

## LETTER

## Stress and domestication traits increase the relative fitness of crop–wild hybrids in sunflower

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### Abstract

After a decade of transgenic crop production, the dynamics of gene introgression into wild relatives remain unclear. Taking an ecological genetics approach to investigating fitness in crop–wild hybrid zones, we uncovered both conditions and characteristics that may promote introgression. We compared diverse crop–wild hybrid genotypes relative to wild *Helianthus annuus* under one benign and three stressful agricultural environments. Whereas relative fitness of crop–wild hybrids averaged 0.25 under benign conditions, with herbicide application or competition it reached 0.45 and was more variable. In some instances, hybrid fitness matched wild fitness ( $\approx 1$ ). Thus, wild populations under agronomic stress may be more susceptible to introgression. Although ‘domestication’ traits are typically considered unlikely to persist in wild populations, we found some (e.g. rapid growth and early flowering) that may enhance hybrid fitness, especially in stressful environments. Rigorous assessment of how particular genotypes, phenotypes, and environments affect introgression will improve risk assessment for transgenic crops.

### Keywords

Biosafety, competition, crop–wild hybrid, domestication,  $G \times E$  interactions, GM crops, herbicide, introgression, relative fitness, transgenes.

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### INTRODUCTION

The advent of genetically engineered crops has underscored the need to understand the process of crop gene introgression into populations of wild relatives (Ellstrand & Hoffman 1990; Wolfenbarger & Phifer 2000; Hails & Morley 2005; Snow *et al.* 2005; Chapman & Burke 2006). Since many crops are cultivated in locations within the geographic range of sexually compatible relatives, they can hybridize spontaneously (Ellstrand *et al.* 1999). Such crop–wild gene flow can have ecological ramifications ranging

from the production of invasive hybrid lineages (Kareiva *et al.* 1996; Ellstrand 2003; Hedge *et al.* 2006) to the extinction or genetic swamping of wild populations (Boudry *et al.* 1993; Huxel 1999; Wolf *et al.* 2001; reviewed in Ellstrand 2003). The risk of invasiveness could increase if the hybridizing crop possesses ecologically important characteristics, such as insect or disease resistance, or cold or salt tolerance, which might increase fitness (Snow & Morán-Palma 1997). Increased invasiveness or extinction could alter community structure and function (Kareiva *et al.* 1996; Callaway & Maron 2006), and the likelihood of each

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outcome depends on the fitness of the crop–wild hybrid relative to its wild counterpart.

For this reason, studies comparing the fitness of crop–wild hybrids and wild plants have been essential to assessing the potential for genetic alteration of wild populations via gene flow from crops (Ellstrand & Hoffman 1990; Darmency 1994; Hauser *et al.* 1998; Campbell *et al.* 2006; Mercer *et al.* 2006b). Further research addressing how frequency-dependent processes, assortative mating, and population demography may affect crop gene introgression has deepened our understanding of ecological and evolutionary dynamics in crop–wild hybrid zones (Cummings *et al.* 2002; Hauser *et al.* 2003; Hooftman *et al.* 2005; Campbell & Snow 2007). Fitness of inter- and intraspecific plant hybrids in natural hybrid zones has been shown to vary depending on the parental genotype (Waser & Price 1989; Byers 1998), the testing environment (Emms & Arnold 1997; Brock & Galen 2005), and their interactions (Campbell & Waser 2001; Johnston *et al.* 2001; Whitney *et al.* 2006). Such variability in fitness of crop–wild hybrids could have important implications for biosafety policy, especially if their effects were predictable.

Crop–wild hybrids may possess ‘domestication’ traits, or traits inherited from the crop that have been directly (e.g. seed retention) or indirectly (e.g. large seedling size) selected for by plant breeders. ‘Domestication’ traits that are agronomically useful are assumed to reduce fitness in natural environments (Stewart *et al.* 2003). However, some traits bred into crops, such as high growth rates, high fecundity, or transgenic traits (e.g. insect resistance; Snow *et al.* 2003), could be more broadly beneficial and enhance fitness of plants in the wild. Wild populations show considerable variability for phenotypic traits (Waser & Price 1989; Nagy & Rice 1997; Etterson 2004), which may influence the ecological impact of some ‘domestication’ traits. For instance, sunflower crop breeders have selected for the production of a single head and against branching, whereas variability among wild populations for amount of branching is considerable (Mercer 2005). Therefore, we would expect that the fitness effects of such a crop trait and the subsequent strength of selection for or against it in a crop–wild hybrid zone will depend on the particular phenotype of the wild population. Selection can also vary under different biotic and abiotic conditions (Etterson 2004; Whitney *et al.* 2006), which could alter the possibility for introgression accordingly. Yet evidence regarding these dynamics is lacking in crop–wild hybrid zones.

