1.0)	23.8 (22.2)	6.8 (5.4)	66.5 (44.5)

Note.- In parenthesis standard deviations; Rs=*Raphanus sativus*; CAwr=California wild radish; Rr= *Raphanus raphanistrum*; CAwr includes six Cst plants and one In1, Rr includes two MX1 plants and one NE9, and Rs with two BSp plants.

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Table 2.7

TOTAL SEED DAMAGE
PERCENTAGES

Lineage	no debris	debris
Rs	19.61	19.61
N=2	(17.70)	(17.71)
CAwr	23.58	21.04
N=6	(24.82)	(19.74)
Rr	6.21	6.17
N=4	(7.67)	(7.63)

CHAPTER 3

Within-Fruit Seed Characteristics and Paternal Fitness of a Hybrid Plant and its two Progenitor Lineages, Genus *Raphanus*

ABSTRACT

The hybrid derived California wild radish has outcompeted and replaced its two progenitor lineages in California. All three plants have pod-like fruits but in the hybrid, these are sired by multiple fathers and positioned non-randomly with respect to paternity, which also determines seed size. These unusual seed characteristics may be a key feature in explaining the competitive superiority of the hybrid. Here I address this hypothesis by directly comparing patterns of seed size and paternity from the progenitors, which have not previously been studied, and the hybrid. Fitness variables including fecundity and phenology were also assessed. The seeds came from three pollination treatments: open pollinated control plants, single and mixed hand pollinated crosses. These experiments demonstrated that the progenitor lineages, like the hybrid, have multiple paternity of the seeds within a fruit. Paternity significantly affects seed weight and reproductive output in all three lineages. In other respects, the three pollination treatments did not produce identical results. The position of the seeds within the fruit was correlated with seed weight in the open-pollinated controls but not in either of the hand-pollinated crosses. Of the two progenitors, the cultivated radish *R. sativus* performs poorly when crossed with the hybrid compared to the wild *R. raphanistrum* progenitor. Plant phenological patterns were strongly influenced by maternity. These results indicate that the multi-seeded fruits

of three *Raphanus* lineages provide an advantage of enclosing seeds with various genotypes and traits that maintain high polymorphism in the population and may enhance fitness in stressful and competitive environments. In natural conditions the hybrid derived CAwr has larger seeds at the peduncular end. This characteristic coupled with their fruit defensive structure appears to be an important component, which I suggest counteracts any potential negative effect from granivores, playing an important role in the success of this hybrid-derived invasive plant.

INTRODUCTION

During the invasion process, the ability of a species to successfully persist and evolve depends on spreading its propagules (Richardson et al. 2000; Sakai et al. 2001; Lambrinos 2004). These offspring carry the resulting effects of biotic and abiotic selective forces including both natural and sexual selection. High reproductive rate characterizes successful invaders (Burton et al. 2010) because it translates into thriving demographics allowing dispersal and range expansion (Richardson et al. 2000). However, reproductive investment alone does not always explain invasiveness (Hayes and Barry 2008) such as when natural selection has the potential to modify this over time (Mooney and Cleland 2001; Bossdorf et al. 2005; Siemann et al. 2006). In multi-seeded fruits, understanding the effects of within-fruit seed characteristics is an opportunity to explore the effects of sexual selection. Sexual selection effects, coupled with natural selection, may influence the success of an invasive lineage (Maron and Vilà 2001, Noonburg and Byers 2005).

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Seeds are the propagules of sexually reproduced plants (Harper et al. 1970) and the majority of plant invaders rely on their dispersal (Pysĕk 1997, Pergl et al. 2011). Most of the studies on seed or fruit traits in invasive plants either emphasize the effects of high fecundity (Rejmánek 1996) or the rate of dispersal and life history traits (Moravcová et al 2005, Bradshaw et al. 2008). However other seed characteristics such as seed size (Stanton 1984 and 1985; Choe et al. 1988), seed paternity and maternity (Marshall and Ellstrand 1986 and 1988; Marshall and Whittaker 1989) and within-fruit seed position (Wang et al. 2010) are variables that have been shown to influence seedling establishment, plant growth, adult plant size, ecology and reproductive output in non-invasive species (Stanton 1984 and 1985; Mazer et al. 1986, Moles et al. 2005, Moles and Westoby 2006, but Moles et al 2003).

Intraspecific seed size variation is considered to be stable and tends to vary under density-dependent competitive situations (Harper et al. 1970). Variation in seed size also occurs within fruits and this is particularly apparent when the ovules are arranged in a linear arrangement within the fruits. In cucumber (*Cucumis sativus*), the higher the fruit position on the mother plant and the within-fruit peduncular seeds were the slower to reach maturity and lowest dry weight (Jing et al. 2000). However, differences in fruit and ovule positions were compensated over time, when seeds had more time to mature inside the fruit (Jing et al. 2000). Seed size, from a broad phylogenetic (Moles et al. 2005) to specific ecological interactions (Harper et al. 1970; Stanton 1984; Mazer et al. 1986; Marshall and Ellstrand 1988; Venable 1992; Zhang 1996; Moles et al. 2003 and 2005; Venable and Rees 2009) is a significant trait in most plant's lifetime performance.

Therefore, within-fruit seed size and other characteristics such as seed position and paternity, can have a relevant effect on invasive hybrid derived lineages. Therefore, I investigated how those within-fruit seed characteristics compare between a hybrid lineage relative to its progenitors.

In particular, the present study compares the within-fruit seed characteristics and fitness among the invasive hybrid-derived California wild radish (CAwr) and its cultivated *Raphanus sativus* (Rs) and wild *R. raphanistrum* (Rr) progenitors in a non-competitive setting. At the seeded portion, *Raphanus* fruits hold up to 13 seeds. Mature seeds have no endosperm but only diploid embryo tissue (Ellstrand 1984). A relatively fine seed coat covers *Raphanus* seeds and its integral presence induces dormancy (Cousens et al. 2010). Some of the features of the hybrid-derived CAwr reproductive biology may strongly influence its ability to adapt to new environments. The hybrid derived lineage fruits have multiple paternity (Ellstrand 1984) and multiple lines of evidence suggest that the seed siring occurs in a non-random mating manner (Stanton 1985, Marshall and Ellstrand 1986, Marshall and Whittaker 1989). Thus, the identity of the pollen donor is an important determinant in the sequence of the fertilization and seed placement within the pod-like fruit (Marshall and Ellstrand 1986, Marshall 1988, Marshall and Ellstrand 1988, Marshall 1991). Inside the fruit, seeds vary in size (Stanton 1984), with a general tendency to be heavier at the attached or peduncular end (Marshall and Ellstrand 1988). The order of ovule fertilization in the *Raphanus raphanistrum* (Rr) follows two patterns: (1) ovules in stylar section first, followed by middle ones and lastly peduncular ones, or (2) middle ovules first followed by stylar ones and peduncular ones

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(Hill and Lord 1986). These patterns are explained by gamete selection at prezygotic mechanisms level as well as by the gynoecium internal structure (Mazer et al. 1986; Hill and Lord 1987). A central transmitting tissue structure, called septum, allows compatible pollen tubes to grow and by-pass ovules at stylar positions (Mazer et al. 1986; Hill and Lord 1987). However, I do not know if all three lineages have the same within-fruit seed characteristics.

Our objectives are (1) to compare seed weight among all three *Raphanus* lineages and their populations, (2) to determine if within-fruit seed positioning influences seed weight, fecundity, and other morphological as well as fitness related characteristics, (3) to compare if single and to mixed hand pollination crosses influence fitness values, (4) to document the occurrence of multiple paternity in all three lineages, and (5) to assess maternal and paternal effect on seed weight and other fitness related variables.

MATERIALS AND METHODS

Seed sources - The plants that produced the seeds used to breed the mother plants and the control fruits in these experiments were reared in a common garden during Spring 2005 and Winter 2006. The seed sources for those maternal and control plants are described in table 3.1. The seeds are the result of natural open pollination in common gardens at the Agricultural Experiment Station at the University of California-Riverside (AgOps-UCR). More details on how the plants were grown can be found in Ridley and Ellstrand (2009).

Control vs. mixed and single crosses - We measured seed characteristics from seeds extracted from: (1) fruits obtained from open pollinated plants (Ridley 2008) herein

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called control plants: at random I selected five mothers within each of three populations for each lineage and randomly chose three fruits for each mother plant for a total of 135 fruits, and (2) from the offspring resulting hand pollinations from mix and single pollen hand pollination crosses performed in 2010. In total I performed 595 crosses, 336 mixed pollen crosses and 259 single pollen crosses, in a total of 83 plants: 23 CAwr, 30 Rr and 30 Rs, for more details on these crosses see "Paternity".

Viable seeds common garden - Fruit from control, mixed and single crosses were carefully opened with a cutting knife (x-acto precision knife with exchangeable blades). Inside each opened fruit, seeds in all positions were examined. We counted the total number of seeds or seed set, including viable and unviable seeds as well as empty seed compartments. Seed viability was initially determined by visually inspecting the seed coat and by putting pressure on each seed between the thumb and the index fingers; when unviable, seeds had black and/or wrinkled seed coats and disintegrated easily.

Before planting in soil, all "viable" seeds were soaked in 600-ppm giberellic acid solution and germinated in large trays lined with distilled water damped Whatman filters. The trays were placed in the dark of the laboratory and visited twice daily to record germination. After 5 days all seeds were transplanted in seedling trays with UC Soil mix III and moved into a temperature-controlled greenhouse. The greenhouse was visited daily to continue monitoring for germination or any other changes.

After 53 days, the plants that survived were transplanted to common garden plots at the University of California-Riverside (UCR) Agricultural Operations (AgOps), where I monitored daily for floral buds emergence and first opening flower as well as any

changes in the plant condition including signs of herbivory. The common garden design consisted of four plots of 11 m x 11 m at AgOps-UCR, where I planted the seedlings spaced by 1 m in all directions. The plants were watered once daily for 5 min with a sprinkler system until they all started to flower. Plants that survived to the end of the experiment were measured for total number of fruits produced and final plant height (cm), and collected to obtain final plant weight (g).

Seed characteristics

Seed weight - Starting from the peduncular end (i.e. basal fruit section), each viable seed was weighed to the 0.01 mg with an analytical balance (Mettler-Toledo AB135-S/FACT). The seeds were always extracted in the same order, and sequentially placed by columns in a labeled 96 well plate recording the row and column in a spreadsheet. Once all "viable" seeds of a fruit were placed in a plate, the section assigned to the fruit was securely taped and labeled with the lineage, population, mother plant and fruit ID's to be stored for further analysis. This arrangement kept the seeds in order and prevented any translocations among positions and fruits. Lastly, the seeds were planted in a common garden to test fitness, always maintaining the same order of extraction from peduncular, middle, to stylar (i.e. distal fruit section) ends.

Within-fruit seed characteristics - Three within-fruit seed characteristics were tested: (1) seed weight, (2) within-fruit seed weight percentages, and (3) relative within-fruit seed fecundity. Within-fruit seed weight percentages were calculated by dividing a given seed weight by the sum of all seed weights in a given fruit and multiplied by 100. Within-fruit relative fecundity was calculated by dividing the seed fecundity by the

overall fruit fecundity where fecundity is defined by the total number of fruits produced.

Within-fruit seed position was considered in two different ways: the seed positions *per se*

recorded during the seed extraction process, and as seed position bins. To make the

different fruit positions comparable, I divided fruit positions into three seed position bins

corresponding to the peduncular, middle and stylar positions as described in figure 3.1.

These seed position bins were based on our own observations and on the way I divided

the fruits as I opened and removed the seeds. Within the seeded portion of the fruit, I

divided the fruit in two. This central point was defined as the middle portion (m). Next,

2/3 away from that central point toward the basal part of the fruit I marked the peduncular

portion (p). Then, 2/3 away from the same central point, this time towards the distal part

of the fruit, I determined the stylar (s) portion of the fruit. These variables included (1)

days to germination after planting, which constituted the time baseline for all other life

cycle variables, such as, (2) the days to floral bud emergence, and (3) the days to first

opened flower.

Paternity - To assess the occurrence of multiple paternity on the three lineages I

performed mix and single pollen crosses on mature unopened flower buds. Any mature

flower bud with a corolla that was not tightly closed or that presented any opening as a

result of damage was discarded. Immediately after performing hand pollinations

(described bellow), pollinated stigmata, fully covered with pollen, were covered with

labeled tulle bags until the end of the experiment. The purposes of the tulle bags were to

avoid unwanted pollen transfer, to protect the fruit from any predation and to allow

proper maturation without losing the fruit once dried.

At the beginning of May 2010, I collected the pollen sources plants for our hand pollinations from a natural well-established population of CAwr. The five already flowering plants were found in Hemet in the San Jacinto Valley in Riverside County, California. The chosen plants had different flower colors looked healthy, and had no pests or signs of herbivory. These plants were individually transplanted to gallon pots and then placed in a temperature-controlled greenhouse at UCR. In the greenhouse the five plants were watered daily, trimmed to prolong their flowering and maintained pest free until the end of the experiment. Mix pollen load crosses included different combinations of four pollen donors, and in one case three, out of the set of five described above. At a given time, the mixture of pollen donor plants depended on: 1) the outcome of initial single crosses that verified compatibility with the pollen receivers and also 2) pollen donor flower availability at a particular time. Single crosses were also used as a reference through the microsatellite analyses. Once a mother plant was pollinated with a particular pollen mix, the same mix was used to pollinate the rest of the flowers on that same mother plant.

