

LETTER

Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment

Lesley G. Campbell,^{1*} Allison A. Snow¹ and Caroline E. Ridley²

¹Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH 43210, USA

²Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124, USA

*Correspondence: E-mail: campbell.633@osu.edu

Abstract

Crop-wild hybridization may produce offspring with lower fitness than their wild parents due to deleterious crop traits and outbreeding depression. Over time, however, selection for improved fitness could lead to greater invasiveness of hybrid taxa. To examine evolutionary change in crop-wild hybrids, we established four wild (*Raphanus raphanistrum*) and four hybrid radish populations (*R. raphanistrum* × *Raphanus sativus*) in Michigan (MI), USA. Hybrid and wild populations had similar growth rates over four generations, and pollen fertility of hybrids improved. We then measured hybrid and wild fitness components in two common garden sites within the geographical range of wild radish [MI and California (CA)]. Advanced generation hybrids had slightly lower lifetime fecundity than wild plants in MI but exhibited *c.* 270% greater lifetime fecundity and *c.* 22% greater survival than wild plants in CA. Our results support the hypothesis that crop-wild hybridization may create genotypes with the potential to displace parental taxa in new environments.

Keywords

Artificial populations, common garden, crop-wild hybridization, fitness, genetic engineering, genotype by environment interaction, pollen fertility, population dynamics, *Raphanus raphanistrum*.

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INTRODUCTION

Weed populations can evolve rapidly when confronted with novel environments (e.g. Clements *et al.* 2004), and a better understanding of the mechanisms and rates of weed evolution could aid in limiting or at least anticipating this process. Hybridization may contribute to adaptive evolution, and specifically weedy plant evolution, in two ways (e.g. Anderson & Stebbins 1954; Ellstrand & Schierenbeck 2000). First, hybridization may generate novel adaptations via transgressive segregation. When segregating hybrids exhibit extreme phenotypes, rapid and adaptive phenotypic shifts may enhance the fitness of weedy hybrid lineages in certain environments (Rieseberg *et al.* 1999; Lexer *et al.* 2003a). Second, hybridization may transfer adaptations that could lead to range expansions and extensive weed invasions (e.g. Ellstrand & Schierenbeck 2000) and/or increased fecundity and weediness of local populations (Ellstrand *et al.* 1999; Snow *et al.* 2003).

Many studies have attempted to quantify the fitness implications of hybridization between crops and weeds (reviewed by Ellstrand 2001; Hails & Morley 2005). However, inferences about the fitness of advanced hybrid generations often remain tentative because most experimental studies have used F₁ hybrids, which may exhibit transient heterosis or hybrid breakdown (Arnold & Hodges 1995; Burke & Arnold 2001; Lexer *et al.* 2003b; Rhode & Cruzan 2005). Ideally, experiments should evaluate fitness components of F₂- and later-generation hybrids under natural conditions. Such experiments may provide better predictions of crop allele persistence in wild populations because they incorporate the effects of natural selection, while accounting for partial genetic barriers to introgression such as outbreeding depression (Burke & Arnold 2001).

The development and adoption of transgenic crops with novel fitness-related traits has raised awareness of the potential for increased weediness after crop-wild

hybridization (Ellstrand 2003; Pilson & Prendeville 2004; Snow *et al.* 2005). Although some argue that the introgression of domesticated traits creates maladapted crop-wild hybrids with low fitness (e.g. Stewart *et al.* 2003), conventional crop alleles are known to persist in weed populations (e.g. canola: Hansen *et al.* 2001; sunflower: Whitton *et al.* 1997; radish: Snow *et al.* 2001). Theory predicts such alleles will become common if they provide a fitness advantage or rare if they are deleterious (e.g. Barton 1993; Haygood *et al.* 2004). The introgression of single-gene transgenic traits, such as herbicide tolerance, insect resistance and disease resistance, may lead to even greater fitness advantages in hybridizing populations than conventional crop traits (Davis *et al.* 1999; Desplanque *et al.* 2002; Snow *et al.* 2003). Therefore, it is important to understand the fitness implications of crop gene introgression into natural weed populations over many generations. However, replicated, long-term experiments that test for fitness consequences of crop-to-weed introgression are generally lacking. As a result, it is currently difficult to predict whether the introgression of conventional crop traits (much less transgenic traits) could lead to the evolution of weedier populations (Snow *et al.* 2003).

Here, we present the first long-term, experimental study of naturally evolving crop-wild hybrid populations. We established artificial populations of wild and hybrid radishes in agricultural fields in northern Michigan (MI), USA, in 2002. For 4 years, we quantified their population growth rates and documented pollen fertility and flower petal colour evolution under natural conditions. We then compared lifetime fecundity of F_1 and advanced generation hybrids relative to non-hybridized wild radish in a local common garden experiment in MI. In addition, we measured relative fecundity of wild radish and advanced generation hybrids in

southern California (CA), representing a disparate location within the geographical range of wild radish (Fig. 1). Our results demonstrate the value of following the evolution of constructed weed populations to evaluate the role of hybridization in adaptive evolution, and the importance of measuring fitness components in multiple locations to assess potential ecological risks.

METHODS

Study system

Raphanus raphanistrum (wild radish or jointed charlock) is a cosmopolitan, annual weed of Eurasian origin that occurs in agricultural fields, disturbed areas and coastal beaches (Holm *et al.* 1997; Snow & Campbell 2005). With its long-lived seed bank, high genetic variability, and early emergence after soil disturbance, *R. raphanistrum* is a difficult weed to manage, especially in cereal crops (Warwick & Francis 2005). The cultivated relative of wild radish, *Raphanus sativus*, is an open-pollinated crop often selected for large, colourful roots, delayed flowering and high levels of seed production. Long-distance seed dispersal of weedy radish seeds is common (Kercher & Conner 1996), potentially allowing hybrid genotypes to reach novel environments.