To evaluate the joint influences of genetic, phenotypic and environmental variation on the ecological and evolutionary consequences of crop–wild gene flow, we produced 36 diverse cross types of crop–wild hybrid and wild

*Helianthus annuus* L. (sunflower) seed. We grew them in an agricultural field in Minnesota (USA) under three common stress conditions: with interspecific competition from *Triticum aestivum* L. (wheat) and with an herbicide applied at two concentrations. We also grew them in a control environment, free of herbicide and competition. We tested the wild and crop–wild hybrid sunflowers under these particular stresses for three reasons. First, both interspecific competition and herbicide application are stresses common to the agroecosystems where crop–wild hybrids are generated. Second, we chose wheat as the competitor because it is often found in rotation with sunflowers, so naturally produced crop–wild hybrid sunflowers may be found in competition with wheat. Third, acetolactase synthase inhibiting (ALS) herbicides are commonly used in wheat production, so would be likely used against crop–wild sunflower hybrids. In addition, cultivated sunflowers have now been bred to possess ALS resistance, and hybrids with this cultivar may have a fitness advantage under herbicide application. Analyses of subsets of this and similar experiments have revealed genetic differences among cross types in seed germination (Mercer *et al.* 2006a), plant survival, and fecundity (Mercer *et al.* 2006b), as well as other phenotypic characteristics (K.L. Mercer, unpublished data). We have consistently found strong genotype by environment interaction effects on fitness components.

In this manuscript, we extend these analyses to investigate the influence of three common stressful conditions and the genetic composition of parental populations on the fitness of crop–wild hybrids relative to wild sunflower. We focus here on relative fitness because it directly influences the early stages of introgression. Then we investigate how certain ‘domestication’ traits may ultimately promote crop gene introgression into wild populations. These findings reveal considerable variability in the potential for crop gene introgression across crop–wild hybrid zones.

## MATERIALS AND METHODS

### Study system

Native *Helianthus annuus* L. (wild annual sunflower) grows in disturbed habitats, including in agricultural areas where domesticated sunflower (also *H. annuus*) is grown for seed and oil (Burke *et al.* 2002). Crop sunflower, which is cross-compatible with its wild conspecific, is nevertheless highly differentiated from it due to breeding for ‘domestication’ traits, such as the rapid production of a single head and large seeds. Some sunflower crop varieties have been developed with transgenic insect and disease resistance (Burke & Rieseberg 2003; Snow *et al.* 2003), but only varieties with non-transgenic herbicide resistance – a trait originally found

in weedy wild populations (Al-Khatib *et al.* 1998) – have been commercialized to date.

### Plant materials

The USDA-ARS sunflower breeding program (Fargo, North Dakota), provided the three sunflower inbred crop lines, two of which carry resistance to different acetolactase synthase inhibitor (ALS) herbicides. These three lines – conventional, imidazolinone resistant (Imi-R) and sulfonylurea resistant (SU-R) – were used as pollen donors (Mercer 2005). The use of herbicide resistant lines allowed us to assess the fitness effects of herbicide application (below) on crop–wild hybrids possessing known resistance alleles. Here we do not focus on the herbicide resistance trait *per se*; we simply consider the hybrids from both conventional and herbicide resistant crop lines as examples of different crop–wild hybrid genotypes. We utilized inbred lines as the crop parents rather than hybrid varieties to reduce the genetic variability of the resulting progeny. Thus, had we used varieties, we would have expected even more variability within cross types. From the USDA germplasm bank we acquired nine wild sunflower populations; here, each accession is designated by its state of origin. Using hand pollinations, we produced 36 cross-types – 27  $F_1$  crop–wild hybrid crosses and nine wild–wild crosses – each including seed pooled from 20 maternal families (for further information see Mercer 2005 and Mercer *et al.* 2006b).

### Field experiment

In 2003, we planted the 36 sunflower cross types into a split-plot design at the Saint Paul Experiment Station of the University of Minnesota. To each of the whole plots, one of the four treatments (see below) was assigned at random. Each subplot comprised four individuals from a given cross type. The entire design was replicated seven times. Conventional and SU-R hybrids were planted in all four treatments, while Imi-R hybrids were not planted in the two herbicide treatments. Each cross type was represented by approximately 28 individuals in a given treatment, to include a total of 3528 plants.

We had two classes of treatments designed to act as common stresses found within agricultural settings. First, for the two herbicide treatments, we applied an ALS herbicide – a sulfonylurea called tribenuron methyl – at a high field rate ( $1\times = 18.2$  g active ingredient/ha) or at three times the field rate ( $3\times$ ) 2 weeks after planting the experiment. When the herbicide was applied, sunflower seedlings were at the 2–4 leaf stage at which point they should have been susceptible to this ALS herbicide used to control dicot weeds (Tranel & Wright 2002). Second, for the

competition treatment, we imposed interspecific competition by planting germinated sunflower seeds into recently established stands of wheat (for more information see Mercer 2005 and Mercer *et al.* 2006b).

Two and five-weeks after planting, we measured seedling height (cm). Early height was defined as height at 2 weeks and early growth was the difference between the two height measures (height at 5 weeks – height at 2 weeks). We noted the first day of flowering for each plant and whether it survived to produce seeds. All plants were openly cross-pollinated by the local pollinator community. We measured fecundity by counting the number of seeds per head on a representative sub-sample of measured sunflower heads and then used measurements of head size and head number to estimate total seed production per plant (Mercer 2005; Mercer *et al.* 2006b). We calculated the overall means and standard errors for these characteristics of the wild plants, conventional hybrids and SU-R hybrids in the control, herbicide and competition treatments.

### Analysis of relative fitness

Estimates of fitness were obtained for each cross type within each replication and treatment by averaging fecundity values of the four plants representing that cross type, including fecundities of zero. By including fecundities of zero for plants that did not survive to reproduce, we included plant survival in this estimate of fitness. We calculated the fitness of hybrids produced from each crop line and wild population relative to wild plants from the same wild population and growing in the same conditions. The fitness of hybrids relative to wilds was calculated for each replication (i), treatment (j), wild population (k) and crop line (l) as:

$$\text{Relative fitness}_{ijkl} = \text{hybrid fitness}_{ijkl} / \text{wild fitness}_{ijk}$$

We explored the relative fitness data in two ways. First, we calculated average relative fitness values for the three hybrid types under the four experimental treatments. We assessed the variability among relative fitness means within a hybrid type using the Kruskal–Wallis test, a non-parametric one-way ANOVA (PROC NPARIWAY; SAS Institute 2001).

