Original Article

# The role of intraspecific crop-weed hybridization in the evolution of weediness and invasiveness: cultivated and feral radish (*Raphanus sativus* L.) as a case study

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**Short title:** Intraspecific crop-weed hybridization in *Raphanus sativus*

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**PREMISE:** Crop-wild/weed hybrids usually exhibit intermediate and maladapted phenotypes compared to their parents; however, hybridization has sometimes been associated with increased fitness, potentially leading to enhanced weediness and invasiveness. Since the ecological context and maternal genetic effects may affect hybrid fitness, they could influence the evolutionary outcomes of hybridization. Here, we evaluated the evolutionary potential of reciprocal early-generation crop-weed hybrids using intraspecific weedy and cultivated radish (*Raphanus sativus* L.) as a model system.

**METHODS:** Using experimental hybridization and common garden experiments under field conditions, we assessed the differences in time to flowering, survival to maturity, plant biomass and reproductive components between reciprocal crop-weed hybrids and their parents, under two contrasting ecological conditions, agrestal (wheat cultivation, fertilization, weeding) and ruderal (human-disturbed uncultivated area) over two years.

**RESULTS:** Crop, weeds and reciprocal hybrids overlapped -at least partially- during the flowering period, indicating a high probability of gene flow. Reciprocal hybrids survived to maturity at rates at least as successful as their parents and showed higher plant biomass and fecundity, which resulted in higher fitness compared to their parents in both contrasting environments, without any differences associated with the direction of the hybridization.

**CONCLUSIONS:** Intraspecific crop-weed hybridization, regardless of the cross direction, has the potential to promote weediness and invasiveness in the invasive feral *R. sativus* weed both agrestal and ruderal environments, increasing the chances of the introgression of crop alleles into weed populations. This is the first report of intraspecific crop-weed hybridization in *R. sativus*.

**KEY WORDS:** Brassicaceae; crop-wild hybridization; gene flow; hybrid fitness; invasives; weedy radish; weeds.

## INTRODUCTION

Hybridization is increasingly recognized as an important evolutionary phenomenon that can influence the evolution of weeds and invasives (Ellstrand et al., 2010, 2013; Vercellino et al., 2022). When hybridization occurs, the hybrid lineages harbor alleles from both progenitors, and the process of introgression will depend on the fitness of the hybrids relative to their parents (Mercer et al., 2007; Presotto et al., 2019). Hybrids commonly exhibit intermediate phenotypes and reduced fitness compared to their parents (Mercer et al., 2006; Sahoo et al., 2010; Hooftman et al., 2015; Martin et al., 2019; Yue et al., 2021), with nonviable or sterile offspring in extreme cases (Rieseberg et al., 2007). If novel alleles reduce hybrid fitness, these alleles may not prevail or introgress. However, hybridization can also lead to adaptive evolution through evolutionary novelty, increased genetic variation, hybrid vigor (i.e., heterosis) and dumping genetic load, increasing weediness and invasiveness (Schierenbeck and Ellstrand, 2009; Hovick and Whitney, 2014). Spontaneous hybridization between crop and their wild/weedy relatives has increased the weediness and invasiveness of existing weeds and invasives, e.g., weedy *Brassica rapa* (Ureta et al., 2017; Pandolfo et al., 2018b) and California wild radish (Snow et al., 2010), and has resulted in the evolution of new weeds and invasives, e.g., European weed beet (Bartsch, 2010), weedy rice (Vigueira et al., 2019), European teosinte (Le Corre et al., 2020) and weedy sunflower (Presotto et al., 2017; Hernández et al., 2022). In addition, in some cases, hybrid populations have replaced their progenitors locally (Hegde et al., 2006). Of course, in many instances, hybridization has not affected weediness and invasiveness (Magnussen and Hauser, 2007; Whitney et al., 2009; Martin et al., 2019; Page et al., 2019).

Hybrid performance relative to their parents may be influenced by the environment (Campbell and Snow, 2007; Arnold and Martin, 2010; Hovick and Whitney, 2014; Presotto et al., 2019).

Due to this environmental dependence, evolutionary outcomes of hybridization in agrestal (i.e., agricultural) environments may differ from those in ruderal (human-modified uncultivated area) habitats (Mercer et al., 2006; Presotto et al., 2019; Mitchell et al., 2021). The fitness of wild/weeds could be enhanced by the acquisition of crop traits under agricultural environments. This is especially true for traits conferring resistance to herbicides, insects or diseases, or tolerance to abiotic stresses (Xia et al., 2016; Ureta et al., 2017; Pandolfo et al., 2018b; Nam et al., 2020; Kreiner et al., 2022), but also for other typical crop traits, such as early flowering, high growth rate and seed production (Campbell and Snow, 2007; Presotto et al., 2017; Le Corre et al., 2020). However, the acquisition of crop traits which have been shaped to suit the agricultural environment by artificial selection, frequently create maladapted crop-wild/weed hybrids with lower fitness under less selective environments (e.g., ruderal environments), limiting the adaptive evolution of weeds and invasives (Mercer et al., 2007; Hovick et al., 2012; Presotto et al., 2019; Vercellino et al., 2021a).

Numerous studies have investigated the ecological and evolutionary consequences of crop-to-wild/weed hybridization (Campbell et al., 2006; Mercer et al., 2006, 2007; Snow et al., 2010; Hovick et al., 2012; Presotto et al., 2019). However, much less attention has been paid to hybridization in the opposite direction (i.e., wild/weed-to-crop hybridization) and how maternal genetic effects –defined as the contribution of the phenotype or genotype of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987; Pace et al., 2015; Hernández et al., 2021)– may influence the fitness of their offspring immediately after hybridization (Kimball et al., 2008; Piskurewicz et al., 2016; Singh et al., 2017; Hernández et al., 2021). Common garden studies comparing the fitness of reciprocal crop–wild/weed hybrids relative to both parents under contrasting ecological

conditions are essential to deepen our understanding on the role of crop-wild/weed hybridization on weediness and invasiveness (Mercer et al., 2006; Campbell and Snow, 2007).

In this study, we performed common garden experiments using experimental populations of the crop-weed complex of radish (*Raphanus sativus* L.) as a model system to assess the hypothesis that reciprocal intraspecific crop-weed hybridization increases weediness and invasiveness (Bourgeois et al., 2019). These studies allow the acceleration of natural processes in order to explore local ecological scenarios under which the fitness of crop-weed hybrids may equal or exceed the fitness of their parents, favoring the persistence of crop alleles in wild/weedy populations and consequently enhancing weediness and invasiveness (Mercer et al., 2006; Campbell et al., 2009). The *Raphanus* complex, formed by the cultivated species *R. sativus*, their feral conspecific forms and their weedy related species *R. raphanistrum*, is a well-established model system that has been widely used to evaluate the ecological and evolutionary consequences of interspecific crop-weed hybridization (Campbell et al., 2006, 2009; Hegde et al., 2006; Campbell and Snow, 2007; Ridley et al., 2008; Snow et al., 2010; Hovick et al., 2012; Shukla et al., 2020, 2021). Evolutionary experiments are often necessary for the observation and characterization of evolutionary changes occurring as a consequence of natural crop-wild/weed hybridization processes (Ellstrand et al., 2010; Hovick and Whitney, 2014). We compared the time to flowering, survival to maturity, plant biomass and reproductive components of reciprocal early generation crop-weed hybrids in relation to both parents under two contrasting ecological environments, agrestal (wheat cultivation, fertilization, weeding) and ruderal (human-disturbed uncultivated area) over two years. Despite that the fitness of early generation hybrids does not determine the ultimate fate of all crop alleles, it affects the initial course of introgression through

its effect on the frequencies of crop alleles that are subsequently subject to selection and drift (Snow et al., 1998; Mercer et al., 2006; Presotto et al., 2019).

Our results represent the first experimental study to evaluate the effect of reciprocal intraspecific crop-weed hybridization in *R. sativus* on the evolution of weediness and invasiveness. These findings reveal the potential of reciprocal crop-weed hybridization to promote weediness and invasiveness in natural populations of feral radish under both agrestal and ruderal environments. We discuss the potential implications of these results on the agroecosystem.

