

THE POTENTIAL FOR GENE FLOW BETWEEN CULTIVATED AND WILD SUNFLOWER (*HELIANTHUS ANNUUS*) IN THE UNITED STATES¹

JOHN M. BURKE,² KEITH A. GARDNER, AND LOREN H. RIESEBERG

Indiana University, Department of Biology, Bloomington, Indiana 47405 USA

The transfer of genes from crop plants to their wild relatives via hybridization has emerged as one of the primary risks associated with the commercialization of genetically engineered crops. Although previous studies have revealed relatively high levels of hybridization when crop plants come into contact with their wild relatives, the frequency of such contact across the range of cultivation of any crop taxon is unknown. Here we report the results of a multi-year, range-wide survey of the potential for reproductive contact between cultivated and common sunflower (*Helianthus annuus*). The results of this work indicate that the opportunity for crop–wild hybridization exists throughout the range of sunflower cultivation. Approximately two-thirds of all cultivated fields occurred in close proximity to, and flowered coincidentally with, common sunflower populations. In these populations, the phenological overlap was extensive, with 52–96% of all wilds flowering coincidentally with the adjacent cultivar field. Moreover, there was morphological evidence of hybridization in 10–33% of the populations surveyed within a given year. These findings indicate that crop–wild hybridization is likely across the range of sunflower cultivation in the USA.

Key words: flowering time; *Helianthus*; hybridization; phenology; sunflower.

Hybridization between cultivated plants and their wild relatives provides a conduit for the transfer of genes out of crop lineages and into natural populations. Such gene flow may facilitate the evolution of weedy and invasive plants (Raybould and Gray, 1994). In general, traits introduced into crop lineages via traditional breeding techniques (such as increased seed size, loss of seed dormancy, loss of natural seed dispersal, etc.) might be expected to be maladaptive in the wild. Crop–weed hybridization has, however, been known to produce aggressively weedy crop mimics that are difficult to control because they share a number of traits with their crop progenitor (reviewed in Barrett, 1983). This possibility notwithstanding, the transfer of genes from domesticated plants into their wild relatives was of little concern prior to the advent of modern agricultural biotechnology. Current concerns stem from the fact that some traits that are the target of genetic manipulation—such as disease or insect resistance and tolerance of various abiotic stresses—may confer a strong fitness advantage in the wild. Crop–wild hybridization has thus emerged as one of the primary risks associated with the commercialization of genetically engineered crop plants (Colwell et al., 1985; Goodman and Newell, 1985; Tiedje et al., 1989; Linder and Schmitt, 1994; Ellstrand, Prentice, and Hancock, 1999).

In order for crop genes to be transferred to wild populations via hybridization, crop plants and their wild relatives need to occur sympatrically, overlap in flowering time, and be cross-compatible (Keeler and Turner, 1990). In many cases, these conditions are met and crop–wild hybridization appears to be frequent. For example, there is evidence that 12 of the world's 13 most important food crops hybridize with at least one wild relative in at least part of their range of cultivation (reviewed

in Ellstrand, Prentice, and Hancock, 1999). Furthermore, genetic studies have shown that crop–wild gene flow can occur at an appreciable rate over relatively long distances and that crop alleles can persist in wild populations well after the cessation of contact with the cultivated form (e.g., Kirkpatrick and Wilson, 1988; Klinger, Elam, and Ellstrand, 1991; Arias and Rieseberg, 1994; Whitton et al., 1997; Linder et al., 1998; but see Scott and Wilkinson, 1998). Such studies have, however, focused on a limited number of populations in which the crop and wild forms were already known to co-occur. To date, no studies have examined the potential for crop–wild hybridization across the range of cultivation of any crop taxon.

The weedy, self-incompatible common sunflower (*Helianthus annuus* var. *annuus*) is native to North America and found throughout the USA, Canada, and Mexico. It is particularly abundant in the central and western USA (Heiser, 1951), and its range contains nearly all of the cultivated sunflower (*H. annuus* var. *macrocarpus*) acreage in the USA. Despite being morphologically distinct, cultivated and common sunflower are considered to be members of the same species. Common sunflower, which is frequently observed growing in disturbed habitats, is often found in close proximity to cultivated sunflower fields, and hand-pollinations between the two forms result in fertile hybrids. Both cultivated and common sunflower are pollinated by honey bees, bumble bees, and solitary bees, and, although cultivated sunflower generally matures more rapidly than common sunflower, they often exhibit some degree of phenological overlap. Results of previous research indicate that, where they come into contact, cultivated and common sunflower often hybridize, with as many as 42% of the progeny of wild plants near cultivated fields being hybrids (Arias and Rieseberg, 1994; Whitton et al., 1997; Linder et al., 1998). The frequency of this sort of contact, however, remains unknown. In this paper we report the results of a multi-year, range-wide survey of the potential for reproductive contact between cultivated and wild *H. annuus*.

MATERIALS AND METHODS

Surveys of the degree of phenological overlap between cultivated and wild sunflower were performed during the summers of 1999 (6–9 August), 2000

¹ Manuscript received 12 March 2002; revision accepted 7 June 2002.

We thank two anonymous reviewers for comments on an earlier version of this paper. This work was supported by grants from the USDA (32-00-35300-9244 to JMB) and Pioneer Hi-Bred International, Inc. (to LHR).

² Author for correspondence, current address: Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235 USA (e-mail: john.m.burke@vanderbilt.edu).

(17–26 August), and 2001 (18–26 August). The timing of these surveys coincided with the flowering season of cultivated sunflower in those years. In all three years, detailed quantitative data were taken from an average of 44 individuals in each of 16–20 wild *H. annuus* populations. These populations, which were all adjacent to cultivated sunflower fields, were selected to cover the range of sunflower cultivation in the United States. In 1999, ten were located in North/South Dakota, five were in the High Plains of Kansas/Colorado/Nebraska, and three in western Texas (18 total). In 2000, nine were located in the Dakotas, nine in the high plains, and two in western Texas (20 total). In 2001, seven were located in the Dakotas and nine were in the High Plains (16 total). Over the course of this study, North/South Dakota, the High Plains, and western Texas accounted for approximately 75, 20, and 3%, respectively, of the total acreage of sunflower grown in the United States (USDA National Agricultural Statistics Service). Detailed location information is available upon request.

In each of the selected wild populations, plants were randomly sampled along a transect through the population. Because each site was visited only once during the flowering season, a simple count of the number of flowering individuals, as well as the number of flowering heads per individual, would have underestimated the true level of overlap throughout the season. Therefore, the developmental stages of heads on each plant were scored following the classification scheme of Schneiter and Miller (1981). The developmental stage of the adjacent cultivated field at each site was then estimated by visual inspection. In contrast to wild sunflowers, which produce numerous flowering heads throughout the late summer and fall, entire fields of cultivated sunflower mature, flower, and senesce relatively coincidentally. These data, therefore, allowed us to estimate the number of wild individuals (and the number of heads per individual) that flowered coincidentally with any given cultivated sunflower field over the course of the season. Consider, for example, a particular field that is near the peak of flowering (stage R5.5, when ~50% of the disk florets are open). Clearly, flowering in this field would overlap with all adjacent wild plants that are currently flowering. One can also reasonably assume that all heads on nearby wild plants that just recently stopped flowering (stage R6) also overlapped with the cultivar. Likewise, those heads that are nearly open (stage R4) will overlap with the cultivar before it finishes flowering. The resulting estimates were analyzed by two-way ANOVA, with “region” and “year” as the main effects. Because of the small number of populations surveyed, as well as the missing data in 2001, Texas was excluded from these analyses. Frequency data were arcsine transformed prior to analysis (Sokal and Rohlf, 1995).

In addition to the detailed phenological surveys within the selected populations, flowering time data were collected from all cultivated fields encountered along the routes traveled in the High Plains and the Dakotas during the field trips of 2000 and 2001. (Texas was left out of this phase of the study, as only ~3% of cultivated sunflower acreage in the USA occurs there.) Of the 259 sites visited in 2000, 192 were located in the Dakotas and 67 were in the High Plains. Similarly, of the 316 sites visited in 2001, 243 were located in the Dakotas and 73 were in the High Plains. In order to assess the likelihood of reproductive contact across the range of sunflower cultivation, we recorded: (1) whether or not common sunflower was growing inside of or in close proximity (typically within 50–100 m) to one or more of the four borders of the cultivated field and (2) if so, whether or not the wild and cultivated plants were flowering coincidentally. In order to assess the effects of reproductive contact in past years, we also recorded the presence of any apparent crop-wild hybrids at each location. The presence of crop-wild hybrids was inferred on the basis of morphological intermediacy. In general, cultivated sunflowers have a thick, unbranched stem bearing large leaves and are topped by a single, large inflorescence (Heiser, 1954). In contrast, common sunflower is characterized by a highly branched growth form, the production of multiple, small heads, and comparatively small leaves and thin stems. Early-generation hybrids typically exhibit a combination of these characters and are easily distinguished in the field. During the 2000 field season, a small number of hybrid plants were collected, preserved as herbarium voucher specimens, and deposited in the Indiana University herbarium. The categorical data collected during this phase of the surveys were analyzed via χ^2 tests.

TABLE 1. Extent of coincidental flowering in wild *Helianthus annuus* populations growing adjacent to cultivated sunflower fields in the major regions of sunflower cultivation in the USA. Values are presented as means \pm 1 SE.

Region	Number of populations surveyed	Fraction of wild individuals flowering	Number of flowering heads/individual
1999			
North/South Dakota	10	0.52 \pm 0.08	3.3 \pm 0.7
High Plains	5	0.52 \pm 0.08	3.0 \pm 0.4
Texas	3	0.93 \pm 0.04	16.4 \pm 6.9
2000			
North/South Dakota	9	0.96 \pm 0.02	7.1 \pm 2.1
High Plains	9	0.89 \pm 0.04	6.6 \pm 1.7
Texas	2	0.66 \pm 0.34	4.2 \pm 3.7
2001			
North/South Dakota	7	0.93 \pm 0.03	4.1 \pm 0.7
High Plains	9	0.96 \pm 0.02	4.2 \pm 0.6

RESULTS AND DISCUSSION

The extent of phenological overlap between the selected wild populations and their adjacent cultivated fields was remarkably similar across the Dakotas and the High Plains within all three years (Table 1). In fact, there was no significant variation among regions in terms of either the fraction of wild individuals flowering or the number of flowering heads per plant ($P = 0.84$ and $P = 0.69$, respectively; data not shown). In contrast, there was significant variation among years in terms of both the fraction of wild individuals flowering and the number of flowering heads per plant ($P < 0.0001$ and $P < 0.005$, respectively; data not shown). Although the greatest overlap occurred during the 2000 and 2001 flowering seasons, there was ample opportunity for pollen transfer between cultivated and common sunflower where they came into contact in all three years (Table 1). This result is consistent with previous findings of high levels of gene flow between cultivated and wild sunflower where they co-occur (Arias and Rieseberg, 1994; Whitton et al., 1997). But how often do they co-occur?

Of the 259 cultivated sunflower fields visited in August 2000, 54 (21%) had no evidence of wild *H. annuus* in close proximity and 39 (15%) were located near nonflowering wild *H. annuus* (Table 2). The remaining 166 fields (64%) were

TABLE 2. Frequency of overlap in flowering time between cultivated sunflower fields and adjacent common sunflower populations. Cultivated fields were assigned to one of four categories: (1) no wild *Helianthus annuus* within 50 m of any border, (2) adjacent to a wild *H. annuus* population that did not overlap in flowering time, (3) adjacent to a wild *H. annuus* population that overlapped in flowering time or (4) adjacent to a wild *H. annuus* population that overlapped in flowering time and contained morphologically identifiable crop-wild hybrids.

Region	No wilds	No overlap	Overlap	Hybrids
2000				
North/South Dakota	45	28	100	19
High Plains	9	11	41	6
Total	54	39	141	25
2001				
North/South Dakota	77	24	81	61
High Plains	0	1	30	42
Total	77	25	111	103

adjacent to populations of wild *H. annuus* that contained flowering individuals. Similarly, of the 316 fields visited in August 2001, 77 (24%) had no wild *H. annuus* growing in close proximity, 25 (8%) were adjacent to nonflowering wild *H. annuus* populations, and 214 (68%) occurred in close proximity to wild *H. annuus* populations that contained flowering individuals. In both years, there was evidence of past hybridization. Clearly identifiable hybrids were present at 25 of 259 (10%) and 103 of 316 (33%) sites visited in 2000 and 2001, respectively.

Breaking these data down by region, the potential for reproductive contact (defined as the occurrence of flowering wild *H. annuus* individuals adjacent to a cultivated field) was greater in the High Plains (70%) than in the Dakotas (62%) in 2000, but not significantly so ($\chi^2 = 1.44$, $df = 1$, $P = 0.23$; Table 2). In contrast, there was a significantly greater potential for reproductive contact in the High Plains (99%) as compared to the Dakotas (58%) in 2001 ($\chi^2 = 41.5$, $df = 1$, $P < 0.0001$). Pooled across years, 85% of cultivated sunflower fields in the High Plains and 60% in the Dakotas were adjacent to and flowered coincidentally with wild *H. annuus* populations. This difference was highly significant ($\chi^2 = 29.5$, $df = 1$, $P < 0.0001$). It should be noted that our use of 50–100 m as a cutoff for being “adjacent” to a cultivated field was arbitrarily selected to make this work feasible. In fact, Arias and Rieseberg (1994) showed that cultivated sunflower pollen can be exported over much greater distances, making our estimates of the frequency of overlap quite conservative.

The frequency of populations with evidence of past hybridization was relatively low in 2000, with 9% and 10% of all sites visited in the High Plains and the Dakotas, respectively, containing morphologically identifiable hybrids ($\chi^2 = 0.05$, $df = 1$, $P < 0.82$). In 2001, there was considerably more evidence of past hybridization in both regions, with 58% and 25% of all sites visited in the High Plains and the Dakotas, respectively, containing morphologically identifiable hybrids. This difference was highly significant ($\chi^2 = 26.9$, $df = 1$, $P < 0.0001$). The cause of the increased frequency of populations containing hybrids in 2001 as compared to 2000 is unclear. One possibility is that our ability to identify hybrids in the field improved over time. Alternatively, there could be a good biological reason for it. For example, the lower level of phenological overlap in 1999 could have contributed to the relatively low frequency of hybrids in 2000. Whatever the case, it's clear that hybridization between cultivated and common sunflower is a geographically widespread phenomenon. In fact, because advanced-generation hybrids are often morphologically indistinguishable from one parent or the other (Paige and Capman, 1993; Hardig et al., 2000), our estimates of the frequency of hybridization are likely to be conservative.

The results of this work, combined with prior analyses of the frequency with which cultivated and common sunflower hybridize when they come into contact (Arias and Rieseberg, 1994; Whitton et al., 1997), indicate that crop-wild gene flow is virtually inevitable throughout much of the range of sunflower cultivation in the USA. Thus, the issue of whether or not any particular cultivar gene will be transferred into a common sunflower population becomes a question of when it will happen, rather than if it will happen. At least in sunflower, therefore, research on the risks associated with transgene escape should focus on the fitness consequences of the gene(s)

in question, rather than on the rates of hybridization. This conclusion is supported by theoretical work showing that the rate of spread of a new allele is mainly governed by the fitness effects of the allele, as opposed to the migration rate (Fisher, 1937; Slatkin, 1976).

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