Despite evidence for lack of effect in the amount of pollen on reproductive output (Marshall and Ellstrand 1986; Marshall et al. 2007), I equalized amounts of pollen applied per donor across crosses as follows (Marshall D. L. protocol). For single crosses, I always removed the pollen from a single new flower by gently tapping all six anthers against the bottom of a clean Petri dish. For mixed crosses, I selected two anthers per father, totaling eight anthers for all four fathers. All eight anthers were dissected from new flowers with tweezers and tapped against the bottom of a clean Petri dish. The pollen

collected from all anthers was blended by gently swirling the pollen with a clean piece of folded lab tissue (Kimwipes-EX-L, Kimberly-Clark) held with tweezers. To avoid contamination when handling anthers and pollen, I cleansed our fingers and tweezers by splashing fresh alcohol before and after every use. When possible, each of the mix pollen crosses was replicated at least twice on each plant.

Seed paternity - We collected at most five young leaves when available or any leaves from all father, mother, and offspring plants. The tissue collected in the field or at the greenhouse was immediately packed inside labeled clear plastic envelopes, placed inside a cooler with dry ice and promptly transferred to be stored in -80° C until initiating the DNA extractions.

We determined paternity by genotyping microsatellite loci or short tandem repeats (STRs) previously used for Brassicaceae species (www.brassica.info). Total genomic DNA was extracted from 300 mg of leaf tissue collected using the DNAeasy Plant Mini kit (QIAgen 2006). We followed the kit's instructions only modifying the elution step by reducing the amount of buffer to 50 μl to yield 100 μl of final product. Ten pairs of previously developed primers for Brassicaceae (Suwabe et al. 2002, Szewc-McFadden et al. 1996, Ohsako et al. 2010) were initially tested and screened for amplification and detection of polymorphism among the five fathers. DNA concentration was quantified using a micro-volume UV-vis spectophotometer Nanodrop 2000 (Thermo Scientific). Among those ten primers I chose the four most informative and polymorphic comparatively among the five fathers (tables 3.2 and 3.3).

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Polymerase chain reactions (PCR) amplifications of the four loci were performed in a 20 μL total volume with X 0.3 U of Taq polymerase (Rieseberg lab), 2 μL of 10X buffer (Rieseberg lab), 10 mM dNTP, 10 μM/L primers, 10 μM M13 dye (FAM, VIC, NED or PET) and 1.2 μL of ~5-40 ng total DNA. For each locus, the forward primer had a M13 tail labeled with a fluorescent dye (table 3.3). A pigtail sequence (GTTTCTT) was attached to each reverse primer to avoid scoring problems due to genotyping errors as a result of adenosine addition artifacts (Brownstein et al. 1996).   Amplification was performed as follows: 94°C for 5 min, 30 cycles of 94°C for 30s, 56°C for 45s, and 72°C for 45s followed by 8 additional M13 tail cycles of 94°C for 30s, 53°C for 45s and 72°C for 45s (Schuelke 2000) and a final extension of 72°C for 10 min. Analysis of microsatellite fragment size for all four loci were done in a Big Dye Terminator v3.1 sequencing chemistry (Applied Biosystems).

Data analysis

Within-fruit seed characteristics - Data were normalized when needed and feasible with log-normal or Box Cox transformations (R Development Core Team 2010). Significant probability (*P*) values were adjusted a posteriori with sequential Bonferroni tests to adjust for type I error (Rice 1989). One-way analyses of variance (ANOVA) were used to compare seed weight at the lineage and population level. Significant results were followed by TukeyHSD post hoc tests for multiple paired comparisons of means at the lineage and populations levels.

To compare within-fruit seed characteristics among different seed positions I used a Kruskal-Wallis tests to compare: (1) seed weight, (2) within-fruit seed weight

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percentage, and (3) the relative within-fruit seed fecundity. For the purpose of these tests, I discerned among the three types of crosses: either mix or single hand pollination or the control open pollinated plants. Multiple regressions followed by the associated ANOVA were performed to assess the effect of lineage, population, type of cross, maternity, paternity and seed weight.

Comparisons among cross types (single or mix hand pollen crosses) and paternal siring frequencies at different fruit positions were assessed with goodness of fitness chi-square tests, which were followed by Pearson's chi-square with 10000 permutations the differences among row were lower than 5. All statistical tests were implemented using the R statistical program (R Development Core Team 2008) and extra statistical R packages were downloaded from the Comprehensive R Archive Network (CRAN 2012). *Paternity* - We scored genotypes of father, mother and offspring individuals by visualization of the results in GeneMapper Software 3.7 (Applied Biosystems 2004). A genotype with a single PCR fragment was considered a homozygote having two identical alleles. Visual inspection of allele assignments and manual corrections were systematically done. We employed the exclusion parentage analysis (Ellstrand 1984, Jones et al. 2010) to determine from the pool of fathers used, which one sired a particular seed by comparing the genotype of the three or four father candidates and the known mother to the focal progeny. We determined multiple paternity by comparing the siring fathers at different seed positions within the same fruits. We also assessed whether the fathering occurred in a non-random manner by measuring the frequency at which the siring occurred. Finally, I compared the performance of the fathers by calculating at the

offspring siring times, seed weight, within-fruit seed weight percentage and within-fruit relative fecundity and per fathers.

<center>RESULTS</center>

Control vs. mixed and single crosses

      Seed weight -   Seed weight ranged by an order of magnitude across all samples from a minimum of 1.62 mg, in a fruit from the Rr-NE9, to 14.66 mg, in a fruit from the Rs-BSp. Seed weights differ significantly among lineages ($F_{2,946}$= 66.90, P <0.00001) and among populations ($F_{8,940}$= 23.303, P < 0.00001; appendix G). On average, CAwr seeds are heavier, followed by Rs and Rr with lightest seeds (table 3.4). Average seed weights for Rs populations have similar values across its populations (Tukey post-hoc tests, P > 0.25). In contrast, the two wild lineages have inter-lineage similarities and differences. Comparisons across populations show that DK1-Rr seed weights are comparable to all other populations (Tukey post-hoc tests, P > 0.02) whereas MX1-Rr has the most dissimilar weights (appendix G). We also calculated the within-fruit seed weight proportion for every seed extracted. For a given seed, I divided its weight by the total number of seeds found inside the fruit were the seed in question was extracted from (table 3.4).

      Knowing that lineages and populations have a significant influence on weight, I moved on to compare, within lineages, if the type of crosses and within-fruit seed position influence seed weight, within-fruit seed weight percentage and within-fruit seed fecundity (see Data analysis). We tested this by using Kruskall-Wallis tests independently

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for each seed position and seed position bin. The tests were done for each type of cross individually within each lineage. Figures 3.2, 3.3, and 3.4 graphically show the average values from our data set. Within-fruit seed positioning has no statistical significant influence on weight. A trend for heavier seeds at peduncular positions for the hybrid derived lineage CAwr in control fruits can be visualized in figure 3.2. The opposite trend seems to be true for the wild radish Rr. The cultivar Rs has a sinusoidal trend. In the case of the percentage of within-fruit seed weight, seed position *per se* (figure 3.3): (1) influences significantly control, single and mixed crosses fruits of CAwr, and (2) influences significantly mixed Rr and slightly single Rr fruits. Seed position bins only moderately influence within-fruit seed weight percentage in control CAwr fruits. No effects of seed position were found on within-fruit seed fecundity. One-way analysis of variance (ANOVA) followed by Tukey post-hoc tests were performed to test if seed position bins for each lineage and type of cross had an effect on seed weight (table 3.5). The results suggest that only in the case of CAwr control plants is there a significant effect of the bins on seed weight. In this particular case, the Tukey post-hoc test reveals that it is the stylar end bin compared to the peduncular end were the difference lies (table 3.5) with a significant negative effect on seed weight.

Fecundity and relative fitness -   A total of 540 crosses formed viable fruits out of the 595 crosses that I performed. Among the 949 seeds found viable after extraction and first visual inspection, 312 seeds (33%) were transplanted to the common gardens at AgOps and among those 247 (77%) survived to the end of the experiment. Within lineages, multiplicative fitness functions for mixed and single crosses reveal that

total relative fitness was not significantly different among seeds from either cross for both progenitors and marginally different for the hybrid-derived lineage (table 3.6). For Rs the difference was significant as a result of lower viable seed/pod. When the fitness functions for mix and single crosses were compared within each lineage differences were found in all three cases. Surprisingly, mix crosses had lower fitness than single crosses for Rr and CAwr, and the opposite is true for Rs. Differences in fecundity, number of viable pods per pollination and seed viability, while not always significant, affect the overall fitness.

Paternity - We found evidence of within-fruit multiple paternity, i.e. seed sired by different fathers within the same fruit, for all three lineages: 11 out of 11 fruits for CAwr, 9 out of 11 for Rr and 1 out of 2 for Rs. Fruits from single crosses had all seed sired by the chosen father. As mentioned before, seed viability was an issue for our experiment. Very few fruits with all or most seeds survived to the end of the experiment, in particular for Rs (appendices H.1 and H.2). For this reason I cannot accurately assess if the multiple paternity is or is not random. Also, because I had so few plants from Rs that survived until the end of the experiment, I eliminated them from the rest of the analysis (appendices H.1 and H.2). The percentage of siring, seed weight average, within-fruit seed percentage average, and within-fruit seed fecundity average for CAwr and Rr for mixed and single crosses are represented in appendices I.1, I.2, J.1, and J2.

The siring success of the five CAwr fathers is provided in table 3.7. Each column contains the results by individual father, and each row within a division is the value per seed position bin. All of the values in table 3.7 pertain only to mixed crosses with CAwr

and Rr mothers because there was insufficient sample size to analyze the crosses with Rs mothers. The number of seeds sired in each of the three seed position bins did not differ significantly by father. However, when the number of seeds sired by each father was expressed relative to the number of times a particular father was included in a mixed pollination, the percentages of siring were significantly different. Father 1 and father 2 had higher success siring than the other 3 fathers (chi-square results table 3.7). Their total percentage of siring exceeds 100% because they sired seeds at least twice within same fruits. Father 2 was the most successful siring at both stylar and peduncular portions of the fruits. Seed weight average and within-fruit seed weight did not differ significantly among fathers. The highest average weight was found in seeds sired by father 3 in stylar positions. In contrast, the highest average within-fruit seed weight was found on seeds sired by father 2 at peduncular position. The within-fruit fecundity did differ significantly among fathers. Father 2 had higher fecundity relative to the other fathers, at all seed position bins (table 3.7).

In mixed crosses, when I assessed each father at different seed position bins, I found that father 2 has higher and moderately to significantly different: siring percentage, average seed weight and percentage of seed weight at peduncular portions of the fruits However its within-fruit relative fecundity was the same in all seed position bins. Father 3 has a moderately higher average seed weight at stylar positions and significantly higher within-fruit relative fruit of seed at peduncular positions. In single crosses, the only significantly different performances across seed position bins happened for average seed

weight of seeds sired by father 4 at middle positions and within-fruit relative fecundity of seeds sired by father 4 and father B at peduncular positions.

Father performances vary when pollinating in single and mixed pollen crosses. When I assessed lineage-by-lineage, fathers and seed position bins, I found that CAwr offspring resulting from mixed crosses fruits, father 2 appears to have the highest siring percentage at seeds in peduncular ends, with highest average within-fruit seed weight and fecundity at all sections of the fruit. Father 3 has the highest seed weight at stylar and peduncular ends but high average fecundity only at stylar end seeds (appendices I.1, I.2, J.1, and J2). These results are not replicated in the case of single pollen crosses. In the case of Rr offspring resulting from mixed crosses, father 2 also sired the most seeds but this time at the stylar end with highest average within-fruit seed fecundity. Here also the results were not replicated at the single pollen crosses. Allele frequencies in father, mother and offspring are compiled in appendix K.

Maternal and paternal effects – Maternal effects are significant at phenological life cycle level with significant effect on days to germination, days to first true leaf emergence, and final plant weight. Paternal effects significantly influence reproductive output such as total fruit production and potential reproduction as well as offspring seed weight (table 3.8). Fathers also marginally influence cotyledon width and days to flower buds emergence. Lineage and population influence both morphological as well as fitness related characters including seed weight, which is consistent with our previous results. Seed weight is also influenced by the type of cross but not by seed position bins. Seed weight influences cotyledon width and days to first true leaf emergence.

DISCUSSION

Previous studies have demonstrated non-radom multiple paternity for the hybrid-derived CAwr fruits (Ellstrand 1984; Marshall and Ellstrand 1986, Marshall et al. 2007). Our results show that multiple paternity also occurs in both progenitor lineages. Because very few whole fruits were represented in the offspring that survived until the end of the experiment, I were unable to determine if the distribution of paternal DNA is non-random with respect to seed position within the pod. Across lineages, for mixed crosses only, the father that sired most seeds was the one from which offspring were the most fecund. Mixed and single hand pollinations gave different results for individual fathers at different sections of the fruits. The peduncular section of the hybrid-derived lineage did appear to be where the most successful of the fathers sires its seeds. This is also the section of the fruit were there is a positive effect of seed position and seed weight, in the same lineage in open pollinated fruits. As predicted, pollen donors differ in their ability to sire seeds produced by mixed crosses and the pollen donor identity did influence the offspring fecundity. However, when paternity was not taken into account the relationship between offspring seed weight and fecundity is not so clear. Perhaps, in a competitive environment (i.e. stress, density-dependent conditions), these relationships become more apparent.