## 2. MATERIALS AND METHODS

***Study system-***

Conspecific cultivated and weedy feral radish (*Raphanus sativus* L.) are annual, self-incompatible, insect-pollinated plants, that can hybridize over long distances (Ellstrand et al., 1989; Snow and Campbell, 2005). Cultivated radish is an ancient crop domesticated independently in both Europe and South Asia (Zhang et al., 2021) and selected for multiple purposes, including human consumption as roots (botanically hypocotyl plus root), forage and cover crops (Snow and Campbell, 2005). *R. sativus* is not known in the wild (Warwick, 2011); however, feral populations of radish have been found as a weed infesting crops in several parts of the world, including North America, South America and South Africa (Hegde et al., 2006; Barnaud et al., 2013; Vercellino et al., 2018; Costa et al., 2021; Heap, 2022). In North America, its invasive capacity is attributed to the introgression with a closely related species *R. raphanistrum* (known as California wild radish) (Hegde et al., 2006; Ellstrand et al., 2010). In Argentina, feral radish has been considered an widespread invasive weed since at least the 1930s (Ibarra, 1937). It grows in disturbed environments, such as road margins, rural roads and fence

lines, and on agricultural soils. It is one of the most noxious weeds in cereals, oilseeds and some horticultural and forage crops (Scursoni et al., 2014; Vercellino et al., 2018, 2021b) and has evolved resistance to AHAS (acetohydroxyacid synthase, also known as acetolactate synthase (ALS))-inhibiting herbicides (Pandolfo et al., 2016; Heap, 2022). Argentine feral *R. sativus* populations show many crop-like traits, such as white or purple flowers, pods attached to the mother plant at maturity, and no seed dormancy (Vercellino et al., 2018, 2019). On the other hand, feral populations do not harbor typical traits of *R. raphanistrum* like constricted fruits and yellow flowers (Pandolfo et al., 2018a). However, they have an extended period of seedling emergence due to the requirement of darkness for germination and the mechanical restriction of the pericarp (Vercellino et al., 2019).

In southern Buenos Aires, an area severally infested with feral radish (Scursoni et al., 2014; Vercellino et al., 2018), the use of cultivated radish as horticultural and cover crop has grown rapidly in recent years, leading to the formation of natural crop-weed hybrids in the field.

***Germplasm and crosses-***

In this study, reciprocal crosses (i.e., in both directions, pollen flow from crop-to-weed and weed-to-crop) between weed and cultivated *R. sativus* were used due to the potential bidirectional hybridization in the *Raphanus* complex in Argentina. Weed populations were collected from two sites in southern Buenos Aires: Balcarce (BAL; 37°35'25”S, 58°31'59”W) and Pieres (PIE; 38°24'31”S, 58°35'60”W) (Vercellino et al., 2018) before the introduction of *R. sativus* cover crop cultivars in those areas. Cultivated materials were represented by two commercial cultivars: the European radish ‘Rovi – Round Red Radish with White Tips’ (Garde, Giusti y Chuchuy S.A, Ciudad Autónoma de Buenos Aires, Argentina) and the daikon radish ‘CCS 770 – Daikon Radish’ (El Cencerro, Coronel Suarez, Buenos Aires, Argentina). Reciprocal

crosses were made between weed populations and cultivars (referred to as CROP). The crosses (hereafter referred to as Biotypes) were characterized according to their female and male parents.

To minimize the maternal environmental effects, all the seeds used in each experiment were produced in a greenhouse (20 ± 5°C, natural light) in the Agronomy Department at Universidad Nacional del Sur, Bahía Blanca, Argentina (38°41´38” S, 62°14´53” W). For seed production, 60 plants of each BAL, PIE, and CROP were grown in the greenhouse and seeds were produced under controlled hand-pollination, creating seven different biotypes: three pure parents (BAL, PIE and CROP) and reciprocal F1 crop-weed hybrids (BAL x CROP, PIE x CROP, CROP x BAL and CROP x PIE). CROP was represented by the open-pollinated cultivar ‘Rovi – Round Red Radish with White Tips’ in 2019, and by the cultivar ‘CCS 779 Daikon Radish’ in 2020. In the greenhouse, the weedy and cultivated plants flowered simultaneously. Seeds were produced as follows: at the flowering stage, unopened flower buds from weed plants were opened and emasculated without damaging the pistil by using sharp-edged forceps before pollen dehiscence. Once emasculated, the flowers were hand-pollinated with pollen from sibling plants (weed biotypes: BAL and PIE) or pollen from cultivated plants (weed-crop hybrids: BAL x CROP and PIE x CROP). Likewise, unopened flowers buds from cultivated plants were opened, emasculated and hand-pollinated with pollen from sibling plants (CROP biotype) or pollen from weed plants (crop-weed hybrids: CROP x BAL and CROP x PIE). Prior to emasculation, all other flower buds on the racemes were removed. Immediately after pollination, the racemes were covered with 12 × 6 cm pollination bags to avoid any pollination from other plants. Pod formation from successful hand-pollination could be observed ten days after pollination and, subsequently, the bags were removed from the racemes. The effectiveness of pollination was >99 %. At the end of the growing season, the mature pods were stored at room temperature and less

than 10% moisture until use. For experiments the in 2020 and 2021, seeds were produced during the 2019 and 2020 growing seasons, respectively.

***Experimental design-***

We compared weed, crop, and their reciprocal F1 crop-wild hybrids in two environments (ruderal and agrestal). Common garden experiments were conducted in the Agronomy Department experimental field at the Universidad Nacional del Sur, Bahía Blanca, Argentina, during two winter growing seasons (Jun-Jan 2020/21 and 2021/22). The soil has a well-drained loamy sand texture with 1.1% organic matter, pH 7.7, and 8.4 mg $kg^{-1}$ (0–12 cm) available phosphorus (P) (a typical soil in the southwest of Buenos Aires province). Field experiments were arranged in a split-plot design with eight blocks (subplots). Environments (agrestal and ruderal) were assigned to main plots, and biotypes (CROP, BAL, PIE, CROP x BAL, CROP x PIE, BAL x CROP and PIE x CROP) to subplots of ten plants.

From the radish seeds produced in the crossing design described above, 2-3 seeds per pod from more than 400 pods were bulked together to represent each biotype. Since seeds from both weed and cultivated radish are non-dormant (Vercellino et al., 2019), radish seeds from all seven biotypes were hand-planted in early Jul 2020, 2-3 seeds per planting positions, 2-3 cm deep, at 0.3-m intervals in rows spaced at 0.6-m. The subplots were two rows wide by five planting positions long. Sixty-four subplots ((7 biotypes + 1 control without radish) x 8 blocks) made up each main plot (i.e., each ruderal and agrestal environment). The main plots were surrounded by a border of two rows of weedy feral radish to buffer against possible border effects. At the fully expanded one-leaf stage, radish seedlings were hand-thinned to one per position, reaching the target of 10 plants per subplot. In 2021, seedlings of the seven radish biotypes were established in plastic trays containing potting mix (Grow Mix Terrafertil®), grown in a glasshouse (20 ±

5°C, natural light and automatic watering), and transplanted into the experimental field at the 2-3-leaf stage, in early Aug, as above.

***Description of environments-***

*Agrestal environment*- Before the establishment of the radish biotypes in the field in late autumn (mid-Jun), in the agrestal environment, a wheat (*Triticum aestivum* L.) crop was established as follow: seeds were planted in 0.2-m spaced rows at 3-cm deep, to reach a target of 160 plants $m^{-2}$ (the common stand density in southern Buenos Aires). Wheat rows were arranged to surround rather than overlap the radish plants. The cultivars of wheat used were ACA 360 and KLEIN MINERVA in 2020/21 and 2021/22, respectively. Fertilization consisted of 120 kg $ha^{-1}$ diammonium phosphate at planting, 125 kg $ha^{-1}$ urea at early tillering and 125 kg $ha^{-1}$ urea at mid-tillering. Weeds other than radish were periodically removed by hand. At the end of each experiment, the yield of the wheat crop was estimated by hand-harvesting and hand-threshing four replicates of 1 $m^2$, collected in subplots without radish. The yield of the wheat was 5136 ± 201 and 5190 ± 188 kg $ha^{-1}$ in 2020/21 and 2021/22, respectively.

*Ruderal environment*- In the ruderal environment, we did not establish any crops or fertilize the soil. Biotypes were exposed to naturally occurring weather conditions, competing weeds, herbivores, and pathogens, simulating a human-disturbed uncultivated area (e.g., field margin, fence row).

***Measurements-***

In all ten plants per subplot, we recorded daily time to flowering (days from emergence to first flowering) and survival to reproduction (defined as plants that produced at least one pod). At maturity, the above-ground biomass of the six central radish plants from each subplot was hand-

harvested, dried at 60°C to constant weight and weighed to determine the total aboveground dry biomass per plant (hereafter referred to as 'plant biomass'). After that, pod biomass per plant, pod number per plant, seed biomass per plant, seed number per plant and seed weight were evaluated in the harvested plants following the procedure described in Vercellino et al. (2018, 2021b). Seed number per plant was used as the proxy of fecundity.

