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Competition alters life history and increases the relative fecundity of crop–wild radish hybrids (*Raphanus* spp.)

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Summary

- The evolutionary impact of crop-to-wild gene flow depends on the fitness of hybrids under natural, competitive conditions. Here, we measured the performance of third-generation (F_3) radish hybrids (*Raphanus raphanistrum* × *Raphanus sativus*) and weedy *R. raphanistrum* to understand how competitive interactions affect life history and relative fecundity.
- Three wild and three F_1 crop-wild hybrid radish populations were established in semi-natural, agricultural conditions in Michigan, USA. The effects of competition on life-history traits and fecundity of F_3 progeny were measured 2 yr later in a common garden experiment.
- Third-generation hybrid plants generally produced fewer seeds per fruit and set fewer fruits per flower than wild plants, resulting in lower lifetime fecundity. With increasing competition, age at reproduction was delayed, the relative number of seeds per fruit was reduced in wild plants and differences between hybrid and wild fecundity diminished.
- Competition may enhance the fecundity of advanced-generation hybrids relative to wild plants by reducing differences in life history, potentially promoting the introgression of crop alleles into weed populations.

Key words: artificial populations, hybridization, life-history trade-offs, path analysis, *Raphanus* spp., response surface competition experiment, weed, wild radish.

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Introduction

Spontaneous hybridization among crops and their wild relatives may promote the rapid evolution of weeds (Ellstrand *et al.*, 1999). As recent advances in genetic engineering and breeding have introduced novel, transgenic traits into crops, ecologists have asked whether subsequent gene flow could lead to the introgression of dominant, single-gene traits into populations of wild relatives that occur near crops (e.g. in rice, Gealy *et al.*, 2003; sunflower, Snow *et al.*, 2003; squash, Fuchs *et al.*, 2004). Transgenic traits such as resistance to diseases, herbivores, and insects could enhance fitness in some cases and might allow weedy relatives to become more abundant (Snow *et al.*, 2003). Meanwhile, little is known about the potential for complex, quantitative crop traits to enhance the fecundity of wild relatives. Although hybridization with some cultivated plants may reduce the fitness of weeds in natural environments (Stewart *et al.*, 2003), possible benefits of crop–wild hybridization for weeds suggest this phenomenon should not be overlooked (Boudry *et al.*, 1993; Miura & Terauchi, 2005; Campbell *et al.*, 2006). Studies of advanced-generation hybrids find that some interspecific hybrid genotypes persist for multiple generations under certain environmental conditions and therefore long-term gene introgression is possible (Lexer *et al.*, 2003). Introgression may lead to trait combinations that enhance fecundity, competitiveness, and/or pest resistance, and these advantageous genotypes may be able to invade new habitats because of their superior weediness (Rhymer & Simberloff, 1996; Ellstrand & Schierenbeck, 2000; Hauser *et al.*, 2003; Whitney *et al.*, 2006).

Performance measures of late-generation hybrids under 'realistic' environmental conditions are fundamental to understanding how crop–wild hybridization may alter the phenotype and fecundity of weeds (Lexer *et al.*, 2003). Superior performance of crop–wild hybrids has been detected in a few cases where fecundity, number of flowers, or aboveground biomass were used as estimates of lifetime fitness (Klinger & Ellstrand, 1994; Pertl *et al.*, 2002; Vacher *et al.*, 2004). Although examples of superior performance of hybrids are rare, a growing number of studies show that hybrid fitness depends on genotype, generation, and environment (Campbell *et al.*, 1998; Hauser *et al.*, 2003; Lexer *et al.*, 2003). However, most studies involve only early generation hybrids that may display either heterosis, a transient condition that may overestimate the probability of persistence of crop genes within weed populations (reviewed in Arnold & Hodges, 1995; Arnold, 1997), or outbreeding depression, a transient condition that may underestimate the probability of persistence of crop genes within weed populations (Ellstrand, 1992).

The fecundity and resulting evolutionary impact of crop–wild hybrids may depend on their ability to compete with wild relatives, given that crop allele introgression will inevitably lead to populations composed of both wild and crop–wild hybrid plants (Vacher *et al.*, 2004). Predicting the probability of crop allele introgression and the population dynamics of weeds may be accomplished by modeling plant competition with individual performance values derived from plant competition studies (Pascual & Kareiva, 1996; Damgaard, 1998). Many studies have attempted to estimate hybrid success under competitive conditions (Halfhill *et al.*, 2005; Mercer, 2005; for more examples, see the Discussion) and often conclude that, based on the relative number of seeds produced per plant, hybrids are less competitive or equally as competitive as wild taxa (but see Hauser *et al.*, 2003). Alternatively, the effect of competition on the population growth rates of crop–wild hybrids and wild plants may be predicted using mathematical models to provide insights into the long-term evolutionary impact of introgressed alleles (Volterra, 1926; Damgaard, 1998; Hauser *et al.*, 2003).

Many studies of plant competitive ability, fecundity, and invasiveness have attempted to detect the life-history strategies that contribute to successful competitors and invaders (Rejmánek & Richardson, 1996; Gerlach & Rice, 2003). One approach involves comparative studies of weedy and nonweedy species (Baker, 1965; Crawley *et al.*, 1996; Williamson & Fitter, 1996; Sutherland, 2004). However, it may be more meaningful to examine the life-history traits of a group of closely related species to determine which traits contribute to weediness (Mack, 1996; Grotkopp *et al.*, 2002). Specifically, identification of successful weed life-history strategies may depend on the complex interactions between the weed phenotype and environment. Small differences in the life-history traits of a plant, such as the timing of germination or reproduction, may interact with the competitive environment to influence at a small scale, plant fecundity, and at a larger scale, plant distributions and abundance (Sans *et al.*, 2004).

In this study, we used plants from experimental populations of the weedy annual *Raphanus raphanistrum* and its crop–wild hybrid offspring (*R. raphanistrum* × *Raphanus sativus*) to investigate both the effects of competition on hybrid fitness and how fitness differences between wild and hybrid plants may be altered by variation in the competitive environment. *Raphanus raphanistrum* is a well-established model system in studies of plant evolution and ecology that has been used to evaluate the ecological consequences of crop-to-wild gene flow (Klinger *et al.*, 1991; Snow *et al.*, 2001; Hegde *et al.*, 2006). We estimated the competitive ability of two 'biotypes', wild *R. raphanistrum* and advanced-generation crop–wild hybrids, using a response surface competition experiment. We also explored how life-history traits and fecundity were affected by varying density and biotype frequency. We used a path analytic approach to test a model of causal interactions among life-history traits and fecundity (Shipley, 2000), derived from a combination of previous path analytical studies of *Raphanus* (Scheiner *et al.*, 2002) and our own experience with this system. We discuss the potential implications of these processes for the introgression of crop alleles into weed populations.

