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Authors

Ridley, Caroline E.
Ellstrand, Norman C.

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Evolution of enhanced reproduction in the hybrid-derived invasive, California wild radish (*Raphanus sativus*)

Caroline E. Ridley · Norman C. Ellstrand

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Abstract Evolution is receiving increased attention as a potentially important factor in invasions. For example, hybridization may have stimulated the evolution of invasiveness in several well-known plant pests. However, the mechanism for success of such hybrid-derived lineages remains unknown in the majority of the cases studied. Here we ask whether increased reproductive success (in terms of maternal fitness) has evolved in an invasive lineage with confirmed hybrid ancestry. We compare the relative fitness of the non-native, hybrid-derived California wild radish (*Raphanus sativus*) to that of its two progenitor species in field experiments at different sites and in different years. We found that California wild radish has high survivorship and produces more fruits per plant and more seeds per plant than either of its progenitors in several environments. Furthermore, populations of California wild radish display a strong

genotype-by-environment interaction, indicating that maintenance of genetic and phenotypic diversity between populations may be responsible for the weed's ability to invade a wide breadth of California habitats. Our results suggest that hybridization may contribute the evolution of enhanced invasiveness and, also, that by limiting the introduction and subsequent hybridization of congeners, we may be able to prevent the evolution of new invasive lineages.

Keywords Common garden experiment · Fitness traits · Genotype × environment interaction · Hybridization · Invasiveness · *Raphanus*

Introduction

Evidence is mounting that evolution can be an important factor in the invasion process (Barrett et al. 2008; Cox 2004; Dlugosch and Parker 2008; Keller and Taylor 2008; Lambrinos 2004; Lee 2002). Evolutionary change can be rapid and substantial in introduced populations, in some cases stimulating the creation of novel, highly invasive genotypes (Dlugosch and Parker 2008; Ellstrand and Schierenbeck 2006).

Hybridization, defined as the successful mating of two genetically distinct sources (e.g., Arnold 1997), could be quite common in populations of non-native, sexually reproducing species because introductions

C. E. Ridley · N. C. Ellstrand
Department of Botany and Plant Sciences, University
of California, Riverside, CA 92521, USA

N. C. Ellstrand
Center for Conservation Biology, University
of California, Riverside, CA 92521, USA

C. E. Ridley (✉)
Department of Ecology, Evolution and Behavior,
University of Minnesota, St. Paul, MN 55108, USA
e-mail: ridle026@umn.edu

often originate from multiple and/or geographically diverse source populations (e.g., Bartlett et al. 2002; Durka et al. 2005; Genton et al. 2005; Maron et al. 2004; Marrs et al. 2008; Novak and Mack 2001). Such intraspecific hybridization has been implicated in enhancing invasiveness in several species, including both plants and animals (Facon et al. 2005; Lavergne and Molofsky 2007). Many well-known examples of interspecific hybrid invaders also exist. *Spartina anglica* in Britain is derived from the hybridization of an introduced species, *S. alterniflora*, with the native *S. maritima*, while the hybrid lineage between introduced *S. alterniflora* and the native *S. foliosa* is invading the West Coast of North America (Ainouche et al. 2004; Ayres et al. 2003).

Although several coincidences of hybridization with invasion have been identified (Ellstrand and Schierenbeck 2006), the mechanisms by which hybrid-derived lineages establish and spread have been rarely studied. The simplest explanation might be that hybridization creates genotypes that are more reproductively successful than the pure progenitor genotypes, perhaps as a result of fixed heterosis, the purging of deleterious alleles or the transfer of adaptations (Ellstrand and Schierenbeck 2006; Rieseberg et al. 1999). All other things being equal, a lineage that reproduces more is likely to be more invasive because of the prime importance of propagule pressure during the invasion process (Colautti et al. 2006; Hayes and Barry 2008; Lockwood et al. 2005). Increased genetic diversity relative to progenitor populations might also affect the success of hybrid-derived populations by enhancing their ability to respond to selection and to adaptively evolve (Ellstrand and Schierenbeck 2006; Lee 2002; Parker et al. 2003; Sakai et al. 2001). However, hybrid-derived lineages do not necessarily have to display a reproductive advantage to be more invasive than their introduced progenitors (Bergelson 1994). In theory, they could show one of the other qualities of a good invader, such as superior competitive or dispersal ability. Our system, the California wild radish (*Raphanus sativus*), is an extensively studied non-native lineage that is appropriate for examining the mechanism(s) of hybrid invasive success.

In the mid-1800s, two *Raphanus* species colonized the San Francisco Bay area of California. Cultivated radish *R. sativus* was introduced intentionally as a crop. *Raphanus raphanistrum*, also known as jointed

charlock, arrived accidentally, probably as a weed contaminant of grain (Panetsos and Baker 1967; Robbins 1940). Native to the Mediterranean, *R. raphanistrum* has been listed as one of the world's worst weeds (Holm et al. 1997) and is an agricultural pest in Canada, the northern United States and Australia (Snow and Campbell 2005). Both *R. sativus* and *R. raphanistrum* are annual and self-incompatible. The two species are differentiated by a number of genetically-based traits including root morphology, fruit morphology, flower color and flowering time (Hegde et al. 2006; Panetsos and Baker 1967). They also differ from one another by a single chromosomal reciprocal translocation that results in partial reproductive isolation via 50% reduced fertility in the F_1 generation (Panetsos and Baker 1967). This reproductive barrier is easily overcome, however, such that later generation hybrids have substantially recovered fertility (Snow and Campbell 2005).

