

## Gene flow between cultivated sunflower and *Helianthus petiolaris* (Asteraceae)

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**Abstract** *Helianthus petiolaris* (Asteraceae) native to North America has naturalized in Argentina. The extensive overlapping with sunflower crop regions, the coincidence of life cycles and the common pollinators facilitate interspecific crosses with sunflower, *H. annuus* var. *macrocarpus*. To estimate the occurrence of crop-to-wild and wild-to-crop gene flow, off-type plants in progenies of pure stands of both species flowering in coincidence and the presence of crop alleles in *H. petiolaris* populations were investigated in 26 wild populations and nine crop fields. Morphological traits and RAPD markers were used to attempt hybrid characterization. Off-type individuals were found in frequencies of 0.5 and 0.3% among crop progenies and wild populations, respectively. Off-type plants showed intermediate values for metric morphological traits and low fertility traits. Some off-type plants proved to carry crop alleles based on molecular analysis. The average frequency of cultivar-marker introgression across the

wild populations was very low (0.02). Although observed hybridization rates seem to be low, the extension of crop-wild overlapping in Argentina make hybrid formation a noticeable process. Therefore, the new sunflower varieties and eventually GM varieties would transfer their traits through pollen flow and they would persist in *H. petiolaris* populations.

**Keywords** Crop-wild hybridization · Introgression · Morphological traits · RAPD markers

### Introduction

*Helianthus petiolaris* Nutt. (Asteraceae) known as prairie sunflower is a diploid species ( $x = 17$ ) native to North America which has naturalized in Argentina (Covas 1966). Seeds were presumably introduced as impurities in forage seed lots about 60 years ago (A. Luciano pers. comm.). It rapidly spread in the semiarid environments and sandy soils of the central region of the country and, at present, it is established in four provinces, La Pampa, San Luis, western Buenos Aires and southern Cordoba, between 33°14' and 38°48' S (Poverene et al. 2002; Cantamutto et al. 2008). Morphological description of leaf and head characters of the plants found in Argentina matches *H. petiolaris* ssp. *petiolaris*, according to Heiser (1961).

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The extensive overlapping with sunflower crop regions, the coincident life cycles and the occurrence of common pollinator insects facilitate interspecific crosses between *H. petiolaris* and sunflower, *H. annuus* var. *macrocarpus* (Heiser 1947). Although both species differ in chromosome constitution—only seven out of 17 chromosomes are collinear in both species—and there are important barriers to hybridization (Rieseberg et al. 1995a), hybrid swarms and introgression between both species have been extensively studied in their centre of origin (Rieseberg et al. 1999a) where three new homoploid species have originated from them (Heiser 1947; Rieseberg et al. 1996; Rieseberg et al. 1999b; Rieseberg and Linder 1999; Buerkle and Rieseberg 2001).

Sunflower is one of the main four grain crops in Argentina. In the 2006/07 season, the sunflower area in the provinces with naturalized *H. petiolaris* populations was 1.7 million hectares [[www.sagpya.mecon.gov.ar](http://www.sagpya.mecon.gov.ar)] with almost 650,000 hectares comprising the *H. petiolaris* diffusion area (Poverene et al. 2002). Intermediate individuals have been observed for many years within *H. petiolaris* populations (Covas and Vargas López 1970). These off-type plants could originate from crop pollen flow to wild populations. The likely gene transfer from sunflower crop to *H. petiolaris* populations is of concern regarding the commercialization of new imidazolinone resistant (Clearfield®) hybrid cultivars. Herbicide resistance was transferred with high frequency (79%) to wild plants (Massinga et al. 2003). A similar situation could take place if genetically modified (GM) sunflower varieties were released. In Argentina, about 77 GM sunflower petitions have been authorized for field trials, but no varieties have been commercially released up to date (Cantamutto and Poverene 2007).