Specifically, we asked the following questions:

- What is the lifetime fecundity of crop–wild hybrids compared to their wild relatives, and under which competitive conditions is the relative fecundity of advanced-generation hybrids maximized?;
- Is crop–wild hybrid radish as competitive as its weedy progenitor, as measured by competition coefficients?;
- Does competition affect the relative fecundity of wild and hybrid plants by altering their size or age at reproduction?.

Materials and Methods

Study system

Raphanus raphanistrum L. (wild radish or jointed charlock) is a widespread weed of Eurasian origin that occurs in agricultural fields, disturbed areas, and coastal beaches (Holm *et al.*, 1997; Warwick & Francis, 2005). With its long-lived seed bank, early emergence after tilling, and annual growth habit, *R. raphanistrum* is a difficult weed to manage, especially in cereal crops (Warwick & Francis, 2005). It grows a rosette with a thin, fibrous taproot. In Michigan, the plants 'bolt' within a few weeks after germination, when the primary flowering shoot emerges from the rosette. *Raphanus sativus*, the cultivated relative of wild radish, is an open-pollinated crop with large roots, delayed flowering, and high levels of seed production (Snow & Campbell, 2005).

Raphanus raphanistrum and *R. sativus* are self-incompatible, insect-pollinated, and interfertile (Snow & Campbell, 2005). 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 hybrids produce approximately 50–60% aborted pollen grains (Snow *et al.*, 2001). We found that F_1 hybrids can produce as many seeds as their wild relative (but see Snow *et al.*, 2001), and in some environments the fitness of advanced-generation hybrids may greatly exceed that of their weedy progenitor (Campbell *et al.*, 2006).

Cultivated and wild radish species were introduced into California by the nineteenth 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 of crop-wild hybrids, known as wild or feral *R. sativus*, appear to have displaced the original populations of *R. raphanistrum* in California during the past few decades (Snow *et al.*, 2001; Hegde *et al.*, 2006).

Seed sources for experimental populations

In 2001, we collected seeds from several-hundred plants in a natural population of wild *R. raphanistrum* in an agricultural field in Pellston, MI, USA. In a glasshouse at Ohio State University (Columbus, USA) we germinated and grew 100 wild plants and hand-pollinated them with either wild pollen to create F_1 wild biotypes or crop pollen to create F_1 hybrid biotypes. Crop pollen was obtained from 100 'Red silk' *R. sativus* plants (Harris-Moran Seed Co., Modesto, CA, USA), a common, contemporary variety. Below, we refer to radish biotypes as 'wild' or 'hybrid' based on hybridization in this first generation.

Establishment of experimental populations

In 2002, we established three F_1 wild populations (W_1, W_2 and W_3) and three F_1 hybrid populations (H_1, H_2 and H_3), in Emmett and Cheboygan counties, MI, USA (Fig. 1; part of a larger study described in Campbell *et al.*, 2006). The six populations were separated from each other and from local wild radish populations by at least 1 km, which is far enough to avoid unintended gene flow via pollinators (Ellstrand & Marshall, 1985). On May 20, 2002, wild and hybrid seeds were planted in 300 ml of PRO-MIX 'BX' peat (Premier Horticulture Ltd, Rivière-du-Loup, Canada) in biodegradable fiber pots that were maintained in a glasshouse at the University of Michigan Biological Station (UMBS), Pellston, MI (Jiffy Products of America, Inc., Norwalk, OH, USA). Three weeks later, each field population was created by planting 50–60 seedlings in a recently tilled 15 × 15 m plot fertilized with slow-release Osmocote (19N : 6P : 12K, 22.7 kg per site; Scotts Miracle-Gro Co., Marysville, OH, 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 ranged from 42 to 60 in 2002, and reached several thousand plants per population in subsequent years (Campbell *et al.*, 2006). Each spring through 2004, the plots were tilled, fertilized and hand-weeded for 2 wk to simulate agricultural management and to promote population persistence. Otherwise, the populations experienced natural weather conditions, competing plants, herbivores, pathogens, and pollinators (primarily native bees, European honey bees, and syrphid flies; as in Lee & Snow, 1998).

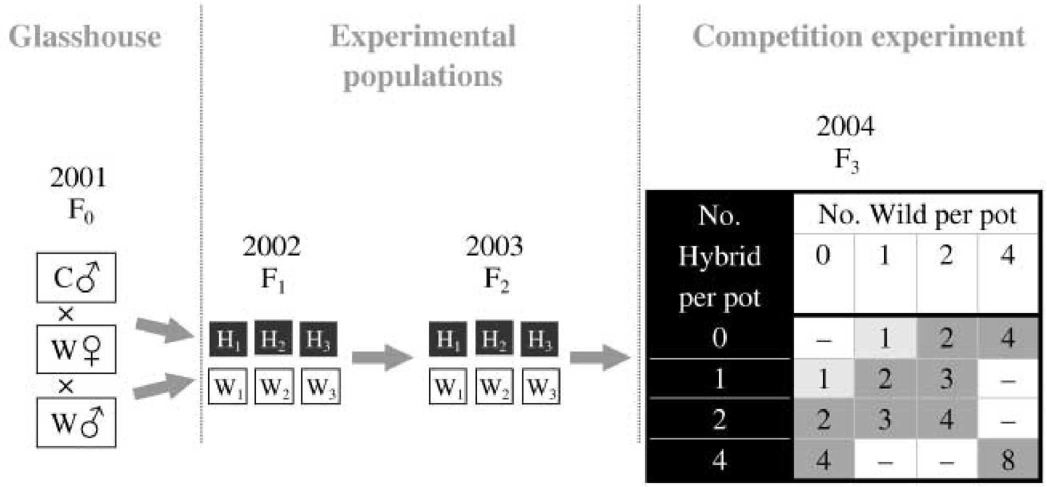


Fig. 1 Schematic diagram of the experiment. The first-generation (F_1) was created by cross-pollinating wild plants (W) with either wild or cultivated (C) radish pollen to create wild and hybrid (H) biotypes. Six isolated field populations of wild biotypes (W_1–W_3) or hybrid biotypes (H_1–H_3) were maintained for 3 yr; small squares represent populations of the two biotypes. In 2004, a response surface competition experiment with plant density (one, two or four plants per biotype) and biotype frequency treatments (interbiotype, intrabiotype$_{\text{Wild}}$, or intrabiotype$_{\text{Hybrid}}$) was composed of F_3 plants from each population. In the competition experiment: dark gray squares, competition treatments; light gray squares, no competition treatments. The numbers within the matrix positions describing the replacement series competition experiment represent the number of plants per growth container.

Competition experiment

Seed sources In 2003, we collected F_3 seeds directly from F_2 plants growing in the three wild and three hybrid populations for the competition experiment (Fig. 1). We collected 21 seeds from 10 fruits on each of 30 mothers in each population to obtain a diverse group of plants in the experiment. Hereafter, we refer to F_3 hybrid plants as 'hybrids' or 'advanced-generation hybrids'.

