When divergent life histories hybridize: insights into adaptive life-history traits in an annual weed

Lesley G. Campbell[1,2], Allison A. Snow[1] and Patricia M. Sweeney[1]

[1]Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH 43210, USA; [2]Present address: Department of Ecology and Evolutionary Biology, Rice University, 6100 Main Street, Houston, TX 77005, USA

Summary

Author for correspondence:
Lesley Campbell
Tel: +1 713 3483054
Email: lgc@rice.edu

• Colonizing weed populations face novel selective environments, which may drive rapid shifts in life history. These shifts may be amplified when colonists are hybrids of species with divergent life histories. Selection on such phenotypically diverse hybrids may create highly fecund weeds. We measured the phenotypic variation, strength of natural selection and evolutionary response of hybrid and nonhybrid weeds.

• We created F_1 hybrids of wild radish, an early flowering, small-stemmed weed, and its late-flowering, large-stemmed, crop relative (*Raphanus* spp.). Replicate wild and hybrid populations were established in an agricultural landscape in Michigan, USA. The consequences of three generations of natural selection were measured in a common garden experiment.

• Hybrid populations experienced strong selection for larger, earlier flowering plants whereas selection was relatively weak on wild populations. Large plant size evolved two to three times faster in the hybrid populations than in wild populations, yet hybrid populations did not evolve earlier flowering. Strong selection on size and phenotypic correlations between age at reproduction and size may have limited the response of flowering phenology.

• Our findings demonstrate hybridization between species with divergent life histories may catalyse the rapid evolution of certain adaptive, weedy traits while tradeoffs limit the evolution of others.

Introduction

As we begin to grasp the full impact of weed invasions, an understanding of the correlates of plant invasiveness has become more valuable. Many workers have analysed floras to understand the phenotypic and life-history traits associated with weediness or invasiveness (Baker, 1965; Daehler, 1998; Sutherland, 2004). A recurring conclusion is that many traits may play fundamental roles in the invasion success of a given species but it is difficult to find trait states that are universally 'invasive'. Moreover, evolutionary processes such as hybridization and rapid evolution may be involved in the success of colonizing weeds (Ellstrand & Schierenbeck, 2000). Hybridization may: (1) increase genetic variation, which in turn can promote adaptive evolution (Anderson & Stebbins, 1954; Stebbins, 1959; Rattenbury, 1962); (2) increase a species' competitiveness or colonization abilities through heterosis (hybrid vigour); (3)

generate novel phenotypes that match the new environment more closely than ancestral genotypes; and/or (4) purge accumulated genetic load, thereby increasing fitness and ultimately invasiveness. Here, we address the first hypothesis by comparing the amount of phenotypic variation, the intensity of natural selection, and the response to selection of hybrid vs nonhybrid populations of a common, agricultural weed, *Raphanus raphanistrum.*

Crop-to-wild gene flow may serve as a model system to evaluate the hybridization between two species and the potential for hybridization to lead to rapid evolution of life history and other fitness-related traits. Cultivated plants are often sexually compatible with their wild relatives and yet exhibit dramatically different life histories. Age at maturity is routinely altered through crop breeding programs depending on the desired agronomic product. If a plant is cultivated to produce seeds, such as annual maize (*Zea mays*), age at maturity is often selected to become earlier

than wild relatives (e.g. one maize progenitor is the perennial *Zea diploperennis*; Wang *et al.*, 1999). Alternatively, if a plant is grown for vegetative portions, such as biennial sugar beet (*Beta vulgaris* ssp. *vulgaris*), age at maturity is usually selected to be delayed relative to wild species (the sea beet, *Beta vulgaris* ssp. *maritima*, is an annual plant; Viard *et al.*, 2002). Although their life histories may differ, many pairs of crop–wild taxa have overlapping flowering phenologies, hybridize easily, and produce relatively fecund offspring (Ellstrand, 2003). Therefore, crop–wild complexes may be useful for furthering our theoretical and empirical understanding of plant life-history evolution.

In addition to altering the life history of hybrid offspring, crop–wild hybridization may also create weedy derivatives, including hybrid offspring with greater fecundity relative to crop or wild progenitors (Ellstrand *et al.*, 1999; Coulibaly *et al.*, 2002; Viard *et al.*, 2002). Several studies have demonstrated long-term introgression of crop alleles into weed populations, such as sunflower (Whitton *et al.*, 1997), canola (Hansen *et al.*, 2001) and radish (Snow *et al.*, 2001), suggesting that crop–wild hybridization may persistently alter the evolutionary trajectory of weed populations. Isolated, invading hybrid populations may also experience strong selection over short time-scales (Sakai *et al.*, 2001; Allendorf & Lundquist, 2003). The interaction of hybridization and selection may significantly alter the population dynamics of these introduced taxa, altering patterns of invasion and establishment.

Evolution in contemporary time (i.e. from years to decades) likely plays an important role in the establishment and success of many introduced species. Furthermore, contemporary evolution might influence how rapidly introduced species spread from their point of origin (García-Ramos & Rodríguez, 2002). In the initial stages of colonization, invading species often persist at low densities, with later population 'explosions' to become invasive at some future time (Schmitz *et al.*, 1997). This pattern might reflect an initial period of adaptation (perhaps slowed owing to small population size) of the population to local conditions before a rapid demographic expansion, or low population growth until suitable environmental conditions arise. Several documented cases of adaptation after colonization suggest that evolution can play a fundamental role in governing the colonization and expansion of invasive species (Maron *et al.*, 2004; Zangerl & Berenbaum, 2005; Lavergne & Molofsky, 2007). Crop–wild hybrid populations are expected to initially possess genetic variation that is either similar to or more diverse than the non-hybridized parental taxa. Subsequently, natural selection could make hybridized populations more similar to their parental taxa or different from them, depending on whether their genetic variation creates phenotypes that are beneficial or deleterious to fitness. If the introduced genetic variation is advantageous, we expect hybridization to lead to long-term introgression.

However, whether or not phenotypes of hybridized weeds tend to converge on those of their non-hybrid weedy ancestors, and at what rate, is understudied (but see Hegde *et al.*, 2006; Whitney *et al.*, 2006).

Research objectives

To examine the effect of hybridization on life-history evolution in a common weed, we described the amount of phenotypic variation created after crop–wild hybridization, the evolutionary influence of trait correlations before and after hybridization, the intensity of natural selection imposed on six phenotypic traits and the evolutionary response of these life-history traits to natural selection. Our previous research compared the lifetime fecundity of wild and hybrid plants after several generations of natural selection in experimental populations (Campbell *et al.*, 2006; Campbell & Snow, 2007). We also documented the potential for rapid evolutionary responses in these populations to strong, artificial selection for two traits of interest, namely, early flowering and large size (Campbell *et al.*, 2009). Here we build on this work to determine the strength and direction of natural selection on these same traits, as well as the evolutionary response of wild and hybrid populations to three generations of natural selection. By using this complementary approach, we take a novel, comprehensive look at life-history evolution in an annual weed.

Materials and Methods

Study organism

As a model system, we used the crop–wild complex of cultivated radish (*Raphanus sativus*), an open-pollinated vegetable selected for large, colourful roots and high levels of seed production (Snow & Campbell, 2005), and its weedy relative, wild radish (*Raphanus raphanistrum*, also known as jointed charlock), a cosmopolitan, agricultural weed that also colonizes disturbed sites and coastal beaches (Warwick & Francis, 2005). These two radish species have emerged as model systems in plant evolutionary ecology and in the assessment of ecological consequences of crop-to-wild gene flow (Mazer, 1987; Stanton, 1987; Conner & Via, 1993; Klinger & Ellstrand, 1994). Although *R. raphanistrum* and *R. sativus* share many phenotypic characters, they exhibit divergent life histories in at least two key traits. First, many *R. sativus* cultivars develop swollen hypocotyls and roots and large rosettes whereas *R. raphanistrum* plants form narrow, branching taproots and reproduce at smaller rosette sizes. Second, although both species are annuals in temperate climates, *R. raphanistrum* flowers earlier than cultivated radish (Panetsos & Baker, 1967). In Michigan, USA, *R. raphanistrum* 'bolt' a few weeks after germination, when the primary flowering shoot emerges from the rosette.