Next, we calculated relative fitness values for the 63 combinations of genetic backgrounds and stressful conditions. For these 63 cases, we judged which hybrid relative fitness values increased, decreased or remained similar under stressful environments when compared with that of the same cross type in the control environment by assessing whether the standard error bars of the mean hybrid relative fitness in the stressful condition overlapped the reference mean of the control group. Where there was overlap, we considered the means similar. Where there was no overlap, we designated the mean under stressful conditions to be higher or lower than the control mean. We used a chi-square

test to compare these data against the expected pattern of equivalent numbers of cases where relative fitness increased, decreased and remained stable with stressful conditions.

### Analysis of the relationship between fecundity and three 'domestication' traits

We assessed the relationship between fecundity of plants that survived to reproduce and three important 'domestication' traits (seedling height, early growth and flowering phenology) by incorporating each trait as a covariate in an ANCOVA. The ANCOVA model also estimated the effects on fecundity (transformed to its fourth root) of treatment, wild population and paternal parent, along with their interactions [similar to Model A described in Mercer *et al.* (2006b), but employing data from all four treatments]. With this analysis, we estimated and tested a given trait's relationship with fecundity (i.e. its regression coefficient), while simultaneously accounting for the effects of experimental factors. In the analysis, the regression coefficients were estimated for each trait in all combinations of treatment and cross type (i.e. hybrid or wild).

To illustrate how 'domestication' traits can ultimately affect relative fitness, for one trait, seedling size, we obtained predictions of relative fitness of the conventional crop–wild hybrid from wild seedling size with linear regression (SAS Institute 2001). Separate analyses were done by treatment using population-level means ( $n = 9$ ). Because this analysis is based on means, rather than raw data, we note that the statistical tests are not rigorously valid.

## RESULTS

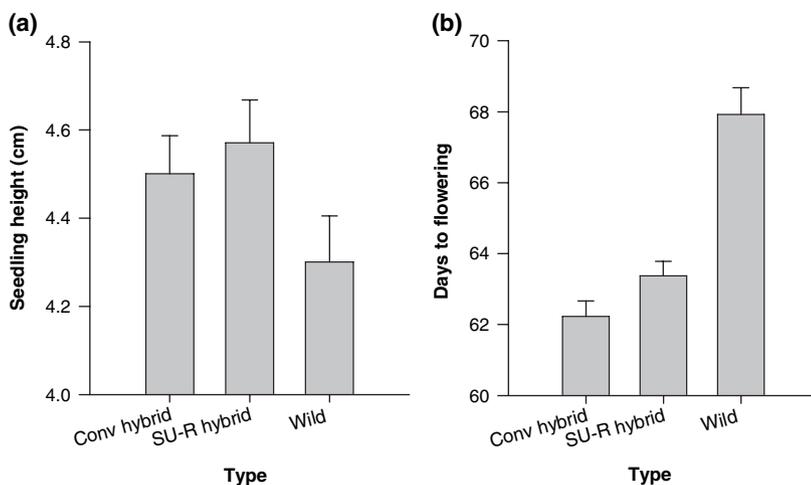
Crop–wild hybrids were larger as seedlings, grew faster, and flowered earlier than wild plants (Figs 1a,b and 2a). They also tended to have higher survival and produced significantly fewer seeds (Fig. 2b,c). Herbicide applications reduced early growth of wilds and crop–wild hybrids, though SU-R hybrids

showed some degree of tolerance (Fig. 2a). Notwithstanding strong negative effects on wild seed production, herbicide applications slightly enhanced hybrid seed production due to compensatory growth (Fig. 2b). Competition with wheat greatly reduced the seed production of both wild and hybrid plants (Fig. 2b). The early negative effects of herbicide on growth and the negative impact of competition on fecundity confirmed that these treatments stressed the plants.