Both *R. raphanistrum* and *R. sativus* are self-incompatible, insect-pollinated and interfertile (Warwick & Francis 2005). Cultivated and wild radish species were introduced into CA by the 19th century, and Panetsos & Baker (1967) suggested that 'introgression of *raphanistrum* characters appears to have been a major factor in converting the erstwhile crop plant, *R. sativus*, into a highly successful weed'. In fact, descendants

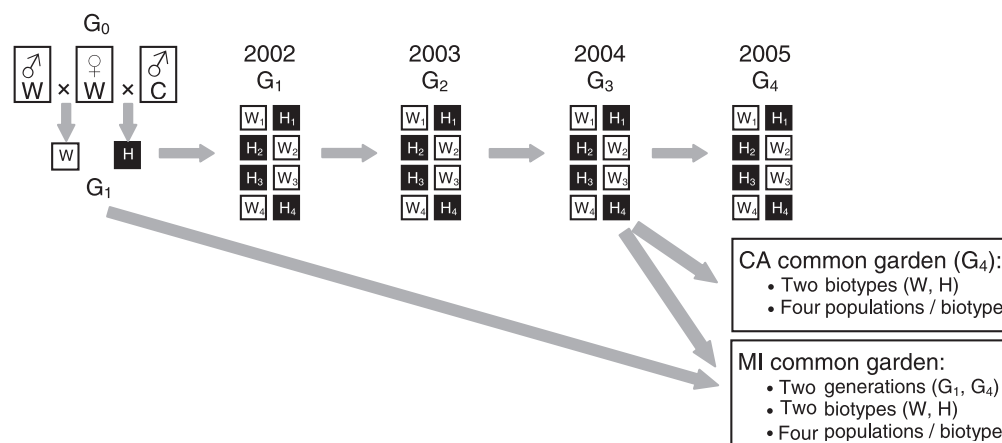


Figure 1 Schematic diagram of the experiment. The first-generation (G_1) was created by cross-pollinating wild plants (W) with either wild or cultivated (C) radish pollen to create wild and hybrid (H) biotypes. Eight isolated field populations of wild biotypes (W_1 – W_4) or hybrid biotypes (H_1 – H_4) were maintained for 4 years; small squares represent populations of the two biotypes. In 2005, common gardens in Michigan and California were composed of G_4 plants from each population. The Michigan common garden also included plants representing G_1 founders of the eight populations.

of crop-wild radish hybrids, known as wild or feral *R. sativus*, appear to have displaced the original populations of *R. raphanistrum* in CA during the past few decades (Snow *et al.* 2001; Hedge *et al.* 2006).

Raphanus has emerged as a model system in plant evolutionary ecology and has been particularly useful for evaluating the ecological consequences of crop-to-wild gene flow (e.g. Klinger & Ellstrand 1994; Snow *et al.* 2001; Hedge *et al.* 2006), although there are no immediate plans to create transgenic radish varieties (Biosafety Clearing-House, <http://bch/bch.biodiv.org/>). Flower petal colour frequencies differ between species and can be used as a crop-specific marker (Snow *et al.* 2001). *Raphanus sativus* has white, pink or purple flowers whereas *R. raphanistrum* generally has yellow flowers or more rarely, white, pink or bronze flowers (Panetsos & Baker 1967; Kay 1976; Kercher & Conner 1996). White flower colour exhibits simple Mendelian dominance over yellow carotenoid pigment (Panetsos & Baker 1967). The genetic basis of pink hues is more complex (Stanton 1987), so this trait was not used as a genetic marker in the current study.

Experimental approach

To investigate ecological and evolutionary consequences of crop-wild hybridization, we created replicated artificial populations of wild and hybrid radishes in agricultural fields in MI, in 2002. We measured changes in population size, pollen fertility and flower petal colour frequencies under natural conditions over 4 years. To quantify evolutionary changes in fitness components, we grew wild plants, F₁ hybrids and advanced generation hybrids in a common garden experiment in northern MI in 2005. In addition, to determine the importance of environmental context to relative fitness of advanced generation hybrids compared with wild plants, we measured their survival and fecundity in a novel and distant location in southern CA, within the geographical range of weedy radishes (Fig. 1).

Seed sources for replicated populations

In 2001, we haphazardly collected seeds from several hundred plants in a natural population of wild *R. raphanistrum* plants (homozygous for the recessive yellow petal colour allele) in an agricultural field in Pellston (MI, USA). In a greenhouse at Ohio State University, we hand-pollinated 100 wild plants with either wild pollen to create F₁ wild biotypes or crop pollen to create F₁ hybrid biotypes. Crop pollen was harvested from 100 'Red Silk' *R. sativus* plants (Harris-Moran Seed Co., Modesto, CA, USA), a common, contemporary variety that is homozygous for white petal colour (as in Snow *et al.* 2001). Below, we refer to radish

biotypes as 'wild' or 'hybrid' based on hybridization in this first generation.