Selective breeding has led to relatively delayed bolting and flowering in *R. sativus* (Curtis, 2003).

Originally native to Europe, both species were introduced into California, USA by the nineteenth century (Panetsos & Baker, 1967). Since then, descendants of the original populations of crop–wild radish hybrids, known as wild or feral *R. sativus*, appear to have displaced the original populations of *R. raphanistrum* in California, to become a regionally important weed (Ball *et al.*, 2000; Hegde *et al.*, 2006; Ridley *et al.*, 2008). The speed with which hybrids evolve likely depends upon the evolutionary divergence of parental taxa, especially when considering hybridization among crops and their wild relatives. Radish is an ancient crop that appears to have multiple origins from several wild species, including *R. raphanistrum* (Ellstrand & Marshall, 1985; Crisp, 1995; Yamagishi & Terachi, 2003). Further, cultivated radish appears to have retained nearly as much genetic variation as *R. raphanistrum*, as measured by allozyme proteins (Ellstrand & Marshall, 1985). Given these facts, we expect crop radish to contribute unique traits to the phenotypic evolution of weedy relatives via hybridization.
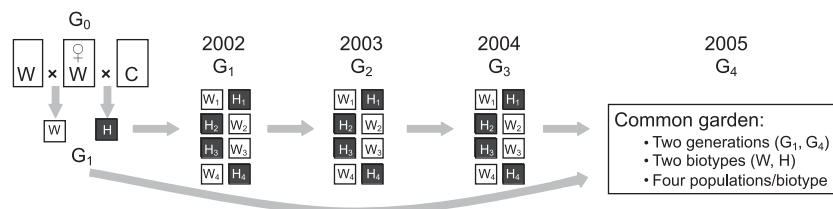
Experimental populations

Detailed descriptions of the wild and hybrid populations are available in Campbell *et al.* (2006, 2009). Briefly, natural selection populations and control populations were generated by hand-pollinating 100 wild *R. raphanistrum* plants with either wild pollen to create F_1 wild biotype populations, or pollen from 100 *R. sativus* var. 'Red Silk' plants (Harris-Moran Seed Co., Modesto, CA, USA) to create F_1 hybrid biotype populations. Based on hybridization in this first generation, we refer to radish biotypes as wild or hybrid. Physical separation and unpollinated control flowers were used to ensure that crosses between these self-incompatible plants were uncontaminated. A representative sample of F_1 wild and hybrid plants was reserved for the common garden described later to estimate the phenotype of F_1 plants before experiencing natural selection or random mating, while also exhibiting maximal heterosis owing to hybridization. Selection in the natural selection experiment was imposed on F_1 plants for three generations whereas we

randomly mated glasshouse-grown, control plants for three generations (F_2–F_5).

The natural selection populations, which were maintained in the field, were established as four F_1 wild populations (W_1, W_2, W_3 and W_4) and four F_1 hybrid populations (H_1, H_2, H_3 and H_4) in pastures or agricultural fields in Emmett and Cheboygan counties, MI, USA in 2002 (Fig. 1). Wild and hybrid populations were paired initially based on the type of agricultural setting surrounding the population (pasture or field). To restrict unintended gene flow, these eight populations were isolated from each other and local wild radish populations by at least 1 km. Each population was started by planting 50–60 seedlings (a minimum of 42 F_1 plants survived to reproduce) in a recently tilled 15 × 15 m fertilized plot (Slow-release Osmocote 19-6-12, 22.7 kg per site; Scotts Miracle-Gro Co., Marysville, OH, USA). To maximize the genetic diversity within each plot, no more than two seeds per maternal plant were planted per plot. Each spring, the plots were tilled to incorporate the previous year's fruits into the topsoil, fertilized and hand-weeded to promote population persistence. Otherwise, the natural selection populations were exposed to naturally occurring pollinators, herbivores, weather, and competition.

Glasshouse-grown 'control' populations that were not exposed to natural selection were initiated in the F_2 generation after 100 individuals from each F_1 biotype were cross-pollinated (Campbell *et al.*, 2009; Fig. 2). In the case of *Raphanus*, early-generation hybrids (F_1) are not an entirely appropriate comparison because advanced-generation hybrids tend to exhibit breakdown by recombination of the linkage disequilibrium created by hybridization. During three generations of random mating (F_5), populations were initiated with 130–200 individuals and were propagated with a subset (10%) of randomly chosen individuals from each replicate each generation. To account for drift as a possible evolutionary mechanism, we created three independent populations for each biotype (wild or hybrid) for a total of six control populations. Variation among control populations was expected to represent the variation in evolutionary trajectory of randomly mated populations that have not experienced selection. We assumed that if these control populations became adapted to glasshouse

Fig. 1 Schematic diagram of the natural selection experiment. As in Campbell *et al.* (2006), eight isolated field populations of wild biotypes (W_1–W_4) or hybrid biotypes (H_1–H_4) were maintained for 4 yr; small squares represent populations of the two biotypes. In 2005, the common garden in Michigan, USA, was composed of G_4 plants from each population and plants representing G_1 founders of the eight populations.
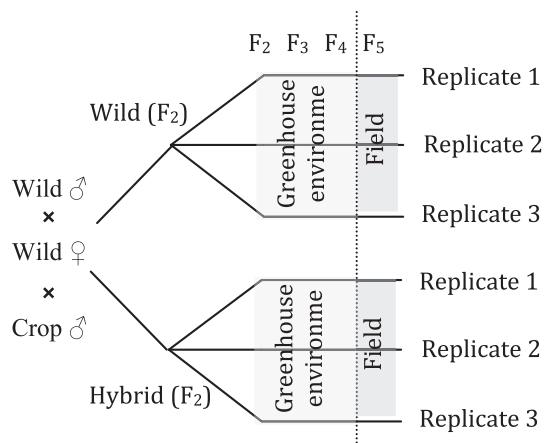
Fig. 2 Design of the randomly mating, control populations. As in Campbell *et al.* (2009), each F_2 plant was randomly assigned to one of three replicates per biotype. Each population in each generation was initially composed of 130–160 plants and 10% of each cohort was randomly mated to produce the following generation.

conditions, this had only minor effects on the phenotypic traits of interest. Thus, we used the control populations to determine the expected variation in traits without selection in advanced-generation hybrid and nonhybrid populations.

Common garden

We measured phenotypic traits of individuals from the wild and hybrid natural selection populations, the control populations and F_1 ancestors of all the populations in one common garden (Table 1). In the selection gradient analysis described below, we also used previously published data on the lifetime fecundity of the wild and hybrid natural selection populations collected from the same common garden (Campbell *et al.*, 2006). Here we report the lifetime fecundity of the control populations (not published previously). As in Campbell *et al.* (2006, 2009), the common garden

Table 1 Summary of wild and hybrid populations included in the common garden

Biotype	Selection treatment	Generations of selection	Generation in common garden	Number of replicate populations
Wild	Ancestors	0	1st	1
	Natural	3	4th	4
	Control	3[1]	5th	3
Hybrid	Ancestors	0	1st	1
	Natural	3	4th	4
	Control	3[1]	5th	3

Ancestors represent the first generation wild and hybrid plants that initiated both the natural selection (Natural) and Control populations.
[1]Note that these populations did not experience selection but rather random mating for three generations.

was located at the University of Michigan Biological Station (UMBS) in Pellston, MI, USA. The proximity of the natural selection populations to the common garden helped to ensure that the phenotypic variation observed was typical.

