

## CAN FERAL WEEDS EVOLVE FROM CULTIVATED RADISH (*RAPHANUS SATIVUS*, BRASSICACEAE)?<sup>1</sup>

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Cultivated plants that cannot survive on their own often have maladaptive domestication traits. Unharvested crop seeds may generate feral populations, at times causing serious weed problems, but little is known about the evolution of ferality. We explored the potential for cultivated radish, *Raphanus sativus*, to become feral, given that closely related taxa (e.g., *R. raphanistrum* and crop–wild hybrids) are well-documented weeds. First, we measured the population growth of five experimental, cultivated, self-seeding radish populations in Michigan, USA, for three generations. Three late-flowering populations went extinct, and two others apparently hybridized with local *R. raphanistrum*. A common garden experiment showed that the two surviving populations had earlier flowering, smaller root diameters, and greater individual fecundity than did nonhybridized populations. We also used artificial selection to measure the evolutionary potential for earlier flowering. After two generations of strong selection, two of three lineages flowered earlier and produced more seeds than control lineages, but insufficient genetic variation prevented dramatic evolution of crop phenotypes. In summary, it seems unlikely that radishes could spontaneously become feral in our study area without gene flow from *R. raphanistrum*. Applying these approaches to other cultivated species may provide a better understanding of mechanisms promoting the evolution of feral weeds.

**Key words:** Brassicaceae; fecundity; ferality; Michigan; naturalized; population dynamics; *Raphanus sativus*; selection experiments; United States; volunteers.

Crop ferality and volunteerism are well-known phenomena in agriculture (Ogg and Parker, 1989; Gressel, 2005). Volunteer populations occur when unharvested seeds from a previous crop germinate and grow in and around agricultural fields. For example, canola harvesting can drop more than a thousand seeds per acre, and volunteers often compete with subsequent crops (Gulden and Shirliffe, 2003). By colonizing field margins, home gardens, ditches, disturbed areas, and other unmanaged habitats, feral plants can reproduce independently of the agricultural management upon which they depended as crop plants (e.g., Heenan et al., 2004; Lumaret et al., 2004; Ulloa et al., 2006). Here, we explore evolutionary pathways that might lead to the evolution of feral radish (*Raphanus sativus* L.).

Weedy species are known to evolve rapidly in response to local selection pressures, and the recent development of genetically engineered crops, especially herbicide-resistant cultivars, highlights the possibility that volunteer and feral plants may create new environmental and crop–weed management risks in agricultural systems (e.g., Smyth et al., 2002). Many cultivated plants have naturalized in introduced areas (e.g., Mack and Erneberg, 2002; Heenan et al., 2004; Tabuti et al., 2004), sometimes becoming invasive (e.g., ornamental plants: Kowarik, 2005; weedy rye: Stump and Westra, 2000). Infestations of fe-

ral rye (*Secale cereale* L.) in wheat fields in the western United States can reduce yield by 30–70% and result in major economic losses annually (Pester et al., 2000). By identifying crops that can produce feral populations, we may anticipate and perhaps avoid this ecological and economic damage.

The extent to which cultivated plants can persist as feral populations is related to heritable variation in domestication traits, such as lack of seed dispersal or seed dormancy, which may limit the plants' ability to persist as free-living populations. For example, canola, cranberry, olive, and many horticultural taxa are relatively undomesticated, and volunteer individuals can easily give rise to feral populations (Pessel et al., 2001; Bervillé et al., 2005; Kowarik, 2005). In rice (*Oryza sativa* L.), few mutations are needed for the evolution of weedy rice from crop plants (e.g., Li et al., 2006). Other crops such as maize (*Zea mays* L. subsp. *mays*) and soybean [*Glycine max* (L.) Merr.] are so strongly domesticated that their progeny rarely persist beyond first-generation volunteers (Gressel, 2005).

In addition to spontaneous mutations and purging of domestication traits by natural selection, gene flow from wild relatives can provide a stimulus for the evolution of feral populations. Gressel (2005) described two mechanisms by which cultivated plants can become feral—endofertility and exofertility. These terms refer to dedomestication without gene flow from other taxa vs. dedomestication that is facilitated by gene flow from other taxa. Both mechanisms could contribute to the evolution of ferality, and they may be difficult to distinguish, especially when breeding practices or spontaneous gene flow from wild relatives allow wild genes to enter the crop gene pool. In sunflower (*Helianthus annuus* L.), for example, off-type crop plants with weedy traits appear to be the result of pollen contamination from wild sunflower in commercial seed nurseries, and seeds from off-type sunflowers are more likely to give rise to feral populations than those from normal volunteer plants (Reagon and Snow, 2006). In general, the persistence of feral populations and the loss of deleterious domestication traits can depend on heritable variation in these traits, the strength of se-

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lection, and rates of gene flow from other taxa such as weedy relatives (Mekuria et al., 2002; Bond et al., 2004).

Several observational studies document the persistence of feral populations in *Brassica* species (e.g., Crawley and Brown, 1995; Pessel et al., 2001; Heenan et al., 2004). More recently, workers have adopted an experimental approach to understanding the potential for crop ferality (Lutman et al., 2003; Harker et al., 2005), but little is known about the potential for ferality to evolve from volunteer populations. Here, we asked whether a widely planted radish cultivar ('Red Silk') can persist as feral populations and evolve more wild-type characteristics. First, we measured population growth rates of five self-seeding, experimental populations over three years in Michigan, USA. Using a common garden experiment, we tested for heritable differences in several phenotypic traits among the surviving populations and compared them to a nonselected control lineage (Fig. 1). Second, we used an artificial selection experiment to measure how much heritable variation the crop cultivar possesses for a key life-history trait, early flowering. We also tested for correlated effects of earlier flowering by examining the lifetime fecundity and other traits of selected and control lineages in a common garden (Fig.

1). We suggest that this combination of natural and artificial selection experiments provides useful insights into the mechanisms involved in the spontaneous evolution of feral weeds.