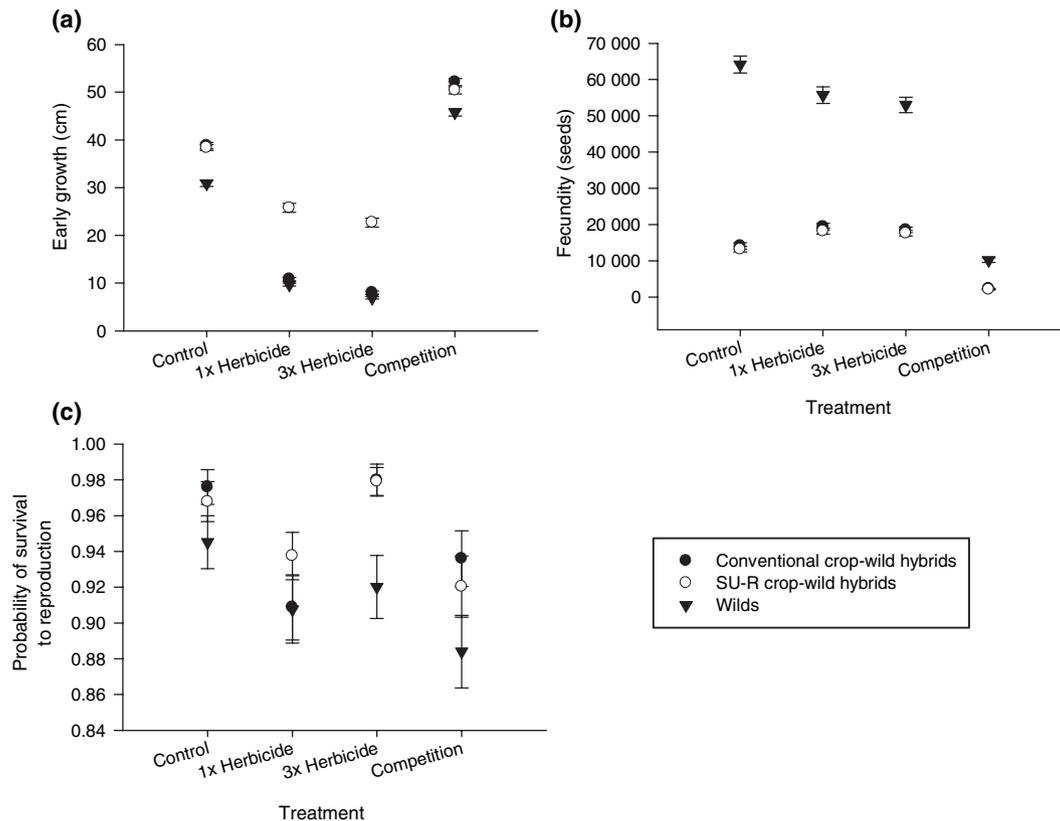
The fitness of hybrids relative to their wild counterparts was higher in stressful environments than under control conditions, but remained lower than one, on average (Fig. 3). This pattern was consistent across the three hybrid types. Moreover, the relative fitness of hybrids showed greater variability among wild and crop lineages in stressful environments and exceeded one, in some cases (Fig. 4). Under control conditions, the relative fitness of hybrids ranged from 0.1 to 0.5 across genetic backgrounds. But under stressful conditions, relative fitness of hybrids ranged more broadly between 0.3 and 1.1 (Fig. 4). Accordingly, the coefficient of variation (CV) for relative fitness was much higher in the stressful treatments than under control conditions (CVs: control = 32.26; competition = 55.60; 1× herbicide = 46.15; 3× herbicide = 42.35).

Despite this variability, the pattern of increased relative hybrid fitness in stressful conditions emerged. In all, we assayed 63 unique combinations of genetic backgrounds and stressful environments for relative fitness values (Fig. 4). In 60% of the cases ( $n = 38$ ), we found higher hybrid relative fitness in stressful than benign environments. In 32% of the cases ( $n = 20$ ), hybrid relative fitness was similar in stressful and benign environments, and in only 8% of the cases ( $n = 5$ ) was hybrid relative fitness lower in stressful environments ( $\chi^2 = 27.8$ , d.f. = 2,  $P$ -value  $\leq 0.0001$ ).

When using the three 'domestication' traits as covariates in separate ANCOVAs, we found that, under competitive conditions, hybrid fecundity increased with faster early growth ( $\beta = 0.051$ ) and with earlier flowering ( $\beta = -0.101$ )



**Figure 1** Differences in morphology and phenology of sunflowers. (a) Heights of seedlings at 2 weeks and (b) days to flower for wild sunflowers and conventional (Conv) and sulfonylurea-resistant (SU-R) crop–wild hybrid sunflowers grown under control conditions at the St Paul Experiment Station of the University of Minnesota, 2003. Bars are standard errors and  $n = 218$ –252 for each mean.



**Figure 2** Differences in growth and fitness components for sunflowers grown under four treatments. (a) Early growth, (b) fecundity of plants that survived to reproduce, and (c) probability of survival to reproduction for wild and crop–wild hybrid sunflower plants grown at the St Paul Experiment Station of the University of Minnesota, 2003. Sulfonylurea herbicide was applied 2 weeks after planting. Bars are standard errors and  $n = 218$ – $252$  for each mean.

(Table 1). Similarly, under stressful conditions (both herbicide and competition), fecundity of wild plants increased with both faster early growth ( $\beta = 0.060$ ,  $\beta = 0.136$ ,  $\beta = 0.084$ ) and earlier flowering ( $\beta = -0.127$ ) (Table 1). For wild plants, larger seedling size prior to the 1 $\times$  herbicide application resulted in higher fecundity ( $\beta = 0.395$ ) (Table 1). Analysis of the linear relationship between wild seedling size and relative hybrid fitness yielded consistently negative relationships in all treatments ( $-0.050 < \beta < -0.199$ ;  $n = 9$ ) (Fig. 5). These among population relationships were strongest in the competition and 1 $\times$  herbicide treatments (Fig. 5). This indicates that wild populations with the largest seedlings tended to produce hybrids that had the lowest relative fitness under stressful conditions.