Since all ten plants per subplot were used to evaluate survival to maturity, but only the six central plants to estimate fecundity, the mean fitness of each subplot was estimated by multiplying survival to maturity by the average fecundity of each subplot in order to calculate the fitness of reciprocal crop-weed hybrids relative to weed plants (hereafter referred to as 'relative fitness'). Relative fitness (RF) was calculated as: RF = reciprocal hybrid (crop-weed or weed-crop)/weed for each block and weed origin (BAL and PIE).

***Statistical Analysis-***

All data were analyzed using generalized linear mixed models (GLMMs) with restricted maximum likelihood in PROC GLIMMIX (SAS University Edition). We analyze the effects of experimental factors in the time to flowering, survival to reproduction, total aboveground biomass, and reproductive components (i.e., pod biomass per plant, pod number per plant, seed biomass per plant, seed number per plant and seed weight). Year (2020/21 and 2021/22), environment (ruderal and agrestal), biotype (CROP, BAL, PIE, CROP x BAL, CROP x PIE, BAL x CROP and PIE x CROP), and their interactions (year by environment, year by biotype, environment by biotype and year by environment by biotype) were considered as fixed effects, while the block nested within environment effect was considered as random. After that, we grouped the seven biotypes into four groups: WEED (BAL and PIE), WEED x CROP (crop-weed hybrids produced in weed plants: BAL x CROP and PIE x CROP), CROP x WEED (crop-

weed hybrids produced in crop plants: CROP x BAL and CROP x PIE), and CROP. To compare groups (WEED, WEED x CROP, CROP x WEED, and CROP), we constructed six a priori orthogonal contrasts: (1) WEED vs. CROP x WEED (2) WEED vs. WEED x CROP, (3) WEED vs. CROP, (4) CROP x WEED vs. WEED x CROP, (5) CROP x WEED vs. CROP, and (6) WEED x CROP vs. CROP.

Since categorical analyses do not allow for the complex model specifications needed in an analysis of a split-plot design, survival to reproduction was analyzed as a continuous response variable, based on averages of radish plants in each subplot. Data were arcsine-square-root transformed prior to analyses to meet assumptions of normality (Mercer et al., 2006). Flowering time analysis only included radish plants that reached flowering, and plant biomass and reproductive component (pod biomass per plant, pod number per plant, seed biomass per plant, seed number per plant and seed weight) analyses only included radish plants that survived to reproduction. To avoid redundancy, we performed a multiple Pearson´s correlation analysis between plant biomass and the reproductive components with PROC CORR in SAS, and for all pairwise comparisons with $r > |0.9|$, and we only retained one variable of each pair. After this filtering, we retained seed number per plant and seed weight (Appendix S1; see Supplemental Data with this article). All traits (i.e., except survival to reproduction which was previously indicated) were square-root transformed to satisfy assumptions regarding the normality of the residuals. Finally, least square means of relative fitness were estimated using PROC GLIMMIX.

## RESULTS

Analysis of the time to flowering, survival to maturity, seed number per plant, seed weight and relative fitness revealed significant effects of at least one kind of interaction involving the year effect (i.e., year by environment, year by biotype or year by environment by biotype interactions)

(Appendix S2; see Supplemental Data with this article). Therefore, all traits were reanalysed by year (Tables 1 and 2).

***Time to flowering-***

In year 1, there were significant biotype, environment, and biotype by environment interaction effects; therefore, we performed contrasts for each environment (agrestal and ruderal) (Table 1). In both environments, the crop flowered later than the weeds, although differences were larger in the ruderal environment (Fig. 1a, Table 1). Overall, hybrids showed a flowering time intermediate to that of both parents. Whereas the crop-weed hybrids produced on weed plants flowered simultaneously with the weeds, the crop-weed hybrids produced on crop plants flowered a little bit later (<5 days) than the weeds, resulting in significant differences between reciprocal hybrids only in the agrestal environment (Fig. 1a, Table 1). In year 2, there were significant biotype and environment effects, but no biotype by environment interaction (Table 2). Crop, weeds and hybrids flowered simultaneously (Fig. 1b), although some contrasts showed statistically significant (<3 days) differences between groups (Fig. 1b, Table 2).

***Survival to maturity-***

In year 1, there were significant biotype, environment, and biotype by environment interaction effects; therefore, we performed contrasts for each environment (agrestal and ruderal) (Table 1). In the agrestal environment, all the weed and hybrid plants survived to maturity, whereas the crop survived significantly less than the weeds and hybrids (Figure 1c, Table 1). In contrast, in the ruderal environment, there was more variation between groups; hybrids (both cross directions) survived significantly more than weeds, and weeds survived significantly more than the crop (Figure 1c, Table 1). There were no significant differences between reciprocal hybrids

(Figure 1a, Table 1). In year 2, most plants (> 95 %) survived to maturity and there were no significant effects of environments, biotypes, nor environment by biotype interaction (Figure 1b, Table 2).

***Fecundity: reproductive components-***

For the set of six variables (plant biomass, pod biomass per plant, pod number per plant, seed biomass per plant, seed number per plant and seed weight), we found positive correlations between seed number per plant and plant biomass (r = 0.95), pod number per plant (r = 0.93), pod biomass per plant (r = 0.96) and seed biomass per plant (r = 0.98), while the seed weight showed no correlation (r < 0.10) with any of these traits (Appendix S1; see Supplemental Data with this article). Therefore, seed number per plant and seed weight were retained for further analysis.

Analysis of the seed number per plant (i.e., fecundity) revealed significant biotype and environment effects, but no significant biotype by environment interaction in either year (Tables 1 and 2). Overall, the radish plants in the agrestal environment showed 84.3 and 60.3 % higher fecundity than the radish plants in the ruderal environment in year 1 and 2, respectively (Figs. 1g-h, Tables 1 and 2). Fecundity of weeds was 167.5 % higher than crop in year 1, but no differences were observed in year 2 (Figs. 1g-h, Tables 1 and 2). Fecundity of the crop-weed hybrids were 47.0 and 169.0 % higher than fecundity of the weeds in year 1 and 2, respectively, with no differences associated with the cross direction, indicating no maternal genetic effect (Figs. g-h, Table 1).

With respect to the seed weight, there were significant biotype and environment (only in year 1) effects, and no significant biotype by environment interaction (Tables 1 and 2). In year 1, the

seeds of the weed plants were 21.3 % heavier than the seeds of the crop plants. However, in year 2, the seeds of the crop plants were 33.4 % heavier than the seeds of the weed plants (Figs. 1e-f, Tables 1 and 2). In both years, the seeds of crop-weed hybrids (regardless of the cross direction) were as heavy as the seeds of the parent with heavier seeds (Figs. 1e-f, Tables 1 and 2).

***Relative fitness-***

In both years, analysis of the relative fitness revealed a significant biotype effect but no significant environment or biotype by environment interaction effects (Tables 1 and 2). The relative fitness of reciprocal hybrids compared to weeds was 1.86 ± 0.21 and 3.11 ± 0.20 in year 1 and 2, respectively, regardless of the environment and the direction of hybridization (Tables 1 and 2).

## DISCUSSION

This study shows that crop-weed hybridization has the potential to stimulate weediness and invasiveness in feral *R. sativus* under both agrestal and ruderal environments. Flowering period largely overlapped between weeds, crop, and reciprocal crop-weed hybrids in both years and environments, suggesting that temporal isolation would not be effective in preventing gene flow in *Raphanus*. Crop-weed hybrids survived at rates that are at least as successful as the rates of both weed and crop plants and produced higher plant biomass that resulted in 47 and 169 % more fecundity than the weeds in year 1 and 2, respectively, in both ruderal and agrestal environments. Relative fitness of hybrids compared to weeds was 1.86 and 3.11 in year 1 and 2, respectively, with no differences between environments. Seeds of reciprocal hybrids were as heavy as the seeds of the parental biotype with heavier seeds. Finally, we did not generally find differences associated with the direction of hybridization, indicating the general absence of

maternal genetic effects. Our study highlights the risk of evolution of weediness and invasiveness after admixture in *R. sativus*.