A potential explanation for the reduction in fecundity and low viability in the cultivated Rs might be that during the time the plants spent in the greenhouse, there was an effect of competition. As seedlings in the greenhouse, the plants were very close together in the seed starting trays. The hybrid-derived (CAwr) and the wild (Rr) lineages

germinated and grew faster than the cultivar. Aboveground competition for light and space during seedling stage might have decelerated the cultivars' growth. Competition has been showed to reduce fecundity in *Raphanus* plants (Campbell and Snow 2006). Similar results were found by Ridley and Ellstrand (2009). Relative to the hybrid-derived CAwr, the cultivar produced fewer fruits and seeds over two years and three replicates period. During their experiments the CAwr, Rs and Rr plants were exposed to open pollination. It is unlikely that the pollination of their cultivar plants (Rs) was restricted among conspecific plants. It is likely that all three lineages were pollen sources, especially CAwr given that it has ephemeral populations at the experimental sites as well as permanent ones in the surroundings. In the present study, CAwr pollen was used for all hand pollinations on all three lineages potentially reducing compatibility and affecting the cultivar's viability and fecundity.

Individual plants with different types of seeds, e.g. different morphology, have seed heteromorphism (Imbert 2002). A known trait in heteromorphic seeds is intraspecific seed size variation within the same mother plant or even within the same fruit. To be adaptive, heteromorphic seeds differ in their ecology and fitness offering an advantage in unpredictable environments (Venable 1985; Venable and Brown 1988). Some ecological consequences of seed size heteromorphism include variation in dispersal ability, dormancy and germination requirements with consequences at sibling competition level, final reproductive output, and final seed density-dependent distribution in time and space (Venable 1985). Under competitive conditions, larger seeds of *Raphanus raphanistrum* growth faster, giving them a competitive advantage over smaller

ones (Stanton 1984). In our study, peduncular seeds in the hybrid-derived plants produced by open pollination (CAwr) were the only situation where seed position and seed size were significantly influenced. Across environmental hetererogeneity, larger seeds of the annual *Cakile edentula* (Brassicaceae) have shorter life cycles, an advantageous trait in unpredictable sand dunes environments (Zhang 1996).

There is evidence of sexual selection in *Raphanus* fruits, manifested in the effect of the pollen-donor identity on fruit set, seeds per fruit, seed abortions and seed weight (Marshall and Ellstrand 1986). Among the fruits and seeds that I was able to genotype and assign paternity, I did find that the fathers that I used as pollen sources did have an effect on seed weight and on final reproductive output. When paternity was determined, seeds at peduncular ends do produce more seeds that will have higher reproductive output. This is not a universal trait, however. Seeds from peduncular portions of the cucumber (*Cucumis sativus*) fruit are the slowest to attain the optimum conditions for germination and desiccation tolerance (Jing et al. 2000). However, effects of within-fruit seed position and within-plant fruit position in cucumber fruits eventually disappear, leveling up final germination rates among all seed positions. In our experiments, I did not find direct effect of seed weight on seed germination and final reproductive output (Jing et al. 2000).

Our data suggest that seed paternity influences seed weight, final plant height and reproductive output. We also found that seed maternity moderately influences reproductive output, final plant weight and life cycle stages. These results support a hypothesis of complementary maternal and paternal effects in all three lineages. The mechanisms involved in ovule fertilization in *Raphanus* fruits are determined by maternal

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choice and paternal identity, which is especially clear in mixed pollinations (Hill and Lord 1986; Mazer et al. 1986; Marshall and Ellstrand 1988). We detected competition among fathers, where single pollination results showed higher reproductive output of some fathers that scarcely were represented in some of the mixed pollinations. When paternity is considered, seeds at peduncular positions in mixed and single crosses produced offspring with higher reproductive output. Combining this result with fruits harder structure at peduncular ends for all three lineages, in particular in the case of CAwr, suggests the intriguing possibility that there is higher protection for higher performing seeds. From an ecological perspective, these results might counteract any potential negative effects of granivores.

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Table 3.1

SEED SOURCES

Lineage	Source / location	Population	ID
Raphanus sativus Rs	Ferry-Morse Seed Co.	Cherry Belle	Rs-ChB
	Ferry-Morse Seed Co.	French Breakfast	Rs-FrB
	Botanical Interests, Inc.	Round Black Spanish	Rs-BSp
CA wild radish CAwr	USA - California	Riverside Riverside County	CA-In1
	USA - California	Lindcove Tulare County	CA-In3
	USA - California	Morro Bay State Park San Luis Obispo County	CA-Cst
R. raphanistrum Rr	Denmark	Roskilde University Botanic Garden	Rr-DK1
	Mexico	Mexico City	Rr-MX1
	USA	Conanicut Island Rhode Island	Rr-NE9

Note.- To eliminate maternal effects, fruits and seeds were reared in the greenhouse from pure lineages of Rr and CAwr (Ridley 2008) and Rs, from purchased seeds. Fruits from CAwr populations comprise one coastal and two inland natural populations.

Within-fruit seed position bins

	peduncular (p)				middle (m)					stylar (s)			
Seed set													
13	1	2	3	4	5	6	7	8	9	10	11	12	13
12	1	2	3	4	5	6	7	8	9	10	11	12	
11	1	2	3	4	5	6	7	8	9	10	11		
10	1	2	3	4	5	6	7	8	9	10			
9	1	2	3	4	5	6	7	8	9				
8	1	2	3	4	5	6	7	8					
7	1	2	3	4	5	6	7						
6	1	2	3	4	5	6							
5	1	2	3	4	5								
4	1	2	3	4									
3	1	2	3										
2	1	2											
1	1												

Figure 3.1. Seed position bins. Schematic representation of the method used to assign peduncular (p), middle (m) and stylar (s) seed position bins for fruits containing different total number of seeds.

Table 3.2

MICROSATELLITE LOCI

Locus	Repeat motif	Sequence of primer (5'-3')	Size range (bp)	Dye
BN35D**	(GA)$_{13}$	F:GCAGAAGGAGGAGAAGAGAGTTGG R:TTGAGCCGTAAAGTTGFTCACCT	218-281 (210-270)	NED
BRMS 005***	(GA)$_{13}$	F:ACCTCCTGCAGATTCGTGTC R:GCTGACCTTTCTTACCGCTC	127-193 (140-163)	FAM
rm25*	(TC)$_{23}$	F:AGTGGACGGTGGAGATTTCTA R:CAAAAGGGATCACACAGTCAAAT	231-270 (258-268)	VIC
rm48*	(AC)$_7$GC(AC)$_6$	F:ACCATGTTCCTGAAATTCAAAGG R:TACTTCCATAGGCAACTTGCTG	278-312 (285-289)	FAM

Note.- The given size ranges were found in the present study and the ones in parenthesis are the ones reported by the literature (***Suwabe et al. 2002, **Szewc-McFadden et al. 1996, *Ohsako et al. 2010).

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Table 3.3

NUMBER OF ALLELES PER LOCUS IN
FATHERS, MOTHERS AND OFFSPRING PLANTS

Locus	Alleles Progenitors					Alleles Offspring							
	Fathers		Mothers			Type of crosses							
						MIXED				SINGLE			
	CAwr	Rs	CAwr	Rr	T	Rs	CAwr	Rr	T	Rs	CAwr	Rr	T
BN35D	4	3	6	8	13	5	7	10	13	3	8	8	11
BRMS005	6	2	5	8	17	6	9	12	16	3	5	11	12
rm25	4	4	4	5	7	4	4	5	5	3	4	5	6
rm48	6	3	3	5	12	7	9	10	12	3	8	8	11

Note.- T=total. Rs=*Raphanus sativus*; CAwr=hybrid California wild radish and Rr=*R. raphanistrum.*

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Table 3.4

OVERALL AND WITHIN-FRUIT SEED CHARACTERISTICS
PER LINEAGE, POSITION AND BIN

Lin	x̄	seed positions										position bins		
		1	2	3	4	5	6	7	8	9	10	p	m	s
							Seed weight (mg)								
Rs	4.5	4.9	4.3	4.5	4.4	4.6	4.1	4.6	3.7	4.8	-	4.7	4.3	4.4
N=148	(2.3)	(2.2)	(2.6)	(2.2)	(2.0)	(2.1)	(2.4)	(2.6)	(2.6)	(2.7)		(2.4)	(2.0)	(2.4)
Cawr	4.6	4.6	4.5	4.9	4.7	4.6	4.8	4.3	3.5	6.6	-	4.7	4.7	4.4
N=296	(2.3)	(2.4)	(2.4)	(2.2)	(2.4)	(2.4)	(2.2)	(2.3)	(2.0)	(1.5)		(2.3)	(2.4)	(2.2)
Rr	3.4	3.0	3.2	3.5	3.8	3.5	3.4	3.4	3.2	3.6	3.7	3.3	3.5	3.3
N=403	(2.1)	(1.8)	(1.9)	(1.9)	(2.0)	(1.9)	(2.0)	(2.1)	(1.5)	(1.4)	(2.0)	(2.1)	(2.1)	(2.2)
						Seed weight proportion								
					(seed weight (mg)/total number of seeds per fruit)									
Rs	0.7	0.9	0.9	0.7	0.6	0.7	0.5	0.7	0.4	0.5	-	0.7	0.6	0.8
N=148	(0.7)	(0.6)	(1.2)	(0.5)	(0.5)	(0.5)	(0.4)	(0.6)	(0.3)	(0.4)		(0.6)	(0.5)	(1.0)
Cawr	0.9	1.1	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.7	-	0.9	0.9	0.9
N=296	(0.7)	(1.0)	(0.8)	(0.7)	(0.6)	(0.5)	(0.4)	(0.3)	(0.2)	(0.2)		(0.8)	(0.6)	(0.7)
Rr	0.5	0.5	0.6	0.6	0.5	0.5	0.4	0.4	0.3	0.3	0.3	0.5	0.5	0.5
N=403	(0.4)	(0.5)	(0.4)	(0.4)	(0.4)	(0.3)	(0.3)	(0.3)	(0.2)	(0.1)	(0.2)	(0.4)	(0.4)	(0.4)

Table 3.4
(continued)

Relative within fruit seed fecundity
(seed fecundity (number of fruit per plant)/highest fruit fecundity)

Lin	$\bar{x}$	seed positions										position bins		
		1	2	3	4	5	6	7	8	9	10	p	m	s
Rs	0.1	0.06	0.12	0.00	0.08	0.10	0.08	0.02	0.00	0.39	-	0.08	0.06	0.08
N=148	(0.2)	(0.2)	(0.3)	(0.0)	(0.2)	(0.3)	(0.2)	(0.1)	(0.0)	(0.5)		(0.3)	(0.2)	(0.2)
Cawr	0.2	0.26	0.24	0.20	0.11	0.24	0.17	0.18	0.25	0.16	-	0.24	0.20	0.17
N=296	(0.4)	(0.4)	(0.4)	(0.3)	(0.2)	(0.4)	(0.3)	(0.3)	(0.4)	(0.3)		(0.4)	(0.3)	(0.3)
Rr	0.2	0.21	0.10	0.21	0.18	0.22	0.20	0.10	0.15	0.01	0.19	0.16	0.21	0.15
N=403	(0.3)	(0.4)	(0.2)	(0.4)	(0.3)	(0.4)	(0.4)	(0.2)	(0.3)	(0.0)	(0.3)	(0.3)	(0.4)	(0.3)

