

## EFFECTS OF COMPETITION ON THE FITNESS OF WILD AND CROP-WILD HYBRID SUNFLOWER FROM A DIVERSITY OF WILD POPULATIONS AND CROP LINES

KRISTIN L. MERCER,<sup>1,2,3</sup> DONALD L. WYSE,<sup>4,5</sup> AND RUTH G. SHAW<sup>3,6,7</sup>

<sup>1</sup>Applied Plant Sciences Program, University of Minnesota, St. Paul, Minnesota 55108

<sup>3</sup>Minnesota Center for Community Genetics, University of Minnesota, St. Paul, Minnesota 55108

<sup>4</sup>Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108

<sup>5</sup>E-mail: wysex001@umn.edu

<sup>6</sup>Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, Minnesota 55108

<sup>7</sup>E-mail: rshaw@superb.ecology.umn.edu

**Abstract.**—Gene flow between crop fields and wild populations often results in hybrids with reduced fitness compared to their wild counterparts due to characteristics imparted by the crop genome. But the specifics of the evolutionary outcome of crop-wild gene flow may depend on context, varying due to local environmental conditions and genetic variation within and among wild populations and among crop lines. To evaluate context-dependence of fitness of F<sub>1</sub> hybrids, sunflower crop lines were crossed with nine wild populations from across the northern United States. These crop-wild hybrids and their wild counterparts were grown under agricultural conditions in the field with and without wheat competition. Hybrids were far less fecund than wild plants, yet more likely to survive to reproduce. There was considerable variability among wild populations for fecundity and the specific crop line used to generate the crop-wild hybrid significantly affected fecundity. The fitness deficit suffered by crop-wild hybrids varied by population, as did the rankings of the crop-wild hybrids from three different crop lines. Wheat competition decreased fecundity and survival considerably and hampered seed production of wild plants more than that of hybrids. Genotype × environment interactions indicated that the response of fitness to competition differed by population. Consequently, the fitness of hybrids relative to wild plants varied considerably among wild populations and was not consistent across environments. Notably, relative fitness of hybrids was greater under competitive conditions. This research is the first study of its kind to demonstrate that the consequences of crop-wild gene flow are context dependent and contingent on the genetics of the specific wild populations and the local biotic and abiotic conditions.

**Key words.**—Competition, context-dependent fitness, crop-wild hybrids, gene flow, genotype × environment interactions, introgression, relative fitness.

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Gene flow is one of the major evolutionary processes shaping plant populations (Arnold 1997; Hartl and Clark 1997; Arnold et al. 1999; Barton 2001). Whether migration occurs via propagule movement or pollen flow, the result is the introduction of new alleles and changes in the frequencies of resident alleles. Inter- and intraspecific hybridization has been shown to promote the evolution of invasiveness in plant populations (Ellstrand and Shierenbeck 2000). With the advent of transgenic crops, gene flow between crop fields and wild populations has emerged as a pressing concern, because transgenes could exacerbate the invasive potential of hybrids (Kareiva et al. 1996; Snow and Morán-Palma 1997; Hails 2000; Ellstrand 2003).

Study of the initial crop-wild gene flow event, in particular the frequency and levels of gene flow (Ellstrand 2003, table 4.1), has led to a better understanding of gene flow risk. Moreover, the study of gene flow between crop and wild plants can serve as a model for hybridization between differentially adapted populations or subspecies. In many cases, crops and their wild relatives are interfertile and can contribute to each other's gene pool (Ellstrand et al. 1999). The ultimate fate of crop alleles that migrate into wild populations depends on their fitness effects (Ellstrand 2003). If the novel alleles decrease fitness in wild conditions, as presumed for alleles that confer trait values favored in the domestication

process (Stewart et al. 2003), their frequency is expected to decline with time, resulting in the loss of crop alleles from the population. Alternatively, if the novel alleles increase fitness, as some transgenes are expected to (Kareiva et al. 1996; Snow and Morán-Palma 1997; Hails 2000), then we would expect the frequency of the novel alleles to increase or introgress into the population as adaptive evolution proceeds. Crop traits that are pleiotropically or physically linked to alleles with strong fitness effects would presumably dissipate or introgress accordingly (Cummings et al. 2002; Stewart et al. 2003).

The F<sub>1</sub> crop-wild hybrids from *Helianthus* (Snow et al. 1998; Cummings et al. 2002), *Raphanus* (Snow et al. 2001), *Oryza* (Oard et al. 2000; Song et al. 2004), and *Cucurbita* (Fuchs and Gonsalves 1997; Spencer and Snow 2001) have lower fecundity than their wild counterparts, whereas that of *Brassica* was equivalent or higher (Hauser et al. 1998; Snow et al. 1999; but see Lefol et al. 1995). Overall, then, the potential for crop-gene or transgene introgression may be lower in some plants than others. Nevertheless, in all cases, hybrids were fertile and produced a substantial number of seeds. The fitness of F<sub>1</sub> hybrids does not determine ultimate introgression of all crop genes, but it affects the initial course of introgression through its effect on frequencies of crop alleles that are subsequently subject to selection and drift.

Specific models of hybrid zone evolution focus on conditions affecting the fitness of hybrid and parental types (for reviews see Arnold 1997; and Johnston et al. 2001). Whereas the tension zone model (Barton and Hewitt 1985) assumes

<sup>2</sup> Present address: Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, Ohio 43210; E-mail: mercer.97@osu.edu.

that hybrid inferiority is constant and results from genetic incompatibilities, other models recognize that the environment (mosaic: Harrison 1986; bounded hybrid superiority: Moore 1977) in combination with genotype (evolutionary novelty: Arnold 1997) can influence fitness differences between hybrids and parentals. These latter three models acknowledge the important role of genotype  $\times$  environment ( $G \times E$ ) interactions in the outcome of hybridization (Johnston et al. 2001): ‘‘Exogenous selection acts as a result of differential adaptation of hybrid and parental genotypes to the complex array of habitats found in natural populations. This selection acts positively or negatively depending on the availability of an appropriate environment for the various hybrid and parental genotypes’’ (Arnold 1997, p. 151).

Hence, in empirical studies of hybridization, it is important to take a comprehensive view of the genetic and environmental factors that could generate variability in  $F_1$  hybrid fitness and, therefore, influence subsequent selection for or against novel alleles in wild populations. Empirical studies of natural hybrid zones have shown that  $G \times E$  interactions can influence the evolution of hybrids between species (Hatfield and Schluter 1999; Wade et al. 1999; Campbell and Waser 2001; Johnston et al. 2001) and may be key to adaptive or ecological evolution of populations (Via and Lande 1985; Arnold 1997; Hatfield and Schluter 1999; Martinsen et al. 2001).