Common garden In a response surface competition experiment, we varied the density and frequency of each biotype independently to estimate the competitive ability of wild and hybrid biotypes by estimating their competition coefficients (Fig. 1; Damgaard, 1998; Inouye, 2001). We measured the lifetime fecundity of F_3 plants in a common garden at UMBS. Plants from each wild population were paired to compete with plants from one hybrid population to determine whether interactions between biotypes depended on the pairs of source populations. Population pairs were haphazardly assigned. The response surface experiment included 11 treatments that varied both plant density and biotype frequency (Fig. 1). The garden was arranged in a complete randomized block design where each treatment was replicated 12 times, once per block; a total of 396 pots and 1621 plants were included in the experiment.

Seeds were planted in 22.3 ml of PRO-MIX 'BX' peat in seed trays in a greenhouse at UMBS in early May 2004. The emergence date of each seedling was noted. The seedling emergence dates of competing plants differed by no more than 1 d, so these data will not be considered further. Once seedlings had developed their first true leaves, each seedling was transplanted into a PVC bottomless tube pot (30 cm tall, 10.16 cm diameter) filled with 1.5 l of local sandy soil and topped with 0.5 l PRO-MIX 'BX' peat, allowing plant roots to grow into local soil. The garden area was cleared of vegetation, leveled and roto-tilled to promote uniform garden conditions. Pots were separated by 30 cm and the use of large tube pots minimized root competition among plants in neighboring pots. In each pot, wild plants were planted at one of four densities: zero, one, two or four plants. In the same pot, hybrid plants were also planted at one of the same four densities. Therefore, total plant density ranged from one plant per pot, with no competition, to eight plants per pot (Fig. 1). This range represents densities seen in natural populations of *R. raphanistrum* (L. G. Campbell & A. A. Snow, unpublished). Six seedlings died within the first week after transplanting and were replaced. If pots contained only one biotype, we refer to them as intrabiotype competition treatments and if they contained both biotypes, we refer to them as interbiotype competition treatments. For the interbiotype competition pots, seedlings were arranged such that neighboring plants were the opposite biotype. In both intrabiotype and interbiotype competition pots, seedlings were planted in a circular pattern.

Plants were watered daily until August 31 and no fertilizer was added. Insecticide (0.0033% esfenvalerate, 20 g per 9.5 l; Scotts Miracle-Gro Co.) was used to control insect herbivory once during the first month after transplantation, when herbivory was highest (primarily flea beetles). Aphids were present at low densities later in the season but did not colonize any plant heavily. Pollinators were very abundant throughout the experiment, as in Lee & Snow (1998). Plants were individually harvested as they senesced until the first hard frost when we harvested all remaining plants (September 16–20). Harvested plants were dried at 60°C.

Pre-competition measurements and analysis

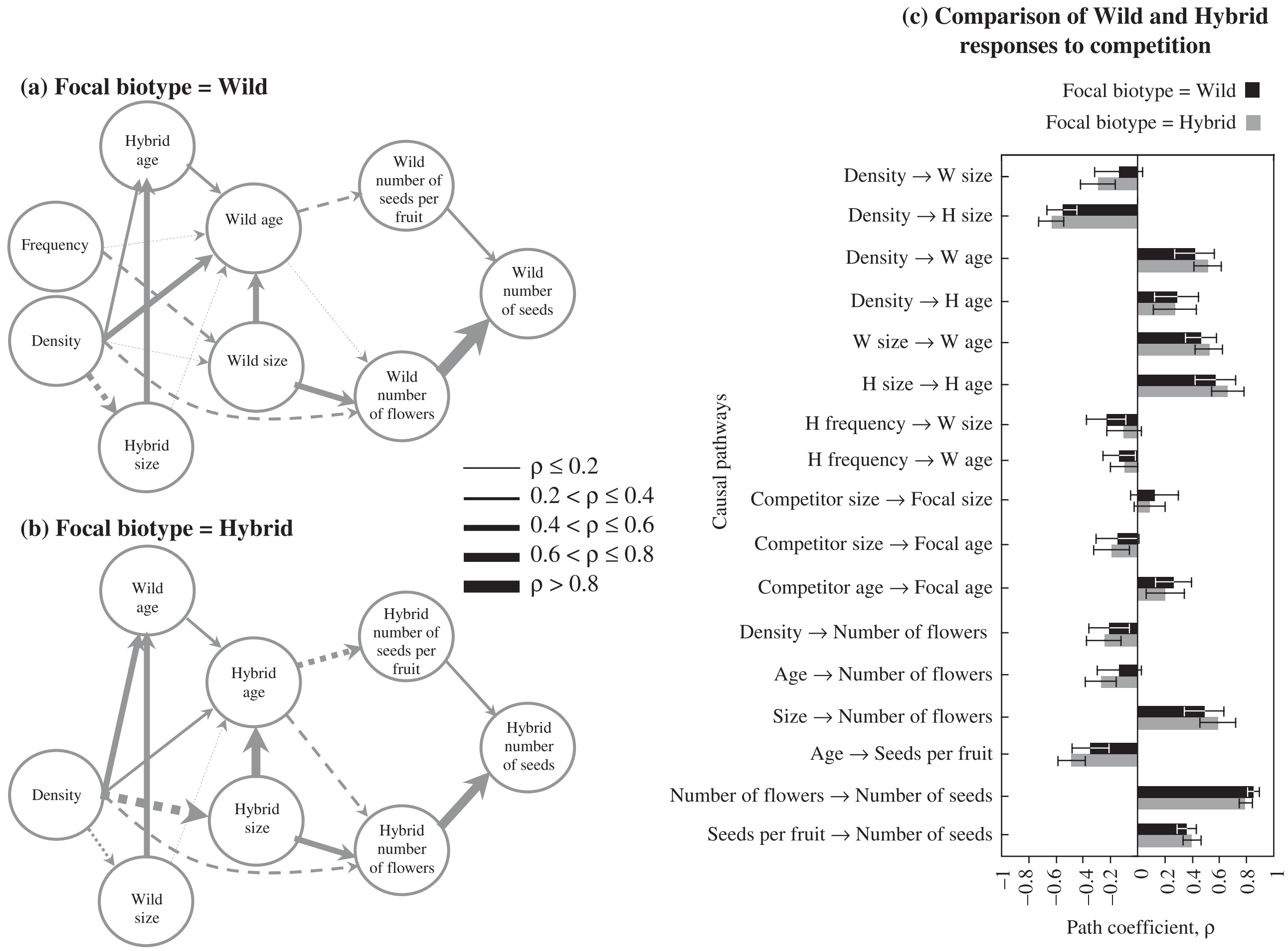
To account for pre-existing conditions before exposure to competition, we asked whether biotypes exhibited differences in seedling biomass by randomly selecting an additional 15 seedlings per wild or hybrid population and harvesting their above-ground biomass before transplanting. Each sample was dried at 60°C and weighed. A nested ANOVA was used to evaluate differences in pretransplantation seedling above-ground biomass between biotypes and populations within biotypes.

