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FITNESS OF HYBRIDS BETWEEN WEEDY AND CULTIVATED RADISH: IMPLICATIONS FOR WEED EVOLUTION

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Abstract. Weed species are known to evolve rapidly with their associated crops. A better understanding of the mechanisms and rates of weed evolution could aid in limiting or at least anticipating this process. Spontaneous hybridization between crops and related weed species can transfer crop genes coding for fitness-enhancing traits to wild populations, but little is known about how easily this takes place in various weed–crop complexes. We studied interspecific hybrids between wild and cultivated radishes (*Raphanus raphanistrum* × *R. sativus*), which often co-occur and share pollinators. To determine whether the F_1 generation represents a strong barrier to subsequent introgression, we compared the fitness of wild plants and wild–crop hybrids. Two experiments were carried out in Michigan, USA, one with potted plants and the other involving four artificially established populations. In the artificial populations, we used white flower color, a dominant, crop-specific allele, to document the persistence of crop genes over time. Wild plants had yellow flowers, which is a recessive trait. F_1 hybrids had lower fitness than wild plants due to lower pollen fertility, fewer seeds per plant, and delayed flowering. Despite these disadvantages, hybrids contributed substantially to each population's gene pool. After 3 yr, frequencies of white-flowered plants in the artificial populations ranged from 8% to 22%, demonstrating that crop genes persisted. Other studies of flower color variation in wild populations of *R. raphanistrum* provide circumstantial evidence for frequent crop-to-wild gene flow. We predict that, if cultivated radish is engineered to possess transgenes coding for traits such as resistance to insect herbivores, disease, herbicides, or environmental stress, these fitness-related crop genes will easily spread to *R. raphanistrum.*

Key words: adaptation; artificial populations; crop; fitness-related trait; flower color; hybridization; introgression; pollen fertility; Raphanus raphanistrum; *transgenic; weed; wild radish.*

INTRODUCTION

Many crops hybridize spontaneously with related weeds, including rice, sunflower, sorghum, squash, canola, radish, and carrot (Ellstrand and Hoffman 1990, Snow and Morán Palma 1997). However, little is known about the extent to which crop-to-wild gene flow fosters adaptive evolution in weeds. One reason little attention has been paid to this question is that useful agronomic traits have often been obtained from wild relatives in the first place, creating the impression that weeds are already very well adapted to local conditions. In addition, certain crop traits such as a short flowering period or lack of seed dormancy would likely be detrimental to wild plants, leading to the conclusion that most crop genes would have deleterious or neutral effects on the fitness of wild or weedy relatives (National Research Council 1989). In some cases, however, agricultural breeding has resulted in crops with single-gene resistance traits that are easily transferred to wild populations lacking these traits. For example, resistance to a fungal disease (*Puccinia* spp.) has been bred into cultivated sunflowers (*Helianthus annuus*) using wild germplasm (Seiler 1992). This trait is not ubiquitous in wild/weedy sunflower populations (also *H. annuus*) and can spread to wild populations via crop-to-wild gene flow (Snow et al. [1998] and references therein).

With the recent commercialization of transgenic crops, it is likely that many more single-gene traits will be available for crop improvement and, inadvertently, the ''improvement'' of associated weedy relatives. Crops with transgenic resistance to viral diseases, insect pests, and herbicides are already being grown commercially, and it is inevitable that these and other fitness-related traits will make their way into wild populations via the dispersal of transgenic pollen and seeds. Concern about the ecological and evolutionary effects of these types of transgenes has been raised repeatedly (e.g., Colwell et al. 1985, Tiedje et al. 1989, Ellstrand and Hoffman 1990, Raybould and Gray 1993, Rissler and Mellon 1996, Snow and Morán Palma 1997), prompting a closer look at the ease with which crop genes spread to wild populations in various crop–wild complexes. Studies of radish, squash, and sunflower, for example, showed that pollinators were capable of transporting crop pollen to related weeds over distances as great as 1 km (Kirkpatrick and Wilson 1988, Klinger et al. 1991, 1992, Arias and Rieseberg 1994). Spontaneous hybridization can occur even when

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the crop and the weed have different chromosome numbers or represent different genera, as in *Brassica napus* × *B. rapa*, *Sorghum bicolor* × *S. halapense*, and *Triticum aestivum* × *Aegilops cylindrica* (Jørgensen and Andersen 1994, Arriola and Ellstrand 1996, Zemetra et al. 1998). Once it is known that this process takes place in a given crop–weed system, it is important to confirm that crop genes actually persist in weedy populations (e.g., Chevre et al. 1997, Whitton et al. 1997, Linder et al. 1998). If this is the case, we also need to assess the impact of fitness-related crop genes on the fitness, abundance, and geographic distribution of wild and/or weedy relatives.