Although *H. petiolaris* is not considered a noxious weed in Argentina, in the last few years we often found it invading summer crops in eastern La Pampa and western Buenos Aires provinces. Gene flow from crops to wild populations has been implicated in several cases of ferality and weed evolution, including new invasive species (Ellstrand 2003; Gressel 2005). Invasiveness has almost always been associated with habitat disturbance (Ellstrand and Schierenbeck 2000) a condition that is closely associated with the occurrence of *H. petiolaris* populations (Cantamutto et al. 2008).

Argentina provides an exceptional scenario with extended sunflower crops overlapping in time and area with small prairie sunflower populations, creating the opportunity for an intense wild-to-crop and crop-to-wild gene flow. In a very wide landscape of arable land, it is common to find isolated *H. petiolaris* plants growing along roadsides among sunflower volunteers. This would highly increase the chance of *H. petiolaris* pollination and fertilization by *H. annuus*.

We hypothesize that in this agro-ecosystem there is a high frequency of reciprocally pollination between *H. petiolaris* populations and sunflower crop. To test this hypothesis we searched for evidence of interspecific hybrids in progenies from pure stands of both species flowering in coincidence, and for the presence of crop alleles in prairie sunflower populations.

The goal of this work was to estimate the occurrence of crop-to-wild and wild-to-crop gene flow, to characterize natural hybrids, and to detect the presence of crop alleles in wild populations. Morphological traits and molecular markers were used to attempt hybrid characterization.

## Materials and methods

### Wild samples

*H. petiolaris* seed was collected in 26 different sites of La Pampa, San Luis and Buenos Aires provinces during flowering coincidence with crop (Table 1). Previous observations verified that heads of the wild species, which shows indeterminate growth habit, flower for 7–10 days, dry during the following 10–15 days and then shatter. When flowering by the same time, the crop reaches the R6–R7 stages (Schneiter and Miller 1981) in that interval. Bulk samples of seed were collected from wild pure populations growing adjacent to sunflower crops in the R6–R7 stages. A subset of seeds was grown in the greenhouse and transplanted to experimental field plots in Bahia Blanca (S 38°41'38", W 62°14'53") in a completely randomized design with two replications. Plots were 20 m long, with plants spaced 0.20 m apart at each side of the drip tape, and the distance between rows was 1.5 m. A plot of cultivated sunflower (Dekalb 3900) was sown at the same time.

**Table 1** Geographical sites of collected *H. petiolaris* populations growing near sunflower crops

Accession	Province	County	Accession	Province	County
0800	La Pampa	Atreucó	<b>1202</b>	La Pampa	Capital
1300	La Pampa	Atreucó	1402	Buenos Aires	A. Alsina
1400	La Pampa	Catriló	1502	Buenos Aires	A. Alsina
<b>0102</b>	Buenos Aires	Guaminí	1602	La Pampa	Quehue
0202	Buenos Aires	Salliqueló	<b>2002</b>	La Pampa	Conhella
0302	Buenos Aires	Salliqueló	2102	San Luis	G. Dupuy
0402	Buenos Aires	Pellegrini	2202	San Luis	G. Dupuy
<b>0502</b>	La Pampa	Catriló	3102	San Luis	G. Pedernera
0602	La Pampa	Atreucó	<b>3202</b>	San Luis	G. Pedernera
<b>0802</b>	La Pampa	Atreucó	3302	San Luis	G. Pedernera
<b>0902</b>	La Pampa	Capital	3602	Buenos Aires	T. Lauquen
<b>1002</b>	La Pampa	Capital	<b>4702</b>	La Pampa	Realicó
1102	La Pampa	Conhella	5002	La Pampa	Catriló

Accessions numbers where off-type plants were identified in the experimental field are in bold

Although wild *H. annuus* is also present in Argentina, this species is not found in the regions where this study was accomplished.

#### Cultivated samples

Seeds were collected in nine highly uniform sunflower crops, without impurities, on fields invaded by *H. petiolaris* when the wild sunflower was between flowering and the shattering phase (Table 2). Heads of 50 cultivated plants in R8 stage were collected at regular intervals and a quarter section of each was sampled. The seed was sown in the experimental field during the next season, as described before.