As late as the 1960s, both morphologically pure populations of each introduced *Raphanus* species and clearly hybrid-derived populations were not uncommon in coastal and inland northern California (Panetsos and Baker 1967). Some 40 years later, in a wide sampling of *Raphanus* throughout the state, hybrid genetic ancestry was found for every population (Hegde et al. 2006; Ridley et al. 2008). Thus, over the last century, the two *Raphanus* species have coalesced into a hybrid lineage that has displaced all natural populations of both parents. Commonly referred to as "California wild radish", this lineage is now found throughout a major portion of naturally disturbed coastal areas and human-disturbed inland sites in California (DiTomaso and Healy 2006; Panetsos and Baker 1967), as well as south into Baja California, Mexico and north into Oregon (Hegde et al. 2006; Ridley 2008). Interestingly, interspecific *Raphanus* hybrids also appear in Europe, but are not invasive (Stace 1975).

Raphanus is an important model system. Plant ecologists, evolutionists and geneticists have all used *Raphanus* for studying pollination biology, life-history variation, ecological genetics, floral evolution and plant-herbivore interactions (e.g., Campbell et al. 2006; Conner 1997; Elam et al. 2007; Ellstrand et al. 1989; Irwin et al. 2003; Marshall and Diggle 2001; Mazer and Schick 1991; Stanton et al. 1991).

Here, we use a common garden field experiment to gain evidence for one mechanism by which

California wild radish has replaced its progenitors on the western coast of North America. Phenotypically, California wild radish tends to show intermediacy to its progenitor species for many traits, including bolting and flowering dates, and root length and width, but it exceeds both progenitors for average fruit weight, a potential fitness correlate (Hegde et al. 2006). In addition, advanced generation artificial *R. sativus* × *R. raphanistrum* hybrids grown in southern California have shown a significant survival and reproductive advantage over the weedy progenitor, *R. raphanistrum* (Campbell et al. 2006). Given this evidence, we hypothesize that the naturally hybrid-derived California wild radish will show enhanced fitness relative to the species that hybridized to create it, and that this could be one mechanism for its successful establishment and spread in the state.

Methods

Plant material

Sources of California (CA) wild radish, *R. raphanistrum* and cultivated radish used in this study are listed in Table 1. In 1998, we conducted a statewide collection of CA wild radish. We chose five populations for this study that represent both the geographic range of wild radish in California and its morphological range varying from appearing crop-like to *R. raphanistrum*-like. Several sources

of *R. raphanistrum* were likewise chosen to represent the species' geographic range, including sites from which plants could have been introduced to California. Seeds from native habitat in Europe (Denmark) and two naturalized populations from North America (Mexico and Rhode Island) were included. The *R. raphanistrum* populations chosen had no evidence of prior introgression from the crop. Cultivars are likely ancestors of the current CA wild radish populations based on molecular analysis (Ellstrand and Marshall 1985; Ridley et al. 2008).

To obtain sufficient seed for multiple years of field studies, seed was multiplied from the original CA wild radish and *R. raphanistrum* populations two times: once in 2004 for a 2005 common garden and again in 2005 for two common gardens planted in 2006. In each year, one seedling from 10 to 12 different maternal families per population was germinated and grown in a 12 cm³ pot of sterilized UC Soil Mix III (Matkin and Chandler 1967) inside an insect-excluded, temperature-controlled greenhouse on the campus of the UC Riverside. Once all individuals of a population reached flowering, they were crossed within each population. This approach has the benefit of eliminating possible maternal environmental effects of field-collected seed. In summer 2004, when local populations of wild radish were no longer flowering, populations were taken out of the greenhouse one by one and exposed to natural pollinators for up to 7 days. Pollinated plants were taken back into greenhouse isolation for at least

Table 1 List of source populations used in the experiment

Species	Population/Cultivar	Source location
<i>R. raphanistrum</i>	Denmark	Roskilde University Botanic Garden
	Mexico	Mexico City
	Rhode Island	Conanicut Island
CA wild	San Mateo County, northern coastal	Half Moon Bay State Beach
	Tulare County, central inland	Lindcove
	San Luis Obispo County, central coastal	Morro Bay State Park
	Riverside County, southern inland I	Riverside
	Riverside County, southern inland II	Hemet
Cultivated	Cherry Belle, round red	Ferry-Morse Seed Co.
	French Breakfast, long pink	Ferry-Morse Seed Co.
	Round Black Spanish	Botanical Interests, Inc.
	White Icicle, long white	Botanical Interests, Inc.

one month until fruits were mature for harvesting. In winter 2005, seeds were germinated and grown as in 2004 and then hand-crossed in the greenhouse. Two dehiscent anthers were plucked from each of three flowers on all individuals within a population and placed in a 1.5 ml microcentrifuge tube. A small paintbrush was then dipped into the mixed pollen load, and pollen was applied to 2–5 flowers per branch on 2–3 branches per plant. The paintbrush was cleaned with ethanol before pollen application to the next plant. Fruits were allowed to mature on the plants under the same greenhouse conditions. In 2004, crosses within one CA wild radish population (San Mateo County) failed and, therefore, this population was not included in 2005 of the common garden experiment.