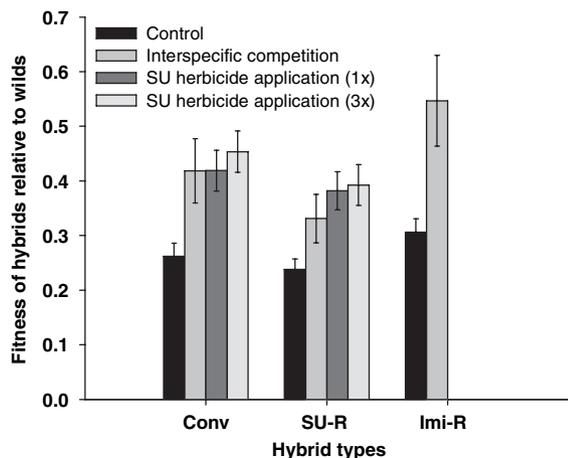
## DISCUSSION

This study shows that the fitness of crop–wild hybrids relative to wild plants increases in stressful conditions. Furthermore, the variability among relative fitness estimates increased in three stressful agricultural conditions. Both of these results appear to be due, in part, to crop-conferred

traits related to domestication. These results invalidate the common assumption that selection against crop traits eliminates or reduces the likelihood of introgression of crop alleles into wild populations. Perhaps, some ‘domestication’ traits may even hasten introgression of other neutral alleles.

## Hybrid relative fitness increases with stress

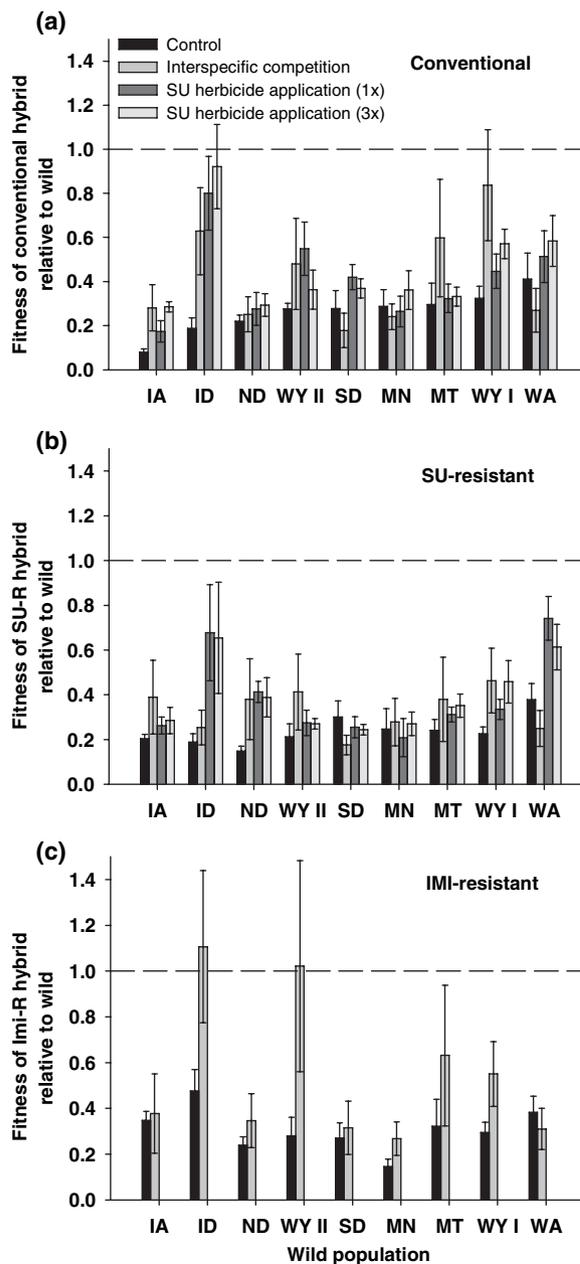
The stressful conditions tested here generally increased the relative fitness of hybrids, indicating that there are important environment-dependent fitness responses that should be considered in crop–wild hybrid zones. In this study, we focused on stresses that are common to conventional agroecosystems: competition and herbicide application. The variable responses of the individual hybrid lineages to these stressful environments indicate even greater complexity. This variability in relative fitness of our hybrid lineages in different environments may result from environment-dependent influences of either the crop or wild parent’s genetics on development. Alternatively, there may be little  $G \times E$  with respect to development, yet the same expressed



**Figure 3** Relative fitness of crop–wild hybrids under four treatments. These treatments include: competition with wheat, a 1× rate or a 3× rate of a sulfonylurea herbicide, or a no competition, no herbicide control. crop–wild hybrids produced by conventional (Conv), sulfonylurea resistant (SU-R), or imidazolinone resistant (Imi-R) crop lines are grouped. Kruskal–Wallis tests indicate significant variability among treatment means within a hybrid grouping (Conv:  $\chi^2_3 = 22.59$ ,  $P < 0.0001$ ; SU-R:  $\chi^2_3 = 25.12$ ,  $P < 0.0001$ ; Imi-R:  $\chi^2_1 = 1.94$ ,  $P = 0.1636$ ). Bars are standard errors and  $n = 60$ – $61$  for each mean.

phenotypes may have different fitness consequences depending on environmental conditions. For instance, the wild populations least resilient to competition had a greater increase in relative fitness of their hybrids under competitive conditions than wild populations that were more resilient to competition (K.L. Mercer, unpublished data). Extensive testing of hybrids under stressful conditions is likely to uncover considerable fitness differences among hybridizing populations and will improve our ability to predict the range of crop gene introgression expected in wild populations. Likewise, this approach can be applied to natural systems to reveal where hybrid zones between particular populations or in a particular set of environmental conditions may be hotspots for the establishment of stable hybrid lineages. Conversely, it could help elucidate where species or subspecies boundaries are less susceptible to introgression due to fitness disadvantages of hybrids. This context-dependent selection deserves further exploration (Campbell & Waser 2001; Mercer *et al.* 2006b).