Competition measurements and analysis

For each plant, we recorded age at first flower, stem diameter at first flower (an index of overall plant size), pollen fertility, flower number, fruit number, number of seeds per fruit, and above-ground vegetative biomass. Survival after transplanting was nearly 100% and will not be considered further. Twenty-five of the 1621 plants in the experiment did not flower during the growing season and those were significantly more likely to be hybrid than wild plants (rank sum test, $P = 5.34 \times 10^{-6}$). Plants that did not flower were removed from the following analyses. Age at first flower was calculated as the number of days between seedling emergence and anthesis. Stem diameter at first flower was used as an index of plant size because it is heritable (L. G. Campbell & A. A. Snow, unpublished) and was strongly correlated with length of longest leaf at flowering ($r_{\text{Wild}} = 0.61$, $P < 0.001$; $r_{\text{Hybrid}} = 0.62$, $P < 0.001$) and flower number (Fig. 2). Stem diameter was measured on the first day of anthesis, using digital calipers at the point of attachment of the cotyledons.

To measure pollen fertility, we collected pollen from one flower on each hybrid plant and 10 wild plants per population. Because wild pollen fertility is uniformly high, a smaller sample size was justified (Campbell *et al.*, 2006). After staining (Alexander, 1969), pollen fertility was assessed with a compound microscope as the proportion of aborted grains in samples of at least 100 grains per plant.

To measure fruit set and fecundity, we counted number of flower pedicles and fruits per plant. Fruit set was calculated as number of fruits produced divided by number of flowers. To



(a) Focal biotype = Wild

(b) Focal biotype = Hybrid

(c) Comparison of Wild and Hybrid responses to competition

Fig. 2 Direct and indirect effects of plant density and biotype frequency on age and size at flowering and lifetime fecundity of F_3 (a) wild and (b) hybrid radish plants. In the solved path diagram for maximum likelihood linear analysis of hybrid relative performance, dashed lines indicate negative coefficients and the width of the arrow indicates the strength of the effect (ρ). Significant effects, black lines; nonsignificant effects, gray lines. (c) Comparison of the strength of the correlation (ρ) between models (a) and (b). Error bars represent the 95% confidence interval (CI) of the mean and nonoverlapping CI indicate a significant difference in the strength of the relationship. This analysis included only interbiotype competition conditions.

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. Finally, all fruits and leaves were removed from the plants and we weighed the remaining above-ground biomass (g) of each dried plant. Above-ground vegetative biomass was strongly correlated with the number of flowers per plant for both wild ($R^2 = 0.95$) and hybrid plants ($R^2 = 0.89$) so we report only the number of flowers per plant (data available from the authors upon request). As plants growing in the same pot were not independent, the data used in our analyses were averages of each fitness component for each biotype within a pot. We transformed data for most measured characters because of deviations from either the assumption of normality or that of homoscedasticity for the analyses below. The transformations were chosen so as to reduce or eliminate these problems, following the suggestions of Zar (1999). The average number of seeds per fruit was normally distributed and required no transformations before analysis. Data on age at first flower, stem diameter at first flower, number of seeds per plant, and number of flowers per plant were natural log transformed and pollen fertility data were arcsine square-root transformed to normalize the residuals. All analyses were performed using SPSS (v. 13; SPSS Inc., Chicago, IL, USA) or SYSTAT (v. 11.00.01; Systat Software Inc., Richmond, VA, USA).

Quantifying competitive ability To summarize plant–plant interactions within and between biotypes, we estimated the competition coefficients for each biotype from a hyperbolic competition model using data from all 11 plant density and biotype frequency treatments. A modified version of Damgaard's (1998) model was used:

$$Y_i = (a_i + b_i(D_i + c_{ij}D_j + e_iD_iD_j))^{-1}$$

(Y_i is the number of seeds per plant type i; D_i corresponded to density of plant type i; c_{ij} is the competition coefficient of biotype j on biotype i; e_i is the correlation coefficient between yield of i and density of i; a_i, and b_i are shape parameters of the effect of density on number of seeds per plant of plant type i). If $c_{ij} < 1$, then intrabiotype competition affects the fecundity of species j more than interbiotype competition. If $c_{ij} > 1$, then interbiotype competition affects the fecundity of species j more than intrabiotype competition (Damgaard, 1998). Maximum likelihood estimates of the parameters were calculated assuming normally distributed residuals using the nonlinear regression function (Levenberg–Marquardt estimation method) in SPSS.

Effect of competitive environment on lifetime fecundity To test for differences in lifetime fecundity between wild and hybrid biotypes across population pairs, plant density and biotype frequency treatments, we used an unbalanced, repeated-measures ANOVA. Wild and hybrid plants within a pot were interdependent; therefore, we used a repeated measures ANOVA, so that biotype and all its interactions were considered within-subject factors. We used a subset of the data, excluding pots with a density of three, because these frequencies were not represented at any other density (Fig. 1). Population pair, biotype frequency, and plant density were considered to be fixed, between-subjects effects, block was a random, between-subjects effect, and the comparison between biotypes within a block was a fixed, within-subjects effect. In a preliminary analysis, block interactions were found to be insignificant and were removed from the final model (L. G. Campbell, unpublished). Variance of the random effect was estimated using restricted maximum likelihood methods and will not be considered further.

Pollen fertility was found to be insensitive to competition in the above analyses so we abandoned the within-pot repeated-measures design of the competition experiment. Instead, we focused on the hierarchical design of the original field experiment; replicate wild and hybrid populations were derived from a common wild or hybrid seed source in the F_1 generation and therefore population effects were nested within biotypes (Fig. 1). We tested for differences in pollen fertility between wild and hybrid plants using a nested ANOVA that included biotype and population nested within biotype as fixed effects and block as a random effect in the ANOVA model. Variance of the random effect was estimated using restricted maximum likelihood methods.

Quantifying the indirect effects of competition Path analysis can be used to test causal hypotheses and judge their ability to predict an observed covariance structure among a set of variables (Shipley, 2000). Models are constructed based on biological knowledge of the study system and are often used to gain insights into the biological mechanisms producing observed phenomena (Conner *et al.*, 1996; Scheiner *et al.*, 2000; Scheiner *et al.*, 2002; Pigliucci & Kolodynska, 2006). The path analytic approach is also compatible with the response surface design of our experiment, allowing us to explore the fitness consequences of a greater diversity of plant densities and hybrid frequencies that are incompatible with the conventional analysis of variance performed above. With this analysis, our goal was to examine how age at flowering, size at flowering, and lifetime fecundity were affected by hybridization and competition treatments.