The purpose of this study was to examine the extent to which genes from cultivated radish (*Raphanus sativus*) can persist in populations of *R. raphanistrum*, which is a major agricultural weed in Eurasia, North America, and Australia (e.g., Thomas et al. 1984, Cheam and Code 1995, Holm et al. 1997, Fischer et al. 1999). We hypothesized that F_1 hybrids would have lower fitness than wild genotypes, but that crop-specific markers that are not linked to deleterious traits would persist in subsequent generations. One phase of our research involved comparing the reproductive success of F_1 hybrids with that of purely wild plants when the plants were grown outdoors in pots. A second approach was to establish isolated, artificial populations of wild and hybrid plants in which genetic markers could be used to track the long-term persistence of crop genes. While the potted plants were convenient for quantifying the reproductive success of the two cross types under uniform conditions, the experimental populations had the advantage of allowing ecological factors such as competition, herbivory, drought stress, flowering phenology, seed predation, and overwintering conditions to influence the persistence of crop genes from one generation to the next. These populations will also permit us to determine the rate at which deleterious traits such as low pollen fertility are purged over time due to natural selection. Here we report results from the potted-plant experiment, the first generation of the field experiment, and persistence of a flower-color marker in the field 3 yr later. Year-to-year changes in allozyme markers, flower color frequencies, and pollen fertility will be described further in a subsequent paper (Uthus, Snow, and Culley, *unpublished manuscript*).

Methods

The study system

Cultivated radish (*Raphanus sativus*) is composed of many varieties that have a swollen edible root, a spongy fruit capsule that crushes easily by hand, and white, pink, or purple petals (but not yellow) with a variety of vein colors (Panetsos and Baker 1967; Table 1). In California, *R. sativus* has escaped from cultivation and colonizes disturbed sites such as roadsides, fields, and coastal sand dunes (Panetsos and Baker 1967; A. A. Snow, *personal observation*). This type of wild radish has been the subject of many investigations of pollination ecology and gene flow, including research on the fitness of F_1 hybrids between wild and cultivated *R. sativus* (Klinger and Ellstrand [1994] and references therein). *Raphanus raphanistrum*, which is also known as wild radish or jointed charlock, has never been domesticated and occurs in agricultural fields and along sheltered beaches (Holm et al. 1997; A. A. Snow, *personal observation*). In the USA, natural populations of *R. raphanistrum* mainly occur in the midwest and eastern states, where feral *R. sativus* is rare or absent (Kercher and Conner 1996; A. A. Snow, *personal observation*). *Raphanus raphanistrum* also occurs in >65 countries on several continents and is ranked as one of the 100 most economically damaging weeds worldwide (Holm et al. 1997).

TABLE 1. Flower color morphs of cultivated *Raphanus sativus*, feral *R. sativus*, and *R. raphanistrum* in the United States (from Kercher and Conner 1996, Stanton 1987).

Plant type	Yellow	White	Pink	Bronze (yellow + pink)
Cultivated *Raphanus sativus*		XX	XX	
Feral *Raphanus sativus*	XX	XX	XX	X
Raphanus raphanistrum	XX	X	X	X

Note: Common color morphs are indicated by XX, and rare morphs are designated by X (frequencies vary among populations).

Raphanus raphanistrum is morphologically similar to wild *R. sativus* and both differ from cultivated *R. sativus* by forming a deep, branching taproot during the rosette stage, and by flowering more quickly than cultivated radish (both species are annuals in temperate climates; Panetsos and Baker 1967). The seeds of *R. raphanistrum* are encased in a woody, segmented fruit capsule (silique) that often has square-edged constrictions between the seeds. Fruit segments eventually break apart and seeds can then be dispersed individually. The seeds germinate readily when the woody fruit segment around each seed becomes cracked and conditions are favorable for germination. In the absence of germination cues, the seeds can remain viable in the soil for many years (Holm et al. 1997).

These two wild radish species, *R. raphanistrum* and feral *R. sativus*, differ in flower color frequencies (Table 1). Most populations of *R. raphanistrum* consist of yellow-flowered plants, occasionally intermixed with white or, more rarely, pink or bronze morphs (bronze is a blend of pink and yellow). In contrast, white, pink, and yellow-flowered plants are all common in feral populations of *R. sativus*. Because yellow flowers are not seen in cultivated radish, it is likely that feral *R. sativus* has hybridized with *R. raphanistrum* (Panetsos and Baker 1967, Stanton 1987, Kercher and Conner 1996; see *Discussion*). Both types of wild radish are pollinated by a variety of insects including bumble

bees, halictid bees, syrphid flies, honey bees, and butterflies (e.g., Stanton 1987, Conner and Rush 1996, Lee and Snow 1998). Cabbage butterflies (*Pieris rapae*) show a strong preference for yellow-flowered plants over white (Kay 1976, Stanton et al. 1986, Lee and Snow 1998), but many other common flower visitors do not (Kay 1976, Stanton 1987, Lee and Snow 1998).

Interspecific hybrids between *Raphanus raphanistrum* and *R. sativus* are vigorous and fertile (both species have $2n = 18$ chromosomes), although F_1 hybrids typically have about 50–60% aborted pollen grains (Panetsos and Baker 1967). Low pollen fertility is due to the fact that hybrids are heterozygous for a reciprocal translocation that affects chromosome pairing during meiosis (Panetsos and Baker [1967] and references therein). *Raphanus raphanistrum* is likely to hybridize with cultivated radishes in areas where the crop is grown for its seed or in fields where it is neglected and eventually bolts (and flowers; A. A. Snow, *personal observation*).