MATERIALS AND METHODS

**Study system**—Radish (*Raphanus sativus* L., Brassicaceae) is a variable and ancient crop that is cultivated around the world for its edible root, seed sprouts, seed pods, leaves, and as forage for livestock (Crisp, 1995; Snow and Campbell, 2005). Many cultivated varieties of *R. sativus* (i.e., European and daikon) are late-flowering annuals or biennials that produce an enlarged, edible hypocotyl/root. In contrast, its wild relative, *R. raphanistrum*, produces a thin tap root before flowering early in the growing season as an annual weed. The relatively early flowering of *R. raphanistrum* may ensure that plants reproduce before frost, drought, or herbivory, and before crop fields are tilled, sprayed with herbicides, or harvested. *Raphanus raphanistrum* (wild radish or jointed charlock) is among the world's worst agricultural weeds, especially in small grain and vegetable crops, and is distributed worldwide (Cheam and Code, 1995; Holm et al., 1997; Warwick and Francis, 2005).

Hybrids between *R. sativus* and *R. raphanistrum* are highly successful weeds in some environments, including coastal dunes, roadsides, and agricultural areas in California (Panetsos and Baker, 1967; Klinger and Ellstrand, 1994; Hegde et al., 2006). European cultivated radishes reportedly became

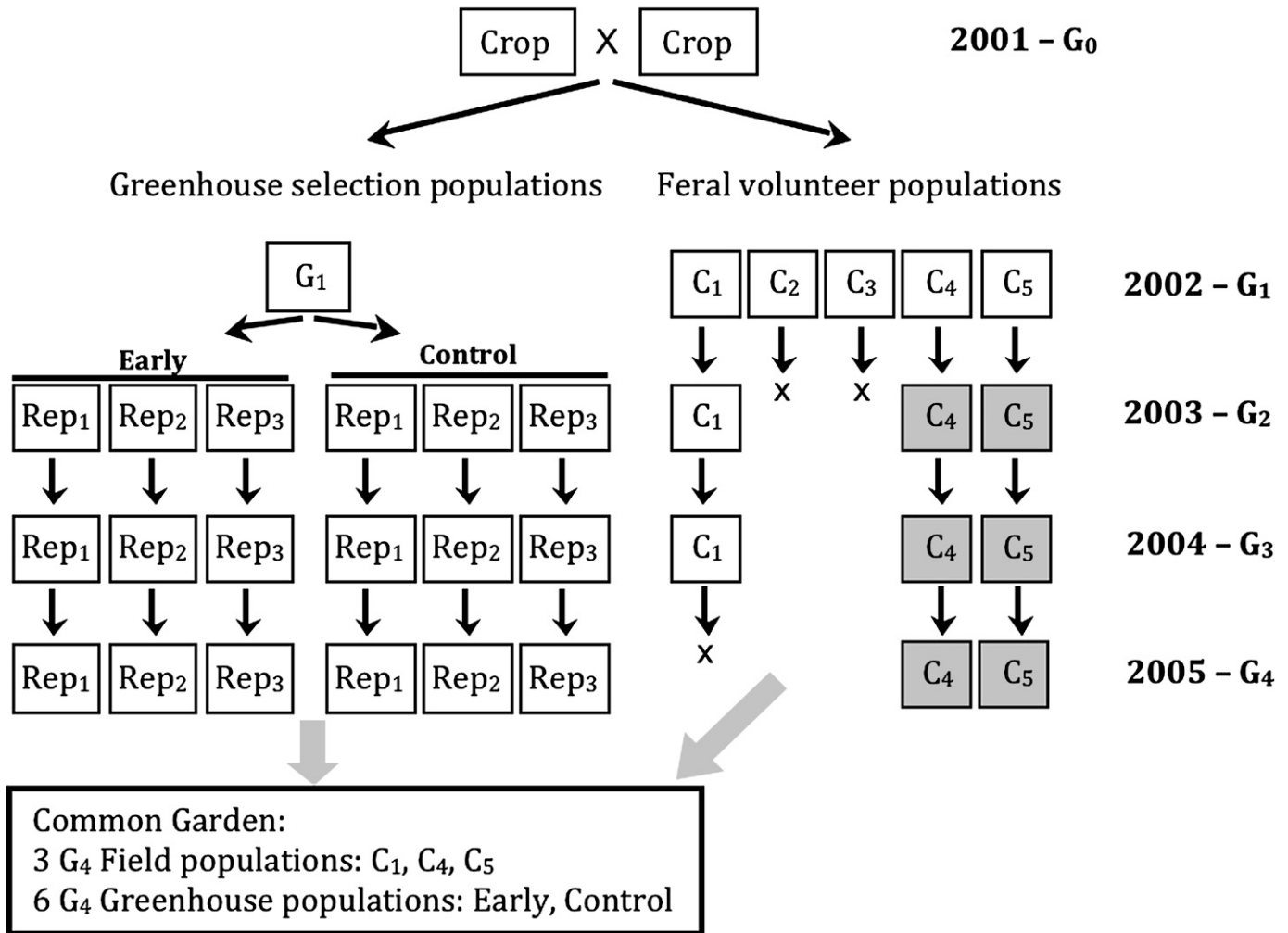


Fig. 1. Schematic diagram of the artificial selection experiment in the greenhouse, the five feral populations under natural selection, and the common garden design. "Early" refers to selection for earlier flowering times. Shaded boxes represent introgressed field populations with yellow-flowered plants; "X" represents population extirpation.

naturalized along the California coast (Panetsos and Baker, 1967), but it is not clear whether this process required hybridization with weedy *R. raphanistrum* or whether endofertility had evolved directly from cultivated radishes. According to Hegde et al. (2006), the “pure” populations of feral *R. sativus* originally reported no longer exist in California. Ridley et al. (2008) found that hybridization between cultivated radish and *R. raphanistrum* has been bidirectional, with both taxa acting as pollen recipients during initial hybridization events.