Similarly, the crop-wild hybrid literature provides a wealth of insights into factors that may influence the fitness of wild and crop-wild hybrids. First, the specific identity of the crop (Hauser et al. 1998; Snow et al. 1998; Oard et al. 2000) and wild genotypes (Hauser et al. 1998; Snow et al. 1998) can influence the fecundity of hybrids, because each has unique sets of alleles or characteristics. Second, specific genes of interest (e.g., transgenes) could provide a selective advantage to hybrids under appropriate conditions, but they could also impose a cost. Mixed results have emerged from studies of insect resistance (Mason et al. 2003; Snow et al. 2003; Vacher et al. 2004), disease resistance (Fuchs and Gonsalves 1997; Bartsch et al. 2001; Spencer and Snow 2001; Burke and Rieseberg 2003), and herbicide resistance (Snow et al. 1999; Oard et al. 2000; Massinga et al. 2003). In environments with high levels of insects, disease, or herbicide, genes deployed to resist these stresses have been shown to increase hybrid fitness above that of hybrids without the gene, and in some cases above that of the wild counterpart (Fuchs and Gonsalves 1997; Bartsch et al. 2001; Massinga et al. 2003; Vacher et al. 2004), but there are exceptions (Burke and Rieseberg 2003; Mason et al. 2003; Mercer 2005). No evidence for a fitness cost of herbicide resistance (Snow et al. 1999; Oard et al. 2000) or for *Bt* insect resistance (Mason et al. 2003; Snow et al. 2003) has been found in crop-wild hybrids, but Bartsch et al. (2001) found evidence of a cost for virus resistance.

In addition to genetic variability that could affect the evolutionary consequences of crop-wild hybridization, variation in environmental effects on life history and fitness is likely to play an important role. The abiotic and biotic environment, including interspecific interactions, can influence selection regimes in the wild (Wade and Kalisz 1990; Weis et al. 1992; Totland 2001; Etterson 2004; Halpern 2004) and, similarly,

environmental conditions may differentially affect survival and reproduction of wild and crop-wild hybrid plants. Responses to interspecific competition shifted relative biomass relationships of hybrids and wilds in *Brassica* (Lefol et al. 1995; Vacher et al. 2004). In crop-wild hybrid sunflower, disease susceptibility was lower (Snow et al. 1998), while predispersal (Cummings et al. 1999) and postdispersal seed predation (Alexander et al. 2001) were higher compared to those of wild sunflowers. Therefore, it is difficult to assess a priori whether hybrids or wild plants would suffer greater fitness reductions under these stresses. Finally, hybridization may alter life-history characteristics, such as germination and dormancy, in ways that influence seedbank dynamics (Mercer et al. 2006). Crop-wild hybridization in *Brassica* altered the responses of seeds to germination cues, which could decrease their fitness potential (Adler et al. 1993). Any of these factors and interactions among them could impinge on the fitness effects of crop alleles and influence their frequencies in wild populations over time.

Previous work by Snow et al. (1998), in which three wild populations and their crop-wild hybrids were grown in pots in Ohio and in the field in Kansas, suggested that environment and source population may be important influences on fitness. This raised the possibility that, as predicted by evolutionary novelty theory (Arnold 1997; Johnston et al. 2001),  $G \times E$  interactions could result in differential relative fitnesses of hybrids and wilds across crop-wild hybrid zones.

To broaden understanding of the potential evolutionary trajectories of crop-wild hybridizing populations, we evaluated the fitness of nine wild sunflower populations and their hybrids with three crop cultivars under controlled competition treatments in an agricultural field. We chose to subject the experimental plants to wheat competition as a relevant environment for two reasons. First, sunflower and wheat are often found together in crop rotations, making crop-wild sunflower hybrids potentially invasive in wheat fields. Second, wheat is a strong competitor; accordingly, we expect our evaluations of absolute invasiveness to be thus conservative. This investigation has implications for natural hybrid systems because it addresses the impact of genetic and environmental variability and  $G \times E$  interactions on the evolution of populations following hybridization between differentially adapted populations or subspecies.

In this study we investigated how genetic and environmental factors and their interactions influence fecundity, survival to reproduction, and relative fitness of hybrid and wild sunflower. In addition, we considered how this informs our understanding of the variability inherent in context-dependent fitness and the evolutionary consequences of crop-wild gene flow. We explored these issues using the most genetically diverse materials to date under relevant conditions. Furthermore, our design allowed us to rigorously and simultaneously test the effects of this diversity and the competitive environment.

## MATERIALS AND METHODS

The native range of *Helianthus annuus* extends throughout most of North America (Rogers et al. 1982). Molecular evidence suggests a primary domestication event in central

TABLE 1. Description of wild sunflower populations used in wild-wild and crop-wild crosses. Xs indicate hand-pollinations performed in 2002 and cross types used in the sunflower fitness experiment, St. Paul Experiment Station, University of Minnesota, 2003.

USDA PI number	Origin of wild populations				Paternal parent			
	State of origin	Abbreviation of population	Latitude (N)	Longitude(W)	Wild	Crop inbred lines		
						Conventional	Imidazolinone-resistant	Sulfonylurea-resistant
586808	North Dakota	ND	47°26'	99°20'	X	X	X	X
531021	Montana	MT	47°30'	115°15'	X	X	X	X
531018	Washington	WA	47°31'	120°29'	X	X	X	X
613745	Minnesota	MN	45°1'	93°18'	X	X	X	X
613721	South Dakota	SD	45°1'	99°57'	X	X	X	X
586822	Wyoming	WY I	44°56'	105°25'	X	X	X	X
597898	Iowa	IA	43°5'	96°11'	X	X	X	X
586836	Wyoming	WY II	43°3'	104°15'	X	X	X	X
531010	Idaho	ID	43°13'	112°20'	X	X	X	X

North America from eastern wild *H. annuus* populations (Harter et al. 2004); previous domestication may have occurred in Mexico (Lentz et al. 2001). Crop and wild annual sunflower are both *H. annuus*; the crop was domesticated to self-fertilize facultatively, germinate easily, and produce a single head with large seeds, all in contrast to native populations. Crop and wild *H. annuus* share insect pollinators, are sympatric, and overlap in flowering time (Burke et al. 2002). The yearly harvest of crop seed and the strongly asymmetrical abundances of crop and wild sunflowers in agricultural areas favor gene flow from crop fields into wild populations. Studies quantifying crop-wild gene flow have shown that hybridization is highest in populations along field margins (27–42% of seeds produced are hybrids), and that hybrid seed production declines with distance (to 2% at 1000 m; Arias and Rieseberg 1994; Whitton et al. 1997). Crop marker alleles persist in wild populations over time, indicating that the fitness decline noted in  $F_1$  hybrids is not a strict barrier to crop-gene introgression (Whitton et al. 1997; Linder et al. 1998; Cummings et al. 2002). Transgenic sunflower germplasm resistant to insects and disease has been tested (Burke and Rieseberg 2003; Snow et al. 2003), but not commercially planted; nontransgenic, herbicide-resistant sunflowers were grown commercially for the first time in 2003.

#### Germplasm and Crosses

To generate seeds as material for fitness comparisons between plants representing pure wild populations and plants representing crop-wild hybrids, we carried out crosses within each of nine wild populations and between each of these populations and three inbred crop lines (Table 1). The wild populations were chosen from the USDA collection of wild *H. annuus* (see Table 1; <http://www.ars-grin.gov/ars/MidWest/Ames/>), and originated from geographically distant locations within the United States between 43°N and 47.5°N latitude. Three crop lines (HA 89, HA 425, and an SU-B line) were provided by the USDA-ARS sunflower breeding program (Fargo, ND). All of the lines were conventionally bred, two with germplasm from a wild Kansas population found to have resistance to two classes of acetolactase synthase inhibiting (ALS) herbicides: imidazolinone (IMI) and sulfonylurea (SU) herbicides (Al-Khatib et al. 1998).