***Evolutionary consequences of reciprocal crop-weed hybridization-***

In our experiments, we found that the cultivated radish started flowering later than the weedy radish in year 1, but both cultivated and weedy radish started flowering simultaneously in year 2. This is probably associated with genetic differences between the radish cultivars. In year 1, we expected that the crop-weed hybrids would have delayed flowering compared to weedy radish, as was observed in California wild radish (hybrids between *R. sativus* and *R. raphanistrum*) (Campbell et al., 2006, 2009). While we found a trend in this direction (especially in the crop-weed hybrids produced on crop plants) (Fig. 1a), the difference was less than five days. Due to the long flowering period of the species, the flowering period of cultivated and weedy radish largely overlapped in both years in the field and in the greenhouse during seed production (data not shown), suggesting that crop-weed hybridization is highly probable if crop and weed grow in sympatry. Crop-wild/weed hybridization resulting in the evolution of weediness has also occurred in several complexes with contrasting flowering time (Bartsch, 2010; Le Corre et al., 2020).

Common garden studies using experimental early generation crop-weed hybrids can provide tentative predictions of the effects of hybridization on the dynamics of weed populations and the likelihood of crop allele introgression into weeds (Campbell et al., 2006; Mercer et al., 2006; Presotto et al., 2019). In contrast to the common assumption (based on previous results) that early generations of crop-wild/weed hybrids suffer a dramatic loss of fitness relative to their wild/weedy counterpart (Snow et al., 1998; Cummings et al., 2002; Mercer et al., 2006; Campbell and Snow, 2007; Sahoo et al., 2010; Hooftman et al., 2015), our results showed at least

equal survival and vastly higher plant biomass and fecundity in the reciprocal crop-weed hybrids relative to their parents in both contrasting environments, which could facilitate the introgression of crop alleles into weedy populations and stimulate the evolution of weediness and invasiveness (Schierenbeck and Ellstrand, 2009; Vercellino et al., 2022). Similar results, i.e., higher fitness of crop-wild/weed hybrids than the parents, have been reported in other Brassicaceae species (Hauser et al., 2003; Campbell et al., 2006).

The assessment of the effect of crop-weed hybridization under contrasting ecological conditions was used to evaluate the environmental dependence of hybridization effects. In previous studies, the weed *R. raphanistrum* showed higher fecundity than the crop-weed hybrids under non-competitive conditions, but the crop-weed hybrids were less negatively affected under intense competition, suggesting that hybrids may be favored under the highly competitive condition imposed by crops in agricultural environments (Campbell and Snow, 2007; Campbell et al., 2009). Similarly, wild sunflower (*Helianthus annuus*) showed higher fecundity than crop-wild hybrids in four contrasting environments, but the relative fitness of hybrids was higher under competitive conditions, possibly because wild sunflower is not adapted to agricultural environments (Mercer et al., 2006, 2007). Contrary to our expectation, our results showed higher plant biomass and fecundity and consequently higher relative fitness in the reciprocal crop-weed hybrids compared to their parents in both contrasting environments, indicating that the result of the crop-weed hybridization was environmental independent. Also, no biotype by environment interaction was found in crop-weed radish hybrids from North America in response to diverse watering environments (Shukla et al., 2020). Because ecological conditions rarely, if ever, reversed the relative performance of hybrids compared with their parents (Campbell and Snow 2007, and references therein), our results suggest that reciprocal crop-weed hybridization has the

potential to increase weediness and invasiveness in a wide range of environmental conditions (e.g., both inside and outside the agricultural environment), and could increase the chances of success when hybrids invade new environments or are exposed to environmental changes as a result of ongoing climate change (Hovick et al., 2012; Clements and Jones, 2021). However, our inference should be studied by further by evaluating the effect of crop-weed hybridization across a greater diversity of locations in order to assess the generality of these results.

Comparison of the direction of crop-weed hybridization was associated with the fact that reciprocal crop-weed hybridization may contribute to the evolution of weediness and invasiveness in feral radish (Ridley et al., 2008), and previous studies have shown differential maternal genetic effects when evaluating crop-wild/weed hybridization (Pace et al., 2015; Singh et al., 2017; Hernández et al., 2021). Contrary to our expectation, the evaluated traits were not generally influenced by the genotype of the maternal parent, suggesting similar evolutionary consequences regardless of the direction of the hybridization. To our knowledge, this is the first study that has evaluated plants of reciprocal crop-weed hybrids in a common garden under field conditions.

Increased fitness in early generation crop-weed hybrids could be a starting point for further adaptive evolution (Mercer et al., 2006; Presotto et al., 2019). Crop-weed hybridization can lead to adaptive evolution in a number of ways, such as dumping genetic load, evolutionary novelty, introgression of beneficial traits, increased genetic variation and heterosis (i.e., hybrid vigor) (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009). As feral *R. sativus* is a widely distributed weed in the Argentine Pampas region, where populations are generally composed of hundreds to thousands of individuals, and gene flow occurs over long distances (Ellstrand et al., 1989; Scursoni et al., 2014; Vercellino et al., 2018), dumping genetic load

appears to be an unlikely cause of higher early generation hybrid fecundity. Considering that the reciprocal hybrids showed a higher plant biomass and fecundity than both parents, the transfer of beneficial crop traits that enhance offspring fitness also seems unlikely, although it could not be completely ruled out. While evolutionary novelty is the most common hypothesis for the role of hybridization in adaptive evolution (reviewed in Ellstrand and Schierenbeck 2000; Schierenbeck and Ellstrand 2009), there does not appear to be a clear evolutionary novelty to explain our results, which could be explained by increased or reshuffled genetic variation that expands the phenotypic variation (Hegde et al., 2006; Ridley et al., 2008; Campbell et al., 2009; Presotto et al., 2019; Palacio-Lopez and Molofsky, 2021). To demonstrate our inference, future studies should be carried out to evaluate the phenotypic variation of functional traits, as well as the strength and direction of direct and indirect selection operating on them, using phenotypic selection analysis to better understand the process of adaptive evolution that may accompany adaptive evolution (Campbell et al., 2009; Exposito-Alonso et al., 2018; Presotto et al., 2019).

A plausible explanation for the increased plant biomass and fecundity of hybrids is heterosis (Ellstrand and Schierenbeck, 2000). Heterosis, defined as the superiority of hybrids relative to their parents, is expected to occur after admixture between divergent populations that experienced genetic bottlenecks (Hovick et al., 2012; van Kleunen et al., 2015). Feral radish populations from Argentina probably experienced strong genetic bottlenecks, first as result of the species domestication, followed by species introduction into Argentina, and then as result of the feralization process (Vercellino et al., 2022). However, if current cultivars and feral populations are divergent enough to explain heterosis effects observed in this study is unknown. Genetic studies are needed to better understand the evolutionary history of feral radish in Argentina. Theory predicts that heterosis of early generation hybrids is only transitory and it would decrease

in the following generations (Rius and Darling, 2014), however, previous studies have shown that hybrid vigor could be maintained or fixed in subsequent generations (Ellstrand and Schierenbeck, 2000). For example, the superiority of interspecific crop-weed (*R. sativus* x *R. raphanistrum*) hybrids was maintained at least after four generations and under several environmental conditions (Campbell et al., 2006, 2009; Campbell and Snow, 2007; Snow et al., 2010; Hovick et al., 2012). Similar results were found in natural interspecific crop-weed radish hybrids (Hegde et al., 2006; Ridley and Ellstrand, 2009). However, additional studies should be carried out to understand the long-term consequences of intraspecific hybridization events (Snow et al., 2010; Mercer et al., 2014; Mitchell et al., 2019).

A possible limitation of our study of reciprocal crop-weed hybridization is that only two crop cultivars and two weed populations (although separated by more than 100 km) were used to create the biotypes. Despite this, we did not find significant differences in any trait as a result of the sources of the weed population, which could somehow support the generality of our results. On the other hand, hybrid fitness may be influenced by factors that were not considered in our common garden experiments, e.g., seed dormancy and seedling emergence (e.g., Hooftman et al. 2015; Mitchell et al. 2019). However, fecundity is the most important trait contributing to hybrid population growth in the *Raphanus* complex of North America (Campbell et al., 2014, 2016; Teitel et al., 2016). Future studies incorporating more life-history traits would be necessary in order to better understand the result of crop-weed hybridization. On the other hand, considering that the AHAS resistant Trp574Leu mutation showed fitness cost (Vercellino et al., 2018, 2021a), an increase in fitness as the result of offsetting the fitness cost of herbicide resistance cannot be ruled out. Finally, our discussion and conclusions are based on field experiments

representing the two main ecological conditions that progenitors originally colonize and where reciprocal hybridization can occur.