Common garden design

Year 1 of the experiment was planted on March 7, 2005 at UC Riverside's Agricultural Experiment Station in Riverside, CA, USA. Fifty seeds from each greenhouse-multiplied source (except San Mateo County) and 50 from each cultivar source were planted in a randomized complete block design, 550 individuals total. This site experiences extreme temperatures, with nearly 100 days per year over 32°C, and typically receives little rainfall during the growing season, 34.0 and 136.4 mm of total precipitation in 2005 and 2006, respectively, during the months of the experiment. Year 2 was planted at the same Riverside site on January 23, 2006 and at UC's South Coast Research and Extension Center in Irvine, CA on January 30–31, 2006. The common garden design from 2005 was replicated at both locations, except that 50 San Mateo County individuals were added at each site. The Irvine location has a contrasting climate to that in Riverside, with more moderate temperatures (fewer than 40 days per year over 32°C) and with higher relative rainfall. During the experiment in 2006, the site received a total of 189.9 mm of precipitation.

For both field seasons and sites, seeds were germinated in the greenhouse at UC Riverside in flats of sterilized UC Soil Mix III (Matkin and Chandler 1967). Seedlings were watered daily with fertilized water (100 ppm 21-5-20 Peters EXCEL). At the one-leaf stage, seedlings were transplanted and watered into the field in 10 complete, randomized

blocks of 55 individuals each in 2005 and 60 individuals each in 2006. In 2005, the Riverside site received 90 min of overhead irrigation per week from March 31 to May 2 in weeks with no precipitation. In 2006, the Riverside site received 30 min of overhead irrigation per day from January 23–27 and 30 min per week from February 3–23 in weeks with no precipitation. The Irvine site received 60 min of overhead irrigation per week from February 3–24. The field sites were hand-weeded and treated for heavy insect infestations on several occasions (Riverside 2005: March 9, Ortho Bug-B-Gon, 0.0033% esfenvalerate and March 21, Crymax Bt, 20 g/2.5 gal; Irvine 2006: March 2, Serin 80s, 80% carbaryl, 1 lb/acre). On June 16–22, 2005 and June 4–8 (Riverside) and June 24–July 16, 2006 (Irvine), after all plants had senesced, aboveground biomass was collected and stored.

Phenotypic measurements

Fitness traits and some phenotypic traits that may directly or indirectly impact fitness were measured on all experimental plants in 2005. In 2006, phenotypic traits were measured on all plants, and fitness traits were measured on a subsample of plants large enough to statistically detect a 30% difference in seed output between species at an α -level of 0.05 with 95% confidence, as determined by power analysis (Sokal and Rohlf 1995). Traits that were measured during the field season included days to flowering, length of longest leaf at flowering (cm) and root crown diameter at flowering (mm). Days to flowering was calculated as number of days from germination in the greenhouse to the date of the first flower opening. At the end of the experiment, three female fitness traits were measured including number of fruits per plant, average number of seeds per fruit and overall seeds per plant. We measured number of fruits by counting enlarged pedicels (which indicate successful pollination and fruit formation) on dried plants and estimated average number of seeds per fruit by opening and visually assessing viable seed number for 10 separate fruits from each plant. Seed number was estimated by multiplying number of fruits by average number of seeds per fruit for each individual. The three fitness traits we measured were used previously to estimate fitness in *Raphanus* (Campbell et al. 2006; Snow et al. 2001). We did not attempt to measure male fitness in this experiment. We do,

however, note that a prior study showed a significant, but biologically weak, relationship between male and female fitness in CA wild radish (Devlin and Ellstrand 1990).

Data analysis

For the purposes of our data analysis, we are treating cultivated radish, *R. raphanistrum*, and CA wild radish as three different “species” types. For each species in each site-year, we tabulated the number of individuals in four categories: (a) died, (b) survived to the end of the experiment but did not flower, (c) flowered but produced no seed and (d) flowered and produced seed. We tested for differences between species in their distribution across categories, combining site-years, using a likelihood ratio chi-square test using JMP 7.0 (SAS Institute Inc 2007).

Each continuous reproduction variable was statistically analyzed using a mixed model analysis of variance (ANOVA) where species, population within species, site and year were fixed effects and block, higher order interactions and error were random effects (SAS Institute Inc 2003). Number of fruits and number of seeds were natural log and natural log (+1) transformed, respectively, to meet the assumptions of normality. When an ANOVA factor was found to explain a significant amount of variance after a sequential Bonferroni correction (Rice 1990), planned mean contrasts were calculated to test for specific differences between species within site-years. Only individuals that survived to produce at least one fruit were included in the ANOVAs for fitness traits.

As it became clear that significant population within species × year and population within species × site interactions were present, we used a simplified relative distances plasticity index (RDPI_s) to compare the phenotypic plasticity in reproduction between populations of the two fittest taxa: *R. raphanistrum* and CA wild radish. RDPI_s is the distance between mean trait values, in our case seed output, in all pairs of environments calculated for each population (Valladares et al. 2006). We used a simple two-sample *t*-test assuming unequal variances to find significant differences in plasticity between species.