During the spring and summer of 2002, wild and crop

seedlings were transplanted into random spatial positions within crossing blocks in the greenhouse and field at the University of Minnesota (St. Paul, MN). Prior to hand-pollinations, flowerheads were protected from cross-pollination by  $10.2 \times 15.2$ -cm cotton or perforated plastic bags. Self-pollination was assumed to be negligible because wild *H. annuus* is sporophytically self-incompatible. For wild-wild crosses we used a cotton swab to transfer pollen from a known individual to receptive stigmas of another plant of the same population. For the crop-wild crosses, the crop parent always served as the pollen donor. Crop pollen was bulked among multiple individuals within each of the three lines. Resulting seeds were stored at room temperature.

#### Experiment Layout and Planting

The field experiment was designed as a split-plot with eight replications. To the whole plots within each block, we randomly assigned the four treatments: (1) wheat competition; (2) SU herbicide application (field rate); (3) SU herbicide application (three times the field rate); and (4) a no competition, no herbicide control. Here, we discuss only results from the control and competition treatments (for response to herbicide application, see Mercer 2005). Split-plots were randomly assigned to the 36 different cross types, that is, progeny from the wild-wild or crop-wild crosses (for cross types, see Table 1). Each split-plot was composed of four individuals representing a given cross type.

In May 2003, a seedbed was prepared on the St. Paul Experiment Station of the University of Minnesota with a mold board plow and a disk; trifluralin (0.75 kg/ha) was incorporated for pre-emergence weed control, and 56 kg/ha of nitrogen was applied in the form of ammonium nitrate. We planted the competition treatment as 10 15-cm spaced rows of the wheat variety *Triticum aestivum* L. Alsen, surrounding the row of sunflower planting positions. The wheat was planted 4-cm deep at a rate of 202 kg/ha (double the normal planting rate, due to wheat's sensitivity to trifluralin).

From the sunflower seeds produced in the crossing design described above, 15 to 20 maternal families were bulked together to represent each cross type. On May 8, seeds from each cross type were prepared for the two-week, 4°C cold stratification treatment by cleaning them in a 10% (v/v) bleach solution for 15 min and placing 25 seeds onto each

100-mm petri dish. From May 26 to 30, the seeds were moved into the growth chamber (12/12 h light/dark, 25°C/15°C) to germinate. On May 30 we began to plant germinated seeds with well-developed radicles into the field. Eight split-plots (32 plants) made up a row, and one or two germinated seeds were planted at each stake, which were set at 1.83-m spacing. Once planting was finished, remnant germinated seeds were planted into peat pellets in the greenhouse to be used later as replacement transplants.

In early June, we sprayed Asana insecticide (esfenvalerate) at a rate of 57 g/ha of active ingredient at 175 L/ha on the emerged seedlings to control a flea beetle population that was devouring emerged cotyledons. Replications 1 and 2 were most heavily affected due to earlier emergence. Plantings were hand-watered until mid-June to soften the soil's crust without damaging small seedlings. Two cohorts of transplants replaced seedlings that did not emerge or had died. A total of 263 transplants were used of 4544 positions (6%). Seedlings were thinned to one per position. One June rainfall event of 15 cm damaged, uprooted, or buried many plants in replication 2. Thus, replication 2 was removed from the analysis. Weed control was attained by cultivation in the control treatment plots and continuous hand-weeding in the wheat competition plots.

#### *Cohorts and Cotyledon Damage*

Due to the uneven development of the germinated seeds planted and the use of transplants, we classified each individual in the experiment as a member of one of six cohorts. The first four were classified by the date by which the original seedling had fully expanded its cotyledons, and the final two corresponded to the date of transplant. In mid-June, we noted the presence of damage to the cotyledons by flea beetles. Cotyledon damage and plant cohort were later used as categorical factors in the analyses.

#### *Estimation of Fecundity per Plant*

The plant response of primary interest in this experiment was individual fitness. Because it was not feasible to count the seeds produced by each plant, we estimated total fecundity per plant by estimating the number of seeds produced per head, counting the number of heads produced, and calculating their product (see Appendix 1 for complete description). Seeds per head were estimated separately for the primary, one secondary, and one tertiary head per plant using area of seed head as a predictor. Different regressions of the number of seeds per head on head area were obtained for hybrids and wilds due to differences in seed size (see Appendix 2). The numbers of primary, secondary, tertiary, and deformed heads per plant were counted separately from September 11 to October 17, 2003. The sum of the estimated number of seeds produced by all pollinated primary, secondary, tertiary, and deformed heads yielded the total fecundity of the plant.

#### *Statistical Analysis*

Using PROC MIXED in SAS, Version 8.2 for Windows (SAS Institute 2001), we employed ANOVA to analyze prob-

ability of survival to reproduction and fecundity. Restricted maximum likelihood methods were used to assess the main effects and the relevant interactions. We elected PROC MIXED because it correctly estimates the errors for split-plot designs (Littell et al. 2002, p. 136), and employed two models. Model A explored the effect of competition treatment, wild population, hybridization, and their two- and three-way interactions on fitness parameters. All wild and hybrid plants were used in this analysis, and the three hybrid types were analyzed jointly. Model B was performed on a subset of the data: only the hybrids. In this analysis, we investigated the effects of competition treatment, wild population, crop line, and their two- and three-way interactions on fitness measures. Cotyledon damage and cohort were included as factors in both analyses, and degrees of freedom were approximated using the Satterwaithe option in PROC MIXED.

Both models were used to analyze fecundity and the probability of survival to reproduction. The fecundity analysis included only plants that survived to reproduction; results should thus be interpreted as influences on fecundity, given that a plant survives to reproduce. Fecundity was transformed to its fourth root to improve the data's fit to the assumption of normality. Survival to reproduction was analyzed as a continuous response based on averages of plants in each split-plot. Categorical analyses do not allow for the complex model specifications needed in an analysis of a split-plot design. Therefore, data were arcsine-square-root transformed and analyzed with PROC MIXED. Due to the small number of plants per split-plot ( $\leq 4$ ), the proportion of plants that survived to reproduce was calculated as (number of plants within split-plot that survived to reproduce + [3/8]) / (number of plants in the split-plot + [3/4]) (Kuehl 1994, pp. 486-487). Although this transformation reduced bias, it limited the range of backtransformed values to 0.08–0.92. For all analyses, least squares means were estimated using SAS.

To calculate fitness of hybrids relative to wild plants, the mean fitness of hybrids and wild plants was estimated for each population, treatment, and replication combination and their ratios were calculated as relative fitness = hybrid fitness/wild fitness. Unlike the previous analyses of fitness components, which were performed separately for fecundity and survival data, the estimates of relative fitness used untransformed fecundity data and included plants that did not reproduce (i.e., had a fecundity of zero). Thus, survival was integrated into the estimate of lifetime fitness. Least squares means of relative fitness were estimated using PROC MIXED.