In northern lower Michigan, where the current study was conducted, *R. raphanistrum* occurs as an annual weed in alfalfa, oats, potato, sugar beet, and other agricultural fields, often leaving a persistent seed bank (L. G. Campbell and A. A. Snow, personal observation). Cultivated radish is grown in home gardens and commercial fields, with the potential to hybridize with the weed when the crop is grown for seed or when fields are neglected and the rosettes bolt to produce flowers. Indirect evidence for crop-to-wild gene flow comes from the presence of crop-specific flower colors (flowers with white or pink-hued petals), as reported for a Bay City, Michigan, population of *R. raphanistrum* by Kercher and Conner (1996). In this population, 55% of the plants had crop-specific flower colors, and we have often observed lower frequencies of putative hybrids in other populations (A. A. Snow, personal observation). In a companion study to the one described here, we followed the population dynamics and evolution of four wild *R. raphanistrum* and four hybrid *R. raphanistrum* × *R. sativus* populations in Michigan, USA (described in Campbell et al., 2006). We found that all hybrid populations persisted for at least three years and had similar intrinsic growth rates to wild *R. raphanistrum*. Therefore, it is clear that feral radish can become dedomesticated via exofertility, as postulated by Panetsos and Baker (1967) and Hegde et al. (2006). To date, however, we are not aware of studies in which the potential for endofertility has been examined in radish.

**Natural selection experiment—Seed source**—In 2001, we cross-pollinated 100 crop plants in a greenhouse, using the self-incompatible *Raphanus sativus* cultivar Red Silk (Harris-Moran Seed Co., Modesto, California, USA), to create first-generation ( $G_1$ ) plants. This “European” cultivar is widely planted in the USA and is similar to other widely consumed cultivars such as “Cherry Belle” and “French Breakfast”. Plants were grown under controlled conditions, including a 16-h daylight schedule with a 23–28°C/20–25°C day/night temperature range. A long-day photoperiod encourages radish flowering (Erwin et al., 2002), and these conditions were maintained during the artificial selection experiment described later. Maternal parents were randomly assigned pollen donors from concurrently flowering plants.

**Population establishment**—In 2002, we established five first-generation radish populations ( $C_1$ – $C_5$ ), one in each of five meadows in Emmett and Cheboygan counties, Michigan (MI) (Fig. 1). The experimental populations were separated from local populations of *R. raphanistrum* by >1 km to restrict unintended gene flow via pollinators or seed dispersal. Flower color provided a useful indicator of whether wild *R. raphanistrum* genes had inadvertently entered the experimental populations. *Raphanus sativus* is homozygous for the dominant, white petal allele, whereas *R. raphanistrum* is homozygous for the recessive, yellow carotenoid pigment (Panetsos and Baker, 1967; Kay, 1976; Campbell et al., 2006). Therefore, the wild-type flower color could be detected in homozygotes but not in heterozygotes at this locus. Inheritance of pinkish petal hues that blend with white or yellow colors is more variable and complex (Irwin et al., 2003). In the context of this study, pink-flowered plants were grouped with white-flowered ones, and bronze-flowered plants were grouped with yellows (as in Snow et al., 2001).

We planted  $G_1$  seedlings in peat-filled biodegradable pots at the University of Michigan Biological Station (UMBS) in Pellston, MI. At each site, we transplanted 60 seedlings to recently tilled 15 × 15 m fertilized plots (as in Campbell et al., 2006). Between 50–60 plants survived the growing season in each population although not all surviving plants flowered. No resident wild radish plants emerged from the seed bank at these plots in 2002 or 2003. Each spring, plots were tilled, fertilized, and hand-weeded for approximately two weeks to simulate agricultural management and promote population persistence. Otherwise, these self-seeding populations were exposed to naturally occurring weather conditions, competing plants, herbivores, pathogens, and pollinators (Lee and Snow, 1998; Campbell et al., 2006). By 2003, two populations ( $C_2$ ,  $C_3$ ) had declined in size to <10 seedlings. In a futile attempt to promote population persistence, we added 25  $G_1$  seedlings from the original seed source to each population ( $C_1$ – $C_5$ ) in 2003. Therefore, it is possible that more than one generation persisted in each population (this could also occur due to seed dormancy).

**Population surveys**—Population size was estimated from direct counts when <1000 plants were present or subsampling larger populations. For the

latter, we determined the average number of plants in 49 1-m<sup>2</sup> quadrats per site and multiplied this value by the total area.

We did not expect to find any yellow-flowered plants in the experimental populations because the crop was homozygous for white flower color. However, two of the five populations ( $C_4$  and  $C_5$ ) inadvertently acquired plants with yellow flowers and other crop–wild hybrid traits from *R. raphanistrum*, as described later. When we first observed yellow-flowered plants in 2004, we began assessing the frequency of yellow-flowered plants in all populations during peak flowering (25 June–4 July). Yellow-flowered plant frequencies were based on direct counts or subsamples as described earlier, and allele frequencies for this recessive trait were estimated using Hardy–Weinberg expectations. Because the yellow petal allele is recessive, we could not determine the original level or timing of this putative introgression, and we cannot rule out the possibility that  $C_1$ – $C_3$  also experienced gene flow from *R. raphanistrum*. Based on flower color frequencies and the presence of hybrid phenotypic traits in the common garden experiment (discussed next), we refer to  $C_4$  and  $C_5$  as “introgressed” populations and  $C_1$ – $C_3$  as “non-introgressed” populations, with the caveat that the evidence for this distinction is tentative.

**Common garden**—To compare fitness-related traits of the populations that were still present in 2004 ( $C_1$ ,  $C_4$ ,  $C_5$ ) and to measure how much each population evolved over the course of three growing seasons, we used a common garden experiment that included unselected control lineages from the artificial selection experiment described later (Fig. 1). In 2004, we collected one seed per fruit of three fruits from haphazardly selected branches on 30 plants per experimental population. The garden included five  $G_4$  individuals from each field population and control lineage in each of 10 blocks, resulting in 150 plants derived from field populations and 150 control plants in a complete block design randomized across populations and lineages.