We also analyzed total relative fitness using a multiplicative fitness function. For each site-year, we multiplied viability by the average fecundity of the three different species and normalized the product to

the highest fitness in each site-year. We then applied a Fisher exact tests to compare the cumulative fitness of California wild radish relative to cultivated radish and *R. raphanistrum* separately (Nason and Ellstrand 1995). We repeated the test for all three site-years.

Results

Survival, flowering and setting seed

Rates of survival for both CA wild radish and *R. raphanistrum* were high across years and sites (Fig. 1). California wild radish consistently flowered and set seed at rates of 95% and above, regardless of year or site. *Raphanus raphanistrum* flowered and set seed at a rate of 100% at Irvine in 2006, and somewhat less in Riverside in both 2005 and 2006. Cultivated radish had the lowest survival rate, rate of flowering, and rate of setting seed in all site-years. Chi-square analysis revealed a highly significant difference between species in their distribution across life stage categories ($N = 1,009$, $df = 6$, $P < 0.0001$). When cultivated radish was removed from the analysis, a highly significant difference still remained between CA wild radish and *R. raphanistrum*, with 96% of CA wild radish individuals flowering and setting seed overall versus 81% of *R. raphanistrum* individuals ($N = 654$, $df = 3$, $P < 0.0001$).

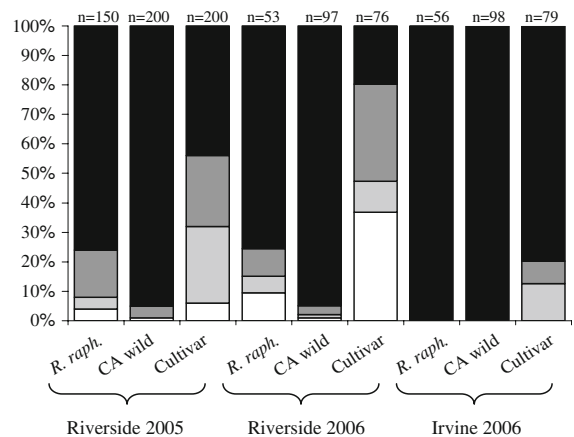


Fig. 1 Percentage of individuals in each species and each site-year within the following life stage categories by the end of the experiment, shown with samples sizes: **a** Died (white), **b** Survived to end of experiment, but never flowered (light gray), **c** Flowered but produced no seeds (dark gray) and **d** Flowered and produced seeds (black)

Table 2 Phenotypic measurements for each species in all site-years

Field site	Species	Days to flowering	Length of longest leaf at flowering (cm)	Root crown diameter at flowering (mm)
Riverside 2005	<i>R. raphanistrum</i>	45.6	12.2	5.4
	<i>n</i> = 137	(43.0–48.2)	(11.1–13.3)	(4.8–6.0)
	CA wild	45.8	17.7	9.6
	<i>n</i> = 196	(43.9–47.7)	(16.1–19.3)	(8.2–11.0)
Riverside 2006	Cultivated	90.9	22.0	52.4
	<i>n</i> = 136	(87.6–93.3)	(20.3–23.7)	(48.5–56.3)
	<i>R. raphanistrum</i>	70.5	17.2	6.9
	<i>n</i> = 136	(67.9–73.1)	(15.6–18.8)	(6.2–7.7)
Irvine 2006	CA wild	80.9	20.8	12.9
	<i>n</i> = 242	(78.2–83.6)	(19.3–22.3)	(11.4–14.4)
	Cultivated	115.3	21.8	42.9
	<i>n</i> = 111	(113.6–117.0)	(19.9–23.8)	(38.6–47.2)
Irvine 2006	<i>R. raphanistrum</i>	68.0	23.7	9.4
	<i>n</i> = 149	(65.6–70.4)	(21.6–25.8)	(8.4–10.4)
	CA wild	74.4	28.8	18.8
	<i>n</i> = 247	(71.7–77.1)	(26.7–30.9)	(16.8–20.8)
Irvine 2006	Cultivated	117.2	21.4	47.0
	<i>n</i> = 171	(115.8–118.6)	(19.2–23.6)	(43.6–50.4)

Numbers represent means and 95% confidence intervals

Traits measured at first flowering

For most traits measured in most site-years, CA wild radish displayed an intermediate phenotype to its progenitor parents, trending towards greater similarity to *R. raphanistrum* (Table 2). At Riverside in 2005, flowering was simultaneous for CA wild radish and *R. raphanistrum*, both significantly earlier than the cultivars. In another exception to intermediacy, in Irvine 2006, CA wild radish had longer leaf length at flowering than both parent species. Additionally, plants generally tended to flower more quickly and at a smaller size in Riverside 2005 compared to both field sites in 2006, though cultivars maintained a similar size at flowering across site-years.

Fruit and seed production

Across years at Riverside, there was a significant difference in seed production between species, as well as a significant difference between populations within species (Table 3). There was, however, no significant effect of year itself. Significant interaction effects between both species and year and population within

species and year indicate a genotype \times environment interaction at the species and population levels. Because of these significant interaction effects, we continued analyzing each year separately.