We analysed the fitness consequences of competition for each biotype separately with distinct hierarchical a priori expectations using linear path analysis (Scheiner *et al.*, 2000; using Procedure RAMONA in SYSTAT). Again, we used the mean phenotype values for each biotype within each pot. We began by considering a model proposed by Scheiner *et al.* (2002) that described the relationships of morphological traits to measures of reproductive success in wild *R. sativus*. In their model, plant size at flowering had a direct effect on two later characters, total number of flowers per plant and number of seeds per fruit. Age at flowering indirectly affected average number of seeds per fruit. Finally, number of flowers per plant and number of seeds per fruit directly influenced the number of seeds per plant. We modified the model to include the particular set of traits we had measured and incorporated variables that described the competitive environment. We allowed plant density and hybrid frequency to directly influence all traits. After an initial analysis, we removed all causal linkages deemed redundant by SYSTAT and nonsignificant paths (P-value > 0.1) with ρ (rho) < 0.1. This improved the fit of our model (see below for a description of how we estimated model fit). The model fit was also improved by incorporating the effect of competitor size and age on the focal biotype size and age, even after including plant density and biotype frequency. The resulting causal model is illustrated in Fig. 2.

We then estimated this causal model for both wild and hybrid datasets. In the analysis, we used data from 222 pots that contained both wild and hybrid plants (i.e. only interbiotype competition conditions) and for which we had records for all traits. To quantitatively summarize the relative importance of the indirect pathways to lifetime fecundity, we multiplied together causally linked path coefficients. In each case, model performance was evaluated by criteria based on the SYSTAT output. The root mean square error of approximation (RMSEA) assesses a given model's fit to the observed covariance matrix while accounting for the number of parameters embedded in the model itself (0 < RMSEA < 1). We also report Akaike's information criterion (AIC) values for two models, our model and a saturated model. The saturated model is similar to the model being tested but it includes all possible paths from each variable to every other variable, yielding an over-parameterized model with the maximum possible fit. If the tested model captured the causal structure sufficiently well, its AIC is expected to fall within the confidence interval of the saturated model.

Results

Competitive ability of wild and hybrid plants

Wild plants generally produced more seeds per plant than hybrids (Fig. 3). Based on lifetime seed production, the competitive coefficient of wild plants on the fecundity of hybrid plants was 1.63 (95% confidence interval (CI) 1.15–2.11), a significantly greater value than the competitive coefficient of hybrid plants on wild plants, which was 0.59 (95% CI 0.41–0.76) (See the Supplementary Material, Table S1 for estimates of other parameter estimates). These values suggest that the competitive ability of one hybrid plant is approximately equivalent to a fraction (≈ 0.6) of one wild plant. In other words, hybrid competitors had a significantly larger negative effect on the fecundity of hybrids than on wild plants, and the same was true for wild competitors.

Response of life-history traits and lifetime fecundity to competition

Before competition occurred, hybrid plants exhibited an early advantage over wild plants. Seedling biomass differed significantly among populations within biotypes (Supplementary Material Table S2, $F_{4,203} = 8.162$, $P = 4.0 \times 10^{-6}$) and advanced-generation hybrid seedlings had greater biomass than wild seedlings (Supplementary Material, Table S2, $F_{1,4} = 7.49$, $P = 0.05$).

The pollen fertility of hybrids was significantly lower than that of wild plants ($F_{1,4} = 12.1$, $P = 0.025$; Supplementary

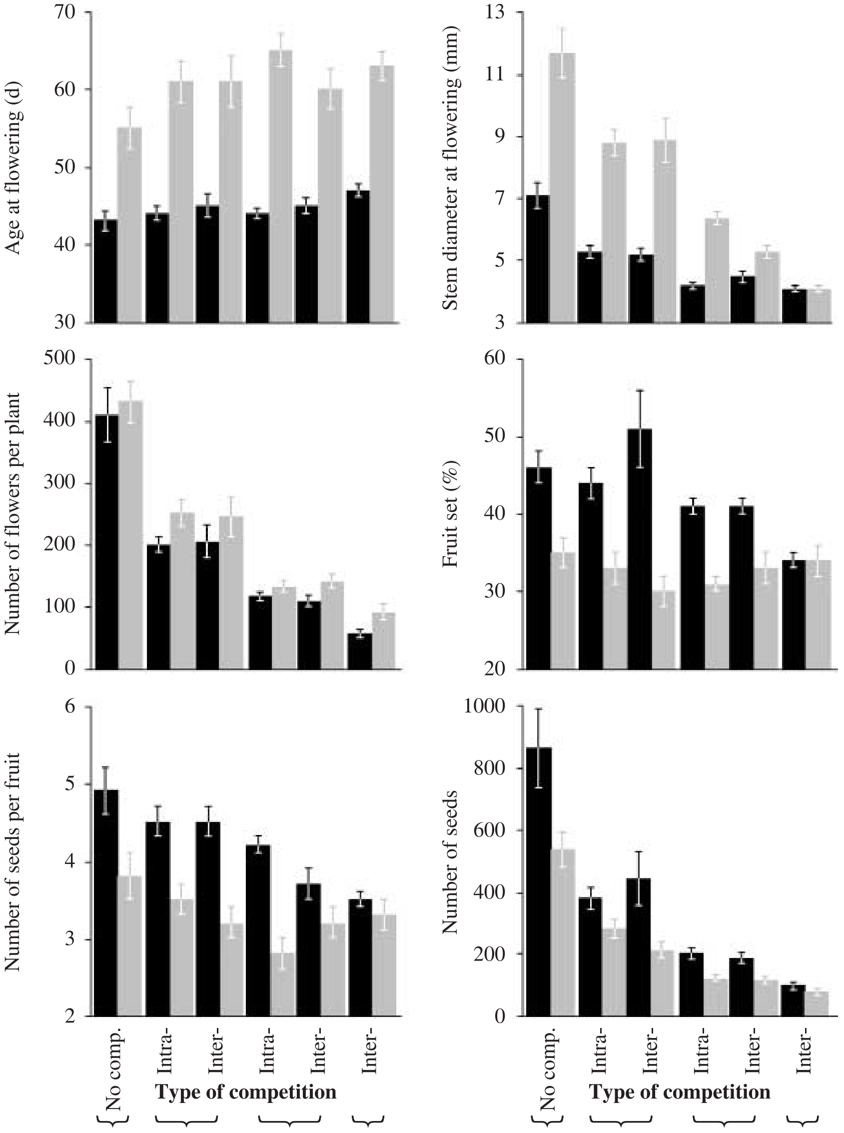


Fig. 3 Comparison of life-history traits and lifetime fecundity of wild (black bars) and hybrid (gray bars) radish plants grown under intrabiotype (Hybrid frequency = 0% or 100%) and interbiotype competition conditions (hybrid frequency = 50%) at four plant densities. Bars represent trait means; error bars represent the SE of the mean. Since populations did not differ significantly for most traits, estimates of relative fecundity are based on averages of 36 pots per biotype where population is pooled ($n \approx 36$ individuals per biotype, biotype frequency and plant density).

material, Table S3). Pollen fertility did not differ among competition treatments (plant density $F_{3,2} = 0.03$, $P = 0.99$; hybrid frequency $F_{1,2} = 0.08$, $P = 0.80$; density × frequency $F_{1,2} = 0.01$, $P = 0.93$) or among source populations within biotypes ($F_{4,261} = 1.43$, $P = 0.16$).