## RESULTS

### *Survival to Reproduction*

Survival varied significantly among wild populations (Table 2). Survival reached 0.90 for two populations (ND, WA) and was limited to 0.83 for two others (IA and ID). Unexpectedly, hybrid plants were generally more likely to survive than wilds (Table 3). The difference between the survival of the hybrids and that of the wild plants varied significantly by population (Tables 2, 4). Hybrid survival varied from 0.86 to 0.92; wild survival varied more broadly, from 0.78 to 0.90

TABLE 2. ANOVAs of survival to reproduction and fecundity in sunflowers performed using maximum likelihood in SAS PROC MIXED. For model A, wild and hybrid plants were analyzed, while the model B analyses only included hybrid plants from the St. Paul Experiment Station, University of Minnesota, 2003.

Source	arcsin(Survival) <sup>1/2</sup>				(Fecundity) <sup>1/4</sup>			
	Num df	Den df	F	Significance <sup>4</sup>	Num df	Den df	F	Significance <sup>4</sup>
Model A <sup>1,2,3</sup>								
Competition (Comp)	1	10	30.85	***	1	7	439.45	****
Wild population (Pop)	8	454	4.05	****	8	469	6.09	****
Hybridization (Hybrid)	1	454	24.49	****	1	477	749.71	****
Comp × Pop	8	454	0.85	ns	8	463	2.68	**
Comp × Hybrid	1	454	2.87	†	1	466	76.82	****
Pop × Hybrid	8	454	3.81	***	8	468	2.58	**
Comp × Pop × Hybrid	8	454	1.58	ns	8	464	1.56	ns
Model B <sup>1,2,3</sup>								
Comp	1	318	30.9	***	1	11	275.67	****
Pop	8	318	0.97	ns	8	304	2.67	**
Crop line (Crop)	2	318	1.32	ns	2	308	8.31	****
Comp × Pop	8	318	1.81	†	8	303	1.42	ns
Comp × Crop	2	318	0.11	ns	2	304	0.49	ns
Pop × Crop	16	318	1.12	ns	16	302	3.49	****
Comp × Pop × Crop	16	318	1.22	ns	16	302	1.54	†

<sup>1</sup> The whole-plot factor, competition, was tested with the rep × comp error term, except in model B for survival. In that case, the component of variance for rep × comp was estimated as zero, and therefore the residual mean square became the denominator for the test.

<sup>2</sup> Split-plot effects were tested with the split-plot error rep × pop × hybrid × split-plot(rep × comp) for model A, and rep × comp × pop × crop for model B.

<sup>3</sup> Cohort and cotyledon damage were used as factors in the analysis (data not shown).

<sup>4</sup> †  $P < 0.10$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$

(Table 4). Wheat competition significantly decreased the probability of survival to reproduction from 0.90 to 0.84 (Tables 2, 3). The survival of the wild plants declined more severely with wheat competition than did that of the hybrids, but this difference was only marginally significant (Table 2). The difference between hybrid and wild survival varied more with than without competition. In competition with wheat, the survival of the wild plants from two populations (IA, ID) dropped below 0.70, while survival in their hybrids remained above 0.85 (data not shown). By contrast, in two populations (ND, MN), wild survival was greater than hybrid survival.

#### Fecundity: Effects of Genetic Factors and Their Interactions

There was significant variability among wild populations for fecundity (Table 2; Fig. 1a). In all cases, the wild for a given population produced more seed than the hybrids (Fig. 1b), six times more, on average (Table 3). But the degree to which a given population's hybrid deviated from the fecun-

dity of the wild differed by population (Table 2). This appeared to be driven by differences in fecundity among the wild plants (Fig. 1b), because as a group the fecundity of hybrids was more uniform than that of the wild plants. The highest wild fecundity was 28,300 seeds (IA), whereas the highest hybrid fecundity was 3500 seeds (WA). The fewest seeds produced by a wild population was 9700 (ID) and for hybrids, 2200 seeds (MN).

Apart from highly significant effects of hybridization and wild population on fecundity as noted in analysis of model A, crop line also significantly affected fecundity (Table 2, model B). On average, the hybrids produced from the SU-R crop line had the lowest fecundity (2600 seeds), followed by the hybrids from the conventional crop line (2800 seeds). The crop-wild hybrids produced by the IMI-R crop line tended to have the highest fecundity (3500 seeds). This pattern was not consistent over the wild populations, indicated by the highly significant interaction between population and crop

TABLE 3. Back-transformed least squares means and 95% confidence limits (CL) for probability of survival to reproduction and fecundity for crop-wild hybrid and wild sunflowers with and without competition. Overall means and CL for the two competition levels and for hybrids and wilds are included. Data were collected at the St. Paul Experiment Station, University of Minnesota, 2003.

	Without competition		With competition		Overall	
	Mean	95% CL	Mean	95% CL	Mean	95% CL
Survival to reproduction						
Wild	0.89	0.86, 0.91	0.80	0.77, 0.84	0.85	0.82, 0.87
Hybrid	0.91	0.89, 0.93	0.87	0.85, 0.90	0.90	0.87, 0.91
Overall	0.90	0.88, 0.92	0.84	0.81, 0.87		
Fecundity						
Wild	44,778	37,729, 52,771	4276	3123, 5721	16,401	13,585, 19,633
Hybrid	7205	5642, 9073	801	517, 1188	2788	2106, 3625
Overall	19,919	16,511, 23,829	2019	1438, 2761		