Table 3 ANOVA results testing the effect of year and site on the number of seeds produced per plant

Site-years compared	Effect	df	F value
Riverside 2005 and Riverside 2006 (<i>n</i> = 557)	Year	1	0.03
	Species	2	4.28*
	Population within species	9	9.83***
	Block within year	12	0.72
	Year \times Species	2	6.69**
	Year \times Population within species	7	4.66***
Riverside 2006 and Irvine 2006 (<i>n</i> = 366)	Site	1	76.67***
	Species	2	5.72*
	Population within species	9	9.44***
	Block within site	6	0.58
	Site \times Species	2	2.75
	Site \times Population within species	8	3.85**

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

Within the second year, 2006, individuals at Irvine produced an average of almost twice as many seeds as individuals at Riverside, and this effect of site was highly significant (Table 3). There was also a significant impact of both species and population within species on total seed production per plant. A marginally significant interaction between species and site and a significant interaction between population within species and site indicated a genotype × environment interaction and led us, as with year, to analyze sites separately.

At Riverside in 2005, species had a significant effect on fruit production, but not on number of seeds per fruit or overall seed production (Table 4). Nevertheless, CA wild radish out-reproduced both *R. raphanistrum* and cultivars by ~300% in terms of fruits per plant and seeds per plant (Fig. 2a, c). Significant variation was also present between populations within a species. Within CA wild radish, San Luis Obispo County and Riverside County I had

relatively high reproductive output compared to Tulare County and Riverside County II (Fig. 3b). *Raphanus raphanistrum* from Mexico and Denmark reproduced more than individuals from Rhode Island (Fig. 3a) and cultivars Black Spanish and White Icicle out-reproduced Cherry Belle and French Breakfast (Fig. 3c).

In 2006 at Riverside, a different picture emerges. Species did not account for a significant amount of variation in the three measurements of maternal fitness, but population within species did (Table 4). *Raphanus raphanistrum* individuals nearly tripled average seed production from 2005 to 2006, driven by an increase in fruit production (Fig. 2a, c). Simultaneously, fruit production and seed production declined in CA wild radish, eliminating any statistical difference between it and *R. raphanistrum*. While *R. raphanistrum* increased relative fitness, cultivated individuals experienced decreased fitness in 2006 compared to 2005 (Fig. 2).

Table 4 ANOVA results for all maternal fitness traits measured in the experiment

Field site	Effect	df	Ln (fruit number) n = 410	Seeds per fruit n = 411	Ln (seed number + 1) n = 410
Riverside 2005	Species	2	7.70*	2.55	4.52
	Population within species	8	15.86***	7.19***	15.00***
	Block	9	1.11	0.44	0.76
	Block × Species	18	1.31	0.44	0.96
	Block × Population within species	68	0.77	0.81	0.79
Field site	Effect	df	Ln (fruit number) n = 146	Seeds per fruit n = 153	Ln (seed number + 1) n = 147
Riverside 2006	Species	2	3.08	0.65	4.01
	Population within species	8	2.68**	6.61**	3.38**
	Block	3	0.09	4.64*	0.14
	Block × Species	6	0.43	1.12	0.83
	Block × Population within species	20	0.67	0.94	0.47
Field site	Effect	df	Ln (fruit number) n = 222	Seeds per fruit n = 219	Ln (seed number + 1) n = 219
Irvine 2006	Species	2	7.26*	1.28	6.30*
	Population within species	9	8.84***	5.54***	11.25***
	Block	3	1.87	1.27	1.30
	Block × Species	6	0.63	1.56	0.72
	Block × Population within species	27	1.15	0.84	1.12

Separate ANOVA's were conducted for each site-year due to significant Species × Year and Species × Site interaction effects. Numbers presented are *F* values

* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001 using sequential Bonferroni correction (Rice 1990)