In 2004, we collected seeds from the wild and hybrid natural selection populations (Campbell *et al.*, 2006). Because radish seeds may remain dormant for several years, we cannot assume each population was composed of only one generation (e.g. all F_4). In a previous publication, we referred to each year's population as G_1, G_2, G_3 and G_4, recognizing that the G_4 (= F_4) may represent a mixture of earlier and later generations (Campbell *et al.*, 2006). All G_4 seeds for the common garden were collected directly from G_3 plants. To simplify our description of the experiment below we refer to G_4 plants using the traditional F_4 notation with the above caveat in mind. Also, in 2004, we collected F_5 seeds from F_4 glasshouse-grown, control population plants (see Campbell *et al.*, 2009).

The garden included F_1 wild and hybrid ancestors of the natural selection and control populations, F_4 wild and hybrid natural selection populations and the F_5 wild and hybrid control populations (see the Supporting Information, Table S1 for final sample sizes of each population). The common garden was organized in a complete randomized design and representatives of each genotype were equally allocated to 21 blocks in the garden. Seeds were planted in 300 ml of PRO-MIX 'BX' peat (Premier Horticulture Ltd, Rivière-du-Loup, Canada) in Jiffy fibre pots in a glasshouse at the UMBS in early May 2005. After the seedlings developed their first true leaves, each fibre pot was transplanted into a PVC bottomless tube pot (46 cm tall) filled with 1.7 l of local sandy soil surrounding the fibre pot, allowing plant roots to grow into local soil. Within each pot of transplanted radishes, we planted companion oat (*Avena sativa*) to provide a uniform level of competition. Four cultivated spring oat seeds (*A. sativa*; Blaskowski's Feed and Seed, Cheboygan, MI, USA) were included in each fibre pot to provide a uniform level of competition, and oat density was thinned to two seedlings per pot. Pots were separated by 30 cm and the use of large tube pots minimized root competition among neighbours. Seedlings that died within the first week after transplanting were replaced. Plants were watered daily for the first month and every other day until August 31. On June 18, 13 mg of fertilizer (Slow-release Osmocote) was added to each pot because the local soil was sandy and nutrient poor. Insecticide (0.0033% esfenvalerate, 20 g/9.5 l; Scotts Miracle-Gro Co., Marysville, OH, USA) was used to control insect herbivory three times during the first month after transplantation, when herbivory was highest. Aphids were present at low densities later in the season but did not colonize any plant heavily. Pollinators were abundant throughout the experiment, as in Lee & Snow (1998).

Plants were individually harvested as they senesced, until the first hard frost (September 16–20), when we harvested all remaining plants. Harvested radish and oat plants were dried at 60°C.

Phenotypic measurements from common garden

Each seed was weighed before planting. The date of germination was recorded daily for the first 2 wk after planting and date of first flowering was recorded daily for each plant. Age at reproduction was calculated as the difference between the date of flowering and the date of seedling emergence. Plant size on the first day of flowering was measured as the stem diameter just below the cotyledons using digital callipers (Chicago Brand Industrial, Inc., Fremont, CA, USA). In addition, we measured the length of the longest leaf, which is correlated with stem diameter (wild: Pearson's $r^2 = 0.39$, $P < 0.001$, $n = 169$; hybrid: Pearson's $r^2 = 0.77$; $P < 0.001$, $n = 313$). We considered stem diameter a better proxy for size as it exhibited higher heritability than leaf length (L. G. Campbell, unpublished.). At harvest, we remeasured root diameter at the thickest point of the root.

In the common garden, survival after transplantation was nearly 100% so survival was not included in the description of individual fitness. As measures of lifetime fecundity, we recorded flower number, fruit number and seeds per fruit. First, we counted number of flower pedicles and fruits on harvested plants. To estimate the number of seeds per plant, we multiplied the average number of seeds per fruit (for 10 randomly chosen fruits per plant) by the number of fruits. Seeds per plant were used as a proxy for individual fitness by numerical dominance, although we recognize that this may not have long-term evolutionary significance (Murray, 1990, 1992).

Statistical analysis

Before analysis, seed mass, age at first flower, stem diameter, leaf length, root diameter, number of seeds per plant and number of flowers per plant data were natural-log transformed to adhere to model expectations of normality. Seeds per fruit data were normally distributed and required no transformations before analysis.

Effect of natural selection on trait means and correlations First, we compared the two life-history traits (stem diameter and age at flowering) across generations, populations and biotypes. To determine the initial consequences of hybridization, we compared F_1 wild and hybrid age and size at reproduction using a multivariate ANOVA. Next, we compared the age and size at reproduction among biotypes (wild and hybrid) for the advanced-generation control and natural selection populations using an unbalanced, nested ANOVA that included biotype and population within biotype as fixed effects and block as a random effect. Variance of random effects was estimated using restricted maximum likelihood methods (REML). In addition, we estimated the phenotypic correlations between traits for each generation of each biotype using Pearson's r.

Directional selection We estimated phenotypic selection on each trait for both biotypes. The individual total number of seeds per plant values was relativized by dividing by population mean fitness (Lande & Arnold, 1983) and standardizing each phenotypic trait to a mean equal to zero and variance equal to one (Zar, 1999). Fitness measures were relativized and traits standardized separately for each population.

Because flower number had an overwhelming importance for fitness, we corrected estimates of phenotypic selection for flower number (as in Caruso, 2000). Correcting estimates of phenotypic selection for flower number has a twofold advantage. First, when flower number is included in the model, the variation attributable to this trait is removed from the error term, thus increasing the power to detect selection and differences in selection. Second, mean selection on flower number in *R. raphanistrum* is consistently stronger than selection on any other floral trait (see Table 2). This selection on flower number could result in strong indirect selection on correlated traits.

Table 2 Phenotypic correlations for six traits of *Raphanus raphanistrum* and *R. raphanistrum* x *R. sativus*

	Seed biomass	Days to flowering	Stem diameter at flowering	Leaf length at flowering	Number of flowers	Root diameter at harvest
Seed biomass		−0.027	0.104	**0.131**	−0.014	0.057
Days to flowering	−0.173		0.751	0.601	0.525	0.717
Stem diameter at flowering	0.148	**0.193**		0.787	0.637	0.876
Leaf length at flowering	**0.181**	−0.009	**0.391**		0.569	0.714
Number of flowers	0.073	**0.285**	**0.298**	**0.162**		0.707
Root diameter at harvest	0.063	**0.457**	**0.485**	**0.345**	**0.628**	

R. raphanistrum (Bottom diagonal, $n = 164$) and *R. raphanistrum* × *Raphanus sativus* (top diagonal, $n = 306$) populations that have experienced natural selection for three generations. Significant correlations are given in bold type.

Correcting estimates of phenotypic selection for flower number allows direct selection on a trait to be partitioned out from indirect selection via flower number. Note that we measured selection after the response to selection. Our conclusions are based on the assumption that selection in earlier generations was more or less constant across generations.