Potted plant experiment

The goal of this experiment was to compare the lifetime fecundity of wild and wild–crop hybrids when grown outdoors under uniform conditions. F_1 hybrids were obtained by crossing 18 yellow-flowered wild plants from Bay City, Michigan, with 12 Scarlet Globe *R. sativus* plants, all of which had white or pink flowers, in a pollinator-free greenhouse in 1996. Scarlet Globe is a common garden variety that is grown in many areas of the USA. To mimic crop-to-wild gene flow, wild plants were used as pollen recipients. Emasculation was not necessary because *R. raphanistrum* is self-incompatible (Sampson 1964). Several flowers on each wild plant received pollen from another wild individual, and an equal number of flowers received pollen from *R. sativus*, which was homozygous for the white petal allele. Seed set from both pollen sources was very high (A. A. Snow, *personal observation*). Nevertheless, we did not have enough seeds to test for possible differences in seed dormancy or survival under natural winter conditions, so this phase of the plants' life cycle was necessarily omitted from fitness comparisons. Seeds were placed in starter cells under a greenhouse mister on May 1996. The seeds germinated synchronously within 4–6 d, and differences between cross types in percentage germination or time to emergence were not significant (*data not shown*). Seedlings were transplanted to pots one week later, for a total of 40 potted plants from each cross type (one plant per pot). All parental plants were represented by 1–4 progeny.

Plants were grown outdoors in pots at the University of Michigan Biological Station in northern lower Michigan (Pellston, Emmet County, 42°35′ N, 84°42′ W). The pots were 2.5 L in volume, with mesh bottoms. The bottom half of each pot was filled with local sand, topped with 1 L of standard potting mix (a mixture of peat, vermiculite, organic mulch, and starter nutrients). Pots were placed in a large fenced enclosure where local vegetation had been cleared away to expose the sandy soil beneath. Plants were watered as needed and 3.5 g of slow-release Osmocote fertilizer (14N–14P–14K [Scott's, Marysville, Ohio]) was added to each pot 3 wk after seedling emergence. Every two weeks, pots were haphazardly relocated to equalize possible microsite effects and prevent the plants' roots from penetrating the sandy soil below each pot. About midway through the flowering period, most plants became infested with aphids, which were largely controlled by hand-spraying the plants once or twice with malathion. Local pollinators were abundant (Lee and Snow 1998).

For each plant, we recorded the date flowering began, pollen fertility, ovules per flower, seeds per fruit, and the total numbers of flowers and fruits. Pollen fertility was assessed by collecting pollen from two anthers per plant on a microscope slide, staining the pollen (Alexander 1969), and using a compound microscope to count the proportion of aborted grains in samples of $\geq$200 grains per plant. A few extra hybrid plants were grown to obtain additional data on pollen viability, thereby compensating for hybrids that never flowered. Ovule number was determined from two flowers per plant, and mean seed number per fruit was calculated from 10 fruits per plant (flowers and fruits were chosen haphazardly). Total seed number was estimated by multiplying the number of fruits per plant by the mean number of seeds per fruit.

Experimental field populations

This experiment also involved comparisons between wild plants and F_1 hybrids, but in this case the wild parents came from a population at Ocean Point, Maine, USA, and their progeny were grown in field populations. The source population in Maine was fixed for the yellow flower morph (A. A. Snow, *personal observation*). Wild plants were selected for crosses based on specific alleles of GPI (glucophosphoisomerase) and PGM (phosphoglucomutase) that were distinct from alleles found in the Scarlet Globe plants we used (electrophoretic methods as in Conner et al. [1997]). These two loci are not linked and therefore represent independent genetic markers (Conner et al. 1997). Seeds from our Michigan collection could not be used for this experiment because the GPI and PGM alleles that were rare in the crop were not sufficiently common in Michigan. We assumed that switching to wild genotypes from Maine would not affect the outcome of the experiment, in part because patterns of genetic variation within and among populations of *R. raphanistrum* suggest little variation among regions (Kercher and Conner 1996).

In addition to the two allozyme loci, flower color provided a third crop-specific genetic marker. The presence of yellow carotenoid pigment is a recessive Mendelian trait, with white dominant over yellow (Kay

TABLE 2. Characteristics of the four experimental populations in Michigan and frequencies of a dominant, crop-specific *Raphanus* flower color marker (white petals) in 1998 and 1999. In the first year (1996), each population consisted of 100 wild plants and 100 F_1 hybrids.

Site name	Local vegetation	Relative size of plants in 1996	Estimated size of population (number of flowering plants)			Proportion with dominant crop marker§	
			1997†	1998‡	1999	1998	1999
Biological Station (BS)	forest	very large (>1.0 m tall)	~2805	~14 380	~101 000	0.15 (3177)	0.15 (955)
Riggsville Road (RR)	forest	large	~2127	~3 770	~86 000	0.17 (1009)	0.22 (1004)
Greenstar Meadow (GM)	meadow, young forest	medium small	303	164	~3 592	0.07 (164)	0.08 (457)
Pellston Plains (PP)	grassland, very dry	small (<0.5 m tall)	237	58	~1 123	0.07 (58)	0.10 (361)

† Only half of the area at each population was rototilled in 1997.