Seeds were planted in 300 mL of peat in fiber pots (3–10 May 2005) with four oat seeds (*Avena sativa*) in a greenhouse at UMBS. Cultivated oats were added as a standard competitor, and they were thinned to one oat plant per pot. Once the radish seedlings developed their first true leaves, each fiber pot was moved into a 4-L plastic pot (Hummert International, Earth City, Missouri, USA) filled with a mixture of 3.7 L of local sandy soil surrounding the fiber pot. The plastic pots were placed on level, recently tilled soil and were separated by 30 cm to minimize competition between experimental plants (roots could grow through the pot bottom). Seedlings that died within one week after transplanting were replaced, and all plants survived to reproduce. Plants were watered daily for the first month and every other day until 31 August. Insect herbivory was minimal due to two early applications of an insecticide (0.0033% esfenvalerate, Scotts Miracle-Gro Co., Marysville, Ohio, USA). 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 and Snow (1998). Plants were individually harvested at senescence until the first hard frost when all remaining plants were harvested (16–20 September).

For each plant, we recorded age at flowering, pollen fertility, stem diameter, flower number, seed production, and aboveground vegetative biomass. Age at flowering was calculated as the difference between the dates of emergence and flowering. Pollen fertility was quantified because hybrids were present in two of the populations, and hybrids between *Raphanus raphanistrum* and *R. sativus* are known to be heterozygous for a reciprocal translocation that affects chromosome pairing during meiosis (Panetsos and Baker, 1967). Typically,  $F_1$  crop–wild hybrids produce approximately 40–50% aborted pollen grains, whereas wild *R. raphanistrum* produce less than 20% aborted pollen grains (e.g., Snow et al., 2001; Campbell et al., 2006). We collected pollen from two flowers per plant and assessed pollen fertility using a compound microscope and Alexander’s stain (Alexander, 1969) to count the proportion of aborted grains in stained samples of at least 100 grains (Campbell et al., 2006). As a correlate of plant size, we measured stem diameter at the widest point at the base of each plant at harvest. We also counted the total number of flower pedicels and fruits per plant. Fruit set was calculated as number of fruits produced divided by the total number of flowers. Number of seeds per plant was estimated by multiplying the number of fruits by the average number of seeds per fruit, estimated from 10 randomly chosen fruits per plant. Finally, we weighed the vegetative biomass (g) of the competing oat plants.

Percentage fruit set required no transformations prior to analysis. Number of seeds, flowers per plant, and oat biomass were log-transformed, and pollen fertility was arcsine-square-root transformed. All analyses were performed using SPSS (v.13, SPSS, Chicago, Illinois, USA). To test for differences in lifetime fecundity and relative competitiveness among the experimental field populations and the control lineages, we ran a mixed model ANOVA for each trait.

Population was a fixed effect and block was a random effect. Variance of the random effect was estimated using restricted maximum likelihood.

**Artificial selection experiment—Seed source and selection**—The  $G_2$  generation was the base generation for the selection experiment and was produced from 100  $G_1$  crop  $\times$   $G_1$  crop crosses in the same room and under similar conditions to those of the parental generation (in a greenhouse at OSU, Fig. 1). We randomly assigned  $G_2$  plants to one of two selection treatments (earlier flowering vs. the unselected control) and one of three replicate lineages per selection treatment, for a total of six lineages. The three replicate lineages allowed us to exclude drift as the evolutionary cause of the changes in flowering phenology. Each lineage in the  $G_2$  generation included 140 plants.

Plants were grown in Cone-tainers (Steuwe and Sons, Corvallis, Oregon, USA), filled with standard potting soil (PRO-MIX BX peat, Premier Horticulture Ltd., Rivière-du-Loup, Quebec, Canada). The replicate lineages served as blocks in the experiment and pairs of selection treatments of each replicate occupied two adjacent greenhouse benches. We randomly repositioned plants within the greenhouse every two weeks to reduce the effects of environmental variation in the greenhouse.

Each generation, we recorded the dates of germination and flowering for each plant. To produce the following generation, we imposed truncation selection for early flowering plants in three lineages by selecting 10% of each lineage that flowered earliest. For the three control lineages, we randomly selected 10% of the plants, regardless of flowering times. Selected plants were cross-pollinated in a complete diallel. For the second and third generations, every lineage was initiated with at least 225 seeds. Two weeks later, we reduced the size of every lineage to the size of the smallest lineage to maintain equally sized lineages. Each  $G_2$ ,  $G_3$  and  $G_4$  lineage included 140, 130, and 50 plants produced by 100  $G_1$ , 16  $G_2$ , and 13  $G_3$  parental plants.

**Common garden**— $G_4$  plants from the early and control lineages were grown in a common garden to assess differences in flowering time, fecundity, and other phenotypic traits. Control lineages were expected to represent variation in the evolutionary trajectory of randomly mated populations that have not experienced selection. If these control lineages had adapted to greenhouse conditions, we assumed this had minor effects, if any, on measured traits because germination rates were high and plants were mated randomly in each generation.

Plants from the artificial selection experiment were planted in the same common garden described earlier, using identical methods, and were interspersed with plants from the field populations. Each of the 10 blocks included five individuals per artificial selection lineage, for 150 early flowering and 150 control plants in the complete randomized block design. To represent each lineage, we haphazardly collected one seed per fruit from three fruits per plant.

**Data collection and analysis**—As before, we noted age at flowering, stem diameter, flower number, seeds per fruit, and seed number. All plants survived to reproduce. To determine if plants responded to selection, we compared the age at flowering of selected and nonselected paired replicate lineages using a repeated-measures ANOVA of  $G_4$  plants. To explore if plants exhibited a correlated response to selection in plant size, we compared the stem diameter of selected and nonselected paired replicate lineages using a repeated-measures ANOVA. In both analyses, selection treatment was the repeated measure, and replicate and block were random effects. Age at flowering and stem diameter were log-transformed prior to analysis. To test for differences in lifetime fecundity in selected and nonselected paired replicate lineages, we compared seeds per fruit, flower number, and seed number using a multivariate, repeated-measures ANOVA. Selection treatment was the repeated measure, and replicate and block were random effects. Number of seeds and flowers per plant were log-transformed.