Establishment of replicated populations in Michigan

In 2002, we established four first-generation wild populations (W₁, W₂, W₃ and W₄) and four first-generation hybrid populations (H₁, H₂, H₃ and H₄) in agricultural fields in Emmett and Cheboygan counties, MI (Fig. 1). To restrict unintended gene flow, these eight populations were separated from each other and from local wild radish populations by at least 1 km. First, wild and hybrid seeds were planted in PRO-MIX 'BX' peat (Premier Horticulture Ltd, Rivière-du-Loup, Canada) in Jiffy fibre pots (Jiffy Products of America, Inc., Norwalk, CT, USA) in May 2002, in a greenhouse at the University of Michigan Biological Station (UMBS, Pellston, MI, USA). Three weeks later, each population was started by planting 50–60 seedlings in a recently tilled 15 × 15-m plot fertilized with slow-release Osmocote (19N-6P-12K, 22.7 kg/site; Scotts Miracle-Gro Co., Marysville, USA). No resident wild radish plants emerged from the seed bank at these plots. The number of surviving experimental plants that reproduced at each plot was > 42. Each spring through 2005, the plots were tilled, fertilized and hand-weeded for *c.* 2 weeks to simulate agricultural management and to promote population persistence. Otherwise, the populations were exposed to naturally occurring weather conditions, competing plants, herbivores, pathogens and pollinators (primarily native bees, syrphid flies and honey bees; as in Lee & Snow 1998).

Yearly surveys of replicated populations in Michigan

We estimated population size, frequency of white-flowered plants and pollen fertility of each population annually. Estimates of population size were based on direct counts when < 1000 plants were present or subsampling when populations were larger. For the latter, we determined the average number of plants in 49, 1-m² quadrats per site and multiplied this value by the total area. Annual population growth (*r*) was calculated as the difference in natural log-transformed population size (*N*) for year_{*t*} and year_{*t*-1}.

As in Snow *et al.* (2001), flower colour provided a crop-specific genetic marker. During peak flowering (25th June to 4th July), we estimated the proportion of plants with white flowers in each hybrid population. White-flowered plant frequencies were based on direct counts or subsamples as described above. We grouped pink-flowered individuals with white-flowered plants and pink–yellow-flowered individuals with yellow-flowered plants (as in Snow *et al.* 2001).

Interspecific hybrids between *R. raphanistrum* and *R. sativus* are heterozygous for a reciprocal translocation that affects chromosome pairing during meiosis (Panetsos & Baker

1967). Typically F_1 crop-wild hybrids produce *c.* 50–60% aborted pollen grains (Panetsos & Baker 1967; Snow *et al.* 2001). To monitor pollen fertility evolution, we collected pollen from one flower on each plant at each site in 2002. In 2003 and 2004, we divided each population into 10 parallel transects and sampled 10 plants at equidistant intervals along each transect. In 2005, pollen was collected from a random subsample of plants involved in the MI and CA common gardens (see below). After staining (Alexander 1969), pollen fertility was assessed using a compound microscope to count the proportion of aborted grains in samples of at least 100 grains per plant.

To test whether population growth rates and evolutionary trajectories for flower colour frequency and pollen fertility were similar among hybrid populations over 4 years, we ran a Type III repeated-measures ANOVA in which population was a fixed effect and year was the repeated measure. We defined population as a fixed effect to make explicit comparisons among populations within biotypes (defining population as a random effect did not change our conclusions). Both pollen fertility and white flower colour frequency were arcsine square root transformed to normalize data, and population growth rates were normally distributed.

Common garden experiments

In 2005, we measured the lifetime fecundity of individuals from the four wild and four hybrid populations in two common gardens (Fig. 1). The MI common garden was located at UMBS in Pellston, MI (42°35' N, 84°42' W) and the CA common garden was located at the Agricultural Experiment Station of the University of California at Riverside in Riverside, CA (33°58' N, 117°17' W). The gardens differed most conspicuously in their growing season and moisture availability, with less water available to plants grown in CA (see Table S1 for a summary of differences). In addition, MI plants were grown in open-bottomed pots with two competing oat plants in each pot; in CA, however, plants were grown directly in local soil with 30 cm between plants (see below). Therefore, differences in the results from the two common gardens could be due to both local abiotic and biotic conditions, as well as differences in below- and above-ground competition environments. At both sites, plants were arranged in a randomized, complete block design.

Common garden seed sources

In 2004, we collected seeds from the four wild and four hybrid populations for the common garden experiments. Because radish seeds may remain dormant for several years, we cannot assume that each population was composed of only one generation of hybrids (e.g. all F_4). Therefore, we refer to each year's population as G_1 , G_2 , G_3 and G_4

(Fig. 1), recognizing that each generation beyond G_1 (F_1) may represent a mixture of earlier and later generations. G_4 seeds for the common garden studies were collected directly from G_3 plants. We collected one seed per fruit from six fruits on each of 30 plants in each wild population, and 12 fruits per plant from 30 plants in each hybrid population. We used twice as many seeds from hybrids because we expected their pollen fertility to be variable, due to the reciprocal translocation, and this could obscure fitness differences between biotypes. Seeds were equally divided between the two common gardens.

Michigan common garden

This experiment included two individuals per G_4 wild population and four individuals per G_4 hybrid population in each of 21 blocks, resulting in 504 G_4 plants. The garden also included 42 G_1 wild plants and 42 G_1 hybrids, two plants per biotype per block, to determine the fitness differences among wild and hybrid biotypes during the first generation.