‡ Seedlings at GM and PP were grown ex situ for 1–2 mo to protect them from insect herbivores. Manure was added to these two sites in September 1998.

§ Number of plants counted for flower color frequencies is shown in parentheses.

1976, 1978, Stanton 1987; the genetic basis of the pink hue is more complex). Both pink and white-flowered plants share the dominant allele for lack of carotenoid pigment. As above, the crop plants used in our crosses were homozygous for the dominant allele, while wild plants were homozygous for the recessive allele, and all F_1 hybrids produced white or pink flowers. Preliminary studies indicate that flower color is not linked to the reciprocal translocation locus or to genes for delayed flowering in the crop (K. L. Uthus and A. A. Snow, *unpublished data*).

After identifying wild and crop plants with mutually exclusive isozyme alleles, we made wild × crop and wild × wild crosses. Seeds were obtained from 17 wild plants (presumably unrelated) and eight crop pollen donors, which became the founding parents of the artificial populations. The seeds were harvested in late June 1996 and were immediately cracked out of their fruits, planted in biodegradable pots (8 × 8 × 8 cm) filled with potting soil, and placed in a greenhouse to germinate.

Four field populations were established at the University of Michigan Biological Station, at elevations of 212–236 m. The field sites are located on open, unshaded sites that have not been farmed for ≥50 yr, if at all, so it is unlikely that wild radish seeds were present in the soil (R. Vande Kopple, *personal communication*). Due to the sandy, infertile soils in this region, *R. raphanistrum* is uncommon and appears to be restricted to fertilized agricultural land (typically land in rotations of potato, corn, and alfalfa; A. A. Snow and K. L. Uthus, *personal observation*). Therefore, it was possible to create isolated populations that are unlikely to spread beyond limits of their artificially amended soil. The populations were separated by ≥2 km and were ~5 km from the nearest naturally occurring *R. raphanistrum* population. We have not seen flowering *R. sativus* within this 5-km radius, most of which is forested. Characteristics of each of the four sites are listed in Table 2. Abbreviations for the populations are BS (Biological Station), RR (Riggsville Road), GM (Greenstar Meadow), and PP (Pellston Plains).

Each experimental population was established in the following manner. First, a bulldozer was used to clear off existing vegetation (grass, brush, small trees) and remove the top ~30 cm of soil and roots from an area >15 × 15 m. The excavated area was then filled with local Kalkaska series topsoil, which was rototilled a few days before planting, and a 3 m high fence was erected around the area to exclude deer. Two-week-old seedlings were planted with their biodegradable pots during the first week of July. This planting date was ~1 mo later than the time when seedlings of *R. raphanistrum* begin to emerge in Emmet County, but was well within the range of natural germination times in local agricultural fields (A. A. Snow and K. L. Uthus, *personal observation*).

Each population consisted of 10 rows with 20 plants per row, half of which were F_1 wild–crop hybrids and half of which were wild. Within rows, ten plants per cross type were randomly assigned to positions that were 60 cm apart, with ~1.5 m between rows. We watered the seedlings at the time of planting and the few that died due to transplant shock were immediately replaced with plants of the same cross type. A layer of oat straw 3–5 cm thick was spread over the remaining bare soil to reduce evaporation and inhibit other weeds. This mulch did not completely inhibit interspecific competition, however, because the straw contained oat seeds that germinated and took hold. Therefore, a 10-cm radius was cleared around each experimental plant to encourage successful establishment. Each plant also received 3.5 g of slow-release Osmocote fertilizer on 6 July and 22 July. Large invading plants such as bracken fern (*Pteridium aquilinum*) were spot-treated with herbicide (glyphosate), which was also applied to a buffer zone outside the fenced areas. Any volunteer radishes were removed as seedlings (~10–15 individuals were found at each site). These unexpected volunteers probably came from the mulch or the layer of new topsoil rather than from local soils. In any case,

frequencies of unwanted seeds were low and all that germinated in 1996 were eradicated.

The experimental plants were not watered again by hand, nor were pesticides used to reduce herbivore damage. When the first experimental plants flowered in early August, we began recording the flowering status of each plant at regular intervals (weekly for the BS population, and less frequently at the other three sites). Several times per week we noted which pollinator species were present at each site. Pollen fertility was quantified for 20 wild and 20 hybrid plants, using the methods described above and plants from two of the four sites. We also counted the number of seeds per fruit from 67 wild and hybrid fruits, each from a different plant at the BS population. We did not attempt to sample total flower or fruit production per plant because of the prolonged period over which plants senesced and shed their capsules (September–November).

In 1997, 1998, and 1999, we rototilled each population in late May or early June to encourage seedling recruitment and reduce the abundance of other plant species. Wild radish seedlings emerged soon after tilling and dominated the vegetation at each site. During the growing season, large weeds were removed by hand, but many individuals of other species grew intermixed with the radishes. Two of the populations, GM and PP, had small plants in 1996 and poor recruitment in 1997 (Table 2). In 1998, grasshoppers destroyed nearly every seedling that emerged at these two sites, but we rescued 164 seedlings at GM and 58 seedlings at PP by moving them to pots at another site. The GM plants were replanted prior to flowering, whereas the PP plants began flowering at an isolated location ~0.5 km from their original site and were replanted in September. In September, manure was added to GM and PP to improve soil fertility. This resulted in a dramatic improvement in 1999, when survivorship of wild radish seedlings at these two sites was high and most plants were large (1.0–1.5 m tall).

Here we report flower color frequencies in 1998 and 1999, and estimates of population sizes for each year. In 1998, flower color frequencies were determined by counting all plants in 56 haphazardly positioned quadrats (each 625 cm^2) at BS and GM; for the other two sites, where plants were temporarily removed to protect them from herbivores, we recorded flower color for every plant. In 1999, color frequencies were determined for 35 quadrats (each 0.25 m^2) and two 5-m linear transects at BS and RR, and by counting all plants in haphazardly chosen patches at GM and PP, where the populations were much smaller (see Table 2 for sample sizes). Individuals with pale pink flowers were grouped with white-flowered plants, while those with bronze flowers were grouped with yellow. These plants will be referred to as ''white'' or ''yellow'' hereafter (in 1999, <2% of the plants had pink or bronze petals). Estimates of population size were based on direct counts when <400 plants were present or by subsampling when the population was larger. For the latter, we determined the average number of plants in 20–56 quadrats per site (quadrat size was 0.25 m^2 or 1.0 m^2, depending on plant densities) and multiplied this value by the total area covered by wild radish.

In 1996, we collected a sample of seeds from wild experimental plants to check for introgression of crop-specific markers. A total of 96 progeny were screened for GPI, PGM, and flower color alleles (one offspring from each of 54 haphazardly selected plants at BS and 42 plants at RR). This rather small sample was not intended for characterizing allele frequencies, but rather as a means of confirming that F_1 hybrids were able to backcross with wild plants.

Results

Potted plant experiment

Hybrid plants grew well but remained in the rosette stage longer than wild plants. The average time from germination to flowering was 44 ± 1 d for wild plants vs. 60 ± 1 d for hybrids (mean ± 1 SE, $N = 39$ and 24 individuals, respectively; <0.001, *t* test). This delay was also manifest in the fact that 15% of the hybrids never bolted to flower, as compared to 2% of the wild plants ($P < 0.05$, *G* test). Aphids appeared on the potted plants ~2 wk after flowering began and were sprayed at regular intervals with malathion. None of the wild plants suffered major aphid damage, but 18% of the hybrids that flowered were heavily infested, perhaps because they flowered later and/or were more susceptible to aphids. Plants that sustained heavy aphid damage or never bolted were excluded from comparisons of flower, fruit, and seed number below. Syrphid flies, halictid bees, and bumble bees were the most common pollinators, making ~80% of the observed visits, and each flower appeared to receive many visits per day (Lee and Snow 1998). All of the wild plants had yellow flowers, while the F_1 hybrids were white or pale pink, as expected for progeny from wild plants crossed with cultivated plants that were homozygous for the dominant white petal-color allele.

In hybrids, on average 63% of pollen was viable, as compared to 92% of pollen in wild plants (Fig. 1). Pollen viability was quite variable, however, and three hybrid plants had >79% pollen viability, while two wild plants had <67% viable pollen. The number of ovules per flower was similar in wild vs. hybrid plants, averaging 7.0 ovules in both groups ($N = 39$ and 28 plants, respectively), but hybrids produced only half as many seeds per fruit as wild plants (Fig. 2). This had a major effect on the number of seeds per plant, which was also 50% lower in hybrids. Hybrids produced ~30% more flowers than wild plants, but the number of fruits per plant did not differ significantly (Fig. 2). Hybrid plants senesced by midAugust, well before the end of the growing season and during the time when

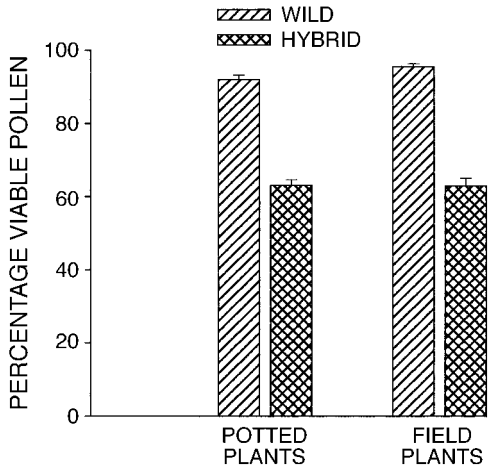

FIG. 1. Comparison of pollen fertility of wild vs. hybrid radish plants (*Raphanus raphanistrum* × *R. sativus*) in the potted-plant experiment ($N = 40$ plants per cross type; wild plants derived from a Michigan population) and the field experiment ($N = 20$ plants per cross type; wild plants derived from a Maine population). All plants were grown outdoors. Data are means and 1 SE. In each experiment, differences between wild and hybrid plants were significant at $P < 0.0001$ (t tests).

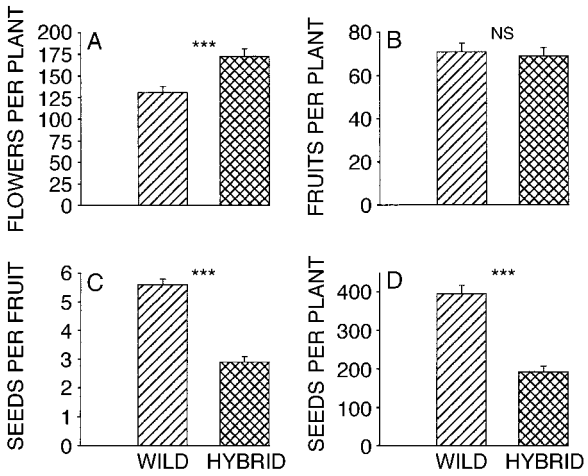

FIG. 2. Comparisons of reproductive success between wild and F_1 hybrid plants that flowered in the pot experiment. Means and 1 SE are shown; $N = 38$ for wild plants and 27 for hybrids. Significance of differences between pairs of means is indicated by *** ($P < 0.001$) or NS (not significant), based on t tests.

pollinators were still abundant. Diminishing numbers of plants in flower and low pollen fertility may have limited the availability of viable pollen toward the end of the hybrids' blooming period, but pollinator visits continued unabated.

Experimental field populations

Fitness of F_1 *hybrids*.—Survivorship was very high in the field populations (Table 3), despite the fact that some plants sustained heavy damage due to grasshoppers, flea beetles, aphids, and occasional larvae of *Pieris rapae*. Plants that were severely affected by herbivores continued to produce new leaves, and most eventually recovered. Plants began to flower ~5–12 August and many continued flowering through November, stopping only after repeated hard frosts finally killed them. Plants that delayed flowering until October produced few or no fruits. Because cabbage butterflies (*Pieris rapae*) are known to prefer yellow-flowered *R. raphanistrum* over white-flowered plants (Kay 1976), we noted which pollinators were present. Pollinator composition in the four populations shifted during the 1996 flowering period. Syrphid flies and halictid bees were the only pollinators seen from early August until 17 August, when bumble bees were first observed: bumble bees gradually became more common. In early September we occasionally observed cabbage butterflies and honey bees, but the great majority of flower visitors continued to be syrphids, halictids, and bumble bees (A. Snow, *unpublished data*). Similar pollinator

TABLE 3. Survival, phenology, and fruiting status of *Raphamus* plants in the experimental populations in Michigan in the first year of the study (1996).

Status	Sites							
	BS		RR		GM		PP	
	Wild	Hybrid	Wild	Hybrid	Wild	Hybrid	Wild	Hybrid
Dead	3	1	1	0	1	0	0	0
Percentage flowering								
5 Aug 1996	14	0	18	0	23	0	23	0
15 Aug 1996	83	34	85	14	79	16	63	12
6 Sep 1996	90	75	75†	69	95	64	98	29
Total percentage flowered (9 Nov 1996)	97	87	99	89	99	78	100	74
Total percentage with fruits (9 Nov 1996)	95	78	97	71	95	62	92	60

Notes: The percentage of all initial plants in each category is shown ($N = 100$ initially and $N = 97$–100 after accounting for plants that died). Site abbreviations are defined in Table 2.

† Some of the wild plants finished flowering early at this site, so there were fewer in flower on 6 Septermber than on 15 August.

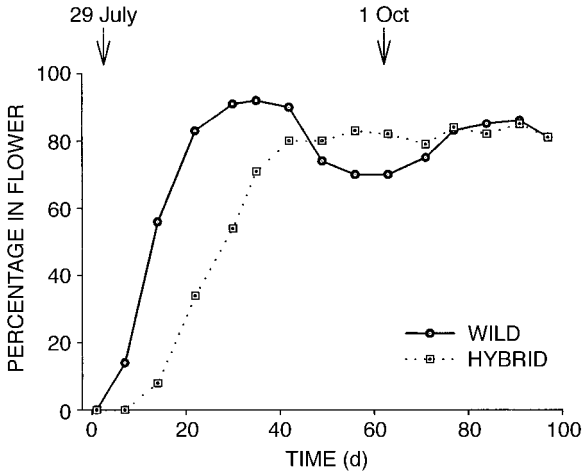

FIG. 3. Flowering phenology of wild and hybrid plants in the Biological Station population in 1996. Sample sizes were 97 wild plants and 99 hybrids.

taxa were observed in 1997–1999, but bumble bees were common throughout these flowering periods. Cabbage butterflies and honey bees were extremely uncommon in the experimental populations.

Wild plants reached peak flowering by late August, whereas hybrid plants flowered considerably later, especially at PP (Table 3, Fig. 3). Hybrids also produced fewer seeds per fruit than wild plants (3.7 ± 0.2 vs. 4.9 ± 1.3 seeds/fruit, $N = 67$ fruits; $P < 0.01$), and fewer viable pollen grains (Fig. 1). At the RR and PP sites, respectively, 0.11 and 0.26 of surviving hybrids never flowered (Table 3). In contrast, all of the wild plants that survived (>97%) also bolted and flowered. A large proportion of hybrids never produced fruits (0.22 at BS, 0.29 at RR, 0.38 at GM, and 0.40 at PP), while 92–97% of the wild plants produced fruits (Table 3).

Plants were smallest and slowest to flower at GM and PP, whereas they performed better at RR and best at BS (Tables 2 and 3). The poor growing conditions at GM and PP appeared to exaggerate differences between wild and hybrid plants in each population, with hybrid plants having lower relative reproductive success at GM and PP and greater success at BS and RR. In later years, populations at GM and PP remained much smaller than those at BS and RR (Table 2).

Persistence of crop-specific alleles.—The founding populations consisted of 50% white-flowered plants, all of which were heterozygous for crop-specific alleles. In 1996, some seeds of wild plants were sired by F_1 hybrids, based on their allozyme genotypes and flower color (recall that wild plants were homozygous recessive for yellow petals). For the 96 progeny examined, frequencies of diagnostic crop alleles were 0.7 GPI, 0.4 PGM, and 0.12 flower color, respectively. Only PGM was significantly different from the expected frequency of 0.125, based on chi-square tests and assuming random mating in the founding population ($P < 0.05$; data from BS and RR were pooled). This sample of progeny was likely too small to be representative of the next generation, however. The purpose of scoring these seeds was simply to document the occurrence of backcrossing between F_1 and wild plants.

Three years later, in 1999, white-flowered plants were still present in all four populations (Table 2). The highest frequencies of white-flowered plants were 0.22 at RR and 0.15 at BS. At GM and PP, where fewer hybrids flowered in the first year of the study, frequencies of white-flowered plants were only 0.08 and 0.10, respectively. Similar frequencies were seen in 1998 (Table 2). At RR, the proportion of white-flowered plants increased significantly from 0.17 in 1998 to 0.22 in 1999 ($G = 8.19$, $P < 0.005$); changes at other sites were not statistically significant. Because flower color is a dominant marker, we do not know whether the increase at RR represents a change in allele frequencies and/or a change in the relative proportions of homo- and heterozygous plants. Another possible source of variation in crop allele frequencies was the soil seed pool, which was not examined.

DISCUSSION

Our results show that after hybridization has occurred, crop genes are likely to persist in weedy populations of *Raphanus raphanistrum,* despite the fact that F_1 hybrids had lower fitness than wild plants. The main differences between wild and F_1 hybrid plants were seen in flowering times, pollen fertility, and seeds per fruit, as documented in both potted plants and the experimental populations. Crop genes caused the plants to produce thickened taproots and large rosettes, and some plants never flowered despite being healthy and vigorous. In the field populations, delayed development was most pronounced at GM and PP, the two sites where the plants and population sizes were smallest. The average pollen viability of hybrids was only 0.63, as compared to 0.92–0.96 for wild plants (Fig. 1). For potted plants, lifetime seed production of hybrids was 49% that of wild plants, reflecting the fact that hybrids had fewer seeds per fruit (field-grown hybrids produced ~76% as many seeds per fruit as wild plants). Both pollen abortion and fewer seeds per fruit were likely due to heterozygosity for a reciprocal translocation in the F_1 hybrids (Panetsos and Baker 1967).

The combined effects of delayed flowering and reduced fecundity inhibited transmission of the white flower color allele to later generations. Nonetheless, crop genes persisted in all four populations. By 1998, the frequencies of white-flowered plants were 0.15 and 0.17 at BS and RR, while only half as many plants had white flowers at GM and PP. These frequencies changed little from 1998 to 1999. We have not estimated allele frequencies for flower color because it is a dominant trait and it is unlikely to be selectively neutral. Polli-

nators sometimes prefer yellow-flowered *R. raphanistrum* over white (e.g., Stanton et al. 1986, Lee and Snow 1998), and insect herbivores may also discriminate among flower color morphs (S. Strauss, *unpublished data*). Cabbage butterflies (*Pieris rapae*) and a syrphid (*Eristalis arbustorum*) in England strongly prefer yellow-flowered plants (Kay 1976, Stanton et al. 1986). The most common pollinators at our populations were syrphid flies (Syrphidae), halictid bees (Halictidae) and bumble bees (*Bombus* spp.). Several of these species show a slight preference for yellow-flowered plants when the frequency of whites is greater than ~0.15, but this preference disappears when whites are less common (Lee and Snow 1998; A. Snow and K. Flinn, *unpublished data*). Therefore, pollinator preferences for yellow-flowered plants could have reduced the fitness of F_1 hybrids somewhat, especially in 1996, when the frequency of whites was highest. Allozyme data should provide more accurate estimates of the frequencies of crop genes in each generation. In 1998, frequencies of crop-specific isozyme alleles ranged from ~0.15 to 0.20 in these populations, confirming that introgression has occurred (K. L. Uthus, A. A. Snow, and T. M. Culley, *unpublished manuscript*).

Regardless of the exact frequencies of crop-specific genetic markers, it is obvious that crop genes can persist in natural populations of *R. raphanistrum* for at least three generations and probably much longer. Previous investigators have mentioned the possibility that nonyellow petal morphs and smooth, unconstricted fruit capsules in populations of *R. raphanistrum* may be evidence for past hybridization with the crop (Kay 1976, 1978, Stanton et al. 1989, Kercher and Conner 1996, Lee and Snow 1998). Rare mutations could also introduce flower color polymorphisms into natural populations (e.g., Kay 1976, Waser and Price 1981). However, the white morph is sometimes common in *R. raphanistrum,* with no obvious fitness advantages, so we suspect that its frequency is often influenced by hybridization with *R. sativus.*

Flower color frequencies in natural populations suggest that hybridization between *R. raphanistrum* and *R. sativus* may have occurred repeatedly in both directions and to varying degrees throughout many temperate regions of the world. In California, feral populations of *R. sativus* often include individuals with yellow or bronze petals or >40% aborted pollen, most likely due to past hybridization with *R. raphanistrum* (Panetsos and Baker 1967). Populations of *R. raphanistrum* in northern lower Michigan typically have 1–2% white, pink, or bronze-colored petal morphs, as well as rare individuals with low pollen fertility (Uthus and Snow, *unpublished data*). Kercher and Conner (1996) reported high frequencies of 49% white and 6% pink or bronze in Bay City, Michigan, and 36% white-flowered plants in a population from Hamden, Connecticut, while a New York population was pure yellow. Populations of *R. raphanistrum* along beaches in Connecticut and Maine are usually fixed for the yellow allele, whereas inland populations often have low frequencies of the white petal morph (A. A. Snow, *personal observation*).

In Europe, *R. raphanistrum* appears to be more variable than in the USA. Tutin et al. (1964) described several European subspecies of *R. raphanistrum* based on differences in flower color, flower size, fruit diameter, and leaf shape. These include a yellow-flowered subspecies that occurs along maritime coastlines, a pale pink-flowered subspecies found along Aegean shorelines, a white or reddish-flowered subspecies in cultivated fields of Spain, Portugal, and the Azores, and a subspecies known as *raphanistrum* with white or yellow flowers in cultivated fields throughout Europe. Kay (1976) reported that white morphs of *R. raphanistrum* often predominate in southern and eastern Britain, while the yellow-flowered plants predominate or form monomorphic populations in northern and western Britain. These studies also provide circumstantial evidence for past hybridization between cultivated or wild *R. sativus* and *R. raphanistrum,* although nonyellow morphs of *R. raphanistrum* could also evolve spontaneously.

Crop–wild hybridization has been implicated as a factor contributing to the weediness of feral *R. sativus* in California. Both the crop and *R. raphanistrum* were introduced into California by the 19th century, and Panetsos and 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 California''. Although there is no hard evidence for this claim, it seems likely that feral *R. sativus* could benefit from *raphanistrum* traits and/or heterosis. Remarkably, it now appears that wild *R. sativus* has hybridized extensively with California populations of *R. raphanistrum,* and ''pure'' *R. raphanistrum* populations have not been seen in recent years (N. Ellstrand, M. Stanton, S. Strauss, *personal communication* to A. A. Snow; A. A. Snow, *personal observation*).

It is also possible that evolutionary adaptations in *R. raphanistrum* have been facilitated by gene flow from cultivated *R. sativus,* although this has not been proven and requires further study. Fitness benefits of crop genes were seen by Klinger and Ellstrand (1994), who documented greater lifetime seed production in F_1 hybrids between cultivated and feral *R. sativus* in California. In their study, which involved intra- rather than interspecific crosses, wild and hybrid plants grown in outdoor pots did not differ in other components of fitness (i.e., germination success, flowering time, or paternal transmission of a crop-specific allele to the next generation). Langevin et al. (1990) also found evidence for higher fitness of F_1 hybrids between cultivated and weedy rice (both *Oryza sativa*). Heterosis and/or particular crop genes might also enhance the fitness of *R. raphanistrum* genotypes, despite the fact that some

crop genes and/or chromosomal rearrangements are deleterious to wild plants (e.g., late flowering and heterozygosity for the reciprocal translocation). Beneficial crop genes can potentially introgress into wild populations when they are not linked to traits that would be deleterious to the weed. Worldwide, new varieties of weedy wild radish may be evolving frequently because cultivated plants can spawn feral populations, and both the crop and feral *R. sativus* are capable of hybridizing with *R. raphanistrum.*

Future adaptations in *R. raphanistrum* could be facilitated by the acquisition of transgenes from the crop. Biotechnology companies are currently concentrating on more profitable crops, but if transgenic methods are used to improve *R. sativus,* this species should be treated with caution. To avoid undesirable impacts of fitness-related crop genes that can spread to wild radish, we recommend that transgenic radishes with resistance to herbicides, diseases, herbivores, or stressful environmental conditions not be released if these resistance traits are beneficial to weedy populations (also see Klinger and Ellstrand 1994). Release from abiotic or biotic stresses that limit population growth could potentially allow *R. raphanistrum* and feral *R. sativus* to become even more abundant and widespread than they are today. Similar concerns apply to many other crops that also exchange genes with weedy relatives.

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