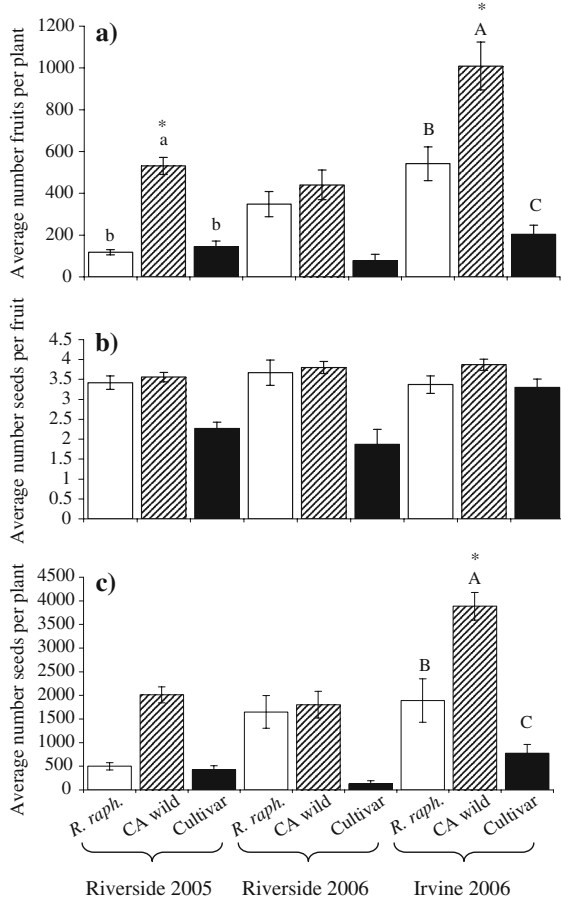


Fig. 2 Individual average **a** number of fruits per plant, **b** number of seeds per fruit and **c** number of seeds per plant for each species in each site-year \pm 1 SE. Asterisk indicates a significant effect of species on the trait within the site-year at the $P < 0.05$ level using a sequential Bonferroni correction. Independent contrasts are also shown, where different letters indicate a significant difference in mean values at the $P < 0.05$ level. Statistics were based on \ln (fruits), seeds per fruit and \ln (seeds + 1), but untransformed values are presented for simplicity

As the significant year \times population interaction suggested in the initial ANOVA, the rank of populations with respect to reproduction changed from year to year, in particular for CA wild radish. Whereas the San Luis Obispo County and Riverside County I populations reproduced the most in 2005, Riverside County II individuals produced the most seeds per plant in Riverside in 2006 (Fig. 3b). Rank order of parental populations and cultivars remain similar to that of 2005 (Fig. 3a, c).

At Irvine in 2006, CA wild radish produced \sim 200 and 500% more fruits than *R. raphanistrum* and

cultivars, respectively (Fig. 2a), and this species effect was significant (Table 4). California wild radish also produced significantly more seeds per plant than either progenitor parent (Fig. 2c). Population within species continued to have a significant effect on all three female fitness traits (Table 4).

Rank of CA wild radish population reproduction changed between field sites, just as it changed between years. In Riverside in 2006, the worst reproducing population was from San Mateo County, but in Irvine in 2006, this population performed the best, showing more than a tenfold difference in seed production (Fig. 3b). Riverside County II, San Luis Obispo County and Tulare County individuals all reproduced more in Irvine, while Riverside County I individuals produced approximately the same number of seeds per plant as in Riverside. Mirroring the trend between years, the between site rank order of parental *R. raphanistrum* populations and cultivars was unchanged (Fig. 3a, c).

The statistical comparison of RDPI_s between *R. raphanistrum* and CA wild radish was consistent with qualitative observations about genotype \times environment interactions: CA wild radish had significantly higher plasticity than *R. raphanistrum* for seed output across all environments (Two sample *t*-test: $df = 20$, $P = 0.02$).

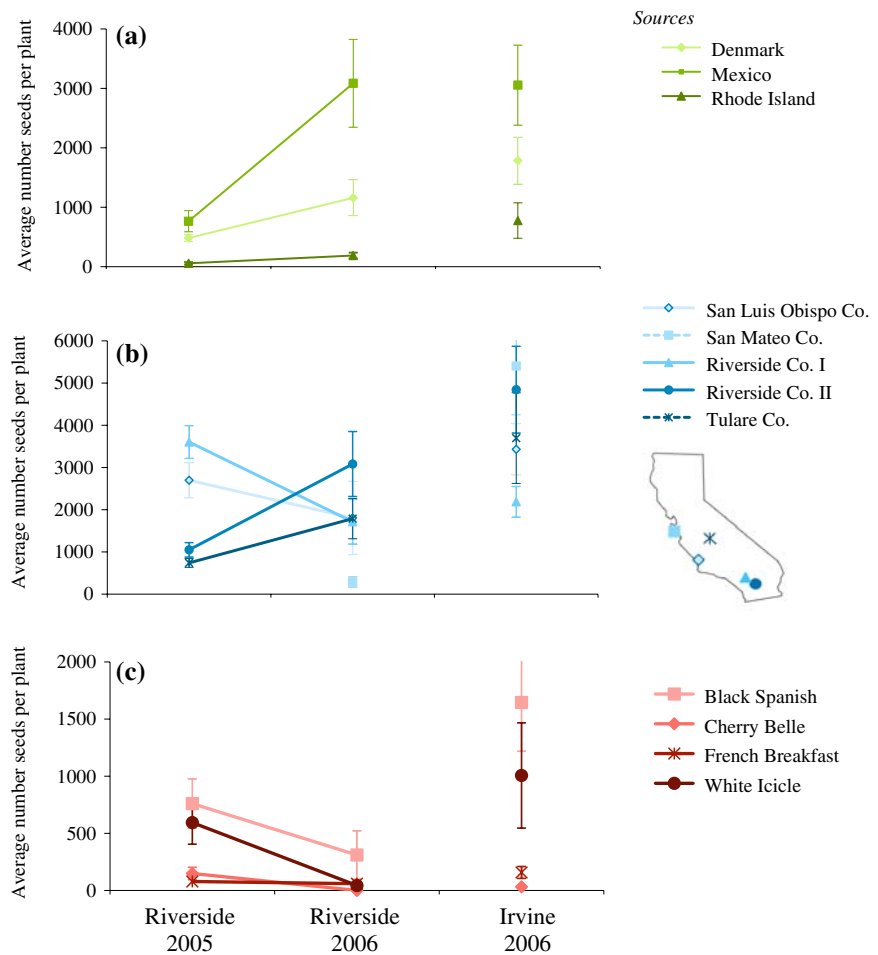
Cumulative fitness

Using a multiplicative fitness function, we found that California wild radish had a significant fitness advantage over both progenitor species in all site-years (Table 5). Even in Riverside 2006 when survival and reproduction were similar between California wild radish and *R. raphanistrum*, the cumulative fitness of California wild radish exceeded that of its progenitor by 20%.