Wild and hybrid plants differed significantly in life history and lifetime fecundity (Fig. 3). Hybrids flowered significantly later ($P < 0.001$), and grew to a larger stem diameter before flowering than wild plants at most densities ($P < 0.001$, Fig. 3, Table 1; Supplementary Material, Table S4). Flower number was not significantly different between wild and hybrid plants ($P > 0.10$). Yet, wild plants produced more seeds per plant than hybrids ($P < 0.001$), perhaps because they set more fruits per flower ($P < 0.001$) and more seeds per fruit than hybrids ($P < 0.001$, Table 1).

As plant density increased, the differences between biotypes in age and size at flowering, and seeds per fruit were diminished (Fig. 3, Table 1, Supplementary material Table S4). Increased density delayed age at flowering in wild plants but not in hybrid plants ($P < 0.001$) and simultaneously reduced size at flowering more in hybrid plants than wild plants ($P < 0.001$). Hybrid plants responded to interbiotype competition conditions by flowering significantly earlier and becoming marginally significantly smaller than in intrabiotype competition conditions whereas wild plants did not (age at flowering $P < 0.001$; size at flowering $0.05 < P < 0.10$). Finally, the number of seeds per fruit and fecundity of hybrid plants was less sensitive to changes in density and competition type than wild plants (seeds per fruit $P < 0.01$; seeds per plant $0.05 < P < 0.10$; Table 1, Fig. 3). This result reveals that hybrid plants responded to competitive conditions differently. The fitness consequences of these life history and fecundity responses to competitive conditions are explored further in the path analysis below.

Indirect effects of competition on lifetime fecundity

As *R. raphanistrum* and wild *R. sativus* are model organisms for plant population biology, it was feasible to build a causal model relating life-history traits to lifetime fecundity in a way that would allow us to predict, with reasonable accuracy, the observed covariance matrices for both wild *R. raphanistrum* and hybrid plants grown across a gradient of competitive environments. Judging from a variety of indices, our models fit the data reasonably well ($\mathrm{RMSEA}_{\mathrm{Wild}} = 0.08$, 95% CI 0.032–0.125; $\mathrm{RMSEA}_{\mathrm{Hybrid}} = 0.075$, 95% CI 0.034–0.114; see the Supplementary Material, Table S5, for other estimates).

The path analyses of wild and hybrid focal plants were very similar (Fig. 2), with generally small, nonsignificant differences in the magnitudes of the path coefficients (Fig. 2c). Further, the magnitudes of path coefficients shared by the two models (e.g. Density → Hybrid age) were statistically similar, again suggesting that the models were robust (Fig. 2c). Increased density generally delayed flowering and decreased plant size. This led to reductions in the number of flowers per plant and the number of seeds per fruit, both of which were positively correlated with the number of seeds per plant.

Although wild and hybrid plants generally responded similarly to competition, path analysis revealed a few key differences (Fig. 2). Density was more strongly correlated with size at flowering in hybrid ($\rho = -0.64$) vs wild plants ($\rho = -0.29$). Further, size at flowering and age at flowering were more strongly correlated in hybrid ($\rho = 0.66$) vs wild plants ($\rho = 0.52$). Yet, density was more strongly correlated

Table 1 A comparison of life-history traits and lifetime fecundity of wild and hybrid radish populations grown in a competition experiment in Michigan, USA

Source	$\mathrm{df}_{\mathrm{H,E}}$	$F_{\text{Age at first flower}}$	$F_{\text{Stem diameter}}$	$F_{\text{Flowers/plant}}$	$F_{\text{Fruit set}}$	$F_{\text{Seeds/fruit}}$	$F_{\text{Seeds/plant}}$
Between-subjects effects:							
Hybrid freq.	1, 10	1.34^{ns}	2.85^{ns}	3.29^{+}	1.76^{ns}	0.22^{ns}	3.93^{+}
Density	3, 10	3.84^{*}	81.55^{***}	93.96^{***}	9.17^{*}	4.63^{*}	134.17^{***}
Pop. Pair	2, 10	4.09^{*}	2.31^{ns}	3.89^{+}	20.85^{*}	0.12^{ns}	2.68^{ns}
Hybrid freq. × Density	1, 10	0.16^{ns}	0.18^{ns}	1.58^{ns}	8.03^{+}	0.07^{ns}	1.70^{ns}
Hybrid freq. × Pop. Pair × Density	10, 184	1.68^{ns}	0.84^{ns}	0.95^{ns}	5.28^{+}	1.49^{ns}	0.71^{ns}
Within-subjects effects:							
Biotype	1, 10	101.73^{***}	150.38^{***}	1.48^{ns}	195.34^{***}	120.79^{***}	32.53^{***}
Biotype × Hybrid freq.	1, 10	11.00^{***}	3.76^{+}	0.08^{ns}	5.41^{ns}	2.37^{ns}	0.69^{ns}
Biotype × Density	3, 10	4.42^{***}	9.00^{**}	0.50^{ns}	3.78^{ns}	1.87^{ns}	0.05^{ns}
Biotype × Pop. Pair	2, 10	0.70^{ns}	0.70^{ns}	2.32^{ns}	1.42^{ns}	0.58^{ns}	1.97^{ns}
Biotype × Density × Hybrid freq.	1, 10	7.35^{**}	3.61^{+}	0.14^{ns}	6.04^{+}	8.72^{**}	4.32^{+}
Biotype × Density × Hybrid freq. × Pop. Pair	10, 184	0.20^{ns}	0.82^{ns}	1.33^{ns}	2.05^{ns}	0.54^{ns}	1.10^{ns}

We performed a repeated measures ANOVA for each trait for two biotypes (Within-subjects effect). Three populations of wild plants were paired to compete with three populations of hybrid plants (Pop. Pair). Plants were exposed to variation in plant density (Density = one, two, four, or eight plants per pot) and hybrid frequency (Hybrid freq. = 100% hybrid plants per pot or 50% hybrid plants per pot). *F*-statistics are presented to indicate significant differences: ns, $P > 0.10$; +, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

with age at flowering in wild ($\rho = 0.52$) vs. hybrid plants ($\rho = 0.27$). Finally, age at flowering was more strongly correlated with number of seeds per fruit in hybrid ($\rho = -0.49$) vs wild plants ($\rho = -0.35$). The differences observed between wild and hybrid path diagrams may provide biological mechanisms to explain the relative increase in hybrid fitness with increased density found with the above analysis of variance. Specifically, several life history traits of wild and hybrid plants differed in their response to competition.

The path analysis revealed direct effects of competition conditions on the life history of wild and hybrid plants and direct and indirect effects of changes in life history on their lifetime fecundity (Fig. 2, Table 2). In wild plants, the magnitude of the direct effect of density was larger on age at reproduction than size at reproduction, whereas the opposite was true for hybrid plants. Interestingly, for both biotypes, the trait least sensitive to changes in density had the largest direct effect on lifetime fecundity. Indirect effects of age and size at reproduction on lifetime fecundity were small for both wild and hybrid plants. Consequently, the total effect of age and size at reproduction on lifetime fecundity largely reflected their direct effects on lifetime fecundity (Fig. 2, Table 2).