In contrast to the common expectation that crop–wild hybrids suffer dramatic fitness loss (Cummings *et al.* 2002; Stewart *et al.* 2003), in 6% of our cases, hybrid fitness in the stressful environments was indistinguishable from that of the wild counterpart (i.e. relative fitness was not different from one; Fig. 4). In other crop–wild complexes, examples of high hybrid fitness relative to wild plants have been similarly noted, though not necessarily associated with



**Figure 4** Relative fitnesses of diverse crop–wild hybrids under four treatments. These treatments include: competition with wheat, a 1× rate or a 3× rate of a sulfonylurea herbicide, or a no competition, no herbicide control. Separate panels illustrate relative fitness values for hybrids from different crop lines: (a) conventional, (b) sulfonylurea-resistant (SU-R), and (c) imidazolinone-resistant (Imi-R) crop–wild hybrids. Wild populations used in the crosses are indicated on the  $x$ -axis and are ordered by increasing relative fitness of conventional crop–wild hybrids under control conditions. Wild population abbreviations designate: Iowa (IA), Idaho (ID), North Dakota (ND), Wyoming II (WY II), South Dakota (SD), Minnesota (MN), Montana (MT), Wyoming I (WY I) and Washington (WA). Bars are standard errors and  $n = 4$ – $7$  for each mean.

**Table 1** Regression coefficients for four covariates. Coefficient of each covariate (height of sunflower seedlings, early growth and days to flower) was estimated from an ANCOVA model predicting fecundity of plants that survived to reproduce (transformed to its fourth root).

Treatment	Cross type	Regression coefficient	SE	d.f.	<i>t</i> -value	Significance
<i>Height of seedling at 2 weeks (cm)</i>						
Control	Hybrid	-0.061	0.078	2723	-0.78	ns
	Wild	0.151	0.119	2697	1.27	ns
1× Herbicide	Hybrid	0.015	0.066	2474	0.22	ns
	Wild	0.395	0.119	2839	3.33	***
3× Herbicide	Hybrid	-0.006	0.069	2708	-0.09	ns
	Wild	0.048	0.112	2809	0.43	ns
Competition	Hybrid	-0.012	0.037	2535	-0.31	ns
	Wild	0.050	0.059	2530	0.84	ns
<i>Early growth (cm)</i>						
Control	Hybrid	-0.031	0.011	2527	-2.81	**
	Wild	0.058	0.017	2518	3.38	***
1× Herbicide	Hybrid	-0.007	0.007	2610	-1.10	ns
	Wild	0.060	0.030	2854	1.99	*
3× Herbicide	Hybrid	-0.012	0.006	2668	-1.93	*
	Wild	0.136	0.041	2840	3.27	***
Competition	Hybrid	0.051	0.010	2796	5.28	***
	Wild	0.084	0.015	2835	5.45	***
<i>Days to flower (days)</i>						
Control	Hybrid	-0.001	0.020	2817	-0.04	ns
	Wild	-0.007	0.023	2796	-0.28	ns
1× Herbicide	Hybrid	-0.005	0.016	2808	-0.33	ns
	Wild	-0.029	0.027	2704	-1.06	ns
3× Herbicide	Hybrid	0.026	0.015	2852	1.77	†
	Wild	0.013	0.029	2781	0.45	ns
Competition	Hybrid	-0.101	0.018	2812	-5.50	***
	Wild	-0.127	0.020	2830	-6.34	***

We report regression coefficients for each treatment and cross type combination using data collected at the St Paul Experiment Station at the University of Minnesota, 2003.

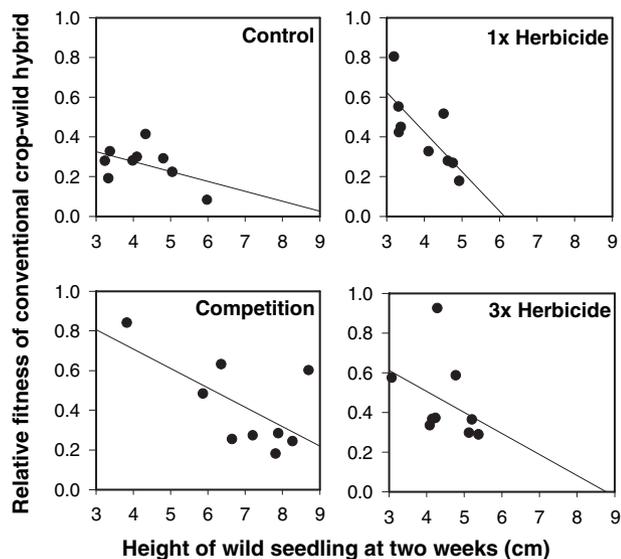
ns, not significant; \**P*-value < 0.05; \*\**P*-value < 0.01; \*\*\**P*-value < 0.001; †*P*-value < 0.10.

stressful environments (see review in Hails & Morley 2005; *Raphanus*: Klinger & Ellstrand 1994; Campbell *et al.* 2006; *Sorghum*: Arriola & Ellstrand 1997; *Oryza*: Song *et al.* 2004; *Cucurbita*: Fuchs *et al.* 2004). There are studies that have evaluated crop-wild fitness under stress, especially with competition (see review in Campbell & Snow 2007). Some greenhouse studies support our findings by showing higher relative growth of crop-wild *Brassica* hybrids with more intense competition (Lefol *et al.* 1995; Vacher *et al.* 2004). Recent work provides further support with results of greater relative fecundity of crop-wild *Raphanus* hybrids under field competition (Campbell & Snow 2007) and in novel field environments (Campbell *et al.* 2006).