Seeds were planted in 300 mL of PRO-MIX 'BX' peat in Jiffy fibre pots in a greenhouse at UMBS in early May 2005. Four cultivated spring oat seeds (*Avena sativa*, Blaskowski's Feed and Seed, Cheboygan, MI, USA) were included in each fibre pot to provide a uniform level of competition, and oat density was thinned to two seedlings per pot. The garden area at UMBS was cleared of vegetation, levelled and rototilled twice. After the seedlings developed their first true leaves, each fibre pot was transplanted into a polyvinyl chloride bottomless tube pot (46-cm tall) filled with 1.7 l of local sandy soil surrounding the fibre pot, allowing plant roots to grow into local soil. Pots were separated by 30 cm and the use of large tube pots minimized root competition among neighbours. Seedlings that died within the first week after transplanting were replaced. Plants were watered daily for the first month and every other day until 31 August. On 18 June, 13 mg of fertilizer (Slow-release Osmocote) was added to each pot because the local soil was sandy and nutrient poor. Insecticide (0.0033% esfenvalerate, 20 g/9.5 L, Scotts Miracle-Gro Co.) was used to control insect herbivory three times during the first month after transplantation, when herbivory was highest. Aphids were present at low densities later in the season but did not colonize any plant heavily. Pollinators were abundant throughout the experiment, as in Lee & Snow (1998). Plants were individually harvested as they senesced, until the first hard frost (16–20 September), when we harvested all remaining plants. Harvested radish and oat plants were dried at 60 °C.

California common garden

The CA garden included 10 randomized blocks of five individuals per G_4 wild population and 10 individuals per G_4 hybrid population, totalling 600 individuals. On 31

December 2004, seeds were planted in individual cells containing 22.7 cm³ of dry, sterilized UC Soil Mix III, a sand/peat moss mix supplemented with micronutrients (Blackmore 128DS Flats; Blackmore Co., Belleville, USA; Matkin & Chandler 1967). The flats were placed in a greenhouse and were watered with a dilute nutrient solution (100 p.p.m., 21-5-20 Peters EXCEL with N; Grace-Sierra Horticultural Products Company, Milpitas, CA, USA). At the two-leaf stage, seedlings were transplanted into a tilled field at 30-cm intervals in blocks of six rows with 10 plants per row at the University of California Riverside Agricultural Experiment Station. Neighbouring radish plants may have experienced some root competition in this experiment.

Seedlings were watered every other day for the first week to promote survival and the plot received overhead irrigation once a week during weeks without rain (8 March to 2 May). To prevent birds from damaging radish fruits, we constructed an enclosure of metal fence posts with cables to support nylon bird netting. The netting was 19-cm mesh, allowing pollinators but not birds into the enclosure (note: bird predation was not observed in MI). Pollinators were abundant. Blocks were hand-weeded and sprayed with 0.0033% esfenvalerate to control flea beetles on 8th March and with Crymax (2.1 g L⁻¹; Ecogen Inc., Langhorne, PA, USA) to control diamondback moth larvae on 21 March. Plants were harvested and dried after the majority had senesced (10–14 June).

Measurement and analyses of fitness components

For each common garden, we recorded survival, pollen fertility, flower number, seed production and aboveground vegetative biomass per plant. A rank-sum test was used to compare proportion of plants that survived and flowered at each site. Plants that died before anthesis or did not flower before the end of the experiments were removed from the following analyses. During the field season, we estimated pollen fertility for a subset of plants from each garden, as described above. For harvested plants, we counted numbers of flower pedicels and fruits per plant. Fruit set was calculated as number of fruits produced divided by number of flowers. To estimate the number of seeds per plant, we multiplied the average number of seeds per fruit (for 10 randomly chosen fruits per plant) by the number of fruits. Percent fruit set was normally distributed and required no transformations prior to analysis. However, number of seeds per plant and number of flowers per plant were natural log transformed, and pollen fertility data were arcsine square root transformed. Transformations were similar for both common gardens, but for CA the residuals of the number of seeds per plant was normally distributed when log₁₀ transformed. All analyses were performed using spss (v.13; SPSS Inc., Chicago, IL, USA).

To test for differences in lifetime fecundity between G₁ wild and hybrid plants, we ran a linear mixed model ANOVA for each fitness component. Biotype was considered to be a fixed effect and block was a random effect. Variance of random effects was estimated using restricted maximum likelihood.

To test for differences in lifetime fecundity between G₄ wild and hybrid plants grown in the two locations, we used a linear mixed model ANOVA. After detecting a biotype by garden interaction, we ran separate analyses for each garden. For each G₄ common garden, we ran a linear mixed model ANOVA for each fitness component. The unbalanced nested ANOVA included biotype and population within biotype as fixed effects and block as a random effect. Variance of random effects was estimated using restricted maximum likelihood.

RESULTS

Growth rates of wild and hybrid populations

Population growth rates did not differ significantly between wild and hybrid populations between 2002 and 2005 ($F_{1,4} = 0.62$, $P = 0.48$; Fig. 2). In the second year, population growth rate was significantly greater than the third and fourth years for both biotypes ($F_{2,8} = 20.48$, $P = 0.001$), when population sizes reached $c.$ 13 000–109 000

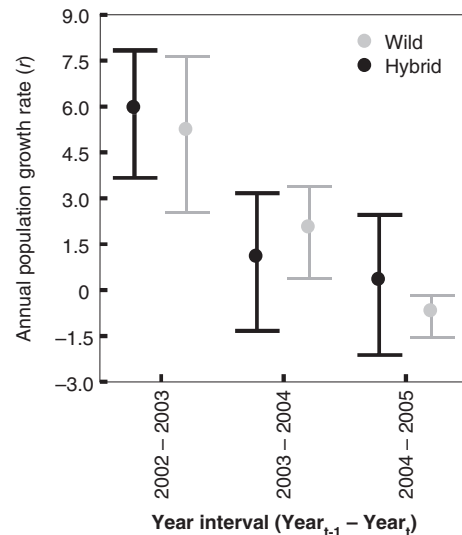


Figure 2 Annual population growth rate (r) of four wild and four hybrid populations grown in isolated agricultural fields in Michigan over three 1-year intervals ($r = \ln(N_t) - \ln(N_{t-1})$, where N_t is population size in year t and N_{t-1} is population size in the preceding year $t - 1$). Error bars represent 95% CI of the mean ($N = 4$).