Discussion

In this study, we demonstrate that a hybrid-derived, invasive lineage of plants can have a higher rate of survival to reproduction, higher reproductive output and higher overall cumulative fitness than its genetically pure progenitors. At the Riverside field site in both years, hybrid-derived California wild radish displayed enhanced survival relative to its

Fig. 3 Individual average lifetime maternal reproductive output \pm 1 SE for each population of **a** *R. raphanistrum*, **b** California wild radish and **c** cultivars. Note that each graph is on a different y-axis scale



progenitors, and in Riverside in 2005 and Irvine in 2006, hybrid-derived California wild radish produced significantly more fruits per plant than either pure progenitor species. At Irvine in 2006, the hybrid-derived lineage also produced significantly more seeds. In all environments, California wild radish showed enhanced cumulative fitness. Since the introduction of *Raphanus* to California, the hybrid-derived lineage has greatly expanded its range throughout the state, replacing its exotic progenitors (Hegde et al. 2006). We suggest that the evolution of enhanced survival and reproduction in this lineage may be a mechanism for the success of the hybrid genotypes.

Our conclusions are based on field experiments where we supplied additional water and controlled herbivory. These ‘altered’ conditions are representative of at least a portion of environments that the progenitors originally colonized and that the hybrid-

derived lineages have since spread to and continue to exploit successfully. Such environments include disturbed agricultural field margins and roadside ditches. We also argue as Baker (1974) did, that the ability of hybrid-derived California wild radish to take greater advantage of favorable conditions than either of its two progenitor parents is a hallmark of a successful weedy invader. We do not exclude the possibility that the differences in survival and rates of flowering favoring hybrid-derived California wild radish that we observed would be more pronounced had unaltered conditions prevailed.

The results we obtained using hybrids created naturally in situ are consistent with the results from studies using advanced generation artificial *Raphanus* hybrids grown in Michigan and California. Replicated populations of F_1 hybrids between *R. raphanistrum* and cultivated radish were allowed to evolve in old fields in northern Michigan for three

Table 5 A comparison of total relative fitness between California wild radish and its progenitors in each site-year

Site-year	Species	<i>N</i>	Proportion survival to reproduction	Relative survival to reproduction (%)	Average seed number	Relative fecundity (%)	Total relative fitness (%)
Riverside 2005	CA wild	150	0.99	100	2,011	100	100
	<i>R. raphanistrum</i>	200	0.92	93	501	25	23**
	Cultivated	200	0.68	69	428	21	15**
Riverside 2006	CA wild	53	0.98	100	1,803	100	100
	<i>R. raphanistrum</i>	97	0.85	87	1,650	92	79**
	Cultivated	76	0.53	54	129	7.2	3.9**
Irvine 2006	CA wild	56	1	100	3,886	100	100
	<i>R. raphanistrum</i>	98	1	100	1,892	49	49**
	Cultivated	79	0.87	87	777	20	17**

All relative rates are normalized to the highest value within a site-year. Relative fecundity is average for all individuals that survived to reproduce

** $P < 0.001$ according to Fisher's exact test

seasons (three generations), and then a sample of the resulting seed was grown in common gardens in northern Michigan and Riverside, California (Campbell et al. 2006). Hybrid-derived lineages had reduced seed production compared to pure *R. raphanistrum* populations in Michigan but produced 250% more seed than *R. raphanistrum* in California. In the present experiment, we also found a significant reproductive advantage of hybrid-derived *Raphanus* in California and the advantage was somewhat environmentally dependent, as highlighted by the significant species \times year interaction effect on average number of seeds produced per plant.

Artificial hybridization can contribute to our understanding of hybridization and invasion by recreating genotypes involved in relatively early post-hybridization scenarios and has the benefit of being able to control genetic history (e.g., Burke and Arnold 2001; Cummings et al. 2002; Lexer et al. 2003). In contrast, the natural hybrids in our experiment represent genotypes up to 150 generations away from the original interspecific hybridization event and have a vastly more complicated genetic history and present genetic constitution. These particular features of our study system have several implications. First, the relative fitness advantage of hybrid-derived *Raphanus* in certain environments is likely not only determined by its hybrid genetic makeup, but also by other evolutionary forces that have acted on the lineage in the last century and a half. While hybridization can create individuals with

an immediate reproductive advantage over their progenitors due to heterosis, continued recombination in the hybrid lineages coupled with natural selection on the resulting diverse and unique hybrid genotypes could also result in the overall adaptive evolution. In *Raphanus* in California, hybridization may have been responsible for initial unique and beneficial combinations of traits and alleles, while natural selection acted in subsequent generations to adaptively 'fine-tune' its reproductive success. Such a situation is plausible given that California wild radish is phenotypically and genetically distinct from its progenitors (Hegde et al. 2006) and that, with its high genetic variability, obligately outcrossing breeding system and annual habit, California wild radish is also potentially highly capable of rapidly responding to natural selection.