***Implications for cover crop and weed management-***

The assessment of the effect of intraspecific crop-weed hybridization under contrasting ecological conditions was initially motivated by concerns about the risk of the evolution of weediness and invasiveness after crop-weed hybridization. We assume that the relative fitness of reciprocal hybrids is well described by early generation studies but we recognize that our discussion is dependent on this assumption. Advanced-generation hybrids were not studied here but have been widely studied for the *Raphanus* complex in North America (Campbell et al., 2009; Ridley and Ellstrand, 2009; Snow et al., 2010; Hovick et al., 2012). Those studies suggest that late-generation hybrids exhibited increased plant biomass and fecundity compared to the weed biotype. Since reciprocal hybrids responded to contrasting environments in a similar way, weed management may be relatively consistent across environments. Here, we discuss the implications of our results for weed evolution and management.

In recent years, *R. sativus* has been included as a cover crop in agricultural systems from several parts of the world (Lawley et al., 2011). In the Argentine Pampas region, radish cover crop is currently cultivated for both seed production and in farm fields in the same areas where feral radish has been one of the most problematic weeds for more than 80 years (Ibarra, 1937; Scursoni et al., 2014). Considering that *R. sativus* is a self-incompatible, insect-pollinated species (Snow and Campbell, 2005) with high gene flow over long distances (Ellstrand et al., 1989), crop-weed hybridization is expected in areas where cultivated and weed radish are sympatric. Therefore, the concern regarding crop-weed hybridization and the subsequent evolution of

weediness and invasiveness is not whether it can happen, but the speed with which humans accelerate this evolutionary process (reviewed in Vercellino et al. 2023).

A management practice to prevent crop-weed gene flow could be the suppression of the cultivated radish prior to the flowering stage. Since this practice could only be carried out in farmer fields, but not in seed production areas where the risk of dispersion is considerably higher (e.g., weedy beet in Europe; Bartsch 2010), we propose that the introduction of cultivated radish in areas with feral radish should be carried out with extreme care, i.e., by planting cultivated radish in feral radish-free plots and by controlling feral radish plants in its surroundings. Considering that weed evolution after the introduction of a new crop in a new area has been demonstrated in several crop-weed complexes, with multiple negative impacts (reviewed in Ellstrand et al. 2010; Vercellino et al. 2023), we recommend a thorough agroecological and environmental risk assessment of the introduction of a new crop (e.g., radish cover crop) before being approved for production and commercialization.

In South America, feral radish developed resistant to AHAS-inhibitors in Chile, Brazil and Argentina which is due to the Trp574Leu mutation (Pandolfo et al., 2016; Costa et al., 2021; Heap, 2022), that showed considerable fitness cost in both agrestal and ruderal environments under field conditions (Vercellino et al., 2018, 2021a). Considering that the radish cover crop is susceptible to AHAS-inhibitors, it could be cultivated in areas infested with AHAS-resistant feral radish to flood their populations with the AHAS-susceptible allele and contribute to reducing the frequency of the AHAS-resistant allele. However, the higher relative fitness of reciprocal hybrids found in this study could lead to the development of more problematic weed and invasive populations (e.g., AHAS-resistant feral radish populations with increased weediness and invasiveness). Therefore, the use of a radish cover crop to reduce the frequency of the

AHAS-resistant allele should be studied in advance prior to the implementation in order to prevent the occurrence of unexpected contrary effects.

Finally, integrated weed management strategies aimed at preventing the evolution of herbicide resistance and gene flow with the crop should be a priority in order to prevent the emergence and spread of novel more problematic feral radish biotypes (reviewed in Norsworthy et al. 2012 and Vercellino et al. 2023).

## CONCLUSION

In summary, our results using experimental populations under field conditions in a common garden show that crop-weed hybridization has the potential to stimulate weediness and invasiveness in the invasive weedy feral *R. sativus* in both ruderal and agrestal environments, regardless of the direction of hybridization. This could promote the introgression and spread of crop alleles -including alleles with resistance to herbicides, insects, pathogens, viruses and tolerance to abiotic stresses (genetically modified or not)- into weed populations, promoting the evolution of novel weed biotypes. We suggest that subsequent long-term full life-cycle experiments in multiple locations are necessary to assess the generality of our results. Further research should also study the phenotypic variation of functional traits on which selection can act, as well as the strength and direction of direct and indirect selection operating on them using phenotypic selection analysis to better understand the process of adaptive evolution that may accompany the evolution of weeds and invasives.

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## AUTHOR CONTRIBUTIONS

AP acquired funding. RBV, FH and AP designed the research. RBV performed hand-pollination. RBV and FH carried out the experiments and collected the data. RBV analyzed the data and wrote the manuscript. FH and AP critically revised the manuscript.

## DATA AVAILABILITY

The data that support the findings of this study are openly available in Figshare at 10.6084/m9.figshare.21787097. The DOI becomes active when the manuscript is accepted.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1**. Correlations among plant biomass and reproductive components of feral radish.

**Appendix S2**. Restricted maximum likelihood for survival to reproduction, time to flowering, seed weight, seed number per plant and relative fitness of crop-weed reciprocal hybrids and their progenitors (weed: BAL and PIE; CROP) in *Raphanus sativus* growing in two contrasting environments (ruderal and agrestal), in two years (2020/21 and 2021/22), performed in SAS PROC GLIMMIX.

## LITERATURE CITED


Arnold, M. L., and N. H. Martin. 2010. Hybrid fitness across time and habitats. *Trends in Ecology and Evolution* 25: 530–536.

Barnaud, A., J. M. Kalwij, M. A. McGeoch, and B. J. van Vuuren. 2013. Patterns of weed invasion: Evidence from the spatial genetic structure of *Raphanus raphanistrum*. *Biological Invasions* 15: 2455–2465.

Bartsch, D. 2010. Gene flow in sugar beet. *Sugar Tech* 12: 201–206.

Bourgeois, B., F. Munoz, G. Fried, L. Mahaut, L. Armengot, P. Denelle, J. Storkey, et al. 2019. What makes a weed a weed? A large-scale evaluation of arable weeds through a functional lens. *American Journal of Botany* 106: 90–100.

Campbell, L. G., and A. a. Snow. 2007. Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). *New Phytologist* 173: 648–660.

Campbell, L. G., A. A. Snow, and C. E. Ridley. 2006. Weed evolution after crop gene introgression: Greater survival and fecundity of hybrids in a new environment. *Ecology Letters* 9: 1198–1209.

Campbell, L. G., A. A. Snow, and P. M. Sweeney. 2009. When divergent life histories hybridize: Insights into adaptive life-history traits in an annual weed. *New Phytologist* 184: 806–818.

Campbell, L. G., Z. Teitel, and M. N. Miriti. 2016. Contemporary evolution and the dynamics of invasion in crop-wild hybrids with heritable variation for two weedy life-histories. *Evolutionary Applications* 9: 697–708.

Campbell, L. G., Z. Teitel, M. N. Miriti, and A. A. Snow. 2014. Context-specific enhanced

invasiveness of *Raphanus* crop–wild hybrids: A test for associations between greater fecundity and population growth. *Canadian Journal of Plant Science* 94: 1315–1324.

Clements, D. R., and V. L. Jones. 2021. Ten ways that weed evolution defies human management efforts amidst a changing climate. *Agronomy* 11: 284.

Le Corre, V., M. Siol, Y. Vigouroux, M. I. Tenaillon, and C. Délye. 2020. Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. *Proceedings of the National Academy of Sciences* 117: 25618–25627.

Costa, L. O., J. Cechin, M. A. Rizzardi, S. L. Martin, C. A. Sauder, F. S. Adegas, and M. F. Grando. 2021. Target-site resistance and cross-resistance to ALS-inhibiting herbicides in radish and wild radish biotypes from Brazil. *Agronomy Journal* 113: 236–249.

Cummings, C. L., H. M. Alexander, A. A. Snow, L. H. Rieseberg, M. J. Kim, and T. M. Culley. 2002. Fecundity selection in a sunflower crop-wild study: Can ecological data predict crop allele changes? *Ecological Applications* 12: 1661–1671.

Ellstrand, N. C., B. Devlin, and D. L. Marshall. 1989. Gene flow by pollen into small populations: Data from experimental and natural stands of wild radish. *Proceedings of the National Academy of Sciences* 86: 9044–9047.

Ellstrand, N. C., S. M. Heredia, J. A. Leak-Garcia, J. M. Heraty, J. C. Burger, L. Yao, S. Nohzadeh-Malakshah, and C. E. Ridley. 2010. Crops gone wild: Evolution of weeds and invasives from domesticated ancestors. *Evolutionary Applications* 3: 494–504.