## RESULTS

**Population dynamics of experimental field populations**—By 2005, three of the five populations ( $C_1$ ,  $C_2$ ,  $C_3$ ) were effectively extinct (no flowering adults, Fig. 2). One of these populations,  $C_1$ , lasted longer and grew to 149 plants in 2003, declining to only nine plants in 2005. In the two populations that persisted through 2005, peak population sizes were 7839 plants for  $C_4$  and 502 plants for  $C_5$ . Both of these populations appeared healthy

at the end of the study (Fig. 2). We observed plants with non-flowering rosettes with large roots in all of the populations, and this domestication trait undoubtedly retarded population growth rates. Populations  $C_4$  and  $C_5$  flowered earlier than  $C_1$  in 2004, unlike in 2002, when these three populations flowered synchronously (L. G. Campbell and A. A. Snow, personal observation).

In populations  $C_4$  and  $C_5$ , yellow-flowered plants appeared in 2004, revealing that these populations had received unintended gene flow from wild populations. Yellow-flower color allele frequencies in these populations suggest that 7–14% of the alleles were of *Raphanus raphanistrum* origin in 2004, given Hardy–Weinberg expectations. This frequency increased to 10–18% in 2005. Thus, the only populations that persisted for more than two generations were the two that apparently hybridized with *R. raphanistrum*. If yellow flower color is used as an indicator of hybridization, then approximately 19% of the  $C_5$  seeds and 32% of the  $C_4$  seeds used in the common garden experiment could have been homozygous or heterozygous for wild-type traits (based on Hardy–Weinberg expectations and the allele frequencies of 10% and 18% [Fig. 2], respectively).

**Phenotypes of field populations in a common garden**—For many phenotypic traits, plants from population  $C_1$  were intermediate between plants from populations  $C_4$  and  $C_5$  (which included yellow-flowered plants) and those from the nonselected control lineages created in the artificial selection experiment (Table 1; for the ANOVA, see Appendix S1 in Supplemental Data with online version). Unlike the plants in the field populations, all plants in the common garden initiated flowering before the end of the growing season. Perhaps field populations were less likely to bolt than common garden plants because they were not confined to pots and could reach larger rosette sizes. In the common garden, plants from population  $C_1$  initiated flowering 3 days earlier than the control population and 5–7 days later than populations  $C_4$  and  $C_5$  ( $F_{3,27} = 83.92$ ;  $P < 0.001$ ). Plants from  $C_4$  and  $C_5$  had significantly smaller stem diameters than either  $C_1$  or the control populations ( $F_{3,27} = 67.62$ ,  $P = 0.001$ ; posthoc comparison:  $P < 0.001$ ), but did not differ from each other (posthoc comparison:  $P = 1$ ). The competing oats of control populations were largest, whereas the oats of  $C_4$  were significantly smaller than those of population  $C_1$  and the control population ( $F_{3,27} = 13.61$ ,  $P < 0.001$ ). This suggests that plants from population  $C_4$  were more competitive than those from  $C_1$ . Plants from population  $C_1$  did not evolve significantly different lifetime seed production than the plants from the control lineages ( $P > 0.05$ ; Table 1).

Differences in seed production per plant among the three populations were marginally significant ( $P = 0.06$ ), and plants from  $C_4$  produced 54% more seeds than plants from  $C_1$ . The tendency for population  $C_4$  to produce more seeds may be because plants from  $C_4$  produced significantly more flowers ( $P < 0.002$ ) and aboveground vegetative biomass ( $P < 0.004$ ) than plants from other populations. This population also had a high frequency of yellow-flowered alleles (18%; Fig. 2). Finally, both  $C_4$  and  $C_5$  had reduced pollen fertility relative to population  $C_1$  ( $P > 0.001$ ), presumably as a result of the reciprocal translocation associated with hybridization between *R. raphanistrum* and *R. sativus*.

**Phenotypic evolution in artificial selection lineages**—The radish cultivar Red Silk responded somewhat to two generations of strong selection for earlier flowering, but the results are equivocal (Fig. 3). Overall, selection for early flowering advanced age at flowering (selection effect:  $F_{1,2} = 40.27$ ,  $P < 0.001$ ). Specifically, posthoc testing revealed that two of the three early flower-