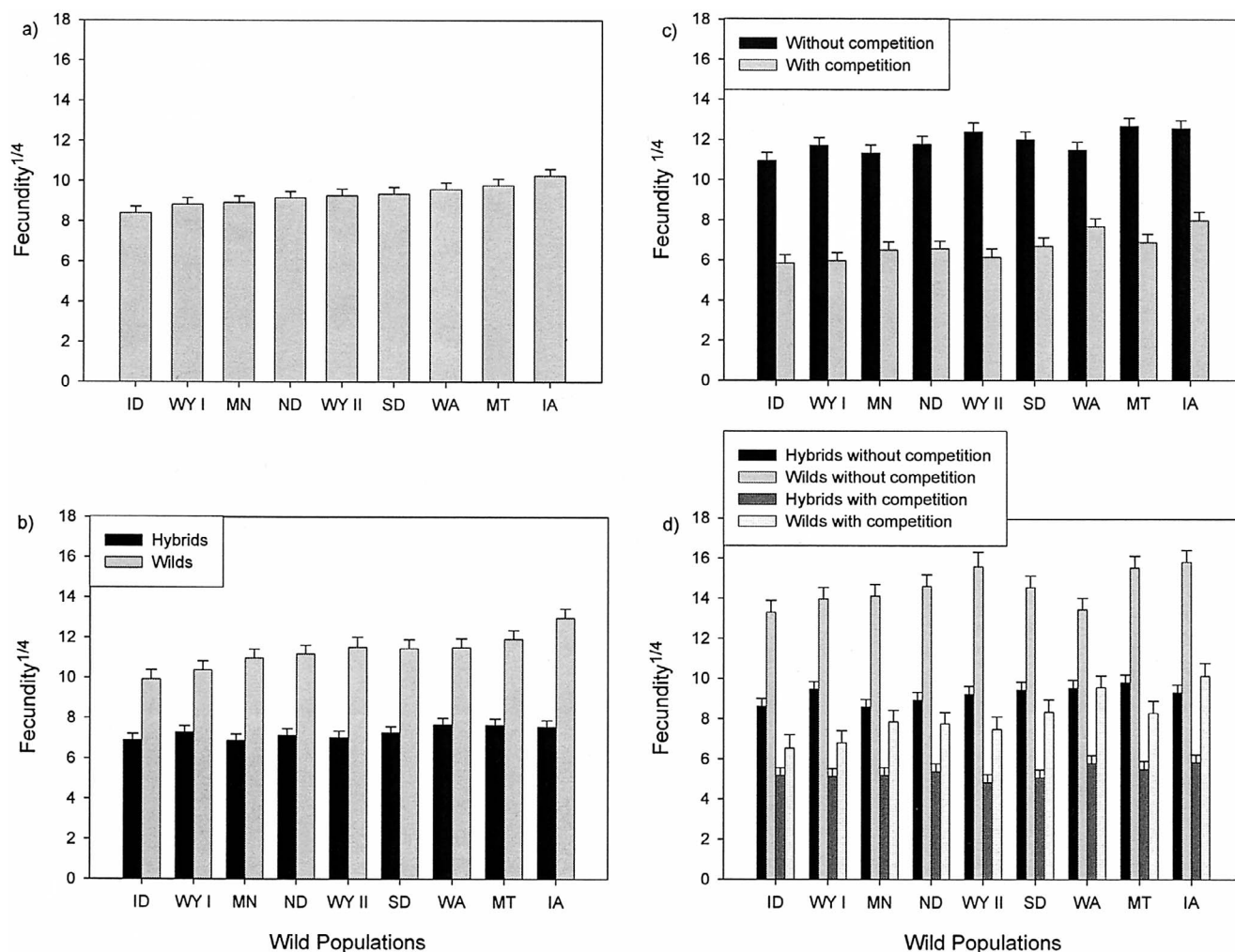


FIG. 1. Least squares means of fourth-root-transformed sunflower fecundity for (a) each wild population, (b) for the wild plants and hybrids from each population, (c) for the plants with and without competition in each population, and (d) for hybrids and wild plants with and without competition from each population grown at the St. Paul Experiment Station at the University of Minnesota, 2003. Bars are standard errors. Abbreviations for wild populations correspond to states of origin (see Table 1 for details).

line (Table 2). For instance, in three populations, the IMI-R line produced plants of higher fecundity than the other lines; in one it produced the least fecund hybrid. Although the rank of the three hybrids across the populations changed, in most cases their fecundities did not differ considerably (Fig. 2). By performing analyses on subsets of data, we found the interaction of population and crop line was highly significant without competition, but nonsignificant with competition ( $P < 0.0001$  and  $P = 0.5983$ , respectively). Thus, differences in fecundity among hybrids with the three crops varied across wild populations under noncompetitive conditions.

*Fecundity: Effects of Competition and Genotype × Environment Interactions*

The wheat competition decreased fecundity severely (Tables 2, 3). A greater reduction in wild than in hybrid fecundity in the presence of wheat contributed to a significant interaction of competition and hybridization (Table 2). The fecundity of plants without competition exceeded that of plants growing with wheat by a factor of 10.5 and 9, for wilds and

hybrids, respectively (Table 3). Competition with wheat increased the similarity in fitness between the hybrids and wilds.

Populations responded differentially to the competition (Fig. 1c), as evidenced by the significant interaction between population and competition (Table 2). Across wild populations there were differences in the reduction in fecundity with competition. Some populations were sensitive to competition, while others maintained their fecundity. Hybrid plants without competition were typically more fecund than wild plants with competition. The exceptions were populations in which the wild plants maintained high fecundity under competition (Fig. 1d).

*Relative Fitness: Effects of Competition, Wild Population, and Their Interaction*

The fitness of hybrids relative to wilds varied considerably. Without competition, the relative fitness of hybrids and wild plants varied from a low of 0.20 (ND) to a high of 0.39 (WA). With competition, the relative fitness varied from 0.21

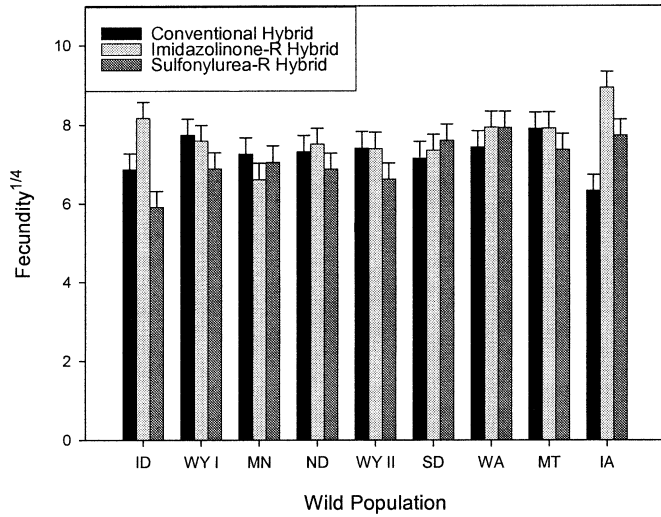


FIG. 2. Least squares means of fourth-root-transformed fecundity of sunflower crop-wild hybrids of different crop origin from each of nine populations grown at the St. Paul Experiment Station at the University of Minnesota, 2003. Bars are standard errors. Abbreviations for wild populations correspond to states of origin.

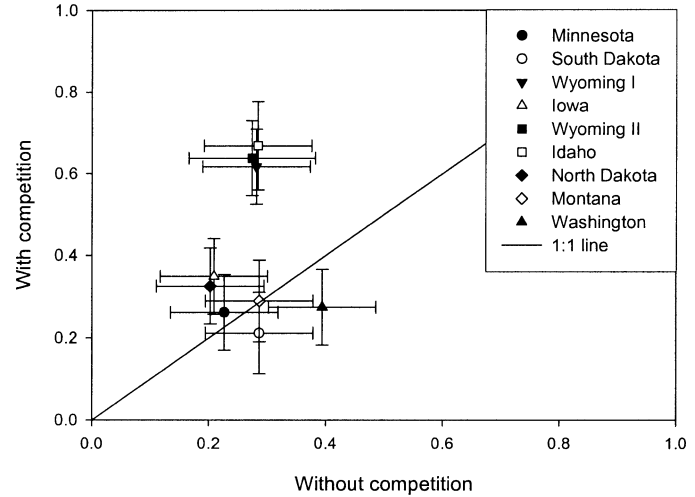


FIG. 3. Least squares means of relative fitness of hybrids and wild plants by population and competition treatment. Fitness was calculated as fecundity and included plants that produced no seeds. Relative fitnesses were calculated for each replication, competition treatment, and wild population combination from plants grown at the St. Paul Experiment Station at the University of Minnesota, 2003. Bars are standard errors.

(SD) to 0.67 (ID). For most populations, the fitness of hybrids relative to wild plants differed among environments (Fig. 3).

DISCUSSION

In assessing evolutionary consequences of crop-wild gene flow, we evaluated how the fitness of hybrids between crop and wild populations of sunflower varied over the genetic diversity that exists on the landscape and also over environmental conditions that realistically represent those at interfaces between crop and wild populations. We documented considerable variation in fecundity and survival among the nine wild populations and among their hybrids with three different crop lines. Hybrid fecundity was lower than that of wild plants, whereas hybrid survival exceeded that of wild plants. Moreover, our experiment revealed interactions between parental genotypes, for example, between crop and wild parents, as well as G × E interactions. All these findings evidence context-dependent selection in crop-wild hybrid zones, such that rates of crop gene introgression may vary considerably.