**Table 2** Geographical sites of collected sunflower seed in crops invaded by *H. petiolaris* plants

Accessions	Province	County
0204	La Pampa	Atreuco
0504	La Pampa	Atreuco
<b>0604</b>	La Pampa	Atreuco
0704	La Pampa	Atreuco
1104	La Pampa	Q. Quemu
1404	La Pampa	Catrilo
2304	Buenos Aires	T. Lauquen
<b>2404</b>	Buenos Aires	T. Lauquen
2504	Buenos Aires	Tres Lomas

Accessions numbers where off-type plants were identified in the experimental field are in bold

#### Morphological characterization

Morphological data were collected on cultivated sunflower, wild *H. petiolaris* plants and their progenies. Off-type plants suspected of being interspecific hybrids were visually identified by morphology intermediary. The following traits were studied: leaf length, leaf width, leaf shape (l/w), disc diameter and bract (phyllary) width. Phenotypic similarity among individuals and species across the metric traits was graphically assessed by principal components analysis (PCA) of character  $\times$  character correlations. Means were compared using the Hochberg test for imbalanced samples. A hybrid index for leaf margin was calculated on a 0–2 scale, being 0 = entire, 1 = serrate, 2 = deeply serrate. Hybrid fertility was estimated through pollen stainability and seed set. Three pollen samples per plant were stained (Alexander 1980). Counts of 400–800 pollen grains per sample gave a mean percentage of viable pollen. Seed set was assessed in 3–5 heads per plant. Fertility data were also collected on four plants of a sunflower commercial hybrid (DK3900) and on four plants of *H. petiolaris*, as controls. Life cycle length was estimated in 10-day intervals from sowing date to senescence.

#### Molecular analysis

Molecular analysis was accomplished in crop-to-wild crosses. It comprised 11 *H. petiolaris* accessions, nine

off-type plants, and six inbred lines of cultivated sunflower (Table 3). Young leaves were lyophilized and DNA was isolated with a CTAB method (Hoisington et al. 1994). RAPD markers were amplified by nine primers (Operon Technologies A2, A3, A4, B4, B5, C4, C5, D2, and D4). The amplifications were carried out in a total volume of 25  $\mu$ l starting with 50 ng of purified DNA template, 30 ng of primer, 1U of Taq DNA polymerase, and a final concentration of 2 mM  $MgCl_2$ , 20 mM buffer, and 50 mM of dNTP 1U. The reactions were placed in a PTC-100 MJ Research Thermal Cycler programmed for one cycle of 6 min at 94°C, 40 cycles of 15 sec at 94°C, 45 sec at 40°C, 1 min at 72°C, and a final extension at 72°C for 7 min. Amplification products were separated by electrophoresis in 1.5%

TAE agarose gels and detected by staining with ethidium bromide.

Numerical analysis of markers was done with Infogen (2006) software. Multivariate and AMOVA analyses on data were done with GenAlex 6 (Peakall and Smouse 2005) software.

## Results

### Crop-to-wild gene flow

Morphological intermediate plants were found in nine out of 26 *H. petiolaris* populations in the field. Frequency of intermediate plants per population ranged from 0 to 0.02. Thirteen presumably hybrid individuals were found among 4,374 wild type plants (0.3%). They were identified by the accession number (Table 1), replication number, and the letter of the plot where they were found: 0102 IID, 0502 IID, 0802 IA, 0802 IID1, 0802 IID2, 0902IID, 1002 IA, 1002IIC, 1202 IIC, 2002 IB, 2002 IID, 3202 IIC, 4702 IID. Off-type plants were highly variable in phenotype, 1.20–1.80 m height, with wide cordate (heart-shaped) leaves but sometimes smaller and lanceolate, mostly long and sometimes very thick petioles. Half of the off-type plants had very large discs and phyllaries and half showed the *H. petiolaris* type.