The second, challenging part of working with natural hybrids derived over many decades is that their genetic history may be complicated with multiple possible parental source populations, multiple hybridization events and different patterns of inter-mating, backcrossing, and recombination. These complications may make experiments comparing progenitors and derivatives difficult to interpret. The essential question with regards our experiment is, have we chosen the most appropriate groups of plants to compare so that we can draw conclusions about hybridization and invasion of *Raphanus* in California. There is ample evidence that California wild radish is descended from *R. raphanistrum* and

cultivated radish (Hegde et al. 2006; Panetsos and Baker 1967; Ridley et al. 2008), so that we can be sure we have at least included the correct progenitor species in the experiment. Unfortunately, more detailed information on the sources of introduction of the two progenitors is still lacking. Isozyme evidence confirms that the cultivars chosen here show high genetic similarity to some California wild radish individuals (Ellstrand and Marshall 1985), while recent cpDNA suggests that at least one introduction of *R. raphanistrum* came from northern Germany, making our inclusion of the nearby Danish *R. raphanistrum* population appropriate (Ridley et al. 2008). Populations of pure *R. raphanistrum* and pure cultivated radish (or feral cultivated radish) from California might also have been a good basis of comparison, but as mentioned previously, all such pure populations are now extinct. Thus, we could not compare the traits of progenitor genotypes and derived genotypes with much specificity. Instead, we took the next best approach, measuring the fitness of a broad sampling of several cultivars, multiple potential sources of *R. raphanistrum* and several hybrid-derived populations from California. Even with the large differences between populations within species that we observed using this broad sampling approach, we were able to show a significantly enhanced fitness in the hybrid lineage in multiple environments.

Other field experiments that explicitly compare the fitness of hybrid invasive lineages derived in situ with that of progenitors are rare. In Germany, natural hybridization between an exotic and a native species of *Rorippa* has resulted in weedy hybrids with fitness equal to or lower than their parents, depending on environment (Bleeker and Matthies 2005). A study of *Carpobrotus edulis*, *C. chilensis* and their invasive natural hybrids in California revealed that hybrids consistently survive better and clonally outgrow their *C. chilensis* parent but not *C. edulis* and, again, that this result can vary across environments (Vila and D'Antonio 1998). These results and our own share the pattern that relative fitness varies with the specific environment. Collectively, they suggest the hybridization-invasion relationship may be idiosyncratic with the taxa and environments involved.

The proximate basis of high reproductive output of hybrid-derived lineages of *Raphanus* in California is not entirely clear and is not necessarily consistent

between sites or through time. In Riverside 2005, it appears that *R. raphanistrum*-like early flowering and an intermediate size at flowering favored higher maternal reproductive success in California wild radish, while in Irvine 2006, an intermediate flowering date and large plant size at flowering correlated with higher fitness in California wild radish. This difference may be due to the later planting in 2005 as compared to 2006. In Michigan a third pattern emerges; selection tends to favor increased size at reproduction and advanced onset of reproduction in artificial, hybrid-derived lineages (Campbell and Snow 2007). Given that California wild radish is uniquely different from either of its progenitor parents by a complex suite of traits (Hegde et al. 2006) and that selection for trait values can vary significantly over space and time, it is perhaps not surprising that the reproductive success of California wild radish is not caused by a single trait or condition. Rather, it could be the diversity in life histories and vegetative-reproductive allocation strategies that can generally account for the hybrids' success.

Biologists have debated whether population-level phenotypic and genetic diversity confer increased colonization success in weeds (Baker 1974; Baker and Stebbins 1965) or increased invasiveness in introduced species (Ellstrand and Schierenbeck 2006). Studies of California wild radish reveal the presence of high genetic diversity in this lineage (Ellstrand and Marshall 1985; Hegde et al. 2006; Ridley et al. 2008). In this experiment, we also show that genotype \times environment interactions characterize the performance of hybrid-derived populations, more so than populations of progenitor parent populations, evidence that hybrid populations maintain between population genotypic and phenotypic diversity. Population-level genetic diversity is a characteristic of a group, not of an individual, however, and as such is not an adaptive trait that can evolve by natural selection. It is a trait, however, that may be enhanced by gene flow (either intra- or interspecific gene flow) and could play a positive role in establishment and/or spread of an invasive species by providing the material upon which natural selection can act to create locally adapted populations, as Baker (1974) suggested. Our future studies of California wild radish will test for the presence of local adaptation and the role it may or may not play in the invasiveness of this lineage.

Conclusions

Here, we present evidence for the evolution of enhanced survival and reproduction in a hybrid-derived invasive lineage. Our results imply, first of all, that using the precautionary principle and limiting the introduction of close congeners or species that have a close congener at the site of introduction could prevent the evolution of more invasive lineages. Second, evolutionary processes should be considered as a possible route to invasion, especially when available ecological explanations fail to fully explain the success of an introduced species. Unfortunately, the difficult reality is that evolving invasive species are moving targets. These populations shrink or grow, expand and adapt, and that means, if we value the communities that invasive species are irrevocably altering, we must understand and appreciate their evolutionary potential.

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