Discussion

Our study shows that competitive conditions may increase the evolutionary impact of advanced-generation crop–wild hybrids through indirect effects on life history traits. These traits include age and size at flowering, and seeds per fruit (i.e. clutch size). Based on lifetime fecundity, hybrids had less competitive impact on wild plants than the competitive effect of wild plants on hybrids (Table 1, Fig. 3). As expected, increased density reduced the fecundity of wild and hybrid plants (Table 1), a result consistent with previous competition studies of *Raphanus* (Uthus, 2001; Wolfe & Mazer, 2005). However, increasingly competitive conditions had a greater negative effect on the lifetime fecundity of wild plants than on hybrid plants (Table 1: Biotype × Density × Hybrid freq. Interaction). Therefore, relative hybrid fecundity was maximized under more intense competitive conditions (Fig. 3).

A common purpose of competition experiments is to anticipate the dynamics of natural plant communities. The probability of species coexistence is estimated using a competition model under a range of simplified environments and is encapsulated by the competition coefficient parameter (Damgaard, 1998; Inouye, 2001). Estimating competition coefficients may improve predictions of persistence of crop alleles within weedy populations across generations and the success of hybrids within weedy populations over a growing season. Yet, despite the abundance of empirical studies on the effect of competition on crop–wild hybrid fecundity (Table 3), only one other study has expressed hybrid competitive ability in terms of competition coefficients (Hauser *et al.*, 2003). Hauser *et al.* (2003), in contrast to our study, found that the competitive ability of F_1 hybrids (*Brassica rapa* × *Brassica napus*) was greater than that of the wild parents ($c_{\text{wild,hybrid}} = 5.80$, $c_{\text{hybrid,wild}} = 0.12$). High competitive ability of early generation hybrids will be important to the early stages of crop gene introgression; however, the persistence of crop genes within weed populations also depends on the competitive ability of advanced-generation hybrids when growing near its wild relative as well as with other weed species (Vacher *et al.*, 2004). Further, the accuracy of competition models in predicting community dynamics will be limited by the ability of experimental designs to represent the complexity of field conditions, including differential herbivory, seed longevity, emergence dates, and multispecies communities.

Highly competitive conditions, such as those one might find in a natural weed population, may facilitate the introgression of crop alleles into weedy populations by increasing the relative fecundity of hybrids (Fig. 3). In the literature, this is a common but under-appreciated result (Table 3). When crop–wild hybrid performance is compared with wild taxa

Table 2 Indirect effects of plant density (Density) and focal plant age (Age) and size (Size) at reproduction on focal plant age and size at reproduction, and lifetime fecundity calculated for wild and F_3 hybrid radish plants using the path analytic models in Fig. 2

	Direct			Indirect			Total		
Causal factors	Age	Size	Lifetime fecundity	Age	Size	Lifetime fecundity	Age	Size	Lifetime fecundity
(a) Wild									
Density	0.417	–0.138	–0.341	–	–	–	0.417	–0.138	–0.341
Age	–	–	–0.244	–	0.466	–0.027	–	0.466	–0.271
Size	0.466	–	0.301	–	–	0.034	0.466	–	0.335
(b) Hybrid									
Density	0.268	–0.635	–0.483	–	–	–	0.268	–0.635	–0.483
Age	–	–	–0.413	–	0.660	–0.062	–	0.660	–0.475
Size	0.660	–	0.192	–	–	–0.004	0.660	–	0.188

Table 3 Summary of 12 competition studies of fecundity of crop-wild hybrids relative to their wild parent

Family	Cultivated spp.	Wild relative	Gen.	Experiment type	Traits measured	Hybrid fitness relative to wild with no/low competition	Hybrid fitness under varying density	Hybrid fitness highest under which type of competition?
Asteraceae	*Helianthus annuus*[1a]	*H. annuus*	F_1	A	F	Reduced	Higher with competition	
	H. annuus[2b]	*H. annuus*	F_1	RP	S	No data		Same
	H. annuus[2b]	*H. petiolaris*	F_1	RP	S	No data		Same
	H. annuus[3]	*H. annuus*	F_1	A	S	Reduced	Higher with competition	
	Echinacea purpurea[4]	*E. purpurea*	F_1	A	B, F	Similar	Highest at medium density	Inter
Brassicaceae	*Brassica napus*[5]	*Raphanus raphanistrum*	BC_{6napus}	A	S	Reduced	Higher with competition	
	B. napus[5]	*R. raphanistrum*	BC_{6raph}	A	S	Similar	Highest at low density	
	B. napus[6]	*B. rapa*	F_1	RS	S, PA	Condition-dependent, Reduced	Frequency-dependent, Higher under low density	See below[e]
	B. napus[7]	*R. raphanistrum*	F_1	A	B	Reduced	Higher without competition	
	B. napus[7]	*R. raphanistrum*	F_1	A	B	Reduced	Highest at low density	
	B. napus[8]	*B. rapa*	F_1^g	RS	S	Condition-dependent	Highest at high density	Inter
	B. napus[8]	*B. rapa*	BC_{1rapa}	RS	S	Density-dependent	Highest at low density	Intra
	B. napus[8]	*B. rapa*	BC_{1napus}	RS	S	Reduced	Highest at low density	
	B. napus[8]	*B. rapa*	F_2	RS	S	Density-dependent	Highest at low density	
	B. napus[8]	*B. rapa*	BC_{2rapa}	RS	S	Reduced	Highest at intermediate density	
	B. napus[9]	*B. rapa*	F_1	A	B	Greater	Highest at high density	
	B. napus[10c]	*B. rapa*	BC_2F_2	A	B	Reduced	Higher with competition	
	B. napus[11]	*B. rapa*	F_2	RP	S	Reduced		Inter
	B. napus[11]	*B. rapa*	F_2BC_1	RP	S	Reduced		Inter
	B. napus[11d]	*B. rapa*	F_2	RP	S	Reduced		Inter
	B. napus[11d]	*B. rapa*	F_2BC_1	RP	S	Reduced		Inter
	R. sativus[12]	*R. raphanistrum*	F_1	A	S	Reduced	Higher with competition	
	R. sativus[12]	*R. raphanistrum*	BC_1	A	S	Reduced	Higher with competition	
	R. sativus[13]	*R. raphanistrum*	F_3^h	RS	S	Reduced	Higher with competition	Same

Studies were included only if they concerned hybrids between a cultivated and wild relative and if they measured hybrid seed production under intra- and interbiotype competition environments. Gen., hybrid generation studied; With/Without, comparisons were made between competitive environments with either no competition or some competition; RP, replacement series; A, additive series (density varied but no frequency treatments); Hexagonal, hexagonal plot design for a neighborhood competition experiment; RS, response surface where density and frequency of biotypes were varied independently of each other. Traits measured: S, number of seeds; F, number of flowers or flower heads; B, above-ground biomass. No data, the comparison was not made in the study; Similar, fitness of hybrid was similar to wild plant; Reduced, hybrid fitness was lower than wild fitness; Greater, hybrid fitness was higher than wild fitness; Intra, hybrid relative fitness was lower under interbiotype competition conditions than under intrabiotype competition conditions; Same, hybrid relative fitness was unaffected by type of competition environment; Inter, hybrid relative fitness was higher under interbiotype competition conditions than under intrabiotype competition conditions.