Estimating the strength of natural selection on weed phenotypes To determine whether the strength of phenotypic selection differed between wild and hybrid populations, we used an ANCOVA (as in Galen *et al.*, 1987, Caruso, 2000), given that ANCOVA is the standard method to test for differences in regression coefficients between groups (Zar, 1999). Data from both biotypes were combined and analysed with a model that included continuous terms for seven phenotypic traits, a categorical term coding for biotype and phenotypic traits × biotype terms. The six phenotypic traits included seed mass before planting, days to flowering, length of longest leaf, stem diameter at flowering, root diameter at harvest and number of flowers. We did not include seeds per fruit or number of fruits in the analysis because they were used to calculate relative fitness, which was the dependent variable. Fitness and standardized phenotypic traits were relativized separately for each biotype. We compared the slope of the fitness–phenotypic trait relationship between wild and hybrid populations using a 1-df contrast on the phenotypic trait × biotype term. All analyses were completed using SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA).

Estimating response to natural selection To determine whether biotype influenced response to natural selection in the two life-history traits, we performed nested mixed model ANOVAS for age and size at reproduction for two treatments (natural selection and control), two biotypes (wild and hybrid) and the replicate populations within each biotype. The unbalanced nested ANOVA included biotype, treatment within biotype and replicate within treatment within biotype as fixed effects and block as a random effect. Advanced-generation wild and hybrid populations were expected to resemble advanced-generation, control populations, if selection had no effect on life history. We interpreted consistent, significantly different, directional deviations from the control populations as a response to selection. Variation among replicates (i.e. W_1 vs W_4) within treatments (e.g. control) was interpreted as variation owing to genetic drift and spatial variation in selection among locations. Finally, differences among biotypes (wild vs hybrid) determine the effect of biotype on the response to selection of the two life-history traits. Although segregation distortion in advanced-generation hybrids cannot be decisively ruled out as a source of phenotypic change, this approach will yield insights into the magnitude of response

that may be expected from 'natural' crop-wild hybrids vs the control populations.

Results

Trait variation and correlation in wild and hybrid populations

To determine if hybrid populations possessed more phenotypic diversity in age and size at reproduction than nonhybrid populations, we compared the phenotypes of the F_1 hybrid and wild plants. First-generation hybrids initiated flowering at a size 1.79 times larger than F_1 wild plants ($F_{1,80} = 79.997$, $P < 0.001$, Fig. 3, Table S1). In addition, initiation of flowering was delayed by 13 d in F_1 hybrids compared with F_1 wild plants ($F_{1,8} = 132.87$, $P < 0.001$). Phenotypic variation (as measured by the coefficient of variation, CV) in age and size at reproduction tended to be higher in G_1 hybrid plants than wild plants (Table S1). Therefore, early-generation hybrid populations possess more phenotypic variation upon which selection may act than nonhybrid populations.

As expected, hybrid F_5 control populations continued to grow to a larger size and flower later than wild F_5 control populations. On average, plants from hybrid F_5 control populations initiated flowering significantly later (12 d) than comparable wild F_5 control plants and tended to have higher CVs in flowering phenology than wild F_5 control plants ($F_{1,4} = 77.02$, $P < 0.001$, Fig. 3, Table S1). Further, hybrid F_5 control plants initiated flowering at a significantly larger size (40% larger) and their size at reproduction had approximately two times higher CoV's than wild F5 control plants ($F_{1,4} = 61.71$, $P < 0.001$). Replicate populations within biotypes differed significantly for both age and size at reproduction (age: $F_{4,77} = 8.98$, $P < 0.001$; size: $F_{4,77} = 16.24$, $P < 0.001$). The variation among replicates of glasshouse-grown control populations is likely caused by genetic drift.

The hybrid F_4 plants from the natural selection populations flowered later and grew larger than analogous wild F_4 natural selection plants. Hybrid F_4 natural selection plants delayed flowering by 8 d relative to wild F_4 natural selection plants ($F_{1,6} = 71.1$, $P < 0.001$, Table S1). Further, hybrid F_4 natural selection plants initiated flowering at a significantly larger size than that of wild F_4 natural selection plants ($F_{1,6} = 88.5$, $P < 0.001$). The CV of age and size at reproduction in F_4 hybrid experimental populations was twice as high as that of F_4 wild experimental populations (Table S1). Replicate populations within biotypes did not significantly differ in age or size at reproduction (age: $F_{6,478} = 0.61$, $P = 0.73$; size: $F_{6,478} = 0.86$, $P < 0.53$). As expected, the variation among replicates within biotypes is lower in the natural selection populations than the control populations.
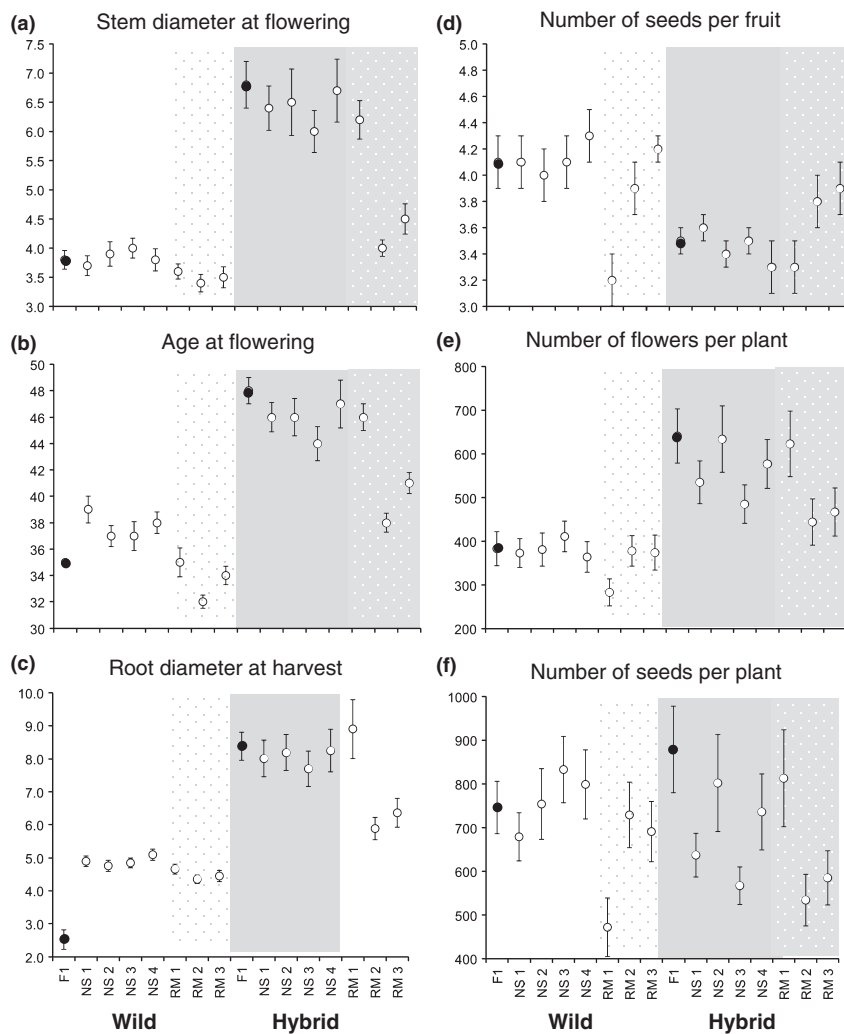
Fig. 3 Mean (± SE) for three life-history traits and three fitness measures of wild radish (*Raphanus raphanistrum*, white background) and hybrid radish (*R. raphanistrum* × *Raphanus sativus*, light-tinted background) from an F_1 population (F_1, closed circle), populations that had experienced natural selection (NS, unpatterned foreground), or control populations that had experienced random mating (RM, hatched foreground).