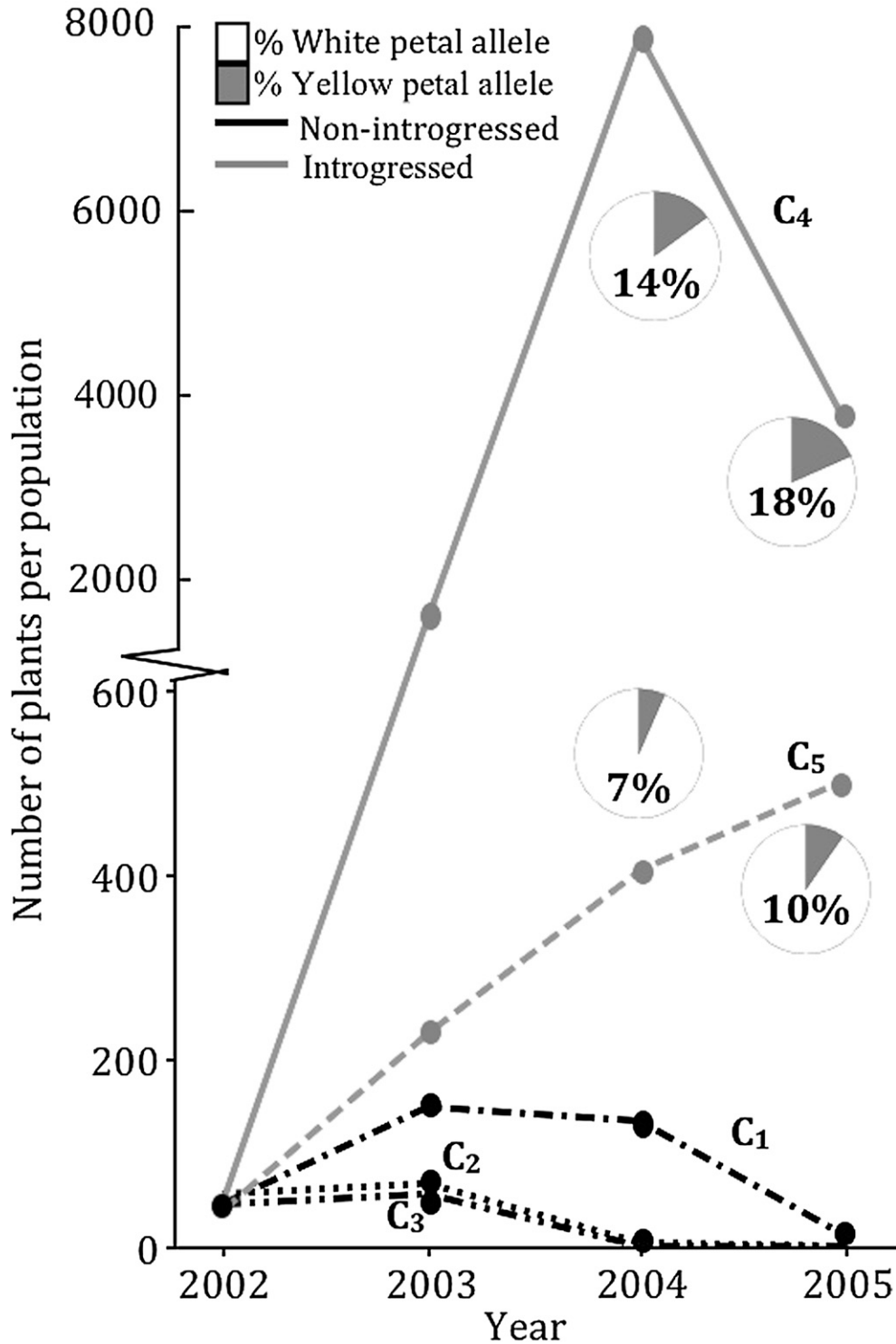


Fig. 2. Annual population size (lines) and frequencies of petal color alleles (pie charts, numeric values) of introgressed (C4, C5) and nonintrogressed (C1–C3) feral *Raphanus sativus* field populations. In 2004, yellow petals appeared in two populations.

ing replicate lineages flowered significantly earlier (5–6 days) than control lineages. However, in the third replicate lineage, control and early flowering lineages did not differ in date of flowering, suggesting that the third lineage in the early selection treatment did not possess heritable variation for age at flowering

(selection  $\times$  replicate effect:  $F_{2,18} = 15.18$ ,  $P < 0.001$ ). Age at flowering was also affected by the main effect block ( $F_{9,18} = 2.86$ ,  $P = 0.004$ ) and the selection  $\times$  block interaction ( $F_{9,18} = 2.05$ ,  $P = 0.04$ ). Therefore, the cultivar apparently possesses a limited amount of heritable variation for earlier flowering.

TABLE 1. Trait means (and standard errors) of plants from control, nonintrogressed, and introgressed populations in a common garden. Control plants were derived from three generations of random mating in a greenhouse, and the field populations underwent three generations of natural selection. Superscripts represent significant differences among field populations ( $P < 0.05$ ; see text for comparisons with control plants); n/a: not available.

Trait	Control greenhouse populations	Field population		
		C <sub>1</sub> Non introgressed	C <sub>4</sub> Introgressed	C <sub>5</sub> Introgressed
<i>N</i>	147	49	50	48
Age at flowering (days)	60 (0.8) <sup>c</sup>	57 (0.6) <sup>b</sup>	50 (0.6) <sup>a</sup>	52 (0.6) <sup>a</sup>
Root diameter (mm)	29.0 (0.2) <sup>b</sup>	23.8 (1.4) <sup>b</sup>	12.7 (0.7) <sup>a</sup>	12.6 (0.8) <sup>a</sup>
Seeds per plant	230.8 (67) <sup>b</sup>	257 (44) <sup>b</sup>	397 (68) <sup>a</sup>	312 (37) <sup>a</sup>
Flowers per plant	271 (55) <sup>b</sup>	194 (21) <sup>b</sup>	397 (43) <sup>a</sup>	225 (25) <sup>b</sup>
Seeds per fruit	3.0 (0.1) <sup>b</sup>	3.9 (0.2) <sup>a</sup>	3.3 (0.2) <sup>a,b</sup>	3.4 (0.2) <sup>a,b</sup>
Fruit set (%)	30.3 (0.01) <sup>b</sup>	38.8 (2.1) <sup>a</sup>	38.0 (2.1) <sup>a</sup>	33.1 (2.1) <sup>a,b</sup>
Pollen fertility (%)	n/a	71.7 (2.1) <sup>a</sup>	60.8 (2.7) <sup>b</sup>	61.7 (3.6) <sup>b</sup>
Oat biomass (g)	2.2 (0.2) <sup>c</sup>	2.1 (0.2) <sup>b,c</sup>	1.6 (0.1) <sup>a</sup>	1.9 (0.1) <sup>a,b</sup>

The plants evolved smaller stem diameters in response to selection on early flowering (Fig. 3; Appendix S2 with online Supplemental Data). Early selected lineages had stem diameters that were 4–6 mm smaller than the stem diameters of control lineages across all three replicates (selection effect:  $F_{1,2} = 23.69$ ,  $P < 0.001$ ). Replicate lineages did not significantly differ in root diameter ( $F_{2,18} = 0.22$ ,  $P > 0.05$ ).

**Correlated fitness consequences of early flowering**—Early flowering plants were much more fecund than control plants in the common garden (Fig. 3; online Appendix S2). Early replicate 1 produced 93% more seeds than control replicate 1 and early replicate 2 produced 87% more seeds than control replicate 2 (selection × replicate effect:  $F_{2,18} = 7.098$ ,  $P < 0.001$ ). These large differences may be due to 72–78% higher flower production (selection effect:  $F_{1,2} = 4.36$ ,  $P = 0.039$ ; selection × replicate effect:  $F_{2,18} = 24.32$ ,  $P < 0.001$ ). Unlike the other two replicates, the lifetime fitness of early replicate 3 did not differ significantly from its control replicate 3 for any trait.