Ellstrand, N. C., P. Meirmans, J. Rong, D. Bartsch, A. Ghosh, T. J. de Jong, P. Haccou, et al. 2013. Introgression of crop alleles into wild or weedy populations. *Annual Review of*

*Ecology, Evolution, and Systematics* 44: 325–345.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences* 97: 7043–7050.

Exposito-Alonso, M., A. C. Brennan, C. Alonso-Blanco, and F. X. Picó. 2018. Spatio-temporal variation in fitness responses to contrasting environments in *Arabidopsis thaliana*. *Evolution* 72: 1570–1586.

Hauser, T. P., C. Damgaard, and R. B. Jørgensen. 2003. Frequency-dependent fitness of hybrids between oilseed rape (*Brassica napus*) and weedy *B. rapa* (Brassicaceae). *American Journal of Botany* 90: 571–578.

Heap, I. 2022. The International Survey of Herbicide Resistant Weeds. Website www.weedscience.org.

Hegde, S. G., J. D. Nason, J. M. Clegg, and N. C. Ellstrand. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60: 1187–1197.

Hernández, F., R. B. Vercellino, I. Fanna, and A. Presotto. 2021. Maternal control of early life history traits affects overwinter survival and seedling phenotypes in sunflower (*Helianthus annuus* L.). *Plant Biology* 23: 307–316.

Hernández, F., R. B. Vercellino, C. Pandolfo, J. R. Mandel, and A. Presotto. 2022. Rapid evolution of seed dormancy during sunflower de-domestication. *Journal of Heredity* 113: 288–297.

Hooftman, D. A. P., J. M. Bullock, K. Morley, C. Lamb, D. J. Hodgson, P. Bell, J. Thomas, and R. S. Hails. 2015. Seed bank dynamics govern persistence of *Brassica* hybrids in crop and

natural habitats. *Annals of Botany* 115: 147–157.

Hovick, S. M., L. G. Campbell, A. A. Snow, and K. D. Whitney. 2012. Hybridization alters early life-history traits and increases plant colonization success in a novel region. *The American Naturalist* 179: 192–203.

Hovick, S. M., and K. D. Whitney. 2014. Hybridisation is associated with increased fecundity and size in invasive taxa: Meta-analytic support for the hybridisation-invasion hypothesis. *Ecology Letters* 17: 1464–1477.

Ibarra, F. 1937. Malezas más comunes del trigo y del lino. *Almanaque del Ministerio de Agricultura.*: 405–410.

Kimball, S., D. R. Campbell, and C. Lessin. 2008. Differential performance of reciprocal hybrids in multiple environments. *Journal of Ecology* 96: 1306–1318.

van Kleunen, M., M. Röckle, and M. Stift. 2015. Admixture between native and invasive populations may increase invasiveness of *Mimulus guttatus*. *Proceedings of the Royal Society B: Biological Sciences* 282.

Kreiner, J. M., S. E. Latorre, H. A. Burbano, J. R. Stinchcombe, S. P. Otto, D. H. Weigel, and S. I. Wright. 2022. Rapid weed adaptation and range expansion in response to agriculture over the past two centuries. *Science* 378: 1079–108.

Lawley, Y. E., R. R. Weil, and J. R. Teasdale. 2011. Forage radish cover crop suppresses winter annual weeds in fall and before corn planting. *Agronomy Journal* 103: 137–144.

Magnussen, L. S., and T. P. Hauser. 2007. Hybrids between cultivated and wild carrots in natural populations in Denmark. *Heredity* 99: 185–192.

Martin, S. L., B. E. Lujan-Toro, C. A. Sauder, T. James, S. Ohadi, and L. M. Hall. 2019. Hybridization rate and hybrid fitness for *Camelina microcarpa* Andrz. ex DC (♀) and *Camelina sativa* (L.) Crantz (Brassicaceae) (♂). *Evolutionary Applications* 12: 443–455.

Mercer, K. L., D. A. Andow, D. L. Wyse, and R. G. Shaw. 2007. Stress and domestication traits increase the relative fitness of crop-wild hybrids in sunflower. *Ecology Letters* 10: 383–393.

Mercer, K. L., D. J. Emry, A. A. Snow, M. A. Kost, B. A. Pace, and H. M. Alexander. 2014. Fitness of crop-wild hybrid sunflower under competitive conditions: Implications for crop-to-wild introgression. *PLoS ONE* 9: e109001.

Mercer, K. L., D. L. Wyse, and R. G. Shaw. 2006. Effects of competition on the fitness of wild and crop-wild hybrid sunflower from a diversity of wild populations and crop lines. *Evolution* 60: 2044–2055.

Mitchell, N., S. A. Chamberlain, and K. D. Whitney. 2021. Proximity to crop relatives determines some patterns of natural selection in a wild sunflower. *Evolutionary Applications* 14: 1328–1342.

Mitchell, N., G. L. Owens, S. M. Hovick, L. H. Rieseberg, and K. D. Whitney. 2019. Hybridization speeds adaptive evolution in an eight-year field experiment. *Scientific Reports* 9: 6746.

Nam, K. H., D. Y. Kim, Y. S. Moon, I. S. Pack, S. C. Jeong, H. B. Kim, and C. G. Kim. 2020. Performance of hybrids between abiotic stress-tolerant transgenic rice and its weedy relatives under water-stressed conditions. *Scientific Reports* 10: 9319.

Norsworthy, J. K., S. M. Ward, D. R. Shaw, R. S. Llewellyn, R. L. Nichols, T. M. Webster, K.

W. Bradley, et al. 2012. Reducing the risks of herbicide resistance: Best management practices and recommendations. *Weed Science* 60: 31–62.

Pace, B. A., H. M. Alexander, J. D. Emry, and K. L. Mercer. 2015. Seed fates in crop-wild hybrid sunflower: Crop allele and maternal effects. *Evolutionary Applications* 8: 121–132.

Page, A., J. Gibson, R. S. Meyer, and M. A. Chapman. 2019. Eggplant domestication: Pervasive gene flow, feralization, and transcriptomic divergence. *Molecular Biology and Evolution* 36: 1359–1372.

Palacio-Lopez, K., and J. Molofsky. 2021. Phenotypic shifts following admixture in recombinant offspring of *Arabidopsis thaliana*. *Evolutionary Ecology* 35: 575–593.

Pandolfo, C. E., A. Presotto, and M. Cantamutto. 2018a. *Raphanus sativus* L. - *Raphanus raphanistrum* L. In Fernández, O. A., E. S. Leguizamón, and H. A. Acciaresi. Malezas e Invasoras de la Argentina, EdiUNS, Bahía Blanca, Argentina, pp 625–636.

Pandolfo, C. E., A. Presotto, F. T. Carbonell, S. Ureta, M. Poverene, and M. Cantamutto. 2018b. Transgene escape and persistence in an agroecosystem: the case of glyphosate-resistant *Brassica rapa* L. in central Argentina. *Environmental Science and Pollution Research* 25: 6251–6264.

Pandolfo, C. E., A. Presotto, F. Moreno, I. Dossou, J. P. Migasso, E. Sakima, and M. Cantamutto. 2016. Broad resistance to acetohydroxyacid-synthase-inhibiting herbicides in feral radish (*Raphanus sativus* L.) populations from Argentina. *Pest Management Science* 72: 354–361.

Piskurewicz, U., M. Iwasaki, D. Susaki, C. Megies, T. Kinoshita, and L. Lopez-Molina. 2016.

Dormancy-specific imprinting underlies maternal inheritance of seed dormancy in *Arabidopsis thaliana*. *eLife* 5: e19573.

Presotto, A., F. Hernández, M. Díaz, I. Fernández-Moroni, C. Pandolfo, J. Basualdo, S. Cuppari, et al. 2017. Crop-wild sunflower hybridization can mediate weediness throughout growth-stress tolerance trade-offs. *Agriculture, Ecosystems and Environment* 249: 12–21.

Presotto, A., F. Hernández, and K. L. Mercer. 2019. Phenotypic selection under two contrasting environments in wild sunflower and its crop–wild hybrid. *Evolutionary Applications* 12: 1703–1717.

Ridley, C. E., and N. C. Ellstrand. 2009. Evolution of enhanced reproduction in the hybrid-derived invasive, California wild radish (*Raphanus sativus*). *Biological Invasions* 11: 2251–2264.