General Effects of Hybridization

As in previous studies on crop-wild hybrids in sunflowers, the hybrids in our study had considerably lower fecundity than the wild plants, particularly in the absence of competition (Snow et al. 1998; Cummings et al. 2002). This low hybrid seed production appears to be due to determinate flowering, decreased branching, and reduced production of heads—all notable crop characteristics. Nevertheless, hybrids show greater survival to reproduction than the wild plants, possibly an advantage also inherited from the crop. The hybrids produced by the different populations appear more uniform in fecundity than their wild counterparts (though there is significant variation among populations in hybrid fecundity; Table 2, model B, population effect). Our wild-wild crosses, performed within the wild accessions, exhibited genetic differentiation among populations. By contrast, the hybrids were produced from genetically divergent wild populations crossed with three distinct crop lines (each inbred line

TABLE 4. Back-transformed least squares means and 95% confidence limits (CL) of probability of survival to reproduction for wilds and crop-wild sunflower hybrids across populations. Data were collected at the St. Paul Experiment Station, University of Minnesota, 2003.

Maternal population	Overall		Hybrid		Wild	
	mean	95% CL	mean	95% CL	mean	95% CL
Iowa	0.83	0.79, 0.86	0.89	0.86, 0.92	0.76	0.69, 0.82
Idaho	0.83	0.79, 0.87	0.90	0.87, 0.93	0.75	0.68, 0.81
Wyoming II	0.84	0.80, 0.88	0.89	0.85, 0.92	0.79	0.72, 0.85
Wyoming I	0.88	0.84, 0.91	0.90	0.87, 0.93	0.85	0.79, 0.90
Montana	0.89	0.85, 0.91	0.91	0.88, 0.94	0.86	0.80, 0.91
Minnesota	0.89	0.85, 0.91	0.87	0.84, 0.90	0.90	0.84, 0.94
South Dakota	0.89	0.85, 0.92	0.89	0.86, 0.92	0.88	0.82, 0.92
Washington	0.90	0.87, 0.93	0.90	0.87, 0.93	0.90	0.85, 0.94
North Dakota	0.90	0.87, 0.93	0.90	0.87, 0.93	0.91	0.86, 0.95
Across populations			0.90	0.87, 0.91	0.85	0.82, 0.87

genetically uniform), such that hybrids sired by a given crop line could be loosely regarded as paternal half-sibs.

#### *Interactions among Genetic Factors*

This is the first study of crop-wild hybrid fitness in sunflower where the distinct effects of diverse wild populations and crop line and their interactions could be revealed through robust joint analyses. Genetic variation among our wild populations evidently responded to or interacted with the crop genomes in a nonuniform manner, resulting in fecundity differences among hybrids produced from different crop and wild genetic combinations.

These findings may reflect interactions between alleles that vary among the wild populations, on the one hand, and among the crop parents, on the other. Wild populations may have differentiated via adaptation to diverse environments or by genetic drift, such that the introduction of particular crop alleles into these differentiated populations may have distinct consequences. Our crop lines differed as well. Two lines possessed herbicide-resistance genes, which can impose fitness costs (Williams et al. 1995; Bergelson and Purrington 1996; Baucom and Mauricio 2004; but see Marshall et al. 2001; Tranel and Wright 2002; Park et al. 2004). Thus, when herbicide is not applied, we might expect that SU-R and IMI-R hybrids would have lower fitness than the conventional hybrids. We found that the SU-R hybrids had the lowest fecundity overall, but IMI-R hybrids had the highest. These fitness differences could result from the different resistance alleles themselves or to their genetic backgrounds. Our estimates of the magnitude of differences among crop lines in their effects on progeny fitness are likely conservative because our three lines were closely related (Mercer 2005) and thus represent a small proportion of the available crop diversity.

One other study has rigorously investigated the importance of the specific crop and wild parents in determining hybrid fitness. Hauser et al. (1998) demonstrated genetic variation in the number of seeds per pod across progeny from crosses between three *Brassica napus* varieties and three wild *B. rapa* populations and that the individual crop plants used within a crop variety affected overall fitness. But they found no significant interaction between crop parent and wild population for any fitness component. In three other studies, multiple crop lines and multiple wild populations were used to explore fitness of wild and crop-wild hybrids (Snow et al. 1998; Oard et al. 2000; Mason et al. 2003), although in each case the experimental design precluded a formal assessment of the interaction among these factors. In sunflower, variability among wild populations in the differences between hybrid and wild seed production and flowering time was suggestive of genetic interactions (Snow et al. 1998), whereas Mason et al. (2003) found little or no variability in reproduction among three *B. rapa* populations and their *Bt* backcross hybrid counterparts.

#### *Genotype $\times$ Environment Interactions*

One salient result of this study was our finding of strong  $G \times E$  interactions. The interaction between hybridization and competition clearly indicated that wild fecundity declined

more severely with competition than did that of the hybrids. Less straightforward were the interactions between population and competition, which resulted in the changes of population rank with competition. For example, the population with the third lowest fecundity in the control plots but the second highest in the wheat (WA) appeared to be relatively insensitive to the wheat competition. By contrast, another population had the third highest fitness without competition, but the third lowest in the wheat (WYII). Reanalysis of the wild and hybrid fecundity data separately revealed a significant interaction of competition and population for the wild plants ( $P = 0.02$ ), but not the hybrids ( $P = 0.31$ ), indicating that hybrids from the different populations responded similarly to competition.

As in this work, previous studies assessing  $G \times E$  interactions involving crop-wild hybridization and an experimental treatment (e.g., disease pressure) have demonstrated that biotic stresses can alter fitness or productivity of hybrids and wilds. Bartsch et al. (2001) assessed total biomass of virus-resistant transgenic and nontransgenic sugar beet  $\times$  Swiss chard intraspecific hybrids at varying levels of disease and weed pressure. They found significant interactions between their genotypes and disease level, with high disease pressure reversing the genotypes' ranking in biomass production to benefit the transgenic over the nontransgenic hybrid. Vacher et al. (2004) reported that the relative biomass of insect-resistant hybrid *Brassica* increased under conditions of high competition and herbivore pressure and that the seed mass deficit suffered by hybrids was eliminated with high herbivore pressure. In a case of nontransgenic hybrids, Snow et al. (1998) witnessed less difference in the number of flower heads between their hybrid and wild sunflowers grown under weedy field conditions in Kansas as compared to those grown in Ohio in well-watered and fertilized pots. They hypothesized an interaction of hybridization and growing conditions.

Recent studies of hybrids in the wild have investigated the environment-dependence of selection (Hatfield and Schluter 1999; Wade et al. 1999; Campbell and Waser 2001; Johnston et al. 2001; Parris 2001). When exploring the ecological basis of hybrid fitness, Hatfield and Schluter (1999) found that  $F_1$  hybrid sticklebacks performed differentially in the littoral and open water habitats of their parental taxa. Campbell and Waser (2001) not only demonstrated  $G \times E$  interactions for fitness in their *Ipomopsis* hybrid zones, they also noted that the direction of hybridization (i.e., which species acted as the pollen parent) influenced the environment dependence of fitness. We have found no studies of natural hybridization that have examined environment-dependence of selection using hybrids between multiple pairs of populations. As in our study, distinct pairings of parental populations could result in differential fitness of hybrids, depending on environment. Such studies could shed further light on natural hybridization processes.