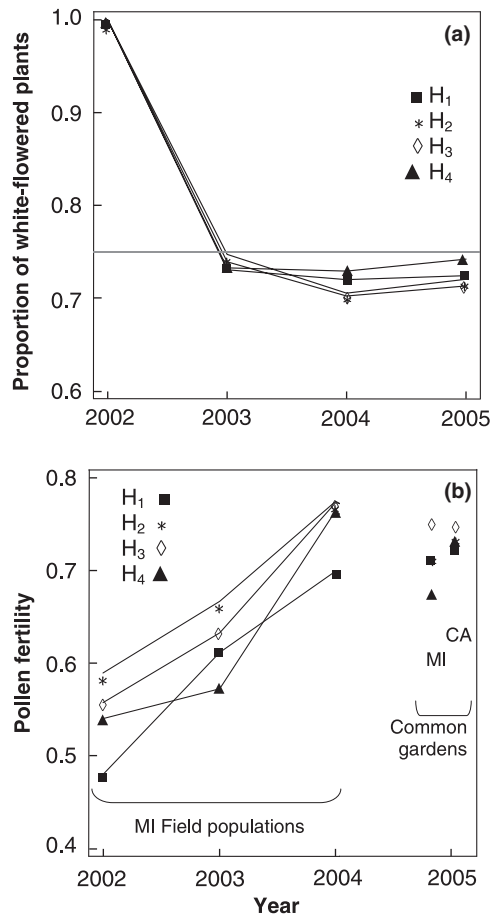


Figure 3 Parallel evolution of hybrid populations (H₁–H₄) over 4 years (2002–2005). (a) Average frequency of white-flowered plants ($N = 1340$ – 4277). Reference line at 0.75 is the null Hardy–Weinberg expectation (after the G₁ generation) of white flower colour frequencies. (b) Average proportion of fertile pollen (sample sizes as in Table 2). From 2002 to 2004, pollen was collected from plants from the artificial field populations. In 2005, pollen was collected from the common garden experiments in Michigan and California. See Table 2 for fertility of wild pollen for G₁ and G₄ plants.

plants. The interaction between year and biotype was not significant ($F_{2,8} = 1.04$, $P = 0.40$).

Evolution of flower colour and pollen fertility in hybrid populations

G₁ hybrid populations had white (or pink) flowers because white is dominant over yellow and the crop parents were homozygous for the white allele. Under Hardy–Weinberg assumptions, we expected subsequent generations to be composed of 75% white-flowered plants and 25% yellow-flowered plants and we found this to be true (Fig. 3a). After the G₁ generation, colour composition remained statistically

constant across populations ($P = 0.30$) and years ($P = 0.37$), with no interaction between population and year ($P = 0.68$).

G₁ hybrid populations had lower pollen fertility than wild plants and their pollen fertility increased over generations (Fig. 3b). Hybrid pollen fertility increased significantly over 4 years ($P = 0.008$) and differed significantly among populations ($P < 0.001$) because one hybrid population (H₁) had significantly lower pollen fertility than H₂ and H₃ (Table 2). There was no year by population interaction for pollen fertility ($P = 0.13$), suggesting that, although pollen fertility differed among populations, the evolutionary trajectory of pollen fertility did not.

Relative fitness of G₁ crop-wild hybrids in Michigan common garden

All G₁ wild and hybrid plants survived to flower. In the garden, G₁ hybrids had 22% lower pollen fertility than G₁ wild plants ($F_{1,58} = 68.2$, $P < 0.001$; Fig. 4, Table 2). However, G₁ hybrid seed production did not differ significantly from that of wild plants ($F_{1,20} = 0.31$, $P = 0.59$). G₁ hybrids had 12% lower fruit set compared with wild plants ($F_{1,83} = 17.4$, $P < 0.001$), 15% fewer seeds per fruit (Table 2 and Table S2) and produced *c.* 170% more flowers than wild plants ($F_{1,63} = 17.4$, $P < 0.001$). Therefore, although G₁ hybrids had lower fruit set than wild plants, they had similar seed production, perhaps due to their greater flower production (Fig. 4). Further, although G₁ hybrids produced 300% more aboveground biomass than wild plants, the companion oat plants growing with G₁ wild and hybrid plants did not differ in biomass (Table 2 and Table S2).

Relative fitness of advanced generation hybrids in common gardens

Environmentally dependent G₄ hybrid fecundity

The combined ANOVA of the G₄ data sets of wild and hybrid plants revealed significant biotype by garden interactions for number of seeds per plant ($P = 0.002$), number of flowers per plant ($P = 0.003$), and percent fruit set ($P = 0.003$), but not for pollen fertility ($P = 0.702$; Table S3). Given the significant interaction, these differences were explored for each common garden experiment in the following analyses.