Ridley, C. E., S. C. Kim, and N. C. Ellstrand. 2008. Bidirectional history of hybridization in California wild radish, *Raphanus sativus* (Brassicaceae), as revealed by chloroplast DNA. *American Journal of Botany* 95: 1437–1442.

Rieseberg, L. H., S. C. Kim, R. A. Randell, K. D. Whitney, B. L. Gross, C. Lexer, and K. Clay. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 129: 149–165.

Rius, M., and J. A. Darling. 2014. How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology and Evolution* 29: 233–242.

Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18: 209–235.

Sahoo, L., J. J. Schmidt, J. F. Pedersen, D. J. Lee, and J. L. Lindquist. 2010. Growth and fitness components of wild × cultivated *Sorghum bicolor* (Poaceae) hybrids in Nebraska. *American Journal of Botany* 97: 1610–1617.

Schierenbeck, K. A., and N. C. Ellstrand. 2009. Hybridization and the evolution of invasiveness in plants and other organisms. *Biological Invasions* 11: 1093–1105.

Scursoni, J. A., R. Gigón, A. N. Martín, M. Vigna, E. S. Leguizamón, C. Istilart, and R. López. 2014. Changes in weed communities of spring wheat crops of Buenos Aires province of Argentina. *Weed Science* 62: 51–62.

Shukla, K., A. E. Laursen, J. Benavides, N. Ejbari, and L. G. Campbell. 2021. Growth and fecundity of colonizing hybrid *Raphanus* populations are environmentally dependent. *American Journal of Botany* 108: 580–597.

Shukla, K., S. Sbrizzi, A. E. Laursen, J. Benavides, and L. G. Campbell. 2020. Hybridization slows rate of evolution in crop-wild compared to wild populations of weedy *Raphanus* across a moisture gradient. *Frontiers in Agronomy* 2: 600346.

Singh, J., J. A. Clavijo Michelangeli, S. A. Gezan, H. Lee, and C. E. Vallejos. 2017. Maternal effects on seed and seedling phenotypes in reciprocal F1 hybrids of the common bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science* 8: 1–13.

Snow, A. A., and L. G. Campbell. 2005. Can feral radishes become weeds?, in Crop Ferality and Volunteerism: A Threat to Food Security in the Transgenic Era?, ed. by Gressel J. Taylor & Francis Group, Boca Raton, FL, pp. 193–207

Snow, A. A., T. M. Culley, L. G. Campbell, P. M. Sweeney, S. G. Hegde, and N. C. Ellstrand.

2010. Long-term persistence of crop alleles in weedy populations of wild radish (*Raphanus raphanistrum*). *New Phytologist* 186: 537–548.

Snow, A. A., P. Moran-Palma, L. H. Rieseberg, A. Wszelaki, and G. J. Seiler. 1998. Fecundity, phenology, and seed dormancy of F1 wild-crop hybrids in sunflower (*Helianthus annuus*, Asteraceae). *American Journal of Botany* 85: 794–801.

Teitel, Z., A. Klimowski, and L. G. Campbell. 2016. Assessing the effects of hybridization and precipitation on invasive weed demography using strength of selection on vital rates. *BMC Evolutionary Biology* 16: 1–13.

Ureta, M. S., F. Torres Carbonell, C. Pandolfo, A. D. Presotto, M. A. Cantamutto, and M. Poverene. 2017. IMI resistance associated to crop-weed hybridization in a natural *Brassica rapa* population: characterization and fate. *Environmental Monitoring and Assessment* 189: 101.

Vercellino, R. B., F. Hernández, C. E. Pandolfo, M. Cantamutto, and A. Presotto. 2021a. Ecological fitness cost associated with the AHAS Trp574Leu mutation in feral *Raphanus sativus*. *Weed Research* 61: 210–220.

Vercellino, R. B., C. E. Pandolfo, G. Breccia, M. Cantamutto, and A. Presotto. 2018. AHAS Trp574Leu substitution in *Raphanus sativus* L.: screening, enzyme activity and fitness cost. *Pest Management Science* 74: 1600–1607.

Vercellino, R. B., C. E. Pandolfo, M. Cantamutto, and A. Presotto. 2021b. Interference of feral radish (*Raphanus sativus*) resistant to AHAS-Inhibiting herbicides in oilseed rape, wheat and sunflower crops. *International Journal of Pest Management* 0: 1–10.

Vercellino, R. B., C. E. Pandolfo, A. Cerrota, M. Cantamutto, and A. Presotto. 2019. The roles of light and pericarp on seed dormancy and germination in feral *Raphanus sativus* (Brassicaceae). *Weed Research* 59: 396–406.

Vercellino, R., F. Hernández, C. Pandolfo, M. S. Ureta, and A. Presotto. 2022. Agricultural weeds: The contribution of domesticated species to the origin and evolution of feral weeds. *Pest Management Science*. DOI: 10.1002/ps.7321.

Vigueira, C. C., X. Qi, B. K. Song, L. F. Li, A. L. Caicedo, Y. Jia, and K. M. Olsen. 2019. Call of the wild rice: *Oryza rufipogon* shapes weedy rice evolution in Southeast Asia. *Evolutionary Applications* 12: 93–104.

Warwick, S. I. S. 2011. Brassicaceae in Agriculture. In Schmidt R., and I. Bancroft [eds.], Genetics and Genomics of the Brassicaceae, 33–65. Springer New York, New York, NY.

Whitney, K. D., J. R. Ahern, and L. G. Campbell. 2009. Hybridization-prone plant families do not generate more invasive species. *Biological Invasions* 11: 1205–1215.

Xia, H., H. Zhang, W. Wang, X. Yang, F. Wang, J. Su, H. Xia, et al. 2016. Ambient insect pressure and recipient genotypes determine fecundity of transgenic crop-weed rice hybrid progeny: Implications for environmental biosafety assessment. *Evolutionary Applications* 9: 847–856.

Yue, J., L. Ze, W. Sheng, Y. Qi, H. Qi, and L. Sheng. 2021. Fitness of F1 hybrids between 10 maternal wild soybean populations and transgenic soybean. *Transgenic Research* 30: 105–119.

Zhang, X., T. Liu, J. Wang, P. Wang, Y. Qiu, W. Zhao, S. Pang, et al. 2021. Pan-genome of

*Raphanus* highlights genetic variation and introgression among domesticated, wild, and weedy radishes. *Molecular Plant* 14: 2032–2055.