To determine whether the evolution of traits may be influenced by correlated evolution, we estimated the phenotypic correlations between traits for each biotype and generation. The three strongest phenotypic correlations within F_4 wild natural selection populations included root diameter and number of flowers ($r = 0.628$, $P < 0.001$), root diameter and stem diameter ($r = 0.485$, $P < 0.001$), and root diameter and days to flowering ($r = 0.457$, $P < 0.001$), with the remaining eight significant correlation coefficients < 0.35 (Table 2). The three strongest phenotypic correlations within F_4 hybrid natural selection populations included stem diameter and root diameter ($r = 0.876$, $P < 0.001$), stem diameter and leaf length ($r = 0.787$, $P < 0.001$), and stem diameter and days to flowering ($r = 0.75$, $P < 0.001$) with seven additional strong phenotypic correlations (i.e. $r > 0.5$, Table 2). Therefore, hybrid trait evolution may be more influenced by phenotypic correlations than wild populations whereas trait evolution in wild populations may be more influenced by a lack of phenotypic variation than in hybrid populations.

The strength of natural selection on weed phenotypes

Mean phenotypic selection varied considerably among phenotypic traits and, to a lesser degree, biotypes (Table 3). On average, selection was strongest for number of flowers, root diameter at harvest and days to flowering, especially for hybrid plants (Table 3). Selection favoured more flowers per plant in both biotypes but was significantly stronger for wild than hybrid plants (Table 3). Further, there was strong selection favouring significantly wider roots at harvest in hybrid than wild plants. Finally, selection on days to flowering differed significantly among biotypes; selection was extremely weak on the flowering time of wild plants whereas selection favoured significantly earlier-flowering hybrid plants (Table 3).

Response to selection

Although we did not detect selection on size at flowering, three hybrid populations and one wild population evolved

Table 3 Standardized directional selection (± 1 SE) for six traits of *Raphanus raphanistrum* and *R. raphanistrum* x *R. sativus* populations

Phenotypic trait	β_{Wild} (SE)	β_{Hybrid} (SE)	F-statistic$_{1,471}$ (P-value)
Seed biomass	−0.008 (0.012)	−0.010 (0.009)	0.848 (0.429)
Days to flowering	0.005 (0.004)	**−0.038 (0.013)**	4.584 (0.011)
Stem diameter at flowering	0.020 (0.014)	0.001 (0.022)	1.029 (0.358)
Leaf length at flowering	0.007 (0.013)	0.011 (0.022)	0.457 (0.634)
Number of flowers	**0.112 (0.016)**	**0.086 (0.013)**	1.669 (0.197)
Root diameter at harvest	0.032 (0.017)	**0.050 (0.020)**	5.043 (0.007)

R. raphanistrum (Wild) or *R. raphanistrum* × *Raphanus sativus* (Hybrid) populations experienced natural selection for three generations. Selection gradients that differ significantly from zero are given in bold type. Selection gradients that differ significantly among biotypes are indicated by the *F*-statistic column.

larger stem diameters under natural selection relative to the control populations (Fig. 3; Table S1, Replicate (Experiment): $F_{1,704} = 6.68$, $P = 0.01$). Furthermore, although selection favoured earlier flowering in two of the four wild populations, we found strong evidence that plants delayed reproduction relative to the control populations and three of the four hybrid populations delayed reproduction (Fig. 3, Table S1, Replicate (Experiment): $F_{1,704} = 4.50$, $P = 0.034$). These unexpected evolutionary trajectories only make sense within the context of phenotypic (and potentially genetic correlations). Given that flowering time and stem diameter are positively correlated with flower number and root diameter (Table 2), apparently the positive selection on flower number and root diameter was stronger than the negative direct response expected in flowering time and produced a positive correlated response in flowering time and stem diameter.

Discussion

This study set out to examine how hybridization and selection on life-history traits interact to drive the evolution of weeds. We used a complementary approach by measuring the magnitude of natural selection imposed on six phenotypic traits, particularly highlighting two life-history traits and the fitness consequences of heritable variation in those two life-history traits. Hybridization created populations that were more phenotypically variable than wild progenitors, immediately shifted the F_1 population mean age and size at reproduction, and created stronger phenotypic correlations among many traits, ultimately resulting in larger plants with delayed reproduction. In the natural selection populations, selection strongly favoured larger plants (in terms of number of flowers and root diameter at harvest) in hybrid populations and hybrid populations apparently responded to this selection. In addition, selection favoured earlier reproduction in hybrid populations. However, over three generations, hybrids evolved delayed reproduction, likely because of stronger selection for large plant size and the presence of strong phenotypic correlations. These results suggest that hybridization between species with divergent life histories may catalyse the rapid evolution of semi-adaptive weed phenotypes.

Does hybridization lead to rapid, adaptive evolution?

The significance of hybridization as a general mechanism in the evolution of natural populations remains unclear (Rieseberg, 1991 and refs. therein). However, relatively rapid evolution has been documented following hybridization. Carney *et al.* (2000) measured the degree of similarity between hybrids and their parental populations, *Helianthus bolanderi* and *Helianthus annuus*. Depending on the location, it was estimated that these populations had evolved at a rate of 6.42–19.68 Darwins (0.0220–0.0486 Haldanes, Bone & Farres, 2001). However, it is difficult to determine whether this rate of evolution occurred because of the normal processes of selection (i.e. because novel gene combinations within hybrid populations allowed evolution to occur rapidly) or phenotypic byproducts of hybridization such as heterosis or outbreeding depression because of the lack of independence of these two factors in the observational experiment. Here we document rates of evolution in the wild radish experimental field populations that varied between 13 and 33 Darwins for size at reproduction (0.06–0.16 Haldanes) and between 22 and 37 Darwins for age at reproduction (0.19–0.30 Haldanes; Table 4). By contrast, hybrid populations evolved at a rate of 53–80 Darwins for size at reproduction (0.21–0.15 Haldanes) and 15–30 Darwins for age at reproduction (0.11–0.16 Haldanes; Table 4). As we calculated the rate of evolution based on the phenotype of control wild and hybrid populations, we suggest that the elevated rate of evolution for stem diameter in hybrid populations under natural conditions may result from the novel genome combination of cultivated and wild radishes and strong selection rather than be a byproduct of hybridization.

Heterosis is a temporary increase the size of a phenotypic trait or fitness caused by an increase in heterozygosity. Many crop–wild hybrids, including radishes, exhibit this increased size (Snow *et al.*, 2001; Campbell *et al.*, 2006). If heterosis is indeed the cause of the increased size in hybrids,

Table 4 Estimated rates of evolution for age and size at reproduction in G_4 wild and hybrid populations relative to a control population

Population	Darwins[1] ($\times 10^{-3}$)		Haldanes[2]	
	Age at reproduction	Size at reproduction	Age at reproduction	Size at reproduction
W_1	36.90	13.39	0.3002	0.0628
W_2	21.77	25.41	0. 2207	0.0968
W_3	26.91	32.88	0.1910	0.1569
W_4	30.28	16.54	0.3004	0.0689
Avg G_4 W	**28.96**	**22.05**	**0.2531**	**0.0963**
H_1	24.49	67.09	0. 2131	0.2130
H_2	22.84	70.37	0. 1595	0.1529
H_3	15.36	53.25	0.1114	0.1770
H_4	30.25	80.06	0.1600	0.1853
Avg G_4 H	**23.23**	**67.69**	**0.1610**	**0.1821**

[1]Darwins = $(\ln(x_2) - \ln(x_1))/t$ (Haldane, 1949) where x_1 is the mean trait value for control populations and x_2 is the mean trait value of selected populations, t is the time in millions of yr (4 yr).
[2]Haldanes = $((x_2/s_p) - (x_1/s_p))/g$ (Gingerich 1983) where s_p is the pooled standard deviation of the populations' trait values and g is the number of generations (four) since the separation of the populations.
The bold values simply highlight the average values across all hybrids or all wilds. There are no significance tests or statistical tests associated with these values.

the effect should be significantly reduced in advanced-generation hybrids in a control population. However, the advanced generation hybrids from the experimental field populations were significantly larger than the control population, suggesting that size differences among wild and hybrid populations were, in part, evolved. Selection in field populations of hybrids may be selecting for one of two things. First, selection may be continuing to favour heterozygous plants, similar to the effects of heterosis seen in F_1 hybrids. Alternatively, hybridization may have led to the introgression of crop traits that produce large plants through correlated evolution. Either scenario suggests that the introgression of crop alleles contributed to the evolution of adaptive weed phenotypes and we recommend that future experimental designs take this possibility into account (as in Rhode & Cruzan, 2005).