Our estimates of relative fitness of sunflower crop-wild hybrids ranged from 0.1 to 1.1 across genetic backgrounds and environments. These estimates are qualitatively similar to other estimates on sunflower which range from 0.15 to 0.80 (Snow *et al.* 1998; our calculations), but much greater than the range of 0.011–0.024 calculated by Cummings *et al.* (2002). Because similar strategies were used to estimate

fecundity in all three studies, it is not easy to understand the discrepancy. The assessment of herbivory in Cummings *et al.* (2002) does not account for the differences. The discrepancy could be primarily due to effects of the different germplasm and field locations and may reflect further effects of G × E interactions on relative fitness values.

Our use of a novel trait (i.e. herbicide resistance) and a selection pressure (i.e. herbicide application) that would be expected to select strongly on the trait allows us to explore the fitness costs and benefits of these traits. The SU-R trait was expected to contribute to higher relative fitness of the SU-R hybrids under the stress of SU herbicide application at the 1× and 3× rates. However, its contribution was not large; there were few cases where the relative fitness of the SU-R hybrids with herbicide was greater than that of the conventional hybrid (Fig. 4). None of the cases with relative hybrid fitness ≈ 1 involved the SU-R trait and the pattern of increased relative fitness under herbicide application was not limited to the SU-R hybrids (Fig. 4). The SU-R trait may have had a limited effect because the crop-wild hybrid



**Figure 5** The relationship between wild seedling height at 2 weeks and the relative fitness of the conventional hybrid for each population. Each symbol represents data from one of the nine wild populations in the control, competition, 1× herbicide, and 3× herbicide treatments. Regression coefficients for each treatment are: control  $\beta = -0.050$ ,  $P$ -value = 0.1799; competition  $\beta = -0.098$ ,  $P$ -value = 0.0627, 1× herbicide  $\beta = -0.199$ ,  $P$ -value = 0.0195; and 3× herbicide  $\beta = -0.106$ ,  $P$ -value = 0.3264.

plants were heterozygous for resistance locus and conventional crop–wild sunflower hybrids apparently possess a degree of tolerance to SU herbicide damage.

Alternatively, there may have been a slight cost of resistance for the SU herbicide tolerant hybrids. The relative fitness of the SU-R hybrids tended to be lower than that of the conventional hybrids under control and competitive conditions (Fig. 3). The pattern for the Imi-R hybrids was reversed; Imi-R hybrids exhibited greater relative fitness than conventional hybrids under control and competition treatments (Fig. 3). However, this pattern is not consistent across populations (Fig. 4). From the literature, we did not expect a fitness cost of ALS-R (Tranel & Wright 2002; Massinga *et al.* 2005; but see Bergelson & Purrington 1996). We suspect that the majority of the effects of crop line on relative fitness resulted from differences at loci other than those involved in herbicide resistance since the three lines we used were not isogenic (Mercer *et al.* 2006b).

#### 'Domestication' traits can promote introgression

Increased relative hybrid fitness under stress appears to result, in part, from the inheritance of certain 'domestication' traits from the crop. Larger seedling size may benefit crop–wild hybrids (Fig. 1b), especially since both herbicide and competition are known to be tolerated better by larger

seedlings (Weiner 1985; Hassan *et al.* 2002). Under competition, hybrid fecundity increased with faster early growth and with earlier flowering (Table 1). Yet whether these 'domestication' traits ultimately introgress into wild populations through a process of adaptive introgression (e.g. Rieseberg & Wendel 1993; Whitney *et al.* 2006) depends on whether they enhance fitness in wild genetic backgrounds. Our data show that they often do. Since the fecundity of wild plants increased with both faster early growth and earlier flowering, we expect these crop traits to introgress into wild populations with little resistance. In other words, the more crop-like values of these traits improve wild fitness, so crop alleles at loci governing these traits should be selected for. However, the polygenic nature of some traits and the potential physical linkage of these traits to other crop alleles with negative effects on fitness could hinder their ultimate introgression. Thus, whereas crop domestication traits are expected to reduce fitness in wild populations (Stewart *et al.* 2003; Hails & Morley 2005), we have shown that some crop-like characteristics enhance wild fitness and could rapidly introgress into wild populations – in some genetic backgrounds and especially under stressful environments. These traits could promote subsequent introgression of other crop alleles via hitchhiking.

Other investigators have found crop traits that are likely to be maintained in crop–wild hybrid zones. Hedge *et al.* (2006) studied *Raphanus* (wild radish) in California and found that wild populations have been completely displaced by invasive crop–wild hybrid lineages. Traits from both crop and wild parents were maintained, while other traits show transgressive segregation (Hedge *et al.* 2006). Campbell & Snow (2007), using path analysis with *Raphanus*, showed that both age and size at flowering affect ultimate levels of fecundity in wild and crop–wild hybrids. As in our study, Campbell & Snow (2007) found that the more hybrid-like phenotype of larger size had a positive effect on fecundity in wild backgrounds, indicating that it would be selected for in crop–wild radish hybrid zones. However, contrary to what we have observed, these authors also found the hybrid-like phenotype of later flowering was not expected to be selected for, due to its negative effect on seed production (Campbell & Snow 2007). Further phenotypic and molecular work with other crop–wild complexes will clarify the circumstances under which crop alleles are most likely to be maintained in wild populations.