[a] In this study, the effect of competition was confounded with the effect of site since the without and without studies were conducted in two different locations.

[b] In this study, the two biotypes were both hybrids – IMI-resistant and IMI-susceptible hybrids of *H. annuus* and *H. annuus* or *H. petiolaris*.

[c] Hybrids contained the Bt transgene.

[d] Hybrids were transgenically mitigated with a dwarfing gene intended to reduce their fitness.

[e] This study included no pure plots of F_1s; however, there were plots that had higher and lower frequencies of hybrids. At high density hybrids had higher fitness under interbiotype competitive conditions and at low density hybrids had lower fitness under interbiotype competitive conditions.

[f] In this study, the effect of competition was confounded with the effect of site since competition was imposed in the field while the without competition study was performed in a glasshouse.

References: [1]Snow *et al*. (1998), [2]Massinga *et al*. (2005), [3]Mercer *et al.* (2006), [4]van Gaal *et al.* (*1998*), [5]Guéritaine *et al*. (2002), [6]Pertl *et al*. (2002), [7]Guéritaine *et al*. (2003), [8]Hauser *et al*. (2003), [9]Vacher *et al*. (2004), [10]Halfhill *et al*. (2005), [11]Al-Ahmad & Gressel (2006), [12]Uthus (2001), [13]this paper.

under increasingly competitive conditions, the difference between hybrid and wild genotypes is often reduced by competition (Snow *et al.*, 1998; Uthus, 2001; Guéritaine *et al.*, 2002; Halfhill *et al.*, 2005; Mercer, 2005). However, in this collection of studies, imposing competition on hybrid plants rarely, if ever, reversed the relative performance of hybrids compared with their wild relative (Table 3). That is, hybrids that possessed reduced relative fitness without competition do not exhibit superior fitness under competitive conditions. These results suggests that hybrid success in natural populations is unlikely to be limited by density alone but that hybrid relative fitness may be promoted by increased density.

Although hybrids were generally poor competitors, as indicated by their competition coefficient, their relative performance was enhanced when grown in mixed pots of wild and hybrid plants rather than purely hybrid pots. This, too, is a common result of competition studies (Table 3). Typically, hybrids of several crop–wild complexes, under at least some densities, tend to be more successful, although not superior, under interbiotype vs intrabiotype competition conditions (van Gaal *et al.*, 1998; Pertl *et al.*, 2002; Hauser *et al.*, 2003; Al-Ahmad & Gressel, 2006). Although our analysis of variance was limited to two frequencies (50% and 100%), the path analysis confirmed this trend continues when hybrids are grown under other hybrid frequencies (33% and 66%). Under interbiotype competition, the hybrids initiated reproduction at a smaller size and assumed a more 'wild-like' appearance than under intrabiotype competition. These findings suggest that the rate of introgression in wild populations will be highest when hybrids grow in mixed populations. This finding supports previous hypotheses about the evolution of weedy *R. sativus* in California. Panetsos & Baker (1967) speculated that hybridization with *R. raphanistrum* allowed cultivated radish to evolve into 'a highly successful weed'. Recently, Hegde *et al.* (2006) used field observations, morphological data, and allozyme frequencies to conclude that hybrid populations of crop–wild genotypes have displaced ancestral populations of weedy *R. raphanistrum* in California. This suggests that the relative fitness of crop–wild hybrids within wild radish populations was sufficiently high so as to promote coexistence of biotypes and crop allele persistence.

Although the effect of competition on the relative fecundity of our crop–wild hybrids was apparent from an analysis of variance, the results of the path analysis contributed several novel insights into the indirect, but causal, consequences of competition on relative hybrid performance via its effects on life history (Fig. 2). While the phenotypic correlations of wild and hybrid plants often responded similarly to density, we were able to detect key differences that may ultimately have led to differences in the response of fecundity to density treatments. Age at flowering in wild plants was more sensitive to changes in density than hybrid plants. The delay in flowering induced by high density in wild plants resulted in a significant decrease in the number of seeds per fruit in wild plants and ultimately a reduction in lifetime fecundity of wild plants. At the same time, size at flowering in hybrids was more sensitive to changes in density than wild plants and was significantly reduced at high densities, suggesting that hybrids tended to advance flowering and increase seeds per fruit and flower production at high density. Therefore, increasing density altered life history, resulting in wild plants that more closely resembled hybrid plants and vice versa. In future studies, it will be important to incorporate more life-history traits, including timing of germination and seedling growth, in order to fully understand the competitive dynamics between wild and hybrid plants within a population (Guéritaine *et al.*, 2003; Hooftman *et al.*, 2005).

Competition had a dramatic effect on the life history of both wild and hybrid plants. Although hybrids began as bigger seedlings they had less competitive impact on neighbors than wild plants. This suggests that competitive dominance may result from different patterns of resource allocation, further reinforcing the idea that changes in life-history are likely to change performance. Another intriguing result, emerging from both path analyses and ANOVA, was that greater density significantly delayed flowering in wild plants but not in hybrids, and that increased density decreases size at flowering more in hybrid plants than wild ones. These results suggest that hybridization may alter both the average phenotype of weeds and the plasticity of those traits (Pigliucci & Kolodynska, 2006).

In summary, we demonstrate through both experimental manipulations and literature review that competition may indirectly impact the relative performance of hybrids and their wild relatives, via its effect on life history, potentially enhancing hybrid fitness in weed populations. We suggest that subsequent competition experiments evaluate those life-history traits most affected by competition and the consequences of plant–plant interactions for the introgression of crop traits into wild populations. Studies that consider the effect of competition on both absolute and relative hybrid fecundity will provide more comprehensive predictions of the ecological consequences of crop gene introgression into wild populations (Damgaard, 1998; Lexer *et al.*, 2003).

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 Maximum likelihood estimates of the competitive coefficients and model estimates of wild and hybrid radish biotypes as measured by the number of seeds produced per plant

Table S2 Pre-competition seedling biomass of wild and advanced-generation crop–wild hybrid radish plants

Table S3 Percentage of pollen grains that are fertile in wild and advanced-generation crop–wild hybrid radish plants

Table S4 Summary statistics of several key life-history traits and fitness components for F_3 wild (W) and hybrid (H) radish plants

Table S5 Summary statistics for the fit of the path analytic models for each radish biotype

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