In experimental field conditions, wild and hybrid populations experienced different selection intensities and differed in their response to those selection pressures (Table 3). Selection was more intense on hybrids perhaps because their phenotypes are maladapted to the environment. The stronger response to selection we observed may result from stronger selection on hybrid than wild populations, because hybrid populations possess more genetic variation than wild populations (as we measured for both age and size at reproduction in artificial selection populations, Campbell *et al.*, 2009), and because biotypes differ in the phenotypic correlations (Campbell & Snow, 2007, data presented here). These changes may be common consequences of hybridization that have important consequences for adaptive evolution of hybrids. Murren *et al.* (2002) found that brassicaceous hybrid species exhibited a higher number of phenotypic correlations than either of their parental taxa. Further, populations that have experienced recent outbreeding events tend to exhibit higher heritabilities than more inbred populations (Swindell & Bouzat, 2005; Syafaruddin *et al.*, 2006). Finally, hybrid populations may exhibit greater responses to selection than parental populations (Carney *et al.*, 2000).

How strong is selection in weed populations?

Our study was rare in its approach to measuring the magnitude of natural selection on life-history traits because of its experimental nature. Instead of measuring selection in natural populations, we explicitly created experimental populations to measure selection. To our knowledge, this is the fourth such selection experiment (*sensu stricto* Reznick & Ghalambor, 2005; e.g. guppies, Endler, 1980; Reznik *et al.*, 1990,; *Anolis* lizards, Schoener & Schoener, 1983; Losos *et al.*, 1997, 2001) and only the second to estimate selection in experimental plant populations (Whitney *et al.*, 2006). Comparable studies, though not formal field selection experiments, have measured selection in association with a dated event and appropriate controls in plant and nonplant populations (e.g. Carroll *et al.*, 2003; reviewed in Bone & Farres, 2001; Kingsolver *et al.*, 2001). Our approach is also rare because, through the process of hybridization, we created phenotypes that did not naturally occur within the region. This method was a useful tool for measuring selection on relatively invariant traits within populations, as suggested by Lexer *et al.* (2003). This work revealed that weedy radish populations would benefit from the introgression of large size (measured by root diameter and flower number),

such as those found in populations of cultivated relatives, but not delayed reproduction.

The magnitude of selection imposed on the phenotypic traits measured in the experimental populations was comparable to other published studies. Kingsolver *et al.* (2001) reported a mean value of $\beta = 0.08$ for estimates of linear selection gradients on life-history and phenological traits. Direct selection on size at reproduction (stem diameter) in this study was smaller than these average values for both wild and hybrid populations ($\beta_{Wild} = 0.02$; $\beta_{Hybrid} = 0.001$). However, selection on the ultimate size of plants (measured by root diameter at harvest) was similar to the average published by Kingsolver *et al.* (2001); $\beta_{Wild} = 0.032$; $\beta_{Hybrid} = 0.05$). Direct selection on age at reproduction was comparable this average value for hybrid populations whereas wild populations experienced weak selection on flowering phenology ($\beta_{Wild} = 0.005$; $\beta_{Hybrid} = -0.038$). However, the relatively weak selection for earlier flowering in hybrid radish populations was countered by the relatively strong selection for large root diameters and more flowers, resulting in total selection favouring an apparently non-adaptive flowering schedule.

Many studies of selection have revealed interannual variation in the magnitude of selection (Inouye *et al.*, 2002; Price *et al.*, 2005; Weis *et al.*, 2005). Our study reflects selection in only 1 yr. However, the pattern of selection across multiple populations was consistent, even though these populations had independently evolved for three generations (Fig. 3). Further, populations generally showed a significant deviation from the expected phenotype (i.e. Control populations) in the direction of selection in the current year's study. This suggests that consistent directional selection had been acting on these traits for several generations. Finally, our results are consistent with previous estimates of selection on various traits in *Raphanus* populations. For example, Conner (1996) found that selection on flower production was always positively associated with female fitness and strong in *R. raphanistrum* ($\beta = 0.70$ in two of three years measured, compared with the current study $\beta*_{Wild} = 0.50$, $\beta*_{Hybrid} = 0.77$). The phenotypic correlations found in this study were also consistent, in magnitude and direction, with those measured in a previous experiment conducted with G_3 plants from the experimental populations a year earlier, in 2004 (Campbell & Snow, 2007). Given that the results of many studies suggests that selection may vary dramatically, this collection of work suggests that the relationship between certain phenotypic traits and fitness is remarkably constant in *Raphanus* across years, locations, populations and laboratories, especially given its environmentally sensitive phenotype (Williams & Conner, 2001; Murren & Pigliucci, 2005; Wolfe & Mazer, 2005).

Although in a previous study we measured genetic correlations among size and age at flowering (Campbell *et al.*, 2009), here we measured only phenotypic correlations.

Therefore, there are two possible explanations for the coincident evolution of late flowering and large size in weedy radishes: pleiotropy or linkage disequilibrium. Depending on which mechanism is involved in the phenotypic evolution of weedy radishes, long-term evolutionary trajectories may differ dramatically. When phenotypic correlations are a result of linkage disequilibrium, alleles present at two or more loci controlling the two correlated phenotypic traits are non-randomly associated (Hartl & Clark, 2007). Although evolution of one trait often results in the correlated evolution of the second trait, independent evolution of these traits is occasionally possible. First, genetic correlations can shift and even reverse when populations encounter novel environmental conditions (a phenomenon reviewed in Sgrò & Hoffmann, 2004). Second, genetic correlations can break down under sustained directional selection allowing the two traits to evolve independently (e.g. Archer *et al.*, 2003; Phelan *et al.*, 2003). When phenotypic correlations are caused by pleiotropy, a single gene controls the phenotype of multiple traits, and thus the evolution of one trait will always result in the correlated evolution of the other trait (Coberly & Rausher, 2008; Latta & Gardner, 2009). Future studies exploring the genetic basis for differences in life histories among crops and their wild relatives may provide insight into which mechanism is more common.