#### *Variation in Evolutionary Trajectories*

From an evolutionary perspective, it is important to quantitatively assess the role that crop-wild hybrids play in the evolution of wild populations under distinct environments and to attempt to predict introgression of crop alleles into a

wild population as recombination between wilds and hybrids proceeds. Introgression levels will depend on dispersal of crop pollen into wild populations and subsequent selection on hybrids and their descendants. In view of the degree of phenotypic differentiation between crop and wild populations, we might expect strong selection against crop migrants and crop-wild hybrids within wild populations (García-Ramos and Kirkpatrick 1997; Lenormand 2002; Hendry 2004). Fitness of hybrids relative to wilds quantifies the initial rate of change in the frequency of crop alleles within wild populations, and its variability sheds light on contingencies of gene introgression.

We found that relative fitness of hybrids was higher with competition than without competition (0.40 and 0.27, respectively) and wheat competition drastically modified the relative fitness of hybrids and wild plants for three populations (Fig. 3). This increase in relative fitness of hybrids in the wheat indicates that under competitive conditions selection against crop alleles diminishes. Thus, natural selection against crop alleles may be weaker and less predictable across the diverse genetic and environmental landscape than has been expected. In particular, for some populations, crop gene introgression would proceed more rapidly in the competitive environments surrounding agricultural fields or within a crop planting.

Taking into account the variability of relative fitness and of levels of gene flow (i.e., frequency with which primary hybrids arise), we can roughly predict frequencies of crop alleles in a wild population during the second growing season after an initial gene flow event (Hartl and Clark 1997, pp. 218–222; Cummings et al. 2002). Our calculations reflect the range of published estimates of crop gene flow into wild populations, that is, the frequency of hybrid seeds produced in year 0 (0.42, 0.17, and 0.02; see Arias and Rieseberg 1994; Whitton et al. 1997) and the range of relative fitness values estimated in this study and others (Snow et al. 1998; Cummings et al. 2002) for sunflower in year 1 (0.02, 0.21, 0.67, and 0.80; see Table 5). Assuming random mating, the frequencies of crop alleles projected in year 2 range from 0.0002 to 0.1834 (Table 5; see Appendix 3 for methods). These estimates are higher than those of Cummings et al. (2002), who predicted crop allele frequencies of 0.030–0.034 in year 2, primarily due to lower and less variable estimates of relative hybrid fitness. Recurrent gene flow would be expected to increase all of these estimates (Wolf et al. 2001; Haygood et al. 2003).

Although this kind of single gene model using data from  $F_1$  hybrids can have utility for conceptualizing initial stages of crop gene introgression, it has important limits. Individual crop alleles will have different fates over time according to their specific effects on fitness, once recombination proceeds (Harrison 1990; Rieseberg et al. 1996, 1999; Martinsen et al. 2001). Segments of the crop genome likely to be purged are those contributing to domestication traits (Stewart et al. 2003) or other characteristics that tend to decrease fitness in nature. Moreover, most traits are polygenic, and selection on multiple traits will affect allele frequencies jointly at many loci, such that the evolutionary change of individual traits introgressing into wild populations is complicated. Wild populations have been shown to differ in characteristics related

TABLE 5. Estimates of sunflower crop allele frequencies in the second year following a gene flow event. Estimates vary depending on the distance of the wild population from the crop field, which determines the frequency of hybrid seeds produced in a gene flow year. Estimates also vary based on relative fitnesses of hybrids and wilds measured in year 1. Frequencies of hybrids and relative fitnesses presented here are taken from estimates in the literature and this study, as indicated.

Relative fitness (hybrid/wild)	Distance from the crop field		
	3 m	50 m	1000 m
	Frequency of hybrids year 1		
	0.42 <sup>1</sup>	0.17 <sup>2</sup>	0.02 <sup>2</sup>
0.02 <sup>3</sup>	0.0071 <sup>6</sup>	0.0020	0.0002
0.21 <sup>4</sup>	0.0660	0.0206	0.0021
0.67 <sup>4</sup>	0.1633	0.0603	0.0067
0.80 <sup>5</sup>	0.1834	0.0704	0.0080

<sup>1</sup> Frequency of hybrid seeds produced in a wild population alongside a crop field (Whitton et al. 1997).

<sup>2</sup> The 50-m and 1000-m estimates of frequencies of hybrid seed produced in a wild population by a gene flow event (Arias and Rieseberg 1994).

<sup>3</sup> Relative fecundity measured by Cummings et al. (2002); combines seed production and predispersal seed predation.

<sup>4</sup> High and low estimates of relative fitness measured in this study.

<sup>5</sup> High estimate of relative fecundity from Snow et al. (1998).

<sup>6</sup> See Appendix 3 for methods of estimation of crop allele frequencies.

to domestication, especially seed germination and dormancy, branching, primary head size, and days to anthesis, and the phenotypic expression of those characteristics has been shown to shift under different environments (Snow et al. 1998; Mercer 2005; Mercer et al. 2006). Consequently, selection against crop chromosomal segments would depend on the phenotypic selection in the environment and genetic background of the wild recipient population.

If relative fitnesses of hybrids are frequently as high as we and Snow et al. (1998) have reported, then the overall predictions of the impact of crop-wild gene flow must acknowledge the high potential for crop gene (including transgene) introgression into wild populations. In addition, it should be noted that our estimates of relative fitness may be biased. In studies of predispersal (Cummings et al. 1999) and postdispersal (Alexander et al. 2001) seed predation, hybrid fitness was reduced more than wild fitness. If published levels of these factors are taken into account, the relative fitness of hybrids reported here decreases by a factor of 0.419.

However, our estimates could also be conservative for two reasons. First, the probability of germination in the field is greater for  $F_1$  hybrids than for wild plants (Mercer et al. 2006). Therefore, relative fitnesses calculated here would increase by a factor (on average) of 1.975 to take this difference into account. Second, we chose to assess relative fitness based on seed number because this relates directly to evolutionary change. An alternative approach is to consider relative reproductive output based on total seed mass. The  $F_1$  crop-wild hybrids produced fewer, larger seeds, while wilds produced more, smaller seeds. The  $F_2$  seeds produced on  $F_1$  hybrid plants were 3.3 times heavier than seeds produced on wild plants (0.0318 g/hybrid seed versus 0.00965 g/wild seed). Where larger seeds impart ecological advantages, such as larger seedling size, the fitness of individuals produced on hybrids could be higher as a result, although  $F_2$  seeds produced on crop-wild hybrid plants may have inherited reduced

overwintering capacity from their crop parents (this has yet to be studied). When seed size is taken into account, calculations of reproductive output of hybrids relative to wild plants (by mass) ranged from 0.67–2.2 across environments and populations in this study. By this measure, hybrid reproductive output was higher than that of the wild plants for five populations in the competitive environment and one population in the noncompetitive environment. In contexts conferring an advantage to larger seeds, effective fitness of hybrids may exceed that of wild plants. In conditions where hybrid fitness does exceed that of wilds, crop gene introgression would be unrestricted and rapid, and the impact of crop alleles on the gene pool of the wild population could be substantial.