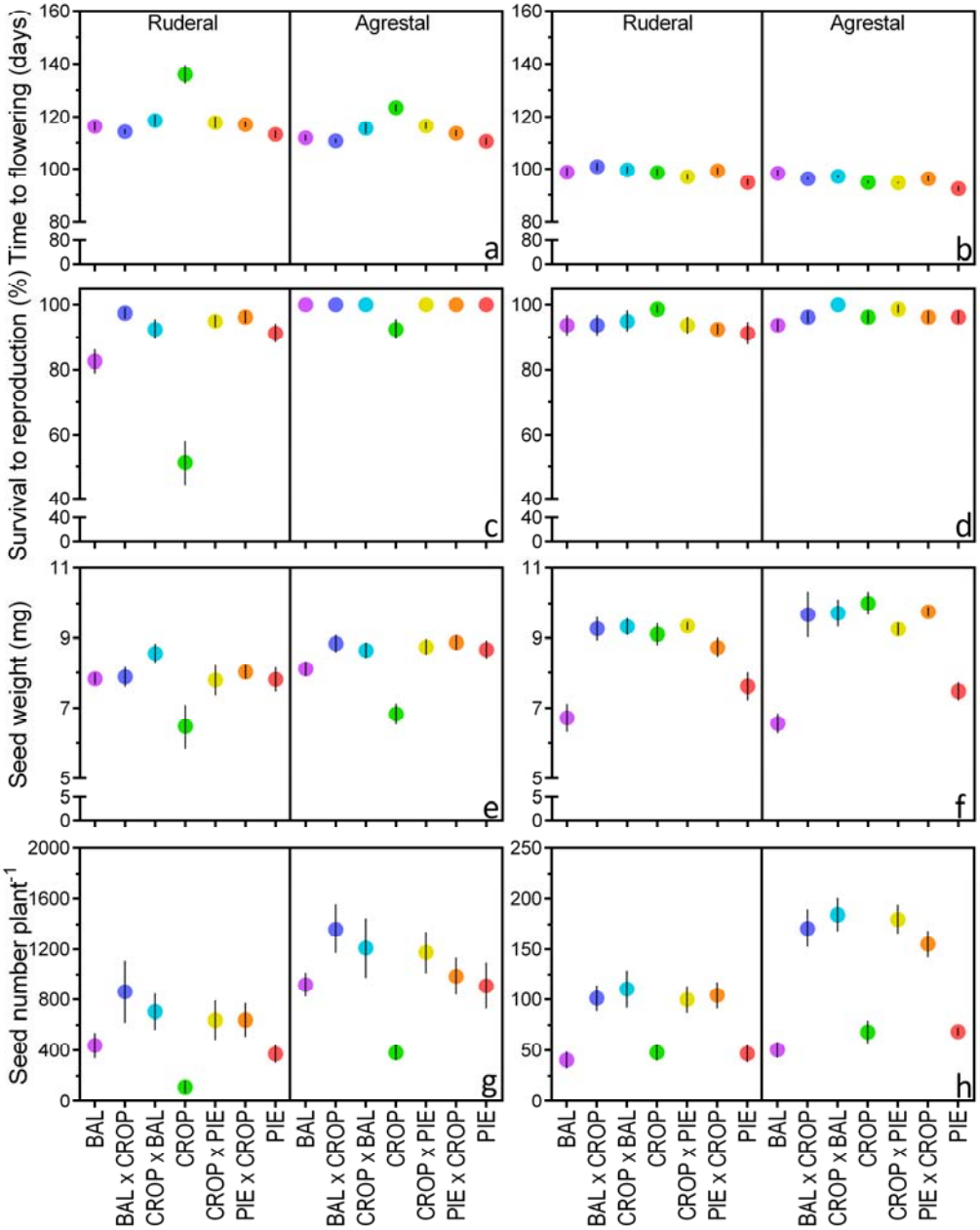


**Figure 1.** Time to flowering (2020/21: a; 2021/22: b), survival to reproduction (2020/21: c; 2021/22: d), seed weight (2020/21: e; 2021/22: f) and seed number per plant (2020/21: g; 2021/22: h) for reciprocal crop-weed hybrids and their parents (weed: BAL and PIE, crop: CROP) of *Raphanus sativus* growing in two contrasting environments (ruderal and agrestal), and in two years. Circles represent mean and vertical bars indicate ± SE. Note the variation in the scale of the y-axis between (h) and (g) to show large differences in seed number per plant between year 1 and 2.

**Table 1.** Restricted maximum likelihood for survival to reproduction, days to flowering, seed weight and seed number per plant in crop-weed reciprocal hybrids and their progenitors (weed: BAL and PIE; CROP) in two contrasted environments (ruderal and agrestal) performed using maximum likelihood in SAS PROC GLIMMIX for year 1. Data were collected in the Agronomy Department at Universidad Nacional del Sur, Bahía Blanca, Argentina. Significant effects are in bold.

| | Time to flowering | | | | Survival to maturity | | | | Seed number plant$^{-1}$ | | Seed weight | | Relative fitness | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fixed effects | *F* | *P* | | | *F* | *P* | | | *F* | *P* | *F* | *P* | *F* | *P* |
| Environment (E) | **11.39** | **0.0045** | | | **84.94** | **<.0001** | | | **25.82** | **0.0002** | **12.53** | **0.0033** | 3.46 | 0.0838 |
| Biotype (B) | **48.55** | **<.0001** | | | **18.47** | **<.0001** | | | **19.9** | **<.0001** | **8.25** | **<.0001** | **2.83** | **0.0224** |
| E x B | **4.09** | **0.0005** | | | **6.12** | **<.0001** | | | 0.4 | 0.8786 | 1.14 | 0.3351 | 0.48 | 0.7885 |
| Random effects | *Z* | *P* | | | *Z* | *P* | | | *Z* | *P* | *Z* | *P* | *Z* | *P* |
| Block (E) | 2.05 | 0.0203 | | | 0 | . | | | 2.17 | 0.015 | 0.87 | 0.1924 | . | . |
| Residuals | 22.73 | <.0001 | | | 7 | <.0001 | | | 17.16 | <.0001 | 16.44 | <.0001 | 6.32 | <.0001 |
| Contrasts[1] | **Ruderal** | | **Agrestal** | | **Ruderal** | | **Agrestal** | | | | | | | |
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| W vs. W x C | 0.45 | 0.5042 | 0.93 | 0.3345 | **10.55** | **0.0023** | 0 | 1 | **23.72** | **<.0001** | 2.19 | 0.1398 | **7.15** | **0.0094** |
| W vs. C x W | **6.55** | **0.0108** | **24.06** | **<.0001** | **4.35** | **0.0431** | 0 | 1 | **20.23** | **<.0001** | 3.63 | 0.0573 | **9.3** | **0.0033** |
| W vs. C | **140.75** | **<.0001** | **104.09** | **<.0001** | **32.67** | **<.0001** | **30.57** | **<.0001** | **37** | **<.0001** | **28.1** | **<.0001** | **-** | **-** |
| C x W vs. W x C | 3.59 | 0.0586 | **15.53** | **<.0001** | 1.35 | 0.2518 | 0 | 1 | 0.16 | 0.6853 | 0.16 | 0.6927 | 0.1 | 0.7559 |
| C x W vs. C | **98.96** | **<.0001** | **39.46** | **<.0001** | **55.04** | **<.0001** | **30.57** | **<.0001** | **88.04** | **<.0001** | **42.77** | **<.0001** | **-** | **-** |
| W x C vs. C | **129.4** | **<.0001** | **88.97** | **<.0001** | **70.02** | **<.0001** | **30.57** | **<.0001** | **93.31** | **<.0001** | **39.06** | **<.0001** | **-** | **-** |

[1]Cross types for orthogonal contrasts: Weeds (W), crop (C), crop-weed hybrids produced on weed plants (WxC), crop–weed hybrids produced on crop plants (CxW).

**Table 2**. Restricted maximum likelihood for survival to reproduction, days to flowering, seed weight and seed number per plant in crop-weed reciprocal hybrids and their progenitors (weed: BAL and PIE; CROP) in two contrasted environments (ruderal and agrestal) performed using maximum likelihood in SAS PROC GLIMMIX for year 2. Data were collected in the Agronomy Department at Universidad Nacional del Sur, Bahía Blanca, Argentina. Significant effects are in bold.

| | Time to flowering | | Survival to maturity | | Seed number plant$^{-1}$ | | Seed weight | | Relative fitness | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fixed effects | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Environment (E) | **5.06** | **0.0412** | 2.77 | 0.12 | **12.35** | **0.0034** | 1.71 | 0.21 | 1.72 | 0.2104 |
| Biotype (B) | **11.52** | **<.0001** | 1.27 | 0.28 | **42.83** | **<.0001** | **25.61** | **<.0001** | **28.74** | **<.0001** |
| E x B | 1.05 | 0.39 | 0.99 | 0.44 | 1.73 | 0.11 | 0.67 | 0.68 | 1.37 | 0.2476 |
| Random effects | *Z* | *P* | *Z* | *P* | *Z* | *P* | *Z* | *P* | *Z* | *P* |
| Block (E) | 2.34 | 0.0096 | 0 | . | 2.1 | 0.0181 | 0.47 | 0.32 | 0.93 | 0.1775 |
| Residuals | 22.67 | <.0001 | 7 | <.0001 | 17.23 | <.0001 | 17.04 | <.0001 | 5.92 | <.0001 |
| Contrasts[1] | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| W vs. W x C | **12.45** | **0.0004** | **-** | **-** | **144.1** | **<.0001** | **99.99** | **<.0001** | **86.54** | **<.0001** |
| W vs. C x W | **3.87** | **0.0495** | **-** | **-** | **166.94** | **<.0001** | **102.11** | **<.0001** | **105.2** | **<.0001** |
| W vs. C | 0.97 | 0.33 | **-** | **-** | 0.92 | 0.34 | **70.51** | **<.0001** | **-** | **-** |
| C x W vs. W x C | 2.51 | 0.11 | **-** | **-** | 0.48 | 0.49 | 0 | 0.99 | 0.91 | 0.3439 |
| C x W vs. C | 0.4 | 0.53 | **-** | **-** | **94.68** | **<.0001** | 0 | 0.95 | **-** | **-** |
| W x C vs. C | 3.7 | 0.06 | **-** | **-** | **81.77** | **<.0001** | 0.01 | 0.94 | **-** | **-** |

[1]Cross types for orthogonal contrasts: Weeds (W), crop (C), crop-weed hybrids produced on weed plants (WxC), crop–weed hybrids produced on crop plants (CxW).