Note.- Lin=lineage, N=total number of plants, ͞=overall average, one-seeded plants were excluded as well as any seed position without at least 2 replicates.
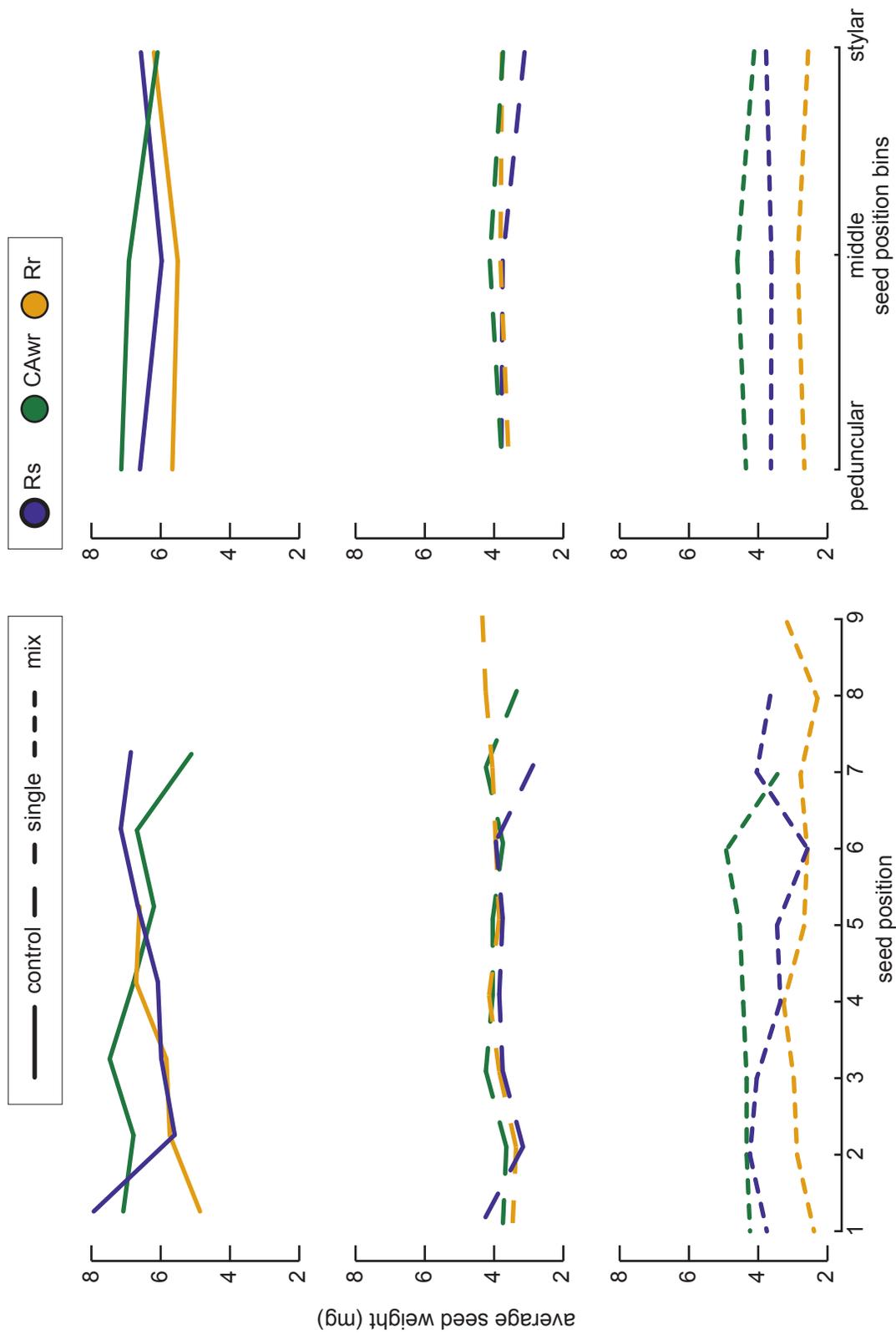
Figure 3.2. Seed weight for each type of cross and lineage per seed position *per se* and seed position bin.
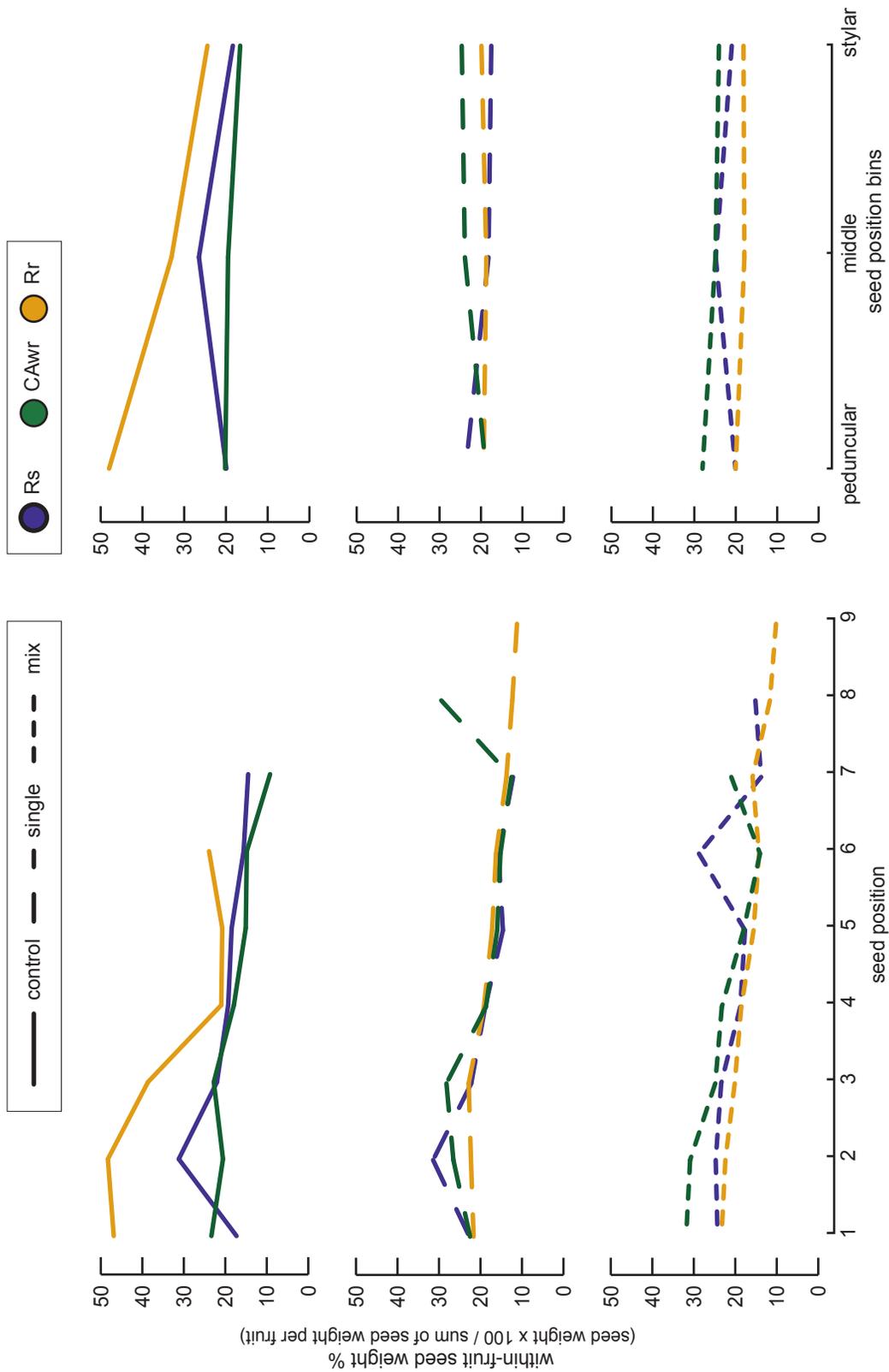
Figure 3.3. Within-fruit seed weight percentage for each type of cross and lineage per seed position *per se* and seed position bins. Values were calculated by dividing a given seed weight by the sum of all seed weights in a given fruit and multiplied by 100.
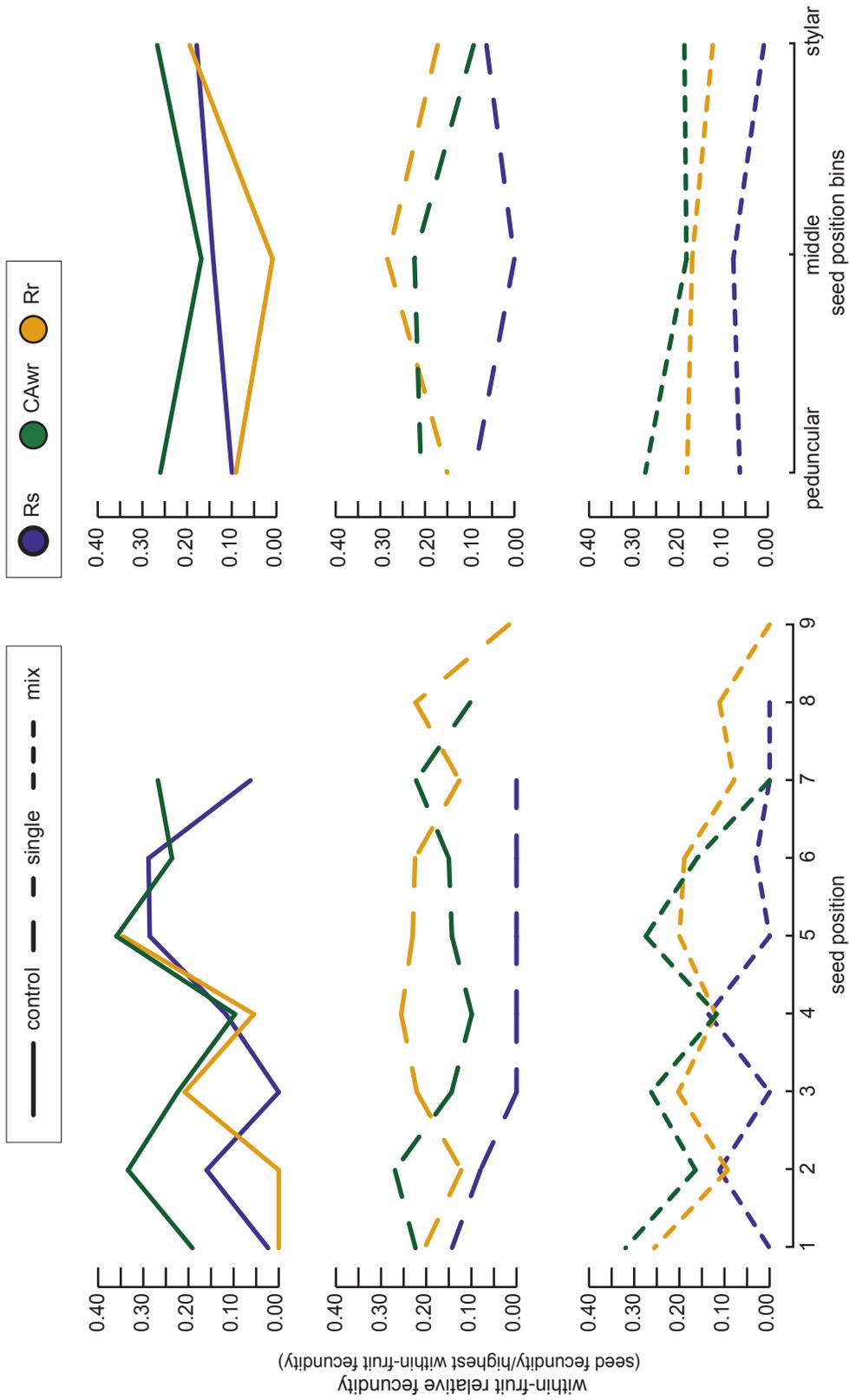
Figure 3.4. Within-fruit relative fecundity for each type of cross and lineage per seed position bins. Values were calculated by dividing a given seed fecundity by the overall fruit fecundity where fecundity is defined as the total number of fruits produced by viable seeds.

Table 3.5

SEED WEIGHT FOR EACH LINEAGE, TYPE OF POLLEN CROSS, AND SEED POSITION BINS
ONE–WAY ANOVA AND TUKEY HSD TESTS

Lineage: Rs

Tests	Pollen crosses														
	Control					Single					Mixed				
ANOVA	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P
Bins	2	3.5	1.7	0.4	0.7	2	4.8	2.4	0.9	0.4	2	0.0	0.0	0.0	1.0
residuals	41	188.8	4.6			52	137.7	2.6			45	167.6	3.7		

Tukey Bins	Control				Single				Mixed			
	Diff	lwr	upr	P	Diff	lwr	upr	P	Diff	lwr	upr	P
2-1	-0.6	-2.6	1.3	0.7	-0.0	-1.3	1.2	1.0	-0.0	-1.7	1.7	1.0
3-1	-0.0	-1.9	1.9	1.0	-0.7	-2.0	0.6	0.4	-0.1	-1.7	1.6	1.0
3-2	0.6	-1.4	2.6	0.7	-0.6	-2.0	0.7	0.5	-0.0	-1.7	1.6	1.0

Lineage: CAwr

Tests	Control					Single					Mixed				
ANOVA	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P
Bins	2	12.3	6.2	3.9	*	2	7.1	3.5	0.6	0.5	2	4.9	2.5	0.5	0.6
residuals	55	86.5	1.6			128	710.3	5.5			107	499.4	4.7		

Tukey Bins	Control				Single				Mixed			
	Diff	lwr	upr	P	Diff	lwr	upr	P	Diff	lwr	upr	P
2-1	-0.2	-1.2	0.8	0.9	0.5	-0.7	1.6	0.6	0.25	-0.9	1.5	0.9
3-1	-1.0	-2.0	-0.1	*	-0.1	-1.3	1.2	1.0	-0.3	-1.5	0.9	0.8
3-2	-0.8	-1.9	0.2	0.1	-0.5	-1.7	0.7	0.6	-0.5	-1.8	0.7	0.6

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Table 3.5 (continued)

Lineage: Rr

Tests	Control					Single					Mixed				
ANOVA	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P	Df	SSq	MSq	F	P
Bins	2	1.8	0.9	0.2	0.8	2	4.4	2.2	0.7	0.5	2	2.0	1.0	0.2	0.8
residuals	23	91.1	4.0			188	615.9	3.3			185	856.9	4.6		
Tukey Bins	Diff	lwr	upr	P		Diff	lwr	upr	P		Diff	lwr	upr	P	
2-1	-0.2	-2.4	2.1	1.0		0.3	-0.4	1.1	0.5		0.2	-0.7	1.1	0.9	
3-1	0.5	-2.0	3.1	0.9		0.3	-0.5	1.0	0.6		-0.1	-1.0	0.8	1.0	
3-2	0.7	-1.9	3.3	0.8		-0.0	-0.8	0.7	1.0		-0.2	-1.2	0.7	0.8	

Note.- Df= degrees of freedom; SSq= sum of squares; MSq= mean of squares; Diff=difference; lwr=lower; upr=upper; Rs=*Raphanus sativus*; CAwr=California wild radish; Rr= *R. raphanistrum*; ****P<0.00001, ***P<0.0001, **P<0.001, *P<0.01. For this analysis we eliminated single-seeded fruits and positions with single values.