Off-type plants showed intermediate values for four metric morphological traits and low fertility estimates (Table 4). High significant differences were found in leaf length and width, disc diameter, and phyllary width among *H. petiolaris*, off-types, and

**Table 3** Accessions and plants subjected to RAPD analysis

<i>H. petiolaris</i> <sup>a</sup>	<i>H. petiolaris</i> <sup>b</sup>	Off-type plants	Sunflower inbred lines
HP 0102	HP 0800	0102 IID	HA89
HP 0802	HP 1602	0802 IA	HA369
HP 0902	HP 2202	0802 IID <sub>1</sub>	HAR2
HP 1002	HP 3102	0802 IID <sub>2</sub>	HAR3
HP 1202	HP 3602	0902 IID	HAR5
HP 2002		1002 IA	RHA274
		1002 IIC	
		1202 IIC	
		2002 IID	

<sup>a</sup> Accessions where hybrids were found

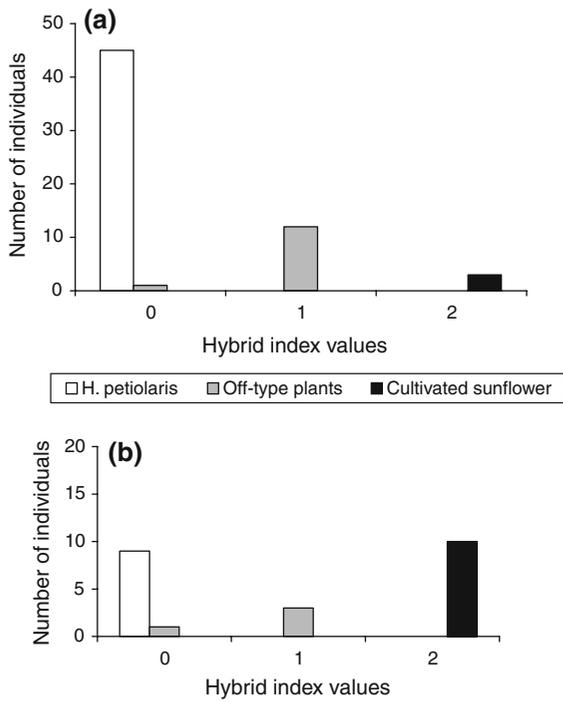
<sup>b</sup> Accessions where no hybrids were found

**Table 4** Morphology and fertility traits in *H. petiolaris* off-type plants suspected to be interspecific hybrids with cultivated sunflower

Character	<i>H. petiolaris</i>	Off-type plants	Cultivated sunflower	Sign
Leaf length	8.24 $\pm$ 1.51(a)	14.59 $\pm$ 2.70(b)	22.83 $\pm$ 4.44(c)	**
Leaf width	5.26 $\pm$ 0.94(a)	12.99 $\pm$ 3.08(b)	21.5 $\pm$ 6.58(c)	**
Leaf shape l/w	1.59 $\pm$ 0.22(b)	1.14 $\pm$ 1.33(a)	1.09 $\pm$ 0.13(a)	**
Disc diameter	2.41 $\pm$ 0.33(a)	4.09 $\pm$ 0.97(b)	13.5 $\pm$ 1.14(c)	**
Bract width	0.45 $\pm$ 0.06(a)	0.90 $\pm$ 0.23(b)	2.83 $\pm$ 0.25(c)	**
Seed set	71.8 $\pm$ 5.26(a)	5.34 $\pm$ 2.6(b)	No data	**
Pollen stainability	82.46 $\pm$ 13.24(a)	26.28 $\pm$ 8.9(b)	No data	**

Mean data and standard deviation, significance ( $P < 0.01$ ) and Hochberg mean comparisons

Means with the same letter are not different at a significance level of 0.01



**Fig. 1** Hybrid index based on leaf margin in *H. petiolaris*, cultivated *H. annuus* and off-type individuals in crop-to-wild (a) and wild-to-crop (b) crosses