These same 'domestication' traits may also play a role in some of the variation we found in relative hybrid fitness among wild populations. The negative relationship between wild seedling size and hybrid relative fitness (Fig. 5) demonstrated that wild populations with smaller seedlings were more likely to have hybrids with high relative fitness. Therefore, relative hybrid fitness may relate, in part, to already existing characteristics of the wild population. This could be

especially important under stressful conditions. For example, in our study, wild populations having relatively larger seedlings may better compete with wheat plants or could have developed the lateral meristems through which they could compensate for herbicide damage. Therefore, these wilds would do relatively better than their smaller counterparts when compared with hybrids with the same advantages. Similarly, we can imagine that existing variability among wild populations for insect resistance could result in an equally variable relative fitness of crop–wild hybrids carrying an insect resistance transgene. In sum, these results suggest that similarities between the phenotypes of the hybridizing wild population and crop variety may reduce the ecological effect (positive or negative) of crop–wild gene flow.

We do not claim that relative fitness of all crop–wild hybrids increases under all stresses. Rather, we propose that under certain environmental stresses certain crop ‘domestication’ traits will enhance fitness and thereby promote introgression. Importantly, not all ‘domestication’ traits can be assumed to be detrimental to fitness in wild populations. For sunflower, fitness increased with large seedling size, fast seedling growth rate, and early flowering under herbicide and competitive stress. The effects of these same traits may differ under other environmental stresses, such as cold stress or herbivory. But other characteristics may become important, such as apical dominance or lack of seed dormancy, and different traits would be expected to be important in different species. For example, the relative survival of crop–wild carrot hybrids did not increase with cold stress (Hauser 2002), presumably because there was no particular crop trait that was beneficial under those conditions. We expect that future work will confirm that the response of relative crop–wild hybrid fitness to a given stress will depend on the tendency of crop traits to confer tolerance or susceptibility to the stress, the gene action of the crop alleles involved, and the distribution of these traits in the recipient wild populations.

### **G × E interactions alter early crop allele introgression**

The genetic and environmental impacts of relative hybrid fitness found here suggest that we should expect initial crop allele frequencies to be substantial in some hybridizing wild sunflower populations, especially under stressful conditions. Thus, the initial phases of introgression could vary radically in different populations growing under diverse conditions. This variability increases if we also consider how variable levels of gene flow into wild populations across the landscape can affect trajectories of introgression (Mercer *et al.* 2006b). In particular, populations closer to crop fields will experience more gene flow from crop fields (Arias & Rieseberg 1994; Whitton *et al.* 1997; Linder *et al.* 1998). Optimally, the prediction of ultimate levels of gene introgression would

require additional information, such as backcrossing rates to the wild population, fitness of future generations, subsequent hybridization rates, the number of genes influencing traits, the effect of individual alleles on fitness, and the degree of assortative mating between genotypic classes (Arnold 1997; Chapman & Burke 2006). Certain crop-conferred traits, such as early flowering, may directly affect assortative mating, altering levels of introgression for some traits. Nevertheless, our findings indicate that the genomic contribution of cultivated sunflower to wild populations could be substantial in the generation following a single bout of hybridization. Since we found that fitness of hybrids grown under stressful conditions matched or exceeded that of wilds (i.e. relative fitness = 1) in 6% of cases, we predict that one of every 20 hybridizing populations growing in stressful environments in or alongside agricultural fields could be expected to have a high frequency of crop alleles. This variability highlights the challenge of predicting the potential for crop allele introgression. Recurrent hybridization and small wild population size could further exacerbate introgression (Goodell *et al.* 1997; Haygood *et al.* 2003), whereas other ecological considerations such as large wild seed banks (Alexander & Schrag 2003; Reagon 2006), or higher predation on hybrid seeds (Alexander *et al.* 2001; Cummings & Alexander 2002), could reduce rates of crop gene introgression. Nevertheless, none of these considerations should change our expectation of considerable variability among crop gene introgression estimates.

We advocate consideration of three aspects of experimental design for future studies of crop–wild hybrid relative fitness. First, these results demonstrate the value of using a representatively wide array of genetic backgrounds and conditions to reveal genetic variability in hybrid fitness among wild populations and crop lines and its interaction with environment. Second, it is important that fitness be assessed comprehensively. Here, we have accounted for differences in survival and reproduction. Ideally, evaluation of life-time fitness encompasses the entire life-history, including dormancy, germination, survival and fecundity (Hails & Morley 2005; Hooftman *et al.* 2005; Mercer *et al.* 2006a,b). Finally, most studies have focused on evaluating relative hybrid fitness in agricultural settings, since crop–wild hybrids may most often be found there. However, it will be worthwhile for future research to evaluate relative fitness in conditions typical of natural areas near agricultural fields in order to best estimate the ecological impacts of crop gene introgression in other environments (Hails & Morley 2005).

In conclusion, variability in relative hybrid fitness is considerable, and hybrid fitness can equal or exceed that of wild plants in certain environments and genetic backgrounds. Some ‘domestication’ traits can even facilitate the introgression of crop alleles into wild populations. Realistic

evaluation of rates of crop gene introgression will depend on further biosafety studies that examine the genetic and environmental influences on hybrid fitness. Current studies assessing fitness of hybrids in one genetic background or solely in a single, benign environment may underestimate true rates of introgression. Studies that identify environments, phenotypic traits and genetic backgrounds conferring the highest relative hybrid fitnesses will have the greatest value in identifying where the likelihood of crop gene or transgene introgression will be highest. Such research would improve prevention and monitoring of transgene introgression into wild populations.

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