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Table 3.6

TOTAL AND RELATIVE FITNESS COMPARISONS
BETWEEN SINGLE AND MIX CROSSES AT LINEAGE LEVEL

Lin	N	Viable pods	Viable seeds	Relative viability	Relative survival %	Relative fecundity %	Fitness function	P	Relative fitness	P	Total relative fitness	P
Rs	23											
M	91	0.13	4.08	58.86	31.81	3.18	54.51	****M	24.48	****	1.02	****
S	86	0.13	0.05	59.84	16.41	2.20	0.22		0.10	****& M	0.36	****
CAwr	30											
M	90	0.32	0.04	71.02	100.00	36.00	42.39	****S	19.04	****	36.37	** & 0.02 S
S	70	0.49	0.04	81.00	98.33	58.68	107.15		48.12	****	58.30	**
Rr	30											
M	119	0.31	0.05	88.24	77.08	96.08	113.27	****S	58.87		74.83	
S	84	0.45	0.05	100.00	98.97	100.00	222.69		100.00		100.00	

Note.- Lin=lineage; N=number of crosses; M=mixed pollen crosses; S=single pollen crosses. Pearson's chi-square among mix and single crosses X^2=45.55, df=2, $P<0.00001$. Values were compared to the highest value within each category and between crosses M=mixed, S=single; ****$P<0.00001$, **$P<0.001$.

Table 3.7

FATHERS SIRING FREQUENCY AND FECUNDITY
MIX CROSSES - CAwr AND Rr

Bins	Fathers				
	1	2	3	4	B
seeds per father					
peduncular	7	6	4	5	4
middle	7	3	4	6	1
stylar	8	7	2	3	2
Total	22	16	10	14	7
siring %					
peduncular	33	40	22	26	29
middle	33	20	22	32	7
stylar	38	47	11	16	14
Total	105	107	56	74	50
seed weight (mg)- average					
peduncular	4.2	4.5	2.7	3.0	4.1
middle	3.6	2.5	4.6	3.8	2.9
stylar	5.1	3.1	5.8	3.2	2.8
Total	4.4	3.5	4.1	3.4	3.6
within-fruit seed weight % - average					
peduncular	17	34	24	11	17
middle	22	18	21	25	12
stylar	18	20	20	22	15
Total	19	25	22	19	16
within-fruit relative fecundity - average					
peduncular	0.6	0.7	0.5	0.6	0.6
middle	0.4	0.8	0.5	0.4	0.0
stylar	0.4	0.8	0.6	0.5	0.6
Total	0.5	0.8	0.5	0.5	0.5

Note.- seeds per father: X^2=5.07, N=79, df=8, P=0.75; siring %: X^2=32.4, N=79, df=8, P<0.00001; seed weight: X^2=2.24, N=79, df=8, P=0.97; within-fruit seed weight: X^2=13.34, N=79, df=8, P=0.1; within-fruit relative fecundity: X^2=59.60, N=79, df=8, P<0.00001.

Table 3.8

MULTIPLE REGRESSION RESULTS FOR
ALL (BLACK) AND ONLY MIX CROSSES (RED) PLANTS

Variables	Lineage	Population	Type Cross	Mother	Father	Seed weight
Seed weight	*** ***	*** ***	*		*	
Final weight	** *	***		*		
Final height	*				*	
Cotyledon width	** ***	*** ***		*** ***		***
Days to germination		*				
Days to first true leaf	*	*** ***	*	** ***		*** **
Days to first flower bud					*	
Days to first flower	***					
Total fruits	*** **			* *	**	
Potential reproduction	*** ***			*	*	

Note.- All crosses (results in black) include single, control and mixed crosses; ***P=0.0001; **P= 0.001; *P=0.01;
*0.05<P<0.08 .

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CONCLUSIONS

The fruits of the hybrid invasive lineage, California wild radish (CAwr), offer better protection to the seeds that will be better competitors, relative to the fruits of both *Raphanus* progenitors. The most favored and prolific fathers in mixed pollen crosses typically sired the seeds at peduncular and middle portions of the hybrid fruits. Those same portions of the hybrid fruits have the hardest fruit walls. Thus, the fruit structure and the effects of mating selection couple in a favorable manner in the hybrid derived lineage. How much of the fruit structure is the result of inter-specific hybridization alone or a combination of it with natural selection is a question that still needs to be answered. The fruit wall hardness appears to be a defensive trait that provides fitness advantages to the hybrid lineage, in particular in the context of pre-dispersal seed predation by the house finch (*Carpodacus mexicanus*). This ecological advantage could have been a significant feature in Southern California leading to successful establishment of hybrids over both progenitors. These results now lead to several questions about how broad these patterns may be: How homogeneous is this mechanism along the current distribution on both coastal and inland sites in California, Baja, Mexico and Oregon? How common is the house finch-hybrid lineage interaction along this geographic range? How homogeneous is this trait within the same mother plant and are fruits within the same plant with weaker fruit structure more heavily predated?

Of the two progenitors, fruits of the wild *Raphanus raphanistrum* (Rr) are much harder than those of the domesticated *R. sativus* (Rs). Patterns in fruit wall hardness of

the *Raphanus* system suggest that it is either a neutral or an advantageous trait. In addition, the results described here implicate another mechanism of defense in the wild progenitor. At maturity the fruits in most Rr plants have a weak connection with their pedicel or the fruit-pedicel abscission zone, a trait already described in this species by Panetsos (1953). This characteristic becomes apparent when mature fruits easily fall off shaky branches of Rr plants. Although some CAwr plants present this trait, it is rare and not as widespread as in Rr plants. The combination of shed or "fall off" in fruits allows mature fruits to escape granivory, with subsequent fruit fragmentation and seed dispersal in single seeded capsules on the ground.

The study of invasiveness, or the evolution of a species' ability to succeed demographically and geographically in a novel environment, goes back over a decade (Mack et al. 2000). A recent multivariate analysis of traits associated with invasiveness concluded that response to damage by herbivores is a common trait among invasive species (Hovick et al. 2012). As in native populations, animal-plant interactions are of fundamental importance to the invasion success of plants (Richardson et al. 2000; Maron and Vilá 2001; Herrera and Pellmyr 2002, Colautti et al. 2004; Richardson and Pyšek 2006; Parker and Gilbert 2007; Mitchell et al. 2006). Furthermore, the complexity of natural communities intensifies when invasive species are added. For example, invasive *Medicago polymorpha* affects the annual local *Lotus wrangelianus* by varying its selection pressures from generation to generation (terHorst and Lau 2012). In some years, *M. polymorpha* directly affects *L. wrangelianus* reducing its fitness, but in other years, the negative effect comes indirectly from increasing the density of the weevil herbivore

Hypera brunneipennis (Lau 2008, terHorst and Lau 2012). In another recent example

from the island of Mauritius, the endemic lizard frugivore *Leiolopisma telfairii*, ingests

the fruits of introduced plants, thereby improving the competitive conditions of local

plants by reducing germination time, increasing local plants seedling survival relative to

the introduced plants (Zuël et al. 2012). In Tahiti, seed dispersal networks in heavily

invaded areas are influenced by introduced fruit trees and introduced frugivores

(Spotswood et al. 2012).

Invasive species impact biodiversity and can also have negative economic

consequences. Specifically, U.S. agriculture weeds have been reported to impact 12% of

the country's crop production. When the cost of herbicide application is added, the final

cost is approximately $27 billion dollars in losses (Pimentel et al. 2005). However,

invasive species also offer large-scale natural experiments to study basic processes in

populations (Sakai et al. 2001; Hierro et al. 2005), proposing hypotheses for

understanding population regulation, niche concepts, competition and species coexistence,

and community assembly and succession. Why some organisms can successfully invade

new habitats is a question that is difficult to answer for a single taxon, and even more

challenging to determine at a general level.

As is the case for all ecological interactions, invasive species exist in a complex

network of interactions, and like most ecological problems, there exists a wealth of

hypotheses for answering this deceptively simple question. Hierro et al. (2005) provide a

summary of the theories pertaining to ecological invasions, and concluded that no single

hypothesis can be unambiguously supported. Furthermore, the authors conclude that the

most salient weakness of past research is a failure to consider the biogeography of both original (native) and novel (exotic) ranges of the focal invasive taxa. This hypothesis has yet to be evaluated, but it is clear that despite steadily increasing research over the last two decades (Ricciardi and MacIsaac 2008; Richardson and Pyšek 2008), two fundamental question have yet to be answered: (1) why do some taxa have the capacity to invade new ranges? and (2) what are the predictors of invasion success? The ability to compare a well studied hybrid derived lineage with its two progenitor lineages, with distinct geographical and domestication history, represents a powerful natural experiment to study evolution of plant traits because it is possible to compare the ancestral condition with a new derived state. Likewise, in invasion biology comparing hybrid derived invaders with their progenitor lineages from their native geographic range provides understanding of the evolution of invasive species and the evolution of invasiveness. Among the most experimentally valuable comparisons are progenitor lineages and the derived invasive when grown side by side (Bossdorf et al. 2005).

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Appendix A. Fruit total length at lineage and population levels.
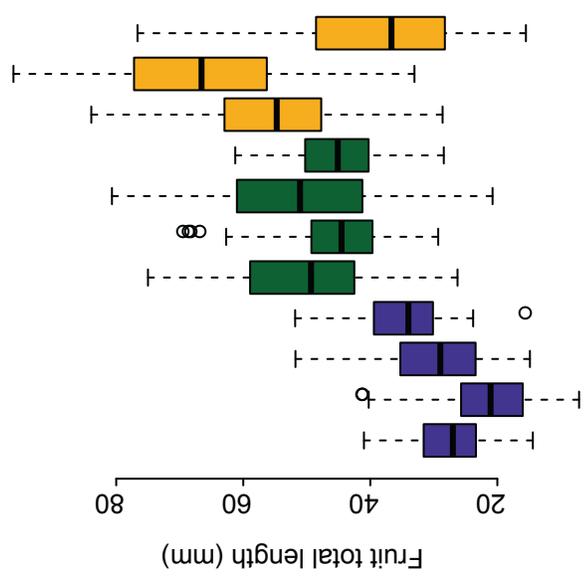
Fruit total length (mm)

ChB FrB BSp Whl In1 In2 In3 Cst DK1 MX1 NE9

Populations

Fruit total length (mm)

Rs   CAwr   Rr

Lineages

Tukey test for fruit total length at population and lineage levels

	FrB	BSp	Whl	In1	In2	In3	Cst	DK1	MX1	NE9
ChB	**	0.8	****	****	****	****	****	****	****	****
FrB		****	**	****	****	****	****	****	****	****
BSp			****	****	****	****	****	****	****	****
Whl				****	****	****	****	****	****	****
In1					**	**	1.0	****	****	0.8
In2						1.0	1.0	****	****	****
In3							***	0.2	****	****
Cst								0.3	****	****
DK1									****	****
MX1										****

Rs ****
CAwr **
Rr

ONE-WAY ANOVAS

	Df	Sum Sq	Mean Sq	F value	P(>F)
Populations	10	170801	17080.1	188.61	****
Residuals	1079	97787	90.6		

	Df	Sum Sq	Mean Sq	F value	P(>F)
Lineages	2	123281	61640	461.35	****
Residuals	1087	145232	134		

Note.- **** $P=0.00001$; *** $P=0.0001$; ** $P=0.001$; * $P=0.01$.

Appendix B. Beak length at lineage and population levels.

Populations

ONE-WAY ANOVAS
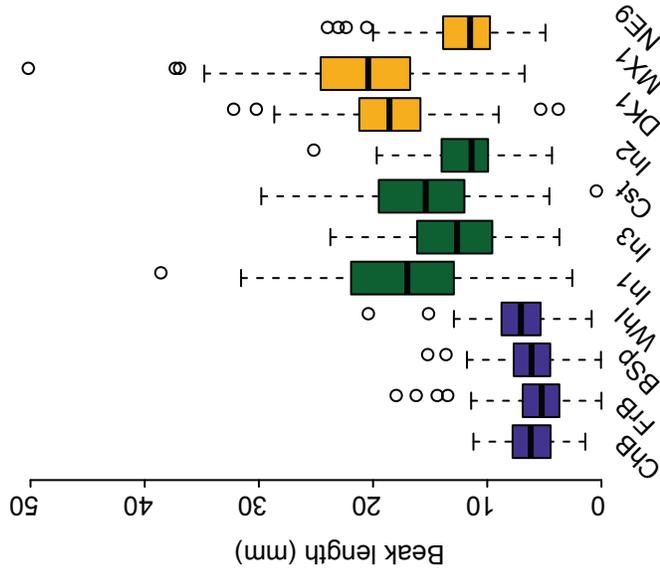
	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Populations	10	26074	2607.42	170.26	****
Residuals	1059	16218	15.31		

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Lineages	2	22207	11103.6	589.87	****
Residuals	1067	20085	18.8		
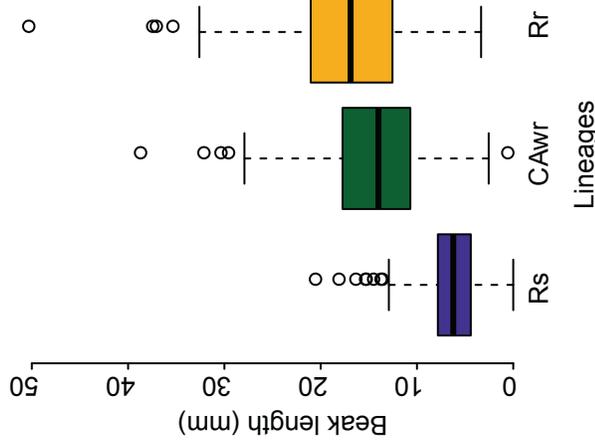
Tukey test for beak length at population and lineage levels

	FrB	BSp	Whl	ln1	ln2	ln3	Cst	DK1	MX1	NE9
ChB	1.0	1.0	0.4	****	****	****	****	****	****	****
FrB		0.9	*	****	****	****	****	****	****	****
BSp			0.7	****	****	****	****	****	****	****
Whl				****	****	****	****	****	****	****
ln1					****	****	0.7	****	****	****
ln2						0.9	****	****	****	****
ln3							*	****	****	1.0
Cst								**	****	0.9
DK1									0.1	****
MX1										****

	CAwr	Rr
Rs	****	****
CAwr		****

Note.- *****P=0.00001; ****P=0.0001; **P=0.001; *P=0.01.