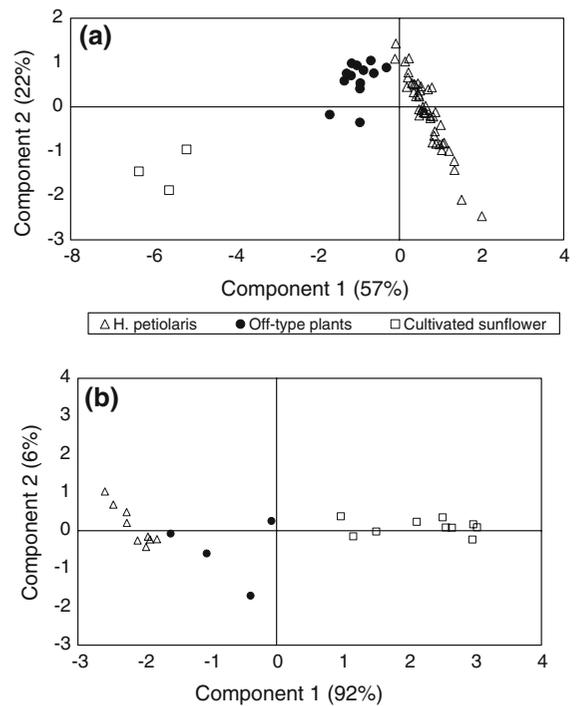
cultivated sunflower. Hybrid measures were significantly different from *H. petiolaris* values, both for morphological and fertility traits.

Hybrid index showed that leaf margin in off-type plants was intermediate between *H. petiolaris* and cultivated sunflower (Fig. 1a). PCA explained 79% of the variation in the first two axes (Fig. 2a). The off-type plants were placed between both parental species mainly based on leaf shape, disc and phyllary size, even though some plants were closer to the wild species. Individuals representing *H. petiolaris* formed a distinct group and differentiated mainly by leaf shape but plants from the same population did not cluster together.

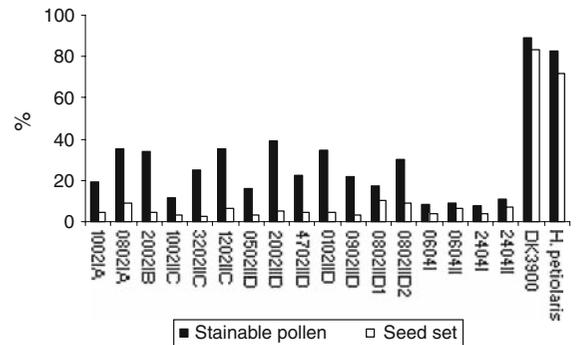
Stainability of off-type pollen was in a range of 11.3–39%, with mean of 26.3%. Seed set was between 2.3 and 10.4%, with mean of 5.3% (Fig. 3).

**Wild-to-crop gene flow**

Morphological intermediate plants were obtained from two out of nine sampled sites from La Pampa and Buenos Aires provinces (Table 2). Frequency of



**Fig. 2** Principal component analysis of metric traits—leaf length, leaf width, leaf shape (l/w), disc diameter and bract (phyllary) width- in *H. petiolaris*, cultivated *H. annuus* and off-type individuals observed in crop-to-wild (a) and wild-to-crop (b) crosses



**Fig. 3** Pollen stainability and seed set in off-type individuals found in *H. petiolaris* populations (first 13 samples), in sunflower crop (samples 14–17), cultivated sunflower (commercial hybrid DK3900), and *H. petiolaris*

intermediate plants per population ranged from 0 to 0.02. Four off-type individuals were found among 851 crop progenies (0.5%). Height of off-types ranged between 1.44 and 1.72 m, and displayed total branching and lack of a main head. Disc flowers were

always red and leaves were cordate or lanceolate. Wide differences were found for petiole length, disc diameter and phyllary size. Hochberg tests sustained highly significant differences among intermediate plants and cultivated sunflower in all the traits other than leaf shape (Table 5).

Hybrid index for leaf margin showed that most off-type plants had intermediate values among their putative parents, though some plants resembled more *H. petiolaris* (Fig. 1b).

Pollen mean stainability of off-type plants was 8.8% (range 7.4–10.7%) and seed set was 5.2% (range 3.5–6.9%) (Fig. 3). PCA placed off-type plants in an intermediate position between parental species, though nearer to *H. petiolaris* (Fig. 2b). The first component explained 92% of variability and was highly correlated to all characters.

### Molecular Analysis

Over 77 analyzed samples comprising the two pure species and off-type plants, four primers (A2, B5, C4 and D2) out of nine revealed 51 bands, only one band being monomorphic. On the whole, analyzed plants showed a very high polymorphism (98%). The primer

A2 rendered the highest number of bands and PIC values ranging 0.23–0.30 (Table 6). The remaining five primers showed very similar patterns among cultivated and wild plants and did not provide specific bands, so the analysis concentrated in the above mentioned primers.

Analysis of molecular variance (AMOVA) for *H. petiolaris* data showed that 91% of the genetic variance was explained by variation among populations and the remainder (9%) was attributed to within-population variation ( $P < 0.001$ ). Among 62 *H. petiolaris* plants, 23 were duplicated samples. Some accessions showed invariant bands and sunflower inbred lines showed the highest monomorphic band ratio (30/51) (Table 7). Over 51 bands, 30 were unique for *H. petiolaris* and seven were characteristic of cultivated *H. annuus*. All sunflower inbred lines differentiated from each other. Four out of nine off-type plants revealed the presence of the crop alleles A2820, A2720 and B5680. At least one of these alleles was found in the four plants, one having two cultivar markers. Four cultivar-specific markers did not introgress into any of the *H. petiolaris* populations. The average frequency of cultivar-marker introgression across the wild populations was very

**Table 5** Morphology traits in sunflower off-type plants suspected to be interspecific hybrids with *H. petiolaris*

Character	<i>H. petiolaris</i>	Off-type plants	Cultivated sunflower	Sign
Leaf length	8.4 ± 0.96(a)	14.85 ± 5.3(b)	25.0 ± 2.91(c)	**
Leaf width	5.65 ± 0.8(a)	12.7 ± 4.2(b)	25.6 ± 4.6(c)	**
Leaf shape l/w	1.5 ± 0.16(b)	1.19 ± 0.23(a)	0.99 ± 0.09(a)	**
Disc diameter	2.3 ± 0.19(a)	3.78 ± 0.50(a)	19.0 ± 3.74(b)	**
Bract width	0.37 ± 0.07(a)	0.90 ± 0.54(a)	3.59 ± 0.54(b)	**

Mean data and standard deviation, significance ( $P < 0.01$ ) and Hochberg mean comparisons

Means with the same letter are not different at a significance level of 0.01

**Table 6** Molecular information per primer obtained from 77 *Helianthus* samples

Primer	Polymorphic bands	Monomorphic bands	Total bands	Polimorphic loci <sup>a</sup>	PIC <sup>b</sup>
A2	22	1	23	0.87	0.3
B5	5	0	5	0.8	0.23
C4	11	0	11	0.91	0.29
D2	12	0	12	1	0.3
Total	50	1	51	–	–

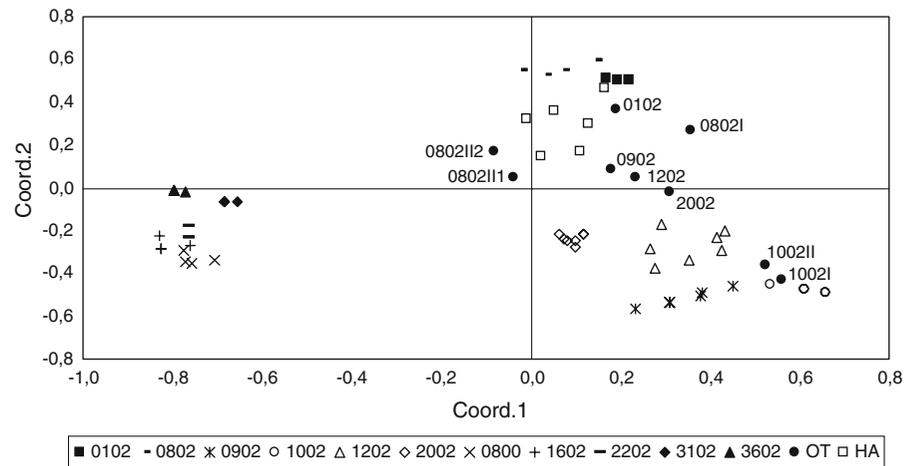
<sup>a</sup> Proportion of polymorphic loci by the 0.95 criterion