Michigan common garden

Within the MI garden, G₄ biotypes did not differ in proportion of plants that survived to flower (Fig. 4). Within each biotype (wild vs. hybrid), populations were not significantly different in pollen fertility, number of seeds per plant, percent fruit set or number of flowers per plant

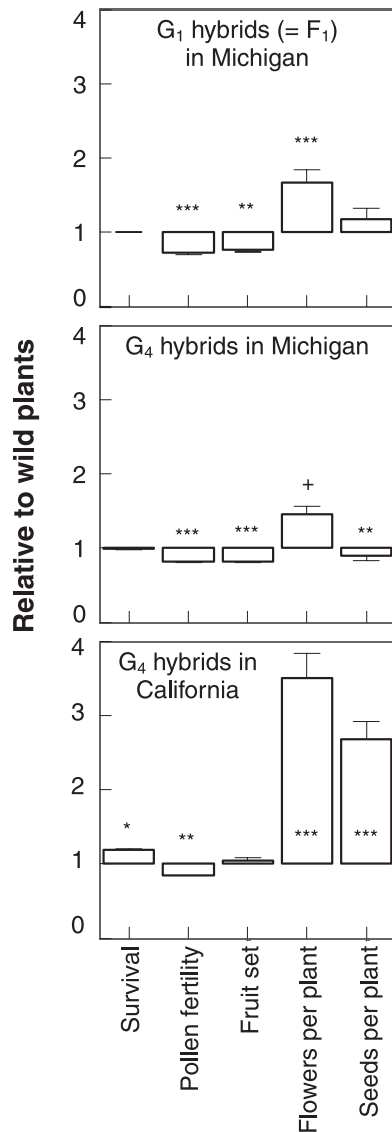


Figure 4 Relative survival and fecundity of wild and hybrid plants grown in two common gardens including G₁ (F₁) hybrids and fourth-generation (G₄) hybrids from the Michigan common garden, and G₄ hybrids from the California common garden. Hybrid trait values were standardized such that wild plants have an average fitness value of unity (reference line). Bars show mean values of mean relative success within experimental blocks; error bars represent ± 1 SE. To indicate significant differences between wild and hybrid fitness, + $P = 0.052$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on ANOVAs (Table 1). Analysis for survival: $N_{\text{Wild}} = 4$, $N_{\text{Hybrid}} = 4$, Michigan – Mann–Whitney U -statistic = 4.0, $P = 0.34$; California – Mann–Whitney U -statistic = 0.000, $P = 0.029$).

(Tables 1 and 2). Therefore, the mean fecundity values summarized below are based on pooled data from the four populations of each biotype.

G₄ hybrids had 15% lower pollen fertility than wild plants (Fig. 4, Tables 1 and 2). Hybrids produced $\approx 11\%$ fewer seeds than wild plants, despite the fact that they produced ≈ 1.5 times more flowers per plant (Fig. 4). Lower fecundity in hybrids was apparently due to lower fruit set than wild plants and fewer seeds per fruit. Hybrids produced 214% more aboveground biomass than wild plants (Table S2), but the biomass of competing oat plants did not differ significantly between biotypes (Table 2).

California common garden

Biotypes differed in several important ways in the CA garden. In CA, G₄ hybrids were 22% more likely to survive to reproduce than wild plants (Fig. 4). Again, within biotypes, there was no significant difference among populations in pollen fertility, number of seeds per plant, percent fruit set or number of flowers per plant (Tables 1 and 2). Unlike the MI garden, G₄ hybrids produced more than twice as many seeds and four times more flowers than wild plants, and G₄ hybrids and wild plants had similar percent fruit set and number of seeds per fruit (Fig. 3, Tables 1 and 2). Similar to the MI garden, G₄ hybrids exhibited significantly lower pollen fertility than wild plants and produced 253% more aboveground biomass than wild plants (Table S2).

DISCUSSION

Evolutionary consequences of hybridization

Our results suggest that crop-wild hybridization can create opportunities for increased fitness by generating evolutionary changes that are advantageous in new environments. Specifically, hybrid lineages had 22% greater survival and produced $\approx 270\%$ more seeds per plant relative to wild lineages in CA. In MI, where they originated and evolved for three generations, hybrids had similar survival to wild plants and produced $\approx 11\%$ fewer seeds per plant. Although we cannot identify the reasons for these differences (Table S1), we were surprised by the strength of genotype by environment interactions on lifetime fecundity. These unexpected consequences of hybridization are consistent with the hypothesis that the evolution of weeds can be stimulated by hybridization among disparate source populations (e.g. Anderson & Stebbins 1954; Ellstrand & Schierenbeck 2000).

G₁ and G₄ hybrid plants produced far more flowers and biomass than wild plants in both common gardens (Fig. 2). Male fitness could be enhanced with more flowers, especially when pollen fertility of hybrids improves over time, as we observed. Greater flower production of crop-wild radish hybrids was reported by Snow *et al.* (2001) and Klinger & Ellstrand (1994), who studied hybrids between CA genotypes of wild *R. sativus* and the crop. Because this

Table 1 A comparison of fitness components for wild and hybrid G_4 populations in common gardens in Michigan and California

Source	d.f. _{Hypothesis}	d.f. _{Error}	<i>F</i>	<i>P</i> -value
Michigan				
A. Number of seeds per plant ^a				
Biotype	1	6	13.25	0.01
Population (biotype)	6	469	0.56	0.77
B. Number of flowers per plant ^b				
Biotype	1	6	5.82	0.052
Population (biotype)	6	473	1.19	0.31
C. Pollen fertility ^c				
Biotype	1	6	59.37	0.0003
Population (biotype)	6	356	1.01	0.42
D. Percent fruit set ^d				
Biotype	1	6	72.29	0.0001
Population (biotype)	6	469	0.86	0.53
California				
A. Number of seeds per plant ^e				
Biotype	1	6	33.50	0.001
Population (biotype)	6	455	1.52	0.17
B. Number of flowers per plant ^f				
Biotype	1	6	105.86	<0.0001
Population (biotype)	6	478	0.98	0.44
C. Pollen fertility ^g				
Biotype	1	6	18.59	0.005
Population (biotype)	6	334	0.72	0.64
D. Percent fruit set ^h				
Biotype	1	6	1.5	0.27
Population (biotype)	6	478	0.78	0.58

We performed linear mixed model ANOVAs for four components of fitness for two biotypes (wild and hybrid), and four populations within each biotype. The plants were equally distributed among 21 blocks within the Michigan garden and 10 blocks within the California garden. ^a $R^2 = 0.327$, ^b $R^2 = 0.336$, ^c $R^2 = 0.429$, ^d $R^2 = 0.450$, ^e $R^2 = 0.148$, ^f $R^2 = 0.183$, ^g $R^2 = 0.172$, ^h $R^2 = 0.179$.

phenomenon is consistent across taxa, location and generation, transgressive segregation is an unlikely cause (Rieseberg *et al.* 1999). One explanation for the persistent hybrid advantage in both gardens may be the introgression of beneficial genes from cultivated radish (e.g. Ellstrand & Schierenbeck 2000). We hypothesize that crop-wild hybrids may be 'pre-adapted' to thrive in new locations, in part because many economically important crops are bred for broad environmental tolerance and hybridization may transfer such traits to crop-wild hybrid progeny (Chloupek & Hrstkova 2005). A second possible explanation for larger size and flower production of G_1 hybrid genotypes is heterosis (Rhode & Cruzan 2005). The lasting advantage of G_4 hybrid plants in the MI garden could be due to heterotic effects of greater allelic diversity and heterozygosity. Heterosis may depend on environmental conditions (e.g. Welcker *et al.* 2005) and may have influenced differences in the relative fitness of hybrids in CA vs. MI. In any case, the hybrid advantage in flower production was much greater when G_4 plants were grown in the CA common garden than in MI (Fig. 2). Further studies are

needed to explore the extent of environmental effects on the relative fitness of crop-wild hybrids. Given the striking effect of environment on hybrid fitness in this study, we expect even larger fitness differences under a wider range of experimental conditions.

Hybrid fitness may be influenced by several important factors not considered in our common garden experiments, including early life history components such as seed dormancy, longevity and seedling establishment (e.g. Hooftman *et al.* 2005), effects of crop and wild parental taxa germplasm diversity (e.g. Ungerer & Rieseberg 2003), and competitive interactions of crop-wild hybrids and their wild parents (including above- and belowground competition as well as pollen competition) (e.g. Vacher *et al.* 2004). Nonetheless, our findings support previous hypotheses about the evolution of weedy *R. sativus* in CA. Panetsos & Baker (1967) speculated that hybridization with *R. raphanistrum* allowed cultivated radish to evolve into 'a highly successful weed'. Also, Hedge *et al.* (2006) used field observations, morphological data and allozyme frequencies to conclude that hybrid populations of crop-wild

Table 2 Summary statistics of fitness components for G₁ and G₄ wild and hybrid plants and their competitor oats

Biotype	Population	N	No. seeds/plant (± SE)	No. flowers/plant (± SE)*	Fruit set (%) (SE)	Aboveground			No. seeds/fruit (± SE)	Pollen fertility (%) (± SE, N)
						radish biomass (g) (± SE)*	Oat biomass (g) (± SE)	Oat biomass (g) (± SE)		
Michigan common garden										
G ₁ hybrid		42	879 (99)	641 (62)	41 (1)	7.5 (1.0)	1.0 (0.03)	3.5 (0.1)	59 (2, 42)	
G ₁ wild		42	746 (60)	383 (39)	53 (2)	2.5 (0.3)	1.1 (0.04)	4.1 (0.2)	81 (2, 29)	
G ₄ hybrid	H ₁	84	637 (50)	535 (49)	43 (2)	6.0 (0.7)	1.0 (0.03)	3.6 (0.1)	71 (3, 55)	
	H ₂	83	802 (111)	634 (76)	41 (1)	6.4 (0.8)	1.0 (0.04)	3.4 (0.1)	71 (2, 58)	
G ₄ wild	H ₃	84	567 (43)	485 (44)	44 (2)	5.0 (0.5)	1.0 (0.03)	3.5 (0.1)	75 (3, 53)	
	H ₄	82	736 (87)	577 (56)	43 (2)	6.3 (0.8)	1.1 (0.04)	3.3 (0.2)	67 (3, 54)	
G ₄ hybrid average		333	685 (39)	558 (29)	43 (1)	6.0 (0.4)	1.0 (0.02)	3.4 (0.1)	71 (1, 220)	
G ₄ wild	W ₁	42	679 (55)	373 (33)	50 (2)	2.7 (0.2)	1.1 (0.05)	4.1 (0.2)	86 (2, 40)	
	W ₂	42	754 (81)	381 (38)	54 (2)	2.8 (0.4)	1.1 (0.04)	4.0 (0.2)	88 (2, 37)	
	W ₃	42	833 (76)	411 (35)	52 (2)	2.8 (0.3)	1.1 (0.06)	4.1 (0.2)	85 (3, 40)	
	W ₄	44	799 (79)	364 (35)	52 (2)	2.7 (0.3)	1.0 (0.04)	4.3 (0.2)	86 (2, 42)	
G ₄ wild average		169	767 (37)	382 (17)	52 (1)	2.8 (0.1)	1.1 (0.02)	4.1 (0.1)	86 (1, 159)	
California common garden										
G ₄ hybrid	H ₁	88	1344 (206)	868 (156)	42 (2)	24 (4.2)	n/a	3.4 (0.1)	73 (2, 71)	
	H ₂	85	707 (105)	526 (65)	41 (2)	18 (2.4)	n/a	3.1 (0.1)	69 (2, 73)	
	H ₃	91	1051 (219)	752 (110)	41 (2)	27 (4.1)	n/a	3.4 (0.1)	72 (2, 82)	
	H ₄	88	1208 (160)	795 (115)	41 (2)	27 (4.9)	n/a	3.9 (0.2)	72 (2, 76)	
G ₄ hybrid average	W ₁	353	1080 (90)	737 (58)	41 (1)	24 (2)	n/a	3.4 (0.1)	71 (1, 302)	
	W ₂	30	272 (73)	161 (29)	43 (5)	8.4 (1.6)	n/a	3.0 (0.3)	90 (3, 11)	
G ₄ wild	W ₃	32	227 (53)	174 (28)	33 (4)	7.3 (1.0)	n/a	2.9 (0.3)	82 (5, 9)	
	W ₄	41	555 (125)	265 (55)	41 (4)	9.8 (2.2)	n/a	3.8 (0.3)	87 (5, 10)	
G ₄ wild average	W ₃	37	484 (111)	217 (39)	41 (4)	11.7 (2.5)	n/a	3.9 (0.3)	78 (7, 10)	
	W ₄	140	401 (52)	209 (21)	40 (2)	9.5 (1)	n/a	3.4 (0.1)	84 (2.5, 40)	