## DISCUSSION

**Persistence of feral radish populations**—Our efforts to establish experimental populations of feral *R. sativus* in Michigan in the absence of crop–wild hybridization were unsuccessful. The three apparently nonintrogressed populations did not survive for more than three generations, whereas the two putatively introgressed populations grew vigorously. Compared with non-selected control plants, the most persistent nonintrogressed population (C<sub>1</sub>) evolved somewhat smaller roots and earlier flowering, but not greater fecundity. Many plants in populations C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub> had swollen roots and failed to flower or set fruit before the growing season ended, allowing other weedy annuals to invade the field plots. In a milder climate than Michigan, such as the coast of California, incipient feral populations with late-flowering and biennial phenotypes might be able to persist, but the delayed flowering trait was clearly maladaptive in Michigan.

To our knowledge, nonhybridized feral *Raphanus sativus* populations occur in Japan and Brazil, and they probably occurred along the California coastline before being displaced by

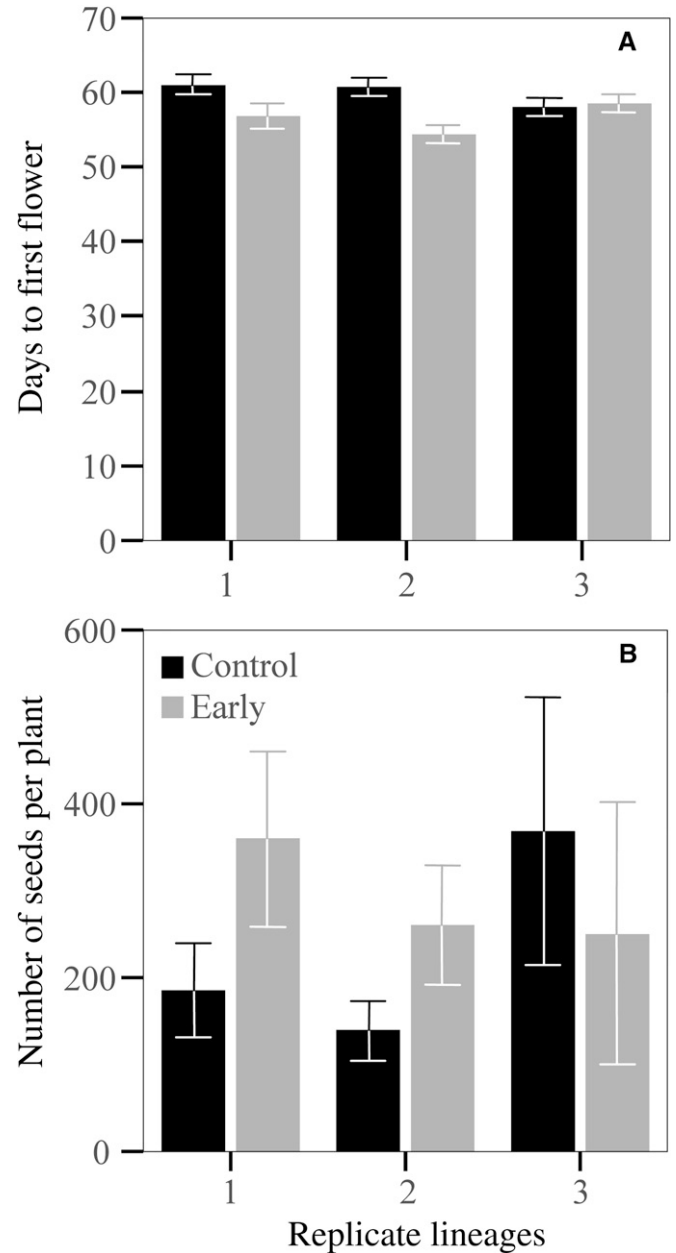


Fig. 3. Effects of artificial selection for early flowering on flowering phenology and the lifetime fecundity of selected lineages relative to control lineages in a common garden. Bars represent the mean value of an early flowering lineage relative to mean value of the paired control lineage; error bars represent the 95% CI; for paired lineages,  $N \approx 50$  per lineage.

crop–wild hybrids (Panetsos and Baker, 1967; Hegde et al., 2006; Ridley et al., 2008). In Japan, feral *R. sativus* populations occur in roadside ditches near fields of cultivated daikon radish, where hybridization with wild plants was not observed (Yamaguchi and Okamoto, 1997). These biennial, feral daikon plants have small, branched roots and are considered by farmers to both “contaminate” and enhance the genetic diversity of landraces (Yoon and Pyo, 1977). In southern Brazil, feral *R. sativus* weeds, apparently derived from cultivated forage radishes, have developed resistance to herbicides (Heap, 2008; G. Theisen, Fundacep Fecotriga, Cruz Alta, Brazil, personal communication). Since 1980, no-till agriculture has predominated

in this region, and a *R. sativus* forage cultivar known as forrageiro was planted widely as a cover crop. Where forrageiro occurred as a volunteer weed in summer crops, it was often treated with various herbicides. By 2001, feral *R. sativus* acquired resistance to several acetolactate synthase (ALS)-inhibiting herbicides in the southern state of Rio Grande do Sul. Based on photographs of the flowers and fruits of these plants (Heap, 2008), and information provided by G. Theisen, feral *R. sativus* does not appear to have the constricted fruits or yellow flower pigmentation common in *R. raphanistrum*, suggesting that these are nonhybridized feral populations.