Appendix C. Peduncular, middle and stylar diameters at lineage and population levels.
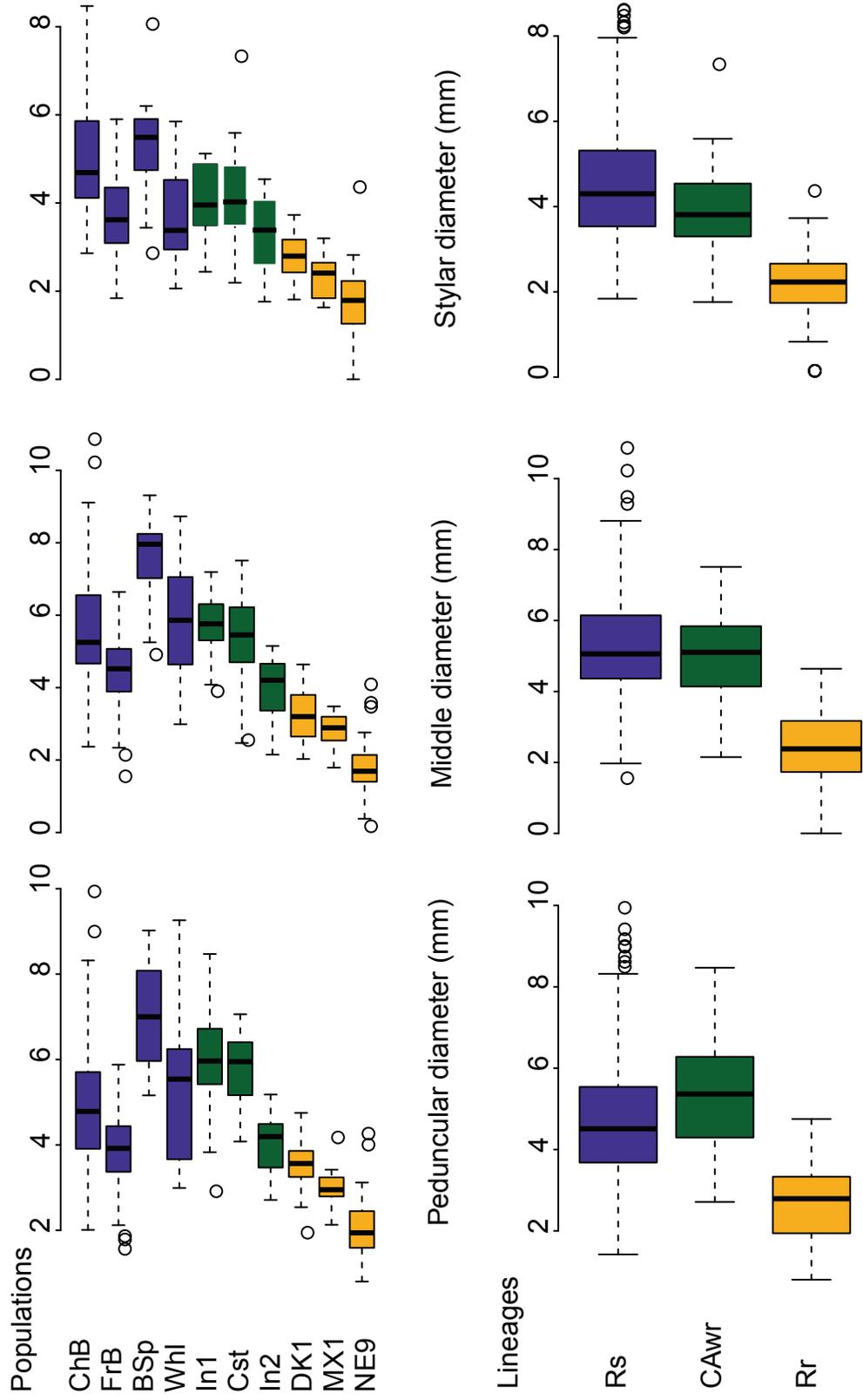
Appendix D. Diameters at different sections of the fruit - Tukey tests

Peduncular diameters at population and lineage levels

	ChB	FrB	BSp	Whl	In1	In2	Cst	DK1	MX1	NE9
ChB		****	****	0.8	*	*	0.1	****	****	****
FrB			****	****	****	1.0	****	1.0	0.1	****
BSp				**	0.2	****	*	****	****	****
Whl					1.0	**	1.0	****	****	****
In1						****	1.0	****	****	****
In2							***	1.0	0.1	****
Cst								****	****	****
DK1									0.9	***
MX1										0.1
NE9										

Lineage level:

	Rs	CAwr	Rr
Rs		0.1	****
CAwr			****
Rr			

Middle diameters at population and lineage levels

	ChB	FrB	BSp	Whl	In1	In2	Cst	DK1	MX1	NE9
ChB		****	****	1.0	1.0	****	1.0	****	****	****
FrB			****	**	**	0.9	0.1	**	****	****
BSp				**	***	****	****	****	****	****
Whl					1.0	***	0.9	****	****	****
In1						***	1.0	****	****	****
In2							*	0.5	*	****
Cst								****	****	****
DK1									1.0	***
MX1										*
NE9										

Lineage level:

	Rs	CAwr	Rr
Rs		0.2	****
CAwr			****
Rr			

Stylar diameters at population and lineage levels

	ChB	FrB	BSp	Whl	In1	In2	Cst	DK1	MX1	NE9
ChB		****	1.0	***	**	****	*	****	****	****
FrB			****	1.0	0.9	0.9	0.6	0.05	****	****
BSp				**	*	****	0.1	****	****	****
Whl					1.0	1.0	1.0	0.3	**	****
In1						0.4	1.0	**	****	****
In2							0.2	0.9	0.1	****
Cst								**	****	****
DK1									1.0	**
MX1										0.4
NE9										

Lineage level:

	Rs	CAwr	Rr
Rs		**	****
CAwr			****
Rr			

Note.- ****P=0.00001; ***P=0.0001; **P=0.001; *P=0.01.

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Appendix E. Fruit volume at lineage and population levels.

ONE-WAY ANOVAS

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Populations	9	21357491	2373055	30.852	****
Residuals	329	25305695	76917		

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Lineages	2	8592294	4296147	37.916	****
Residuals	336	38070892	113306		

Tukey test for fruit volume at population and lineage levels

	FrB	BSp	Whl	In1	In2	Cst	DK1	MX1	NE9
ChB	****	****	0.1	****	0.3	**	0.2	**	****
FrB		****	0.5	****	1.0	****	0.9	1.0	*
BSp			****	0.1	****	0.6	****	****	****
Whl				0.9	**	1.0	***	****	****
In1					****	1.0	****	****	****
In2						***	****	****	**
Cst							1.0	1.0	****
DK1								1.0	*
MX1									0.2

	CAwr	Rr
Rs	***	****
CAwr		****

Note.- ****P=0.00001; ***P=0.0001; **P=0.001; *P=0.01.

Appendix F. Fruit weight at lineage and population levels.

Boxplot: Fruit weight (mg) by Populations — ChB, FrB, BSp, Whl, In1, In3, Cst, In2, DK1, MX1, NE9

Boxplot: Fruit weight (mg) by Lineages — Rs, CAwr, Rr
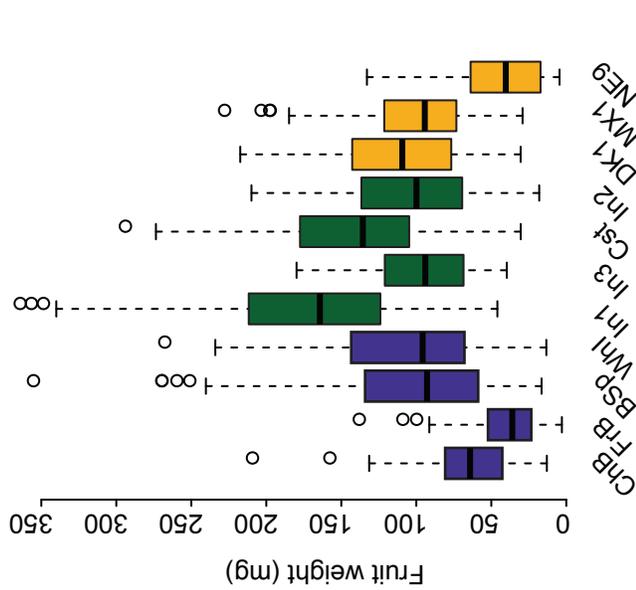
ONE-WAY ANOVAS

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Populations	10	1547302	154730	69.021	****
Residuals	1079	2418891	2242		

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Lineages	2	542810	271405	86.177	****
Residuals	1087	3423383	3149		
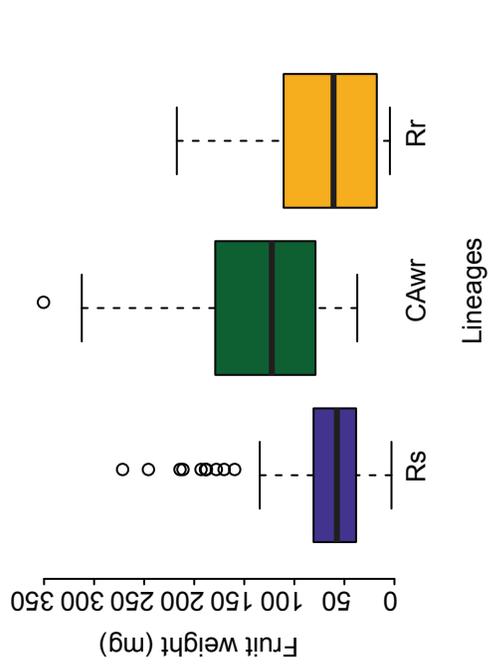
Tukey test for fruit volume at population and lineage levels

	FrB	BSp	Whl	In1	In2	In3	Cst	DK1	MX1	NE9
ChB	**	****	****	****	****	**	****	****	****	0.1
FrB		****	****	****	****	****	****	****	****	1.0
BSp			1.0	****	1.0	0.6	****	1.0	1.0	****
Whl				****	1.0	0.8	****	1.0	1.0	****
In1					****	****	****	****	****	****
In2						1.0	****	1.0	1.0	****
In3							****	1.0	1.0	****
Cst								0.3	****	****
DK1									1.0	****
MX1										****

	CAwr	Rr
Rs	****	0.5
CAwr		****

Note.- ****$P=0.00001$; ***$P=0.0001$; **$P=0.001$; *$P=0.01$.

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Appendix G. Seed weight at lineage and population levels.
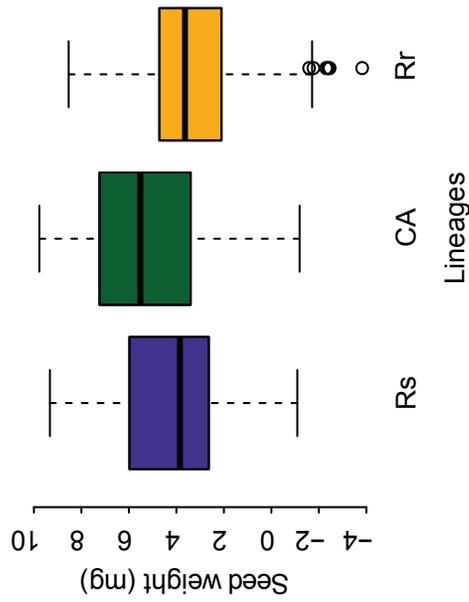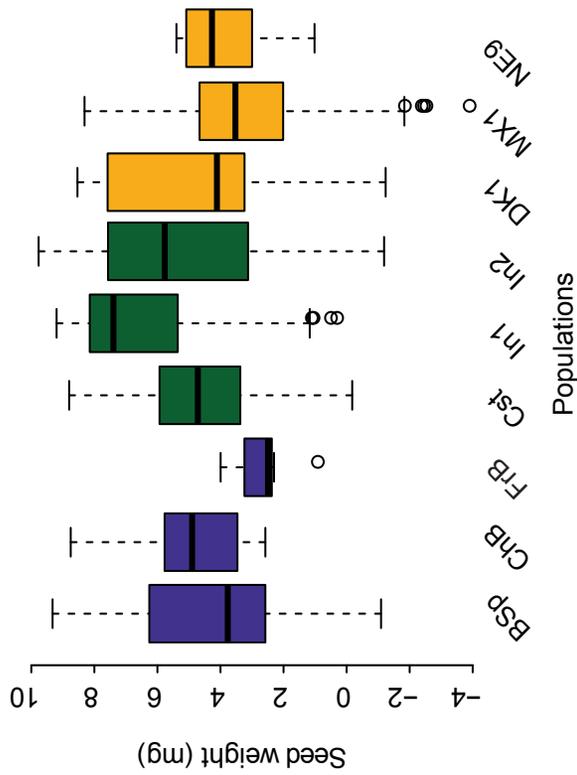
Tukey test for seed weight at population and lineage levels

	FrB	BSp	In1	In2	Cst	DK1	MX1	NE9
ChB	0.25	0.99	0.27	0.99	1.00	1.00	0.02	0.93
FrB		0.40	**	0.02	0.30	0.29	0.99	0.95
BSp			****	*	1.00	1.00	****	1.00
In1				0.13	***	0.02	****	0.02
In2					0.05	0.79	****	0.40
Cst						1.00	****	1.00
DK1							*	0.97
MX1								0.98

	CAwr	Rr
Rs	**	****
CAwr		****

ONE-WAY ANOVAS

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Populations	8	960.4	120.2	23.303	****
Residuals	940	4842.5	5.2		

	Df	Sum Sq	Mean Sq	Fvalue	P(>F)
Lineages	2	719.0	359.52	66.899	****
Residuals	946	5083.8	5.37		

Note.- ****$P=0.00001$; ***$P=0.0001$; **$P=0.001$; *$P=0.01$.

Appendix H.1. *Raphanus sativus* (Rs) mixed crosses - Fathers (a) siring percentage, (b) average seed weight, (c) average within-fruit seed weight percentage, and (d) average within-fruit relative fecundity.
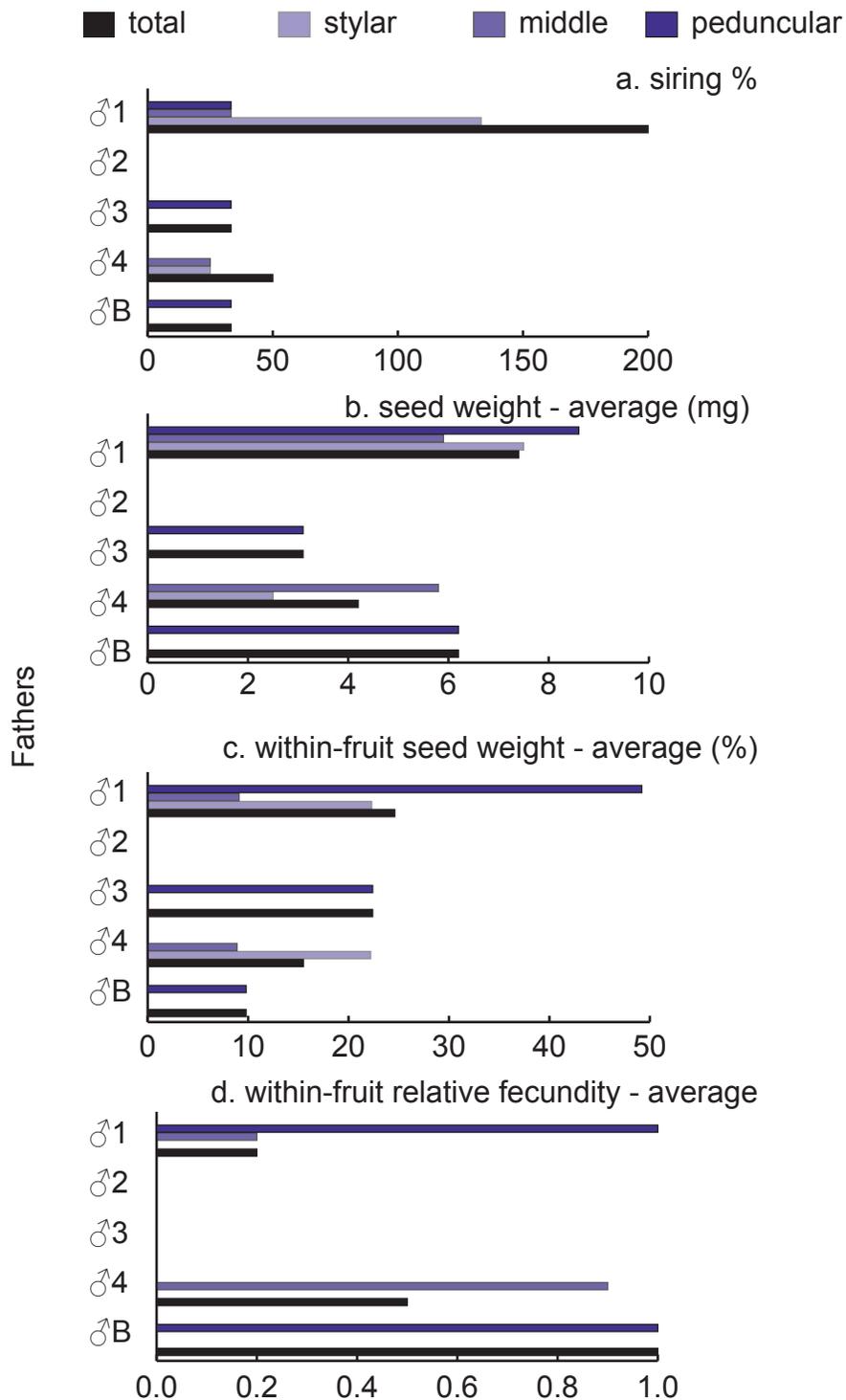
Appendix H.2. California wild radish (CAwr) single crosses - Fathers (a) siring percentage, (b) average seed weight, (c) average within-fruit seed weight percentage, and (d) average within-fruit relative fecundity.
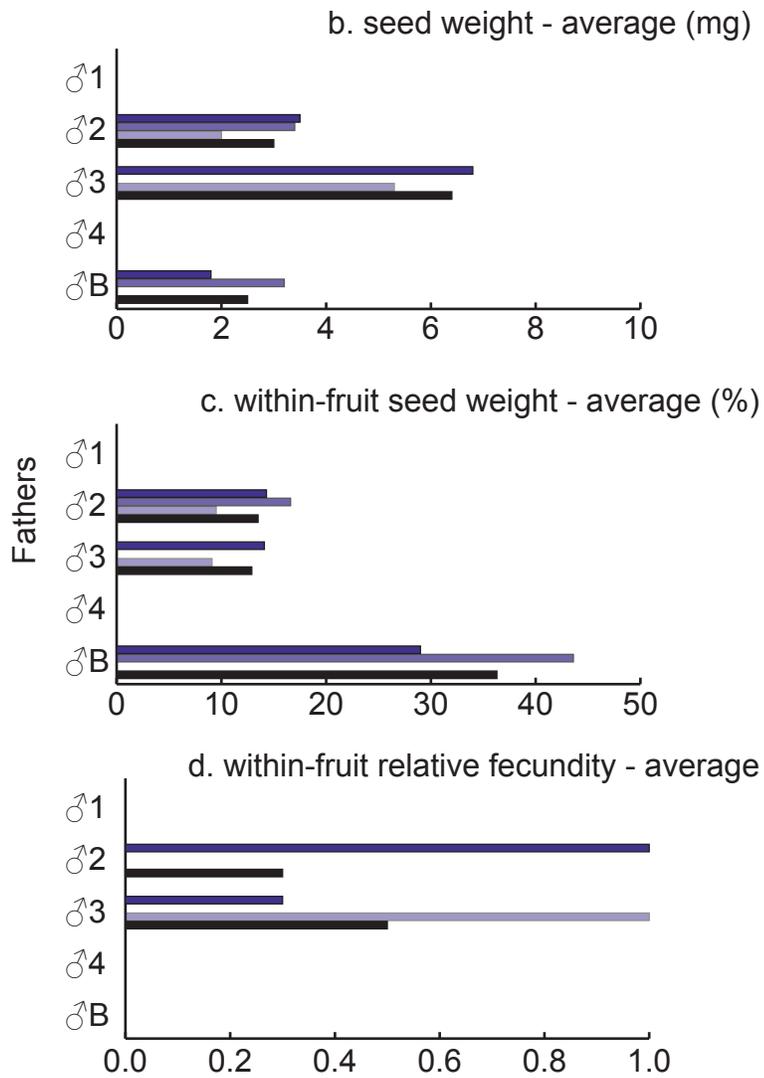
Appendix I.1. California wild radish (CAwr) mixed crosses - Fathers (a) siring percentage, (b) average seed weight, (c) average within-fruit seed weight percentage, and (d) average within-fruit relative fecundity.
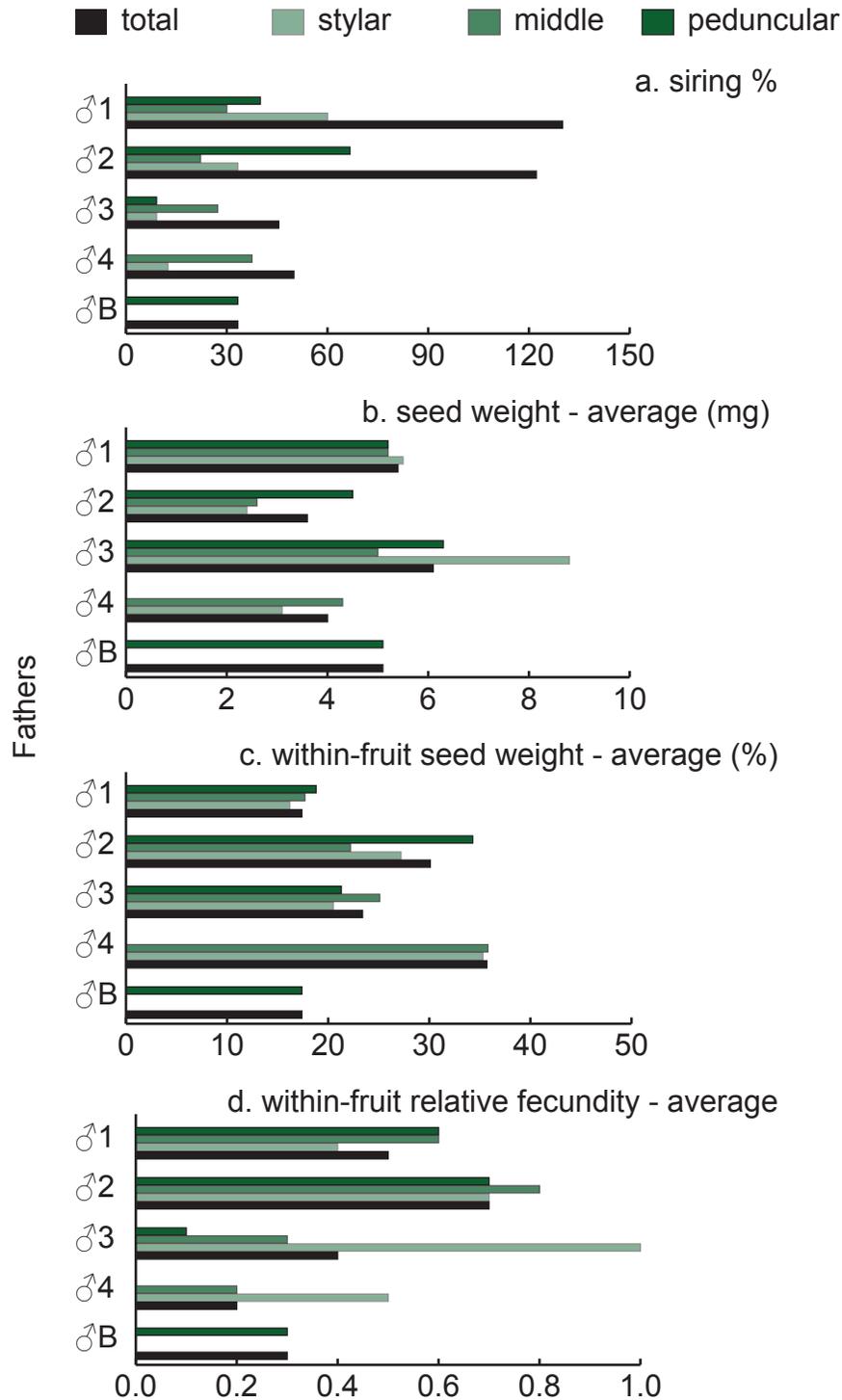
Appendix I.2. California wild radish (CAwr) single crosses - Fathers (b) average seed weight, (c) average within-fruit seed weight percentage, and (d) average within-fruit relative fecundity.
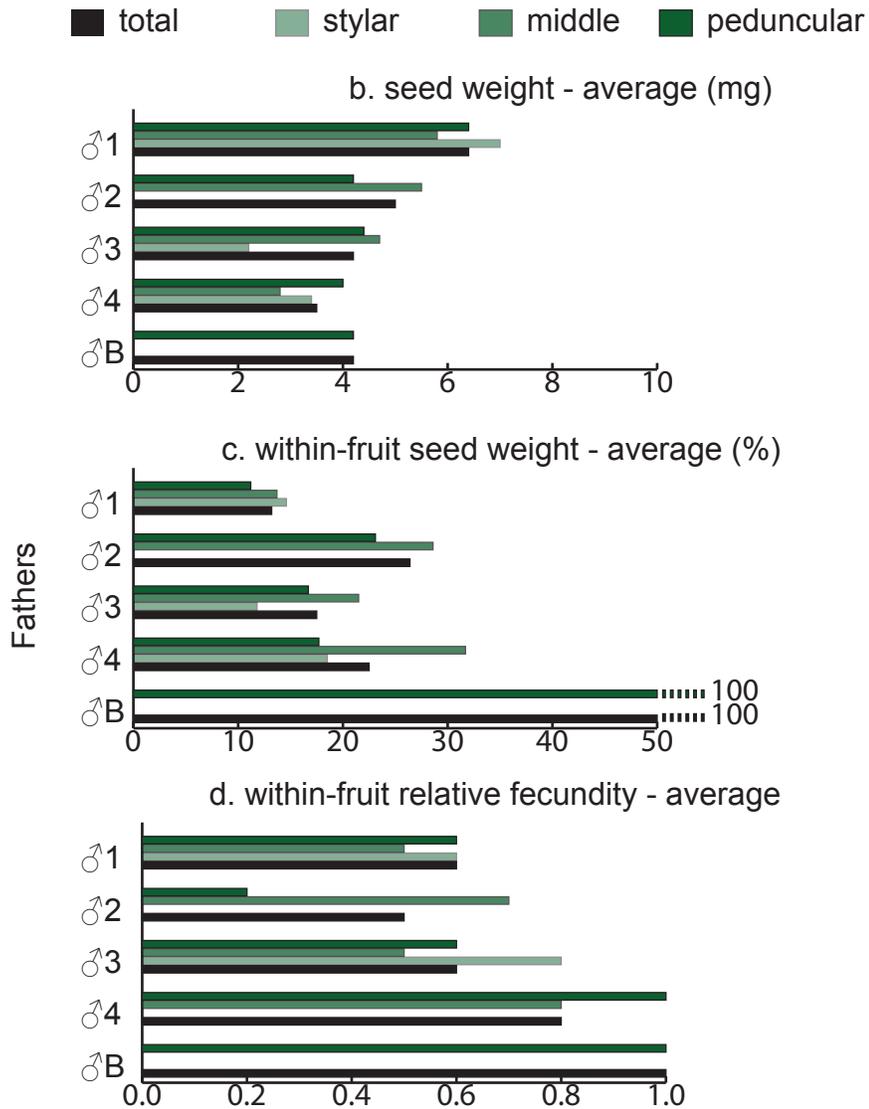
Appendix J.1. *Raphanus raphanistrum* (Rr) mixed crosses - Fathers (a) siring percentage, (b) average seed weight, (c) average within-fruit seed weight percentage, and (d) average within-fruit relative fecundity.
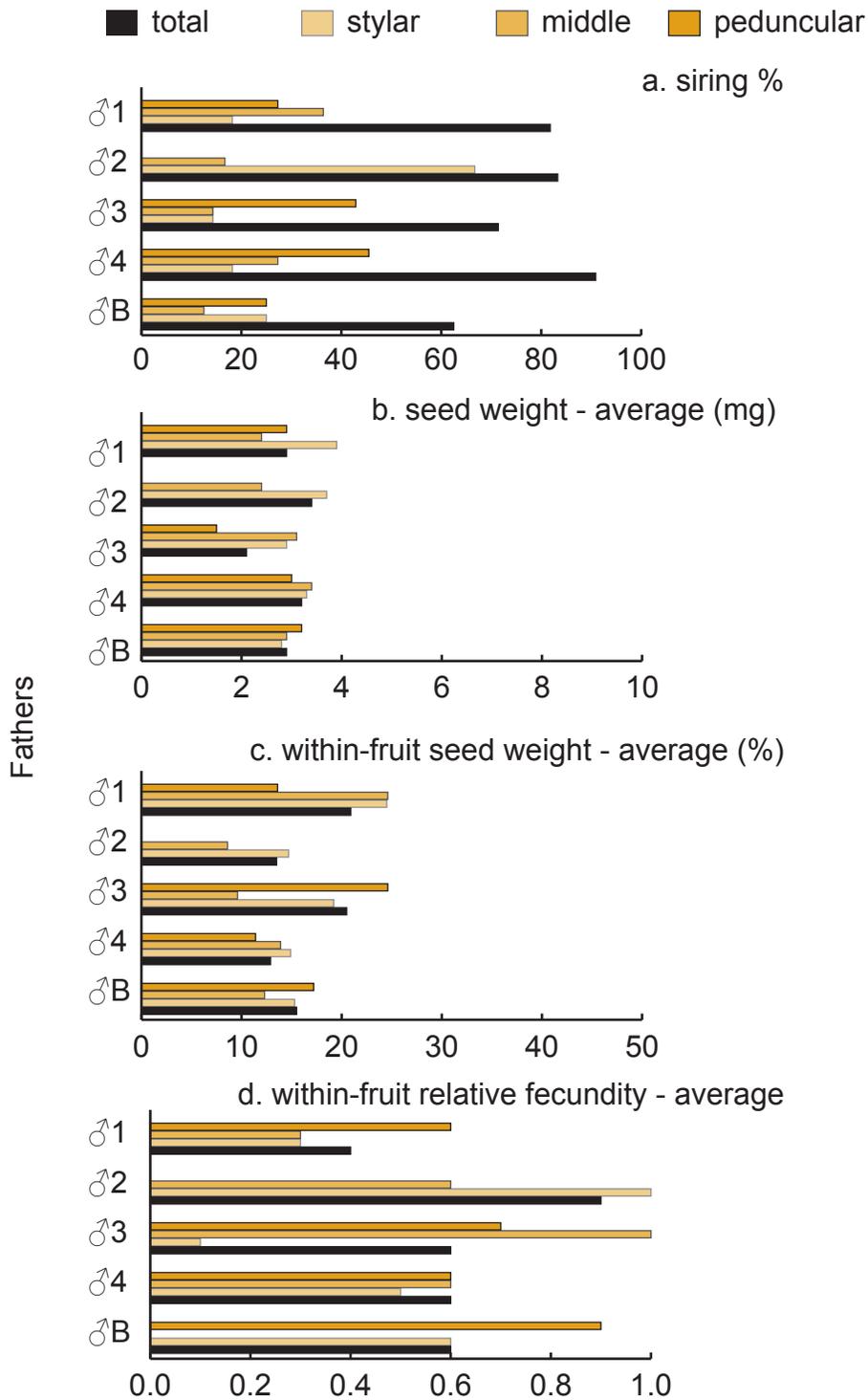
Appendix J.2. *Raphanus raphanistrum* (Rr) single crosses - Fathers (b) average seed weight, (c) average-within fruit seed weight percentage and (d) average within-fruit relative fecundity.

Appendix K

FREQUENCY OF ALLELES FOR FATHERS, MOTHERS AND OFFSPRING FROM MIX AND SINGLE CROSSES.

|	F		M		Offspring-Mix crosses						Offspring-Single crosses					
# of alleles	10	7	12	16	10	10	35	35	34	34	7	7	21	21	24	24
Lineages	CAwr	Rs	CAwr	Rr	Rs	Rs	CAwr	CAwr	Rr	Rr	Rs	Rs	CAwr	CAwr	Rr	Rr
Allele	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
BP																
brms-005																
127	-	-	-	-	0.20	-	-	-	-	-	-	-	-	-	-	-
137	0.40	0.30	0.08	-	-	0.50	0.23	0.37	-	0.53	-	0.57	0.19	0.52	-	0.54
141	-	0.70	0.08	-	0.80	-	0.03	0.06	-	-	1.00	-	-	-	-	0.08
142	0.20	-	-	-	-	0.30	-	0.43	-	0.18	-	-	-	0.33	-	0.13
147	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-
153	-	-	-	0.38	-	-	-	-	0.50	-	-	-	-	-	0.38	-
159	-	-	-	0.06	-	-	-	-	0.03	-	-	-	-	-	0.04	-
163	-	-	-	0.13	-	-	-	-	0.09	-	-	-	-	-	0.29	-
165	0.10	-	0.60	-	-	0.10	0.54	-	-	0.12	-	-	0.81	-	-	-
167	-	-	0.16	-	-	-	0.06	-	-	-	-	-	-	-	-	-
169	-	-	0.08	-	-	-	0.14	-	-	-	-	-	-	-	-	-
173	0.10	-	-	0.06	-	-	-	0.06	0.03	0.09	-	0.43	-	0.05	0.04	-
177	-	-	-	0.19	-	-	-	-	0.12	-	-	-	-	-	0.08	-
187	-	-	-	0.06	-	-	-	-	0.15	-	-	-	-	-	0.08	-
189	-	-	-	0.06	-	-	-	-	0.09	-	-	-	-	-	0.08	-
191	0.10	-	-	-	-	0.10	-	0.03	-	0.06	-	-	-	0.10	-	0.25
193	0.10	-	-	-	-	-	-	0.06	-	0.03	-	-	-	-	-	-

Appendix K (continued)

Lineages	CAwr	Rs	CAwr	Rr	Rs		CAwr		Rr		Rs		CAwr		Rr	
Allele					M	F	M	F	M	F	M	F	M	F	M	F
BP															(23)	(23)
rm48																
278	-	0.43	0.50	0.63	0.40	-	0.74	-	0.79	-	-	-	0.43	-	(23)	(23)
279	0.10	-	-	-	-	-	-	0.14	-	0.06	-	0.29	-	0.05	0.74	-
283	0.10	-	-	-	-	0.10	-	0.03	-	0.06	-	-	-	-	-	-
286	-	-	-	0.06	-	-	-	-	0.09	-	-	-	-	-	0.04	-
287	0.10	-	-	-	-	0.10	-	0.09	-	0.12	-	-	-	0.10	-	0.26
288	0.10	-	-	-	-	0.30	-	0.29	-	0.12	-	-	-	0.19	-	0.26
289	-	-	-	0.06	-	-	-	-	0.03	-	-	-	-	-	0.09	-
293	-	0.14	0.33	0.06	-	-	0.17	-	-	-	-	-	0.48	-	-	0.13
294	0.10	-	-	-	-	0.30	-	0.09	-	0.15	-	-	-	0.24	-	-
296	0.50	-	-	-	-	0.20	-	0.37	-	0.50	-	0.71	-	0.43	-	0.35
304	-	0.43	0.17	-	0.60	-	0.09	-	-	-	1.00	-	0.10	-	-	-
312	-	-	-	0.19	-	-	-	0.09	-	-	-	-	-	-	0.13	-
BN35d																
218	-	-	-	0.06	-	-	-	-	0.03	-	-	-	-	-	-	-
229	-	-	0.08	-	-	-	0.09	-	0.03	-	-	-	0.05	-	-	-
231	-	-	-	0.19	-	-	-	-	0.21	-	-	-	-	-	0.21	-
232	-	0.29	0.25	0.13	0.40	-	0.09	-	0.12	-	-	-	0.33	-	-	0.04
233	0.30	-	-	-	-	0.40	-	0.31	-	0.24	-	0.57	-	0.48	-	0.33
234	0.10	-	0.08	-	-	-	0.09	0.26	-	0.12	-	0.14	0.24	0.05	-	-
240	-	-	-	0.06	-	-	-	-	0.09	-	-	-	-	-	-	-
247	-	0.29	-	-	0.20	-	-	-	-	-	-	-	-	-	0.21	-
251	0.40	0.43	0.25	0.13	0.40	0.50	0.29	0.37	0.12	0.50	1.00	0.29	0.14	0.48	0.08	0.63

Appendix K (continued)

Lineages	CAwr	Rs	CAwr	Rr	Rs		CAwr		Rr		Rs		CAwr		Rr	
					M	F	M	F	M	F	M	F	M	F	M	F
Allele																
BP																
252	-	-	0.17	-	-	-	0.37	-	-	-	-	-	0.14	-	-	-
253	-	-	-	0.25	-	-	-	-	0.29	-	-	-	0.05	-	0.33	-
255	0.20	-	0.17	0.13	0.10	0.10	0.09	0.06	0.12	0.15	-	-	0.05	-	0.08	-
281	-	-	-	0.06	-	-	-	-	0.03	-	-	-	0.08	-	0.08	-
rm25																
233	-	-	-	-	-	-	-	-	-	-	-	-	(20)	(20)	-	-
251	-	0.14	-	-	-	-	-	-	-	-	0.29	-	-	-	-	-
261	0.10	-	-	-	0.30	0.30	0.20	0.20	-	0.09	-	-	-	-	-	0.33
265	0.30	-	0.08	0.13	0.30	0.30	0.20	0.14	0.32	0.41	-	-	0.10	0.15	0.25	0.29
266	0.30	0.43	0.58	0.25	0.10	0.10	0.63	0.34	0.12	0.24	-	0.71	0.65	0.30	-	0.21
269	-	-	-	0.06	-	-	-	-	0.03	-	-	-	0.08	-	-	-
270	0.30	0.29	0.25	0.50	0.30	0.30	0.17	0.31	0.53	0.26	0.71	0.29	0.25	0.35	0.67	0.17
278	-	0.14	0.08	0.06	-	-	-	-	-	-	-	-	-	-	-	-

Note. F=Father, M=Mother, BP=base pairs; when offspring alleles had one or two BP difference with progenitors they were corrected to match the mother or father alleles. Numbers in parenthesis are given when the sample size changed for a specific primer.