Plants were grown in two common gardens (Michigan and California) before (G₁) and after (G₄) experiencing natural conditions as four populations per biotype in Michigan for 3 years. Each site was represented by N plants in the common garden; n/a, not applicable.

*Pearson's correlation coefficient (r) between number of flowers per plant and aboveground biomass was > 0.85 for wild or hybrid G₁ or G₄ plants grown in Michigan or California.

genotypes have displaced ancestral populations of weedy *R. raphanistrum* in CA. Ellstrand & Schierenbeck (2000) speculated that hybrid populations of CA wild radish may be more weedy and invasive than either of their parent taxa (*R. raphanistrum* and *R. sativus*), but studies of demographic processes are needed to test this assumption (Hails & Morley 2005; Snow & Campbell 2005).

Implications for risk assessment of transgenic plants

Our study was motivated by concerns about the evolution of weedy species and the introgression of crop traits (including transgenes) into wild populations. In radish, results of this and past studies (Klinger & Ellstrand 1994; Snow *et al.* 2001; Hedge *et al.* 2006) suggest that: (i) cultivated and wild populations easily hybridize; (ii) first-generation crop-wild hybrids are relatively fecund; (iii) populations of hybrids can persist for many generations and their growth dynamics are similar to those of wild populations; (iv) hybrid populations rapidly evolve increased pollen fertility and produce large quantities of seeds despite retaining high frequencies of crop-specific alleles; and (v) relative hybrid fitness may differ dramatically among environments (e.g. MI vs. CA). These results highlight the importance of risk assessment across environmental gradients and future studies should include a greater diversity of locations in order to assess the generality of these results. Introgression of fitness-enhancing transgenes into wild populations may further enhance the fecundity of hybrid populations (e.g. Snow *et al.* 2003; Fuchs *et al.* 2004).

Measuring the fitness of weedy hybrids across multiple environments is an important goal in assessing the evolutionary effects of (trans)gene flow from crops to their wild relatives. Gene flow between crops and weeds could become more common as human-mediated movement of propagules increases the rate of long-distance dispersal of weed seeds (Ellstrand & Schierenbeck 2000). By comparing wild and hybrid performance in two locations within the geographical range of weedy *Raphanus*, we tested the dependence of relative hybrid fecundity on environmental conditions. Although this is a common practice in studies estimating the stability of natural hybrid zones, using multiple sites to assess crop-wild hybrid fitness is rare (Table S4 lists several studies where genotype by environment interaction appear to affect crop-wild hybrid fitness but this was not tested statistically). Instead it is more common to grow crop-wild hybrids within one location under several experimental treatments (e.g. competition: Hauser *et al.* 2003; Mercer *et al.* 2006; disease pressure: Fuchs *et al.* 2004; or herbicide application: Mercer 2005).

Studies of F_1 hybrids can provide tentative predictions of the effects of hybridization (Arnold & Hodges 1995; Lexer *et al.* 2003b), but our study demonstrates the importance of

including advanced generation hybrid lineages in risk assessment research, especially lineages that are permitted to evolve for several generations under field conditions. The persistence of white-flowered plants and hence a crop-specific allele, at such high frequencies (75% of plants within each hybrid population exhibited the crop-specific trait) was unexpected, given that Snow *et al.* (2001) documented a decline in white-flowered plants in BC hybrid radish populations. Over time, pollen fertility increased, suggesting that natural selection was acting on traits associated with fecundity. Although G_4 hybrid fecundity was somewhat lower than that of their wild relative in the Michigan common garden, yearly population surveys confirmed that hybrid populations can persist in natural environments, and their population sizes were similar to those of the wild biotypes. The fecundity of advanced generation hybrids ($> F_2$ or BC_1) is rarely measured in studies of crop-wild hybrids (Table S4; but see Guèritaine *et al.* 2002; Hauser *et al.* 2003; Halfhill *et al.* 2005), and descriptions of the population dynamics of advanced generation hybrids are less common.

In summary, by incorporating genotype by environment interactions, advanced generation hybrids and measurements of multiple fitness components, we may be better equipped to determine the ecological consequences of crop-wild hybridization in *Raphanus* and other crops. Ultimately, data on both hybrid fitness and growth rates of hybrid populations are needed to determine whether crop-wild hybridization leads to the evolution of more abundant and invasive weeds.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 Differences between the two common garden experiments.

Table S2 Analysis of variance for biomass and seeds per fruit of wild and hybrid radishes.

Table S3 Analysis of biotype × environment interactions in wild and hybridizing radish.

Table S4 Summary of 24 studies of relative fecundity of crop-wild hybrids compared to that of the wild parent.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2006.00974.x>.

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