Finally, our estimates of relative fitness were likely affected by growing wild populations outside their environment of origin. This common-garden experiment allowed us to gain insight into the responses of diverse genotypes to hybridization and competition, as expressed in a particular location. A study in which hybrids and wild plants were compared in their respective sites of origin as well as in a common site would have shown how hybrid effects depend on site, as well as the factors studied here, but would have been considerably more challenging. However, we did not note systematic reductions in fitness of populations originating farther from our field site in MN and the fecundity of the MN population tended to be low (Fig. 1).

### Conclusions

The variability noted in this study in the survival, fecundity, and relative fitness of crop-wild and wild sunflower has broad implications for the study of hybrid zones in managed settings and in the wild. By testing hybrids from a diversity of genetic materials under multiple controlled conditions, we explored the context-dependence of fitness and relative fitness of hybrid and parental genotypes and thus predicted the variability that may be expected in the introgression of novel alleles. By incorporating multiple factors, we were able to discern that there were certain genetic combinations and conditions (in this case, interspecific competition) that are more likely to facilitate introgression in crop-wild hybrid zones. Similar work performed with multiple populations from adjoining species pairs grown under various conditions found along species borders might indicate the conditions most suitable for stabilizing hybrid zones or creating new interspecific lineages. Due to the processes of context-dependent evolution, certain locations and genetic combinations may be found to be hot spots for introgression or speciation.

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Corresponding Editor: S. Kalisz

APPENDIX 1

*Estimating of Fecundity per Plant*

There were three factors complicating estimation of seed number. First, seeds produced by F<sub>1</sub> hybrid plants were two to three times as large as those produced by wild plants (Alexander et al. 2001; K. L. Mercer, unpublished data). Therefore, for these two types, we separately estimated regressions to predict number of seeds per head from head area (see below). Second, in sunflower (especially in crop and hybrid sunflower), the heads produced at different levels of branching (primary, secondary, tertiary) differ in size. To reduce the errors in estimation of fecundity, we measured the diameter of one head of each level per plant, estimated the number of seeds per head for each, and counted the number of heads of each level separately. Third, many of the crop-wild hybrids had deformed or anomalous heads on short stems (<30 cm) with severe developmental deformities, such as fused stems, or fused and incongruous heads. To include them in our estimations, we classified them as small (<4.5 cm), medium (4.5–8.5 cm), or large (>8.5 cm) by a measure at their broadest point. For each, the median number of seeds was estimated.

*Estimating seeds per head*

From July 16 through September 11, we measured the diameter (cm) of sunflower heads twice a week. For each plant we measured one primary, one secondary, and one tertiary flower head across the fully pollinated disk florets. We estimated the number of seeds in each head based on its area, which we calculated from its diameter as  $\pi(\text{diameter}/2)^2$ .

To estimate the number of seeds per head from the diameters measured, we collected a subset of the heads measured and counted their seeds, as follows. After full pollination, heads to be collected were covered with perforated plastic bags to reduce the loss of seed to birds and shattering and harvested once the seeds set. Heads were collected from all plants in replication 3, and from the wild plants in replication 8.

Two separate linear regressions for heads from hybrid and wild plants were performed to predict seeds per head from head area using PROC REG in SAS Version 8.2 for Windows (SAS Institute 2001). In each regression, the intercept was restricted to zero to ensure that the estimation was biologically relevant. Thirty-six to 40 deformed heads from each size class (small, medium, large) were also collected to estimate seed production for each size. The number of seeds per deformed head of a given size class was estimated with PROC MEANS as the median number of seeds per head from the sample of heads of each size.

The regression of number of seeds per head on the area of a seed head for each sunflower type yielded strong predictive relationships (see Appendix 2). Both regressions were highly significant with  $R^2 \geq 0.87$ . The prediction of the number of seeds per deformed head by size successfully discerned high-seed-producing from low-seed-producing heads (see Appendix 2). The slope of the regression from the appropriate sunflower type (wild, crop-wild hybrid) was then multiplied by the area of the heads for each level (primary, sec-

ondary, tertiary) to estimate the number of seeds produced by those representative heads.

*Counting primary, secondary, tertiary, and deformed heads*

Beginning on September 11 we counted all the heads on each plant in the experiment, excluding those in replication 2. The heads were counted in three groups: the normally pollinated heads, deformed heads, and special heads. For normally pollinated heads, we noted the number of primary, secondary, and tertiary heads. We counted the deformed heads as small, medium, or large. The special heads were those that either were not pollinated or were produced on plant parts that were heavily affected by disease or lodging. Within these categories, heads were recorded as either pollinated or not pollinated.

*Calculating total seeds per plant*

The total number of seeds per plant was calculated as the sum of the seeds from primary, secondary, tertiary, and deformed heads, multiplied by the number of each head type. Seeds produced by special pollinated heads were included. Therefore, the number of seeds produced per plant =  $\sum_{i,j} [\text{number of heads}_{i,j} \times \beta_j (\text{area of head})_i] + \sum (\text{number of deformed heads}_k \times \text{median number of seeds per deformed head}_k)$ , where  $i$  is primary, secondary, or tertiary;  $j$  is wild or crop-wild hybrid; and  $k$  is small, medium, or large.

APPENDIX 2

Predictions of number of seeds per head for normal heads (a) using head area as a predictor in linear regression and for deformed sunflower heads (b) using the median number of seeds for each size class.

a. Number of seeds in relation to area of normal heads by plant type			
	Slope <sup>1</sup>	R <sup>2</sup>	N
Crop-wild hybrid	8.25	0.89	924
Wild	14.74	0.87	455
b. Number of seeds per head by size for deformed heads			
	Size (cm)	Median number of seeds per head	N
Small	< 4.5	9	36
Medium	4.5–8.5	50.5	40
Large	> 8.5	124.5	36

<sup>1</sup> Intercepts of the regressions were restricted to zero for biological relevance.

APPENDIX 3

To calculate the crop allele frequencies in year 2, we made three important assumptions. First, we assumed crop seeds are not found in the wild populations and thus disregarded them for this analysis. Second, we assumed the frequency of hybrid genotypes growing in a population in year 1 is equivalent to the frequency of hybrid seed produced in year 0. Third, we assumed random mating. This third assumption is supported by the work of Cummings et al. (2002), whose model for predicting crop allele frequencies in sunflower was not improved by assuming assortative mating.

Frequencies of hybrid (Aa) seed genotypes produced in year 0 were multiplied by representative values for relative fitness of the hybrid genotypes from year 1 (hybrid = 0.02, 0.21, 0.67 or 0.80), yielding a value of S<sub>h</sub> for each of the four cases. The mean fitness,  $w$ , for each case, was obtained by  $S_h + S_w$ , where S<sub>w</sub> is the frequency of wild seed produced in year 0 times 1, the relative fitness of wild progeny. To obtain the frequency of hybrid genotypes in the next generation, S<sub>h</sub> was normalized by dividing by  $w$ . The crop allele frequencies were then calculated as half of this normalized value (Hartl and Clark 1997, pp. 218–222).