Life history strategies of weeds

Our results suggest that, weedy *Raphanus* species benefit more from large root diameters and floral displays than advanced reproduction as selection strongly favoured large plants over plants that reproduced early. While cultivated *R. sativus* routinely experience selection for delayed flowering (Curtis, 2003), we expected selection would favour early flowering in weedy radish populations (Mazer, 1987). Although cultivated *R. sativus* exhibit late flowering and large size and *R. raphanistrum* exhibit early flowering and small size, we have little evidence of a positive correlation between these two traits in nonhybrid populations, presumably because selection has reduced the amount phenotypic variation in both types of non-hybrid populations, limiting the statistical possibility of detecting a correlation (Campbell & Snow, 2009; Campbell *et al.*, 2009). Yet, several other studies have documented a positive correlation between age and size at reproduction in *Raphanus* species and one study has documented similar selection gradients (Jablonski, 1997; Scheiner *et al.*, 2002). Scheiner *et al.* (2002) found evidence for selection for large size in the California wild radish ($\beta* = 0.13$–0.15). Surprisingly, California wild radishes experience selection for delayed reproduction ($\beta* = 0.03$–0.08). Large size appears to provide a significant fitness advantage for this weed in multiple environments. Under natural conditions, large size may also provide individuals with a competitive advantage (Campbell

& Snow, 2007). As a result, delayed flowering appears to be a simple byproduct of both environmentally plastic and genetically inherited correlations with large size (Campbell & Snow, 2007; Campbell *et al.*, 2009). The relative importance of this life history trade-off may be a function of the environment in which the trade-off is evaluated (Reznick *et al.*, 2000). Stronger direct selection for advanced flowering (and a reversal of the direction of total selection) may occur if the environmental conditions in our plots changed, for example in the frequency of disturbance (Meerts, 1995), herbicide application (Mortimer, 1997) or herbivory (Juenger & Bergelson, 2000) Further, environmentally dependent differences in fecundity may result from the effect of context-specific trade-offs. Future work will examine whether a dramatic change in the relative fitness of hybrids when grown in two disparate locations is a consequence of changes in life-history trade-offs (Campbell *et al.*, 2006). Although natural selection favoured large size in both wild and hybrid populations, only hybrids demonstrated a strong response whereas and wild populations did not generally respond to natural selection in this study. These results are consistent with results from an artificial selection study where wild populations exhibited a smaller response to selection for large size than hybrid populations when we imposed extremely strong selection on the trait (Campbell *et al.*, 2009). Therefore, hybridization may facilitate the evolution of a large and successful weed.

Conclusions

This natural selection experiment revealed that adaptive evolution may proceed more rapidly in crop–wild hybrid populations than in populations of their wild progenitors. Given the adaptive evolution demonstrated by the natural selection populations, the evolutionary trajectories of wild and hybrid weedy radish populations are shaped by both specific selection pressures and phenotypic correlations. Future studies that evaluate crop–wild hybrids could provide insight into the adaptiveness of extremely large size and biennial life histories for these weeds. Further, assessing the consequences of variation in life history for population dynamics will be important in determining the fitness and invasiveness of these extreme genotypes (Murray, 1990, 1992; Bergelson, 1994). In summary, we argue that experimental manipulation of life histories provides important insights into the drivers of evolutionary response and should stimulate the development of plant life-history theory.

Acknowledgements

References

Allendorf FW, Lundquist LL. 2003. Introduction: population biology, evolution, and control of invasive species. *Conservation Biology* 17: 24–30.

Anderson E, Stebbins GL. 1954. Hybridization as an evolutionary stimulus. *Evolution* 8: 378–388.

Archer MA, Phelan JP, Beckman KA, Rose MR. 2003. Breakdown in correlations during laboratory evolution. II. Selection on stress resistance in *Drosophila* populations. *Evolution* 57: 536–543.

Baker HG. 1965. Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL, eds. *The genetics of colonizing species*. New York, NY, USA: Academic Press, 147–172.

Ball DA, Cudney D, Dewey S, Elmore CL, Lym RG, Morshita DW, Parker R, Swan DG, Whitson TD, Zollinger RK. 2000. *Weeds of the West*. Laramie, WY, USA: Western Society of Weed Science.

Bergelson J. 1994. Changes in fecundity do not predict invasiveness: a model study of transgenic plants. *Ecology* 75: 249–252.

Bone E, Farres A. 2001. Trends and rates of microevolution in plants. *Genetica* 112–113: 165–182.

Campbell LG, Snow AA. 2007. Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). *New Phytologist* 173: 648–660.

Campbell LG, Snow AA. 2009. Can feral weeds evolve from cultivated radish (*Raphanus sativus*, Brassicaceae)? *American Journal of Botany* 96: 498–506.

Campbell LG, Snow AA, Ridley CE. 2006. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. *Ecology Letters* 11: 1198–1209.

Campbell LG, Snow AA, Sweeney PM, Ketner JM. 2009. Rapid evolution in crop-weed hybrids under selection for divergent life histories. *Evolutionary Applications* 11: 934–943.

Carney SE, Gardner KA, Rieseberg LH. 2000. Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (*Helianthus*). *Evolution* 54: 462–474.

Carroll SP, Dingle H, Famula TR. 2003. Rapid appearance of epistasis during adaptive divergence following colonization. *Proceedings of the Royal Society of London Series B – Biological Sciences* 270: S80–S83.

Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54: 1546–1557.

Coberly CL, Rausher MD. 2008. Pleiotropic effects of an allele producing white flowers in *Ipomea purpurea*. *Evolution* 62: 1076–1085.

Conner JK. 1996. Understanding natural selection: an approach integrating selection gradients, multiplicative fitness components, and path analysis. *Ethology, Ecology and Evolution* 8: 387–397.

Conner JK, Via S. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.

Coulibaly S, Pasquet RS, Papa R, Gepts P. 2002. AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types. *Theoretical and Applied Genetics* 104: 358–366.

Crisp P. 1995. Radish, *Raphanus sativus* (Cruciferae). In: Smartt JS, Simmonds NW, ed. *Evolution of crop plants*. Harlow, UK: Longman Scientific & Technical, 86–89.

Curtis IS. 2003. The noble radish: past, present and future. *Trends in Plant Science* 8: 305–307.

Daehler CC. 1998. The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. *Biological Conservation* 84: 167–180.

Ellstrand NC. 2003. *Dangerous Liaisons? When cultivated plants mate with their wild relatives*. Baltimore, MD, USA: Johns Hopkins University Press.

Ellstrand N, Marshall D. 1985. The impact of domestication on distribution of allozyme variation within and among cultivars of radish, *Raphanus sativus* L. *Theoretical and Applied Genetics* 69: 393–398.

Ellstrand NC, Schierenbeck KA. 2000. Hybridisation as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA* 97: 7043–7050.

Ellstrand NC, Prentice HC, Hancock JF. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539–563.

Endler JA. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34: 76–91.

Galen C, Zimmer KA, Newport ME. 1987. Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution* 41: 599–606.

García-Ramos G, Rodríguez D. 2002. Evolutionary speed of invasions. *Evolution* 56: 661–668.

Hansen LB, Siegismund HR, Jørgensen RB. 2001. Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative. *Genetic Resources and Crop Evolution* 48: 621–627.

Hartl DL, Clark AG. 2007. *Principles of population genetics*. 4th ed. Sunderland, MA, USA: Sinauer and Associates.

Hegde SG, Nason JD, Clegg JM, Ellstrand NC. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60: 1187–1197.

Inouye DW, Morales MA, Dodge GJ. 2002. Variation in timing and abundance of flowering by *Delphinium barbeyi* Huth (Ranunculaceae): the roles of snowpack, frost, and La Niña, in the context of climate change. *Oecologia* 130: 543–550.

Jablonski LM. 1997. Responses of vegetative and reproductive traits to elevated CO_2 and nitrogen in *Raphanus* varieties. *Canadian Journal of Botany* 75: 533–545.

Juenger T, Bergelson J. 2000. The evolution of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*: herbivore-imposed natural selection and the quantitative genetics of tolerance. *Evolution* 54: 764–777.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157: 245–261.

Klinger T, Ellstrand NC. 1994. Engineered genes in wild populations: fitness of weed–crop hybrids of *Raphanus sativus*. *Ecological Applications* 4: 117–120.

Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* 36: 1210–1226.

Latta RG, Gardner KM. 2009. Natural selection on pleiotropic quantitative trait loci affecting a life-history trade-off in *Avena barbata*. *Evolution* 63: 2153–2163.

Lavergne S, Molofsky J. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences, USA* 104: 3883–3888.

Lee TN, Snow AA. 1998. Pollinator preferences and the persistence of crop genes in wild radish populations (*Raphanus raphanistrum*, Brassicaceae). *American Journal of Botany* 85: 333–349.

Lexer C, Randell RA, Rieseberg LH. 2003. Experimental hybridization as a tool for studying selection in the wild. *Ecology* 84: 1688–1699.

Losos JB, Warheit KI, Schoener TW. 1997. Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* 387: 70–73.

Losos JB, Schoener TW, Warheit KI, Creer D. 2001. Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. *Genetica* 112: 399–415.

Maron JL, Vila M, Bommarco R, Elmendorf S, Beardsley P. 2004. Rapid evolution of an invasive plant. *Ecological Monographs* 74: 261–280.

Mazer SJ. 1987. The quantitative genetics of life-history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae) – ecological and evolutionary consequences of seed-weight variation. *American Naturalist* 130: 891–914.

Meerts P. 1995. Phenotypic plasticity in the annual weed *Polygonum aviculare*. *Botanica Acta* 108: 414–424.

Mortimer AM. 1997. Phenological adaptation in weeds – an evolutionary response to the use of herbicides. *Pesticide Science* 51: 299–304.

Murray BG. 1990. Population-dynamics, genetic change, and the measurement of fitness. *Oikos* 59: 189–199.

Murray BG. 1992. The evolutionary significance of lifetime reproductive success. *The Auk* 109: 167–172.

Murren CJ, Pigliucci M. 2005. Morphological responses to simulated wind in the genus *Brassica* (Brassicaceae): allopolyploids and their parental species. *American Journal of Botany* 92: 810–818.

Murren CJ, Pendleton N, Pigliucci M. 2002. Evolution of Phenotypic Integration in *Brassica* (Brassicaceae). *American Journal of Botany* 89: 655–663.

Panetsos CA, Baker HG. 1967. The origin of variation in 'wild' *Raphanus sativus* (Cruciferae) in California. *Genetica* 38: 243–274.

Phelan JP, Archer MA, Beckman KA, Chippindale AK, Nusbaum TJ, Rose MR. 2003. Breakdown in correlations during laboratory evolution. I. Comparative analyses of *Drosophila* populations. *Evolution* 57: 527–535.

Price MV, Waser NM, Irwin RE, Campbell DR, Brody AK. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology* 86: 2106–2116.

Rattenbury JA. 1962. Cyclic hybridization as a survival mechanism in New Zealand forest flora. *Evolution* 16: 348–363.

Reznick DN, Ghalambor CK. 2005. Selection in nature: experimental manipulations of natural populations. *Integrative and Comparative Biology* 45: 456–462.

Reznick D, Nunney L, Tessier A. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution* 15: 421–425.

Reznick DA, Bryga H, Endler JA. 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346: 357–359.

Rhode JM, Cruzan MB. 2005. Contributions of heterosis and epistasis to hybrid fitness. *American Naturalist* 166: E124–E139.

Ridley CE, Kim SC, Ellstrand NC. 2008. Bi-directional history of hybridization in California wild radish *Raphanus sativus* (Brassicaceae) as revealed by chloroplast DNA. *American Journal of Botany* 95: 1437–1442.

Rieseberg LH. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

New
Phytologist

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC et al. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**: 305–332.

Scheiner SM, Donohue K, Dorn LA, Mazer SJ, Wolfe LM. 2002. Reducing environmental bias when measuring natural selection. *Evolution* **56**: 2156–2167.

Schmitz DC, Simberloff D, Hofstetter RH, Haller W, Sutton D. 1997. The ecological impact of nonindigenous plants. In: Simberloff D, Schmitz DC, Brown TC, eds. *Strangers in paradise: Impact and management of nonindigenous species in Florida.* Washington, DC, USA: Island Press, 39–61.

Schoener TW, Schoener A. 1983. The time to extinction of a colonizing propagules of lizards increases with island area. *Nature* **302**: 332–334.

Sgrò CM, Hoffmann AA. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**: 241–248.

Snow AA, Campbell LG. 2005. Can feral radishes become weeds? In: Gressel J, ed. *Crop ferality and volunteerism.* Boca Raton, FL, USA: CRC Press, 193–208.

Snow AA, Uthus KL, Culley TM. 2001. Fitness of hybrids between weedy and cultivated radish: implications for weed evolution. *Ecological Applications* **11**: 934–943.

Stanton ML. 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: I. Pollinator response to color morphs. *American Journal of Botany* **74**: 178–187.

Stebbins GL. 1959. The role of hybridization in evolution. *Proceedings of the American Philosophical Society* **103**: 231–251.

Sutherland S. 2004. What makes a weed a weed: life history traits of native and exotic plants in the USA. *Oecologia* **141**: 24–39.

Swindell WR, Bouzat JL. 2005. Modeling the adaptive potential of isolated populations: experimental simulations using *Drosophila*. *Evolution* **59**: 2159–2169.

Syafaruddin KK, Kobayashi K, Yoshioka Y, Horisaki A, Niikura S, Ohsawa R. 2006. Estimation of heritability of the nectar guide of flowers in *Brassica rapa* L. *Breeding Science* **56**: 75–79.

Viard F, Bernard J, Desplanque B. 2002. Crop–weed interactions in the *Beta vulgaris* complex at a local scale: allelic diversity and gene flow within sugar beet fields. *Theoretical and Applied Genetics* **104**: 688–697.

Wang L, Song YC, Ning SB, Liu LH, Gu MG, Guo LQ. 1999. Identification of *Zea diploperennis* chromosome fragments introgressed to maize via genomic *in situ* hybridization. *Acta Botanica Sinica* **41**: 1264–68.

Warwick S. I., Francis A. 2005. The biology of Canadian weeds. 132. *Raphanus raphanistrum*, L. *Canadian Journal of Plant Science* **85**: 709–733.

Weis AE, Winterer J, Vacher C., Kossler TM, Young CA, LeBuhn GL. 2005. Phenological assortative mating in flowering plants: the nature and consequences of its frequency dependence. *Evolutionary Ecology Research* **7**: 161–181.

Whitney KD, Randell RA, Rieseberg LH. 2006. Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. *American Naturalist* **167**: 794–807.

Whitton J, Wolf DE, Arias DM, Snow AA, Rieseberg LH. 1997. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theoretical and Applied Genetics* **95**: 33–40.

Williams J. L., Conner J. K.. 2001. Sources of phenotypic variation in floral traits in wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* **88**: 1577–1581.

Wolfe LM, Mazer SJ. 2005. Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus:* Brassicaceae). nternational Journal of Plant Sciences **166**: 631–640.

Yamagishi H, Terachi T. 2003. Multiple origins of cultivated radishes as evidenced by a comparison of the structural variations in mitochondrial DNA of *Raphanus*. *Genome* **46**: 89–94.

Zangerl AR, Berenbaum MR. 2005. Increase in toxicity of an invasive weed after reassociation with its coevolved herbivore. *Proceedings of the National Academy of Science, USA* **102**: 15529–15532.

Zar JH. 1999. *Biostatistical analysis.* Upper Saddle River, NJ, USA: Prentice Hall.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Effects of hybridization, no selection, natural selection and random mating populations on five phenotypic traits and three fitness components in wild and crop–wild hybrid populations

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.