**Hybridization as a stimulus for ferality**—Two of our experimental populations appeared to have hybridized with *R. raphanistrum* based on the presence of yellow-flowered plants and other traits such as smaller root diameters, earlier flowering, and lower pollen fertility, which is characteristic of crop–wild hybrids (Panetsos and Baker, 1967). The source of *R. raphanistrum* genes in these populations is unknown. We suspect our own movements among flowering populations of wild, hybrid, and feral plants, which were sometimes visited sequentially on the same day, might be the source of pollen contamination. Other possible sources of pollen contamination seem unlikely. We have never detected evidence of heterozygous crop parents in the thousands of crop and crop–wild hybrids that we have examined; therefore, it is unlikely that the original crop seeds included a few crop–wild hybrids. Also, the original greenhouse crosses were performed under strict isolation to prevent contamination by wild pollen, and no hybrid phenotypes were seen in the first generation or the next two generations, when we examined the flower color of >3000 crop plants grown in the artificial selection study. Because no seedlings of *R. raphanistrum* emerged during the 2002–2003 summers, based on the lack of yellow-flowered plants, it seems unlikely that dormant seeds were present at the field sites. Natural cross-pollination also seems improbable because local populations of *R. raphanistrum* were uncommon and distant, as were constructed hybrid populations (Campbell et al., 2006).

Regardless of their source, the unintentional presence of wild alleles in two of our populations permitted us to explore the weed potential of apparently hybridized feral populations. It is intriguing that the two populations with the yellow petal allele ( $C_4$  and  $C_5$ ) exhibited earlier flowering and greater fecundity than the  $C_1$  population and also had the most rapid population growth in the experimental plots. Many other factors could have influenced population growth rates, such as differences in weedy competitors, soil fertility, pathogens, or deer browsing. Thus, it is not possible to draw strong conclusions from the fact that the only healthy and persistent populations were the two that had wild-type traits. Nonetheless, we suggest that heritable phenotypic differences among the five field populations, due primarily to the presence of wild alleles, may have contributed to the observed differences in population persistence vs. extinction.

Along with other published studies (Panetsos and Baker, 1967; Klinger and Ellstrand, 1994), our results suggest introgression of wild alleles facilitated population persistence and higher growth rates in feral radish populations and allowed the rapid evolution of earlier flowering and increased fecundity as compared to nonintrogressed feral populations. Introgressed populations had lower pollen fertility than nonintrogressed plants, but reduced male fitness is not expected to impede population growth under natural pollination conditions where seed

set is not pollen-limited (Stanton et al., 1986). Our results suggest that infrequent hybridization events can act as an external “trigger” for the evolution of weedy feral populations of *R. sativus*. Therefore, we hypothesize that exoferality has contributed more to dedomestication than endoferality in regions where *R. raphanistrum* is present.

Hybridizing crop–wild radish populations have been reported in Europe (Clapham et al., 1987; Stace, 1991), Asia (Yamaguchi and Okamoto, 1997), and North America (Panetsos and Baker, 1967). Wild *R. sativus* var. *hortensis* f. *raphanistroides*, a biennial plant, is common in Japan and Korea along beaches, cliffs, abandoned fields, and other ruderal areas, and sometimes occurs adjacent to cultivated daikon radishes (Yamagishi and Terachi, 2003), but these populations may be derived from *R. raphanistrum* rather than *R. sativus* (Hegde et al., 2006). Hybridized populations have been particularly well studied in California. Independent populations of feral *Raphanus sativus* and wild *R. raphanistrum* first appeared in California in the 19th century (Panetsos and Baker, 1967). These taxa hybridized so extensively that distinct populations of *R. raphanistrum* seem to have disappeared (Hegde et al., 2006).

**Heritability of earlier flowering in radish cultivars**—To complement results from naturally evolving populations, we examined the potential for earlier flowering to evolve using an artificial selection experiment. Age at flowering is a highly heritable trait in *R. raphanistrum*, *R. sativus*, and crop–wild hybrids (Rabbani et al. 1998; Mazer and Schick, 1991a,b; Campbell et al., 2008). Delayed flowering is a domestication trait that is maladaptive in short growing seasons, and we wanted to determine whether plant breeders had essentially eliminated heritable variation for earlier flowering in the Red Silk cultivar. The evolution of earlier flowering may be one of the first traits required for the establishment of volunteer and feral radish populations (Yamaguchi and Okamoto, 1997; Snow and Campbell, 2005).

Only two of the three lineages responded to selection for earlier flowering and their response was modest—they flowered about 5 days earlier than unselected controls. This lack of response again supports the argument that hybridization seems to be essential for the dedomestication of cultivated radish, rather than ferality via selection on standing variation within the crop. We found this difference in flowering date was correlated with smaller root diameters and a surprisingly large (~90%) increase in flower number and lifetime fecundity relative to control lineages (Fig. 3). Reasons for greater fecundity in the two early-flowering lineages are unclear and do not appear to be related to frost damage or other changes that occurred late in the growing season.

The choice of crop cultivar may influence the available genetic variation upon which selection can act in feral populations. In a survey of 10 radish cultivars, we found that daikon, podding, and forage radishes flowered 8–18 d earlier than the European small-rooted radishes (Campbell, 2007). It is perhaps not coincidental that the reported occurrences of nonhybridizing feral radish involve similar types of daikon and forage radishes (Yamaguchi and Okamoto, 1997; Snow and Campbell, 2005). In California, the co-occurrence of diverse radish cultivars, each possessing unique traits and genetic diversity (Ellstrand and Marshall, 1985; Muminovic et al., 2005; Hegde et al., 2006; Ridley et al., 2008), also may have promoted ferality in radish.

**Conclusions**—Our results suggest that the Red Silk cultivar of *Raphanus sativus* is not capable of establishing feral populations in Michigan without the aid of gene flow from *R. raphanistrum*. In other regions, volunteer radish populations might be considered “incipient” weeds. Some of these populations may evolve into vigorous weeds given an advantageous combination of environmental conditions and genetic diversity, as occurred in no-till rotations in Brazil (Snow and Campbell, 2005), and this process may be accelerated by high rates of gene flow from diverse cultivars or weedy *R. raphanistrum*. Given that agriculture disperses seeds over great distances, feral populations of *R. sativus* may eventually encounter favorable conditions for invasion and rapid expansion. However, for cultivated European radishes to evolve endoferility, key mutations and strong selection may be needed to reduce frequencies of deleterious crop traits such as delayed flowering and increase frequencies of “weedy” traits.

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