<sup>b</sup> Polymorphic information content

**Table 7** Molecular analysis of *H. petiolaris*, cultivated sunflower and off-type plants based on four primers which produced 51 bands

Data description	<i>H. petiolaris</i>	Off-type plants	Sunflower inbred lines
Total samples	62	9	6
Duplicated samples	23	0	0
Duplicated banding pattern	6	21	10
Monomorphic bands	8	9	30
Polymorphic bands (%)	84.3	82.3	41.2

**Fig. 4** Principal coordinate analysis of molecular markers in 12 *H. petiolaris* accessions (numbers in legend), cultivated *H. annuus* (HA) and off-type plants (OT, numbered in figure)



low (0.02). Principal coordinate analysis showed that *H. petiolaris* individuals formed three different groups, one consisted of wild accessions where no intermediate plants were found and two groups comprised wild accessions where morphologically intermediate plants were found (Fig. 4). Among these latter populations some accessions were more related to the cultivated sunflower lines. Intermediate plants were very variable and most were placed close to their original wild accessions.

## Discussion

*Helianthus petiolaris* is very widespread in the provinces of La Pampa, western Buenos Aires, eastern San Luis and southern Cordoba. At present, this is also the main region devoted to the sunflower crop, which has been displaced towards drier and poorer soils by soybean crop (de la Vega et al. 2007). Sunflower commercial seed in Argentina is of high purity and constantly gives rise to highly uniform plant stands.

Off-type plants found among progenies of pure species, originated from seed collected on typical

individuals belonging to each of the parental species, were first generation interspecific hybrids. They could be easily recognized by intermediate morphology among parental species, which were observed in single character, hybrid index, and multivariate analysis. Interspecific crosses involving other *Helianthus* species displayed a similar morphological pattern in hybrid plants (Vischi et al. 2002). Moreover, those findings show the occurrence of bidirectional gene flow between sunflower crop and its relative *H. petiolaris*.

The most appropriate traits for the diagnosis of interspecific hybridity were leaf characters, disc diameter and phyllary width, in agreement with the informative traits reported by Rosenthal et al. (2002). These traits allowed univocal differentiation of hybrids from the true type plants in the populations where they were found, both under crop-to-wild and wild-to-crop gene flow. The presence of red disc flowers among crop progenies was a clear evidence of wild introgression, given that disc flowers in cultivated sunflower are always yellow.

The seed that originated hybrid plants came from three different provinces, where the wild plants grow mostly in disturbed, sandy soils, under a rainfall and

temperature range (Cantamutto et al. 2008). Therefore, hybridization occurs in a wide environment array. A second wild annual species, *H. annuus* has established in Argentina, nevertheless it is not found in the regions involved in this study. Wild-crop *H. annuus* hybrids have been characterized and gene flow was quantified (Ureta et al. 2008).

Considering sunflower crop acreage of 1.7 million ha, 40,000 plants per ha, and a mean of 1,000 seeds per head,  $6.8 \times 10^{13}$  sunflower seeds are annually produced. A wild-crop hybridization rate of 0.5% would render over 100 thousand million hybrids if only one-third of crop acreage were sympatric with *H. petiolaris*. The observed hybrid frequencies suggest that hybridization between cultivated *H. annuus* and *H. petiolaris* could represent a very high number of first generation interspecific hybrids each year. A possible advanced hybrid generation of crop  $\times$  wild crosses would depend on losses during harvest and transportation, but even with few losses this would occur at a high frequency. None of the 159 individuals collected in *H. petiolaris* populations growing adjacent to cultivated sunflower fields in USA and Canada showed morphological indications of hybridization, but crop introgression was afterwards revealed by molecular markers (Rieseberg et al. 1999b). Although both species overlap in flowering time and pollinators, fertilization by intraspecific pollen is selectively favored, thus limiting the formation of hybrids (Rieseberg et al. 1995b). The analysis in Argentine zones where the two species grow in contact gave a similar ratio of first generation hybrids in both directions, with the wild-crop flow slightly superior. Higher hybridization frequencies (4–15%) have been found in natural wild hybrids zones with *H. annuus* as maternal parents (Rieseberg et al. 1998).

Pollen stainability for intermediate plants was significantly lower than those of the parental species and was taken as evidence of hybridization (Jan 1997). Seed set values were as low as those observed by Nikolova et al. (1998). Molecular analyses constitute a useful complement of morphological and fertility observations, considering that hybrid morphology and fertility are highly variable. Intermediate morphology is lost in successive generations and the wild phenotype and fertility are soon recovered (Rieseberg et al. 1996, 1999b).

Analysis of molecular variance showed a very high differentiation among *H. petiolaris* populations.

The populations exhibited enough morphological and molecular variability suggesting that the introduction of this species has not entailed a significant bottleneck (this study and Poverene et al. 2004). *H. petiolaris* has been used as a source of root rot resistance and male sterility (Luciano 1964). Even if there is no evidence, the hypothesis of an escape from experimental fields cannot be ruled out. Though the number of founder strains is unknown, it is evident that they became successful invaders. Unintentional introductions could occur once or only a few times but multiple intentional entries increase the probability of introducing additional genetic variability.

Cross pollination and self-incompatibility are likely to favor gene flow and homogeneity among populations. However, opposite forces like genetic drift due to patchy distribution and a limited number of plants in many populations would be operating. Invariant patterns and duplicated samples within populations can be attributed to inbreeding in fragmented populations. A variable degree of introgression from crop can lead to increased among-population divergence as it was evident in wild accessions where morphologically intermediate plants were found. Though all the wild populations described belong to the typical crop area, it is difficult to estimate accurately the time of wild-crop co-existence in each site.

Nevertheless RAPDs are dominant markers, the presence of a crop specific band within the pattern of a wild plant was considered as an additional proof of the plant's interspecific hybrid origin. These markers allowed identification of some of the intermediate plants as true hybrids. However, both parental species shared most bands.

Multivariate analysis based on morphological data differentiated species in definite groups but distances estimated with DNA markers were able to associate *H. petiolaris* individuals to a particular population, becoming a more discriminative data set, in comparison to morphological traits. *H. petiolaris* populations and hybrid plants showed high molecular variability compared with cultivated sunflower lines.

Crop introgressed marker frequency in *H. petiolaris* populations was 0.02, similar to that obtained by Rieseberg et al. (1999a) and much lower than that of wild *H. annuus* populations in similar situations (Arias and Rieseberg 1994; Linder et al. 1998). *H. annuus* and *H. petiolaris* differ in 11 chromosome

rearrangements, comprising eight translocations and three inversions (Burke et al. 2004). RAPD marker introgression was heavily reduced in rearranged chromosomes compared to collinear ones (Rieseberg et al. 1999b). Also interspecific pollen competition reduces hybridization frequency between both species. These strong reproductive barriers are incomplete since they do not prevent hybridization and introgression but delay gene movement among taxa.

Hybridization between *H. petiolaris* and sunflower crop has been detected on 11 out of 36 studied cases of flowering coincidence. Although the observed rates of interspecific crosses seem to be low, the extension of crop-wild overlapping in Argentina makes hybridization a noticeable process, annually producing several millions of first generation of interspecific hybrids. Therefore, diffusion of new sunflower varieties, i.e. IMI resistant cultivars, and eventually GM varieties would transfer their traits through pollen flow and they would persist in *H. petiolaris* populations. Having quantified hybridizing rates, fitness consequences on wild populations should be estimated. At present, a study on open-pollinated and backcross progenies of first generation hybrids is ongoing, comprising morphology and molecular traits. Fitness will be estimated through germination, flowering time, stainable pollen and seed set for second generation hybrids, backcrosses to *H. petiolaris* and pure *H. petiolaris* plants.

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