

# Natural hybrids between cultivated and wild sunflowers (*Helianthus* spp.) in Argentina

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**Abstract** Two introduced wild species *Helianthus annuus* L. and *H. petiolaris* Nutt. have become widespread in central Argentina and overlap the sunflower crop region. Intermediate off-type plants between the wild and cultivated species are often found, which is of concern because of the recent release of imidazolinone resistant varieties and the likely use of genetically modified sunflower cultivars. The progeny of 33 off-type plants obtained from 14 representative sites of the diffusion area were studied to confirm hybrid origin. Germination, survival, morphological traits and days to flowering confirmed hybridization between crop and both wild species, when compared to eight accessions of typical wild plants. Some progenies were presumably crop–wild *H. annuus* hybrids, some originated from the cross of cultivated plants and *H. petiolaris*, and two were the advanced generation of a cultivated hybrid. Hence,

morphological traits are a good clue for the identification of spontaneous hybrid plants at field. The results indicate that crop–wild hybridization and introgression occur at various places in central Argentina. This fact may represent a way to herbicide resistance escape and future transgene escape if GM sunflower cultivars are released for commercial use.

**Keywords** Fertility · Gene flow · *Helianthus* spp. · Morphology · Sunflower crop · Wild *Helianthus* spp.

## Introduction

Sunflower, *Helianthus annuus* L. is a traditional oil crop in Argentina positioned fourth in world production. In latter years, a remarkable increase of soybean crop displaced sunflower crop towards less adapted southwestern central plains (de la Vega et al. 2007) causing a decline in sunflower production. During the 2006/2007 season, sunflower acreage in Argentina fell to 2.45 million hectares, less than 50% of record area in the last decade. The new crop region greatly overlaps the distribution area of two wild *Helianthus* species which have become widespread through the country in the last 60 years (Covas 1966; Poverene et al. 2002). The use of new imidazolinone herbicide tolerant (IMI) varieties and genetically modified (GM) cultivars could place sunflower again as one

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of the main crops in Argentina. Nevertheless, the release of GM sunflower seems improbable in the next few years. GM soybean, maize and cotton have been commercially released in Argentina, but unlike sunflower, none has naturalized wild relatives. Diffusion of varieties carrying novel traits could modify wild *Helianthus* populations via gene flow. Environmental impact depends on the frequency of trait transference and on its ability to enhance growth and fertility by conferring selective advantages to wild plants (Hails and Morley 2005; Hoofman et al. 2005; Mercer et al. 2006).

The genus *Helianthus* (Asteraceae) is native to North America and comprises 51 annual and perennial species, which are diploids, tetraploids and hexaploids, with basic chromosome number of  $x = 17$  (Heiser 1978; Seiler and Rieseberg 1997; Jan and Seiler 2007). *H. annuus* L. and *H. petiolaris* Nutt. are annual diploid species naturalized over the central part of Argentina (Cantamutto et al. 2008). *H. petiolaris* is more abundant and its botanical description matches subspecies *petiolaris* (Heiser 1961). It grows on sandy soils forming extensive patchy populations. *H. annuus* displays a very variable morphology and corresponds to subspecies *annuus* (Heiser 1954). Both species are sympatric in several localities in the central part of the country and often invade sunflower, maize and soybean crops.

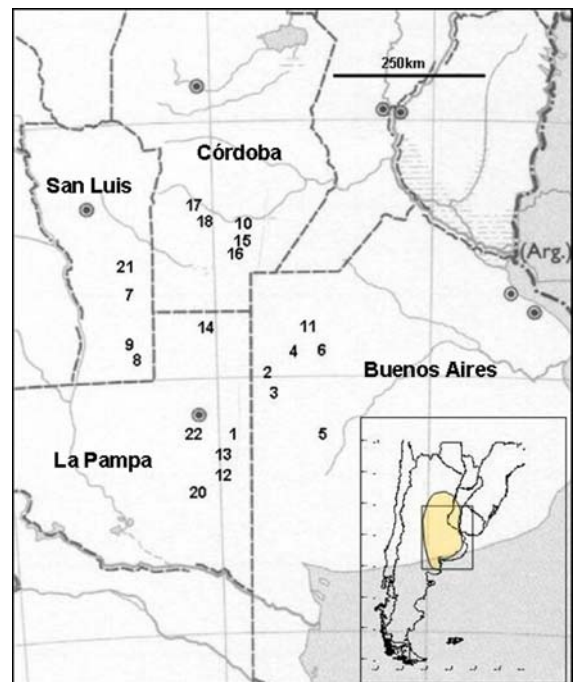
Gene flow among cultivated sunflower and both wild *Helianthus* species has been extensively studied in the center of origin (Arias and Rieseberg 1995; Whitton et al. 1997; Linder et al. 1998; Snow et al. 1998; Rieseberg et al. 1995, 1999a; Burke et al. 2002). In Argentina, Covas and Vargas López (1970) first described intermediate plants between cultivated sunflower and *H. petiolaris*, but there are no detailed studies on the natural occurrence of crop–wild introgression. Plants with intermediate morphological characters are often found in wild populations of both species, along roadsides and in cultivated fields. Those plants could originate from crosses between the cultivated sunflower and the wild species or may represent the advanced generations of a crop cultivar, namely volunteers (Reagon and Snow 2006). Alternatively, they could come from hybridization of both wild species. Morphological characterization of plants constitutes the first step to assess hybrid origin. Trait intermediacy and reduced fertility or fitness in progeny analysis are more reliable

indicators of interspecific crosses or wild–crop gene flow. Different classes of hybrids can be classified using morphological characters, if the areas where intermediate plants occur are considered as hybrid zones (Rieseberg and Carney 1998).

The goal of this study was to confirm hybridization processes between wild species and cultivated sunflower through phenotypic analysis of progenies of morphologically intermediate plants found in central Argentina. We hypothesize that if intermediate plants are in fact of hybrid origin, progeny tests would reveal a segregation of parental characters, a reduced fertility and/or fitness.

## Materials and methods

A number of off-type plants with intermediate morphology were found in different counties of four provinces (Fig. 1, Table 1). Thirty-three healthy plants were chosen for this study in 14 sampled sites: 31 plants were found in *H. petiolaris* populations or growing in rather isolated conditions, in small patches along roadsides. One plant was



**Fig. 1** Wild *Helianthus* sampling sites in four central provinces of Argentina, related to the sunflower crop region (shaded in detail). Numbers refer to Table 1

**Table 1** Off type plants and wild *Helianthus* accessions studied by progeny tests: population of origin, geographic site, and map reference to Fig. 1

Mother plant	Population	Province	County	Map #
<i>Off type plants</i>				
P1, P2	<i>H. petiolaris</i>	La Pampa	Atreucó	1
P3, P4	<i>H. petiolaris</i>	Buenos Aires	Salliqueló	2
P5, P6	<i>H. petiolaris</i>	Buenos Aires	Salliqueló	3
P7	<i>H. petiolaris</i>	Buenos Aires	T. Lauquen	4
P8 to P15	<i>H. petiolaris</i>	Buenos Aires	Guaminí	5
P16, P17	<i>H. petiolaris</i>	Buenos Aires	T. Lauquen	6
P18, P19	<i>H. petiolaris</i>	San Luis	G. Dupuy	7
P20	<i>H. petiolaris</i>	San Luis	G. Dupuy	8
P21	<i>H. petiolaris</i>	San Luis	G. Dupuy	9
A1	<i>H. annuus</i>	Córdoba	J. Celman	10
C1	Sunflower crop	Buenos Aires	T. Lauquen	11
I1, I2	Isolate <sup>a</sup>	La Pampa	Atreucó	12
I3, I4	Isolate <sup>a</sup>	La Pampa	Atreucó	13
I5 to I10	Isolate <sup>a</sup>	La Pampa	Realicó	14
<i>Wild accessions</i>				
WA1	<i>H. annuus</i>	Córdoba	J. Celman	15
WA2	<i>H. annuus</i>	Córdoba	J. Celman	16
WA3	<i>H. annuus</i>	Córdoba	Río Cuarto	17
WA4	<i>H. annuus</i>	Córdoba	J. Celman	19
WP1	<i>H. petiolaris</i>	Buenos Aires	Tres Lomas	20
WP2	<i>H. petiolaris</i>	La Pampa	Utracán	21
WP3	<i>H. petiolaris</i>	San Luis	G. Dupuy	22
WP4	<i>H. petiolaris</i>	La Pampa	Capital	23

Off-type plants were identified according to the population where they were found P for *H. petiolaris*, A for *H. annuus*, C for crop, I for isolate plants. Seed was bulk collected in wild populations, WA for *H. annuus* and WP for *H. petiolaris*

<sup>a</sup> Growing in small patches along roadsides

collected in a cultivated field and another was found in a wild *H. annuus* population. They were representative of many others in these populations, with a phenotype that made them conspicuous among the surrounding plants. Field assays were established with seeds of one to three heads of the 33 off-type plants, a bulk seed sample of wild *H. annuus* and *H. petiolaris* from eight localities, and a sunflower commercial hybrid (DK H3881). The progeny of each off-type plant is described as a family.

Seed of the 33 off-type plants and of eight wild accessions (Table 1) were germinated in Petri dishes in 1 mM gibberellic acid to break dormancy (Seiler 1998), then transferred to a sand and peat mix (3:1 w/w) in the greenhouse. When the seedlings reached about 10 cm height they were transplanted into 9 m

length plots randomly in the field. Each plot comprised a family of 30 plants spaced 0.30 m and distance between plots was 1.4 m. Irrigation and weed control were performed weekly to ensure plant growth.

Germination in Petri dishes was recorded, except for plants P18 and P19, which were sown directly in the field plots, because they were included later in the experiment. After transplanting to the field, the following traits were recorded for every plant in each plot: Seedling survival (%); plant height (m, recorded in intervals); branching (0–4 from no branching to fully branched from the base according to Luczkiewicz 1975); anthocyanin presence in stems and petioles (yes/no); leaf type (*annuus*, *petiolaris*, intermediate); days from transplant to flowering. Leaf morphology was cordate or subcordate with serrate

margins (classified as *annuus*-type), wide or narrow triangular-shaped with cuneate base (classified as *petiolaris*-type) or intermediate forms. The following traits were recorded in three heads per plant: bract (phylary) width (cm); disc diameter (cm); disc color (yellow, purple); seed color (gray, brown, gray and brown, others); seed design (complete, stripes, mottled, both stripes and mottled); seed pubescence (from 1 = glabrous to 4 = very pubescent); seed length (mm); pollen viability (%); and seed set (%). Survival was recorded when plants reached the reproductive stage and completed life cycle, being 100% if the 30 plants transplanted per plot survived. Pollen viability was assessed by differential staining (Alexander 1980). Heads at anthesis were shaken over a clean slide to collect fresh pollen, then a staining drop was added and at least 300 pollen grains were counted on each slide. Three slides were fixed per head. Seed set was determined as the proportion of the filled seed per head over the total number of disk florets per head. Traits of continuous variation were analyzed by principal components analysis (PCA) of character  $\times$  character correlations. Means per plot and standard deviations were graphically represented. A hybrid index was calculated based on the numerical scores of the categorical traits branching type (0–4), anthocyanin presence (0–1), disc color (1–2), and leaf type (1–3). The index of each plant was the sum of scores for the four traits. The highest score was assigned to the wild taxa and the lowest score corresponded to the cultivated genotype. Hybrid indexes were graphically represented as histograms. A consensus graphic was achieved by Procrustes analysis of metric and categorical variables (Gower 1975). Multivariate analyses were performed using the InfoStat (2006) program.

## Results

Most mother plant heads had a high proportion of aborted seeds. Germination ranged from 2% to 60% among families, with some plants showing a slow development. Families I3, P2, P10 and P15 failed to survive in the field and only one plant survived among progeny of P19. Weakness or premature death was observed in families I2, I4, P1, P8, P9 and P14. Dwarfism or fasciation was observed in families P5, P6, P8, P11, P12 and P13. Many surviving plants

among these progenies produced an early head with abnormal rays and bracts and died before anthesis. Mother plants of all these families were off-type plants found within *H. petiolaris* populations or isolate in fields where this species has established (Table 1) and sunflower cropping is widespread, thus presumably interspecific hybrids.

Germination in wild accessions was slow reaching 50–60% in *H. annuus* and 40–50% in *H. petiolaris*. The surviving families showed within-plot segregation of several traits. Healthy plant height varied from less than 0.5 m to almost 3 m. Table 2 shows mean values per family in germination, plant survival and plant height.

A lower variation for metric characters as compared with families was observed in the wild accessions (Fig. 2). Disc diameter, bract width, seed size and days from transplant to flowering in families were almost always intermediate between wild and cultivated sunflower. Families P1, I4, P20 and P21, showed a shorter disc diameter than wild accessions and high sterility. Low pollen viability and seed set indicated reduced fertility in families P1, I1, I3, and P20. Families A1 and P14 had very high fertility, while families I5, I6, I8, I9, and I10 were almost as fertile as wild accessions (Fig. 3). Mother plants I5–I10 were found in fields where wild *H. annuus* has established (Table 1) so except P14, those plants were presumably wild-crop *H. annuus* intraspecific hybrids. In one plant of each family P16, I5, and I6 male sterility was observed.

The principal component analysis based on metric traits showed good separation along the first component. Off-type families showed an intermediate position between the extremes wild *H. annuus* (WA1–4), *H. petiolaris* (WP1–4) and cultivated strain (Cu). Disc diameter and bract width largely determined this first component. The second component segregated *H. petiolaris* hybrids (P1–P21, I1–I4) from *H. annuus* hybrids (I5–I10, A1, C1) mainly based on seed set and days to flowering (Fig. 4).

Branching type, leaf shape, anthocyanin presence, disc color and seed appearance (color, design and pubescence) were different in families from those in wild species accessions. Wild accessions presented branches on the whole plant, without a main head, except for some *H. annuus* plants which had upper branching (type 2) or a main head (type 3). Types 0, 2 and 3 are present in cultivated sunflower, whereas 1 and 4 are typical of wild forms. Most families segregated different branching types.

**Table 2** Germination, survival, and plant height in 29 off-type families and eight wild *Helianthus* accessions

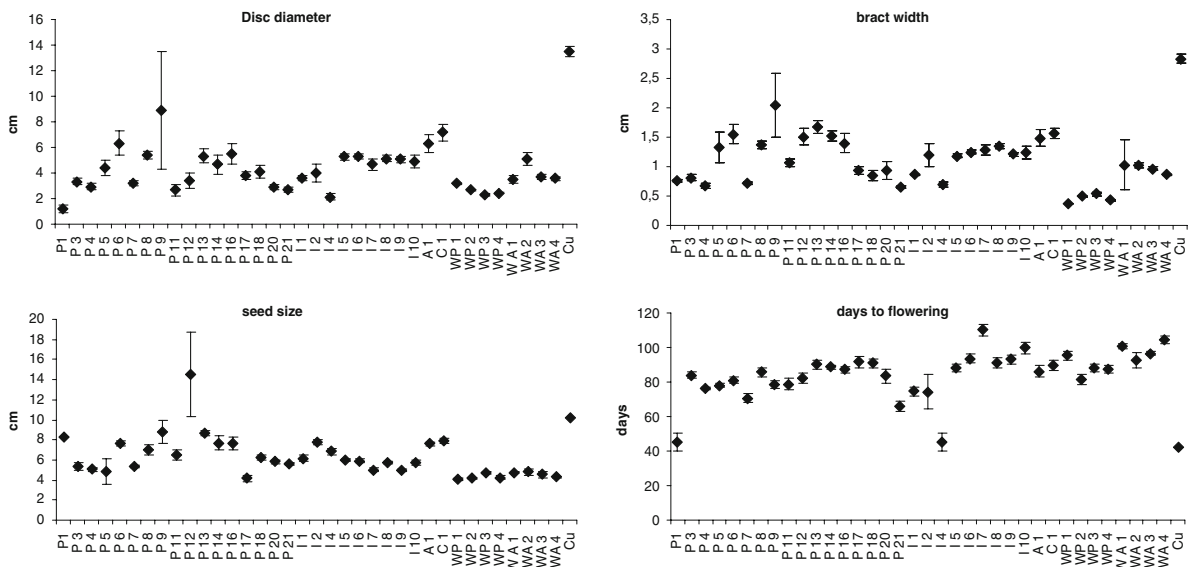
Family	Germination, %	Survival, %	Plant height, m	Family/wild accession	Germination, %	Survival, %	Plant height, m
P1	20	3	<0.5	I1	50	23	0.5–1
P3	5	100	1.5–2	I2	45	10	0.5–1
P4	2	92	1.5–2	I4	2	11	0.5–1
P5	10	78	0.5–1	I5	40	86	1–1.5
P6	10	100	0.5–1	I6	50	100	1.5–2
P7	2	97	1–1.5	I7	20	81	0.5–1
P8	5	100	1–2	I8	45	100	0.5–2
P9	2	56	0.5–1	I9	45	95	1–2
P11	30	75	<0.5	I10	55	60	<0.5
P12	10	90	<0.5	A1	40	92	0.5–2
P13	30	90	0.5–1	C1	60	85	1.5–2.5
P14	30	75	0.5–1	WA1	50–60	96	2–3
P16	5	81	1–1.5	WA2	50–60	83	2–3
P17	10	93	0.5–1.5	WA3	50–60	78	2–3
P18	nd <sup>a</sup>	100	1–1.5	WA4	50–60	96	2–3
P19	nd	One plant	1.5–2	WP1	40–50	100	1.5
P20	20	96	0.5–1	WP2	40–50	82	1–1.5
P21	8	100	0.5–1	WP3	40–50	100	1–1.5
				WP4	40–50	100	1–1.5

Identification numbers as in Table 1

<sup>a</sup> No data

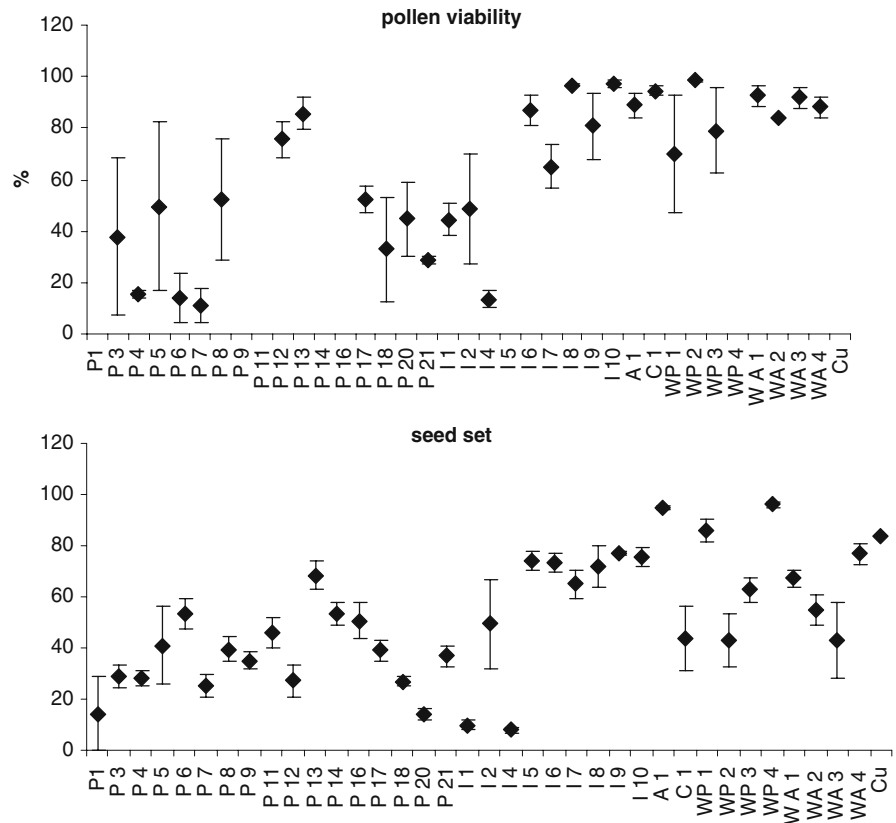
Segregation of leaf types was frequent among progenies within a plot, but one type often predominated. Most wild *H. annuus* and *H. petiolaris* plants

showed red-purple stems and petioles. Among families, three presented anthocyanins in every plant, five presented no colored plant, and the

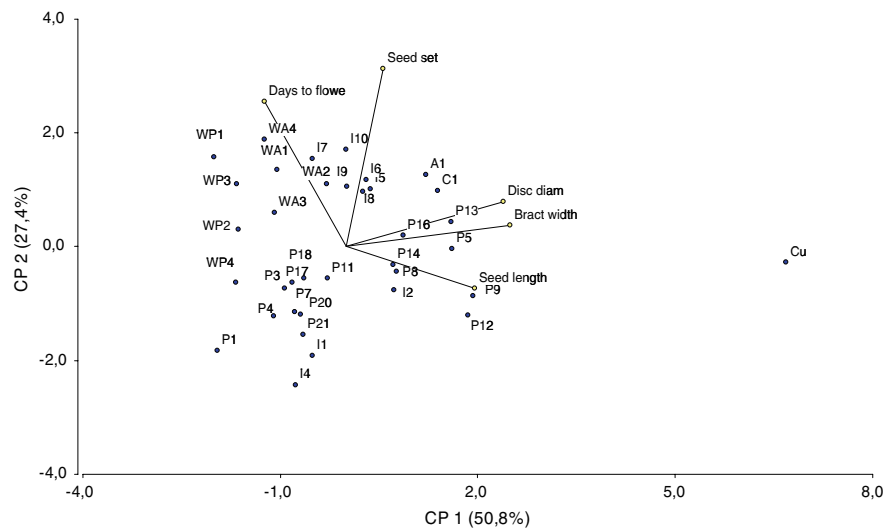


**Fig. 2** Phenotypic metric traits (mean ± SD) in off-type plant families, wild accessions *H. petiolaris*, *H. annuus*, and a representative sunflower cultivar DK3881. Identification numbers as in Table 1

**Fig. 3** Fertility in off-type plant families, *H. petiolaris* and *H. annuus* accessions, and the sunflower cultivar DK3881. Identification numbers as in Table 1



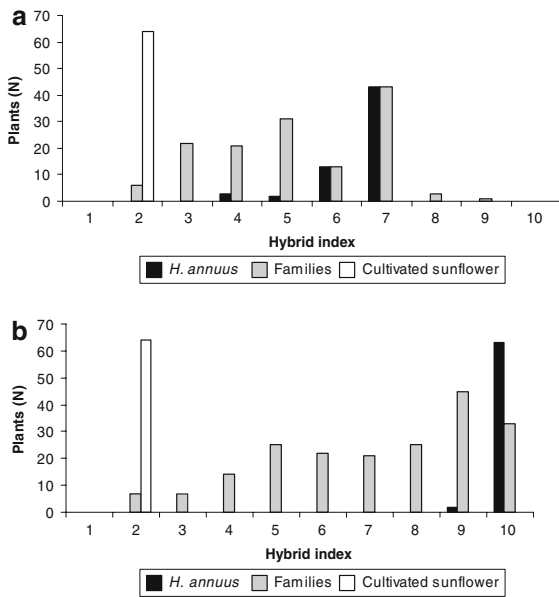
**Fig. 4** Principal component analysis in 29 off-type families, eight wild accessions and a sunflower cultivar. Identification numbers as in Table 1. Two first CP explain over 75% of variability



remaining segregated by anthocyanin presence or absence.

*Helianthus petiolaris* accessions showed an intense purple red color in disc florets, while wild *H. annuus* also had red discs, with less pigment on the corolla lobes. Yellow discs with no anthocyanin-

pigmented florets and black tubular corolla are typical of cultivated sunflower. Among off-type families, seven had yellow discs in every plant; eight had plants with anthocyanin-pigmented lobes and 13 segregated by yellow and red colored discs. Black color in the tubular corolla was not observed, the

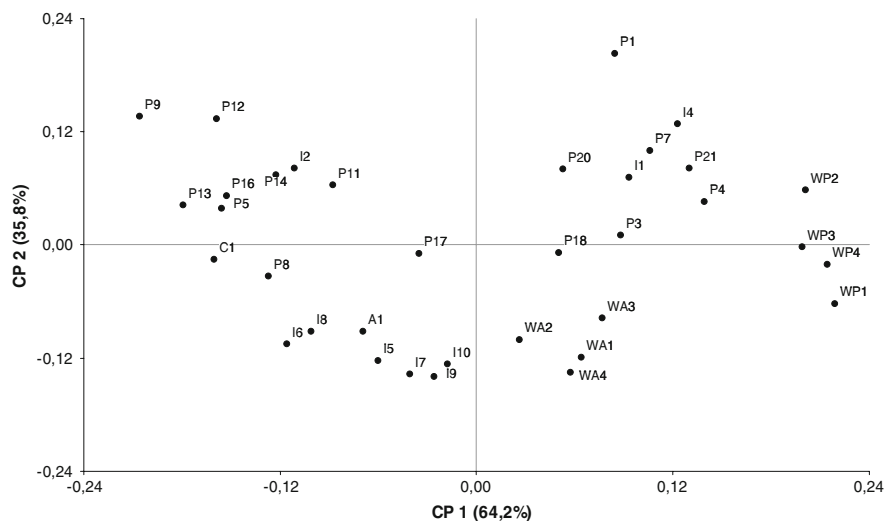


**Fig. 5** Hybrid index based on categorical traits of putative parent species plants and progenies of 29 off-type plants. (a) Wild parent species *H. annuus*. (b) Wild parent species *H. petiolaris*. In both figures, white bars represent cultivated plants, black bars represent typical wild plants, gray bars represent off-type plant progenies

entire corolla being yellow in those yellow-disc plants. Ray flower petals were always yellow in wild accessions and progenies. Anthocyanin pigments in flowers and vegetative parts were associated and both traits segregated in 12 families.

Seed appearance was very variable. In wild accessions, seeds were mostly gray or brown. Off-type

**Fig. 6** Procrustes generalized analysis from metric and categorical data matrix of families and wild sunflower accessions. Identification numbers as in Table 1



families showed black, olive and white seeds as well. Regardless of the color, epidermis was mottled in *H. petiolaris* accessions and presented mottling or stripes in *H. annuus* accessions, whereas families varied from completely colored, mottled, stripes, or both, and many segregated the various types of epidermis. *H. petiolaris* accessions presented a typical dense pubescence, less dense in wild *H. annuus* accessions and progenies intergraded from dense pubescence to almost glabrous, like the cultivated sunflower.

Hybrid index based on four categorical traits showed that most progenies of intermediate morphology had intermediate scores between the extreme parental phenotypes. All plants were graphically represented, including wild and cultivated pure species (Fig. 5).

When metric and categorical traits were combined by Procrustes analysis, off-type families showed an intermediate distribution between wild and cultivated sunflower (Fig. 6) similar to that observed in hybrid index results. The first two axes explained 81% of the variability contained in all the traits. ANOVA showed 82.4% consensus among metric and categorical traits. Families were clustered according to their collection site.

**Discussion**

Wild–crop hybridization can influence the evolutionary ecology of related wild or weedy taxa, especially when they co-occur, have overlapping flowering

periods and share pollinators (Snow et al. 1998). A very conspicuous phenotypic variation characterized most families studied derived from off-type plants as compared with pure wild *H. annuus* and *H. petiolaris* accessions growing in the same conditions. Segregation of categorical traits was considered as evidence of the mother plant hybrid origin, although some variation was expected within wild accessions because the two species are self-incompatible outcrossers. Wild-crop hybridization is frequent in Argentina because of the extensive overlapping among wild *Helianthus* distribution and sunflower crop acreage (Poverene et al. 2004; Ureta et al. 2008, unpublished data).

Poor germination in bulk seeds of wild accessions was ascribed to the typical dormancy of the wild species (Seiler 1998). Most off-type plant seeds germinated earlier, but there was a high failure due to seed inviability, as expected in interspecific hybrid progenies. A reduced dormancy was found in wild-crop hybrids of sunflower by Snow et al. (1998). Many families (i.e. P1-2-8-9-10-14-15, I2-3-4) showed plants with severe delay in development, dwarfism, weakness or premature death which would be a consequence of chromosome or genic imbalance in progenies from interspecific crosses between *H. annuus* and *H. petiolaris* (Rieseberg et al. 1995).

Variability in plant height was within expectable limits, for this trait is a quantitatively inherited character, from 0.2 m to more than 1 m in *H. petiolaris* and 1–3 m in *H. annuus*. Given that height is very variable among commercial hybrids—the putative cultivated parents—no statistical comparisons were attempted.

Variation in metric traits was also higher within and among off-type families compared to wild species. This was first described by Heiser (1947) in natural and artificial hybrids between *H. annuus* and *H. petiolaris*, and other interspecific hybrids in the same genus (Nikolova et al. 1998). Disc diameter, bract width and seed size were good indicators of hybridization as stated by Heiser (1947) and Ferreira (1980) who compared natural and artificial hybrids between these two species. Similar results are shown in Fig. 2.

Progenies of off-type plants may show both parental and intermediate morphological characters (Rieseberg and Carney 1998). The life cycle, represented by days to flowering, was mostly intermediate in families. The two which had a very short cycle

were probably early generation hybrids between *H. petiolaris* and cultivated sunflower; while I6 showed a very long cycle and seemed a *H. annuus* wild-crop hybrid. Life cycle was somewhat distorted because wild accessions had a later emergency compared to families. However, data are valid for comparative purposes. The late flowering of family I7 suggests that life cycle could be transgressive in wild-crop hybrids. If hybrid plants flowered earlier than wild ones, they would primarily intercross originating advanced generation hybrids, not backcrossing progenies. Transgressive hybrids would have an opportunity to establish in slightly different habitats or ecological niches (Lexer et al. 2003). Nevertheless, overlapping life cycles of wild and hybrid plants often occur, as in the sites where mother plants were collected.

Very reduced fertility in some families suggests that mother plants were early generation interspecific hybrids. Recovery of fertility occurs within few generations after hybridization (Heiser 1947; Rieseberg et al. 1999b). Families I5, I6, I8, I9, and I10 with comparatively high fertility may represent advanced hybrid generations or backcrosses to the wild parent, *H. petiolaris*. Higher fertility is expected in wild-cultivated *H. annuus* hybrids, where no chromosome barriers to gene flow occur and crop alleles persist in wild populations for many generations after hybridization (Whitton et al. 1997). Male sterility probably came from cultivated × wild crosses, through volunteers (Reagon and Snow 2006).

Branching has a complex inheritance mode (Luczkiewicz 1975; Miller and Fick 1997). Upper branching or a central head in some families and in wild *H. annuus* plants pointed to gene flow from cultivated sunflower. Intermediate leaf shape and anthocyanin presence was frequent within families. Anthocyanin pigment in stems and leaves is usually dominant and very common in wild accessions. Variation and segregation of these traits among families were considered as evidence of crop hybridization and introgression (Bervillé et al. 2005). Variability in seed coat color and pubescence was also very high. Stripes is a dominant trait characteristic of many old sunflower varieties in Argentina and was present in 25 out of 29 analyzed families.

Wild *H. annuus* populations showed some variation in disc, bract and seed size, and crop traits were observed in two accessions collected in an area



traditionally devoted to sunflower and exposed for many years to crop gene flow. The lower variation observed in *H. petiolaris* accessions, as compared with *H. annuus*, agrees with the species' reports in North America (Heiser 1954, 1961).

Based on seed viability, survival, collection site and morphology, off-type families were assigned to specific wild–crop crosses. There were four family classes according to the pattern of variation among plants. The first was characterized by poor germination, low viability and development abnormalities. In progenies P1, I2, I4, P9, P14 a high plant mortality was observed, with complete loss of families of P2, I3, P10 and P15. Most of the surviving ones died before anthesis or developed abnormally, showing dwarfism or fasciation in families P5, P11, P12, and P13. As all these families originated from *annuus*-like or intermediate plants growing among typical *H. petiolaris*, the mother plants were probably the first generation of an interspecific cross and the abnormalities arose as a consequence of genetic differences between parental species.

The second class showed germination ranging from 2% to 50%, a high phenotypic variation among plants, and sterility. Families I1, P3, P4, P6, P7, P8, P20, and P21 segregated in *petiolaris*-like and *annuus*-like plants, while P18 and P19 showed an intermediate plant type. Their mother plants were mainly of intermediate type growing in *H. petiolaris* populations and may represent second generation hybrids or backcrosses to *H. petiolaris*.

The third family class showed germination between 45% and 60% (except for one having 20%). Families A1, P17, C1, I5–I9 had more restricted variability to *annuus*-like plants, and sterility was not apparent. Mother plants resembled somewhat atypical wild *H. annuus* and probably originated from crosses between wild and cultivated *H. annuus*. The most common situation is fertilization of wild plants by cultivated pollen donors, the reciprocal cross is seldom found in seed production fields (Reagon and Snow 2006). As C1 was representative of a number of similar plants planted in the crop rows, it is possible that they were a consequence of wild pollen contamination in the seed production field.

The last class comprised families P16 and I10. They germinated well and gave a rather uniform progeny, showing characteristic domesticated traits, some plants had male sterility and poor seed set. This class may represent progenies of a volunteer

sunflower and was not the result of a wild-cultivated cross. Volunteers and their progenies are very common along roadsides and railways.

Consensus analysis of metric and categorical traits confirmed these family arrays, grouping each family in a different quadrant, while wild pure species formed two separate clusters. Hybrid index graphically demonstrated that most progenies of the presumably hybrid plants were intermediate between cultivated sunflower and wild species, though this method gives the same hybrid index score to phenotypically different plants (Briggs and Walters 1997) and does not allow differentiation among families.

Partial germination, survival and seed set in the first and second classes show that hybridization and introgression occur between the two species, in spite of chromosomal and genic barriers (Rieseberg et al. 1995, 1999a). Patterns of introgression are similar in natural hybrid zones and in experimental lines, and also in geographically distant zones (Rieseberg et al. 1999b). Natural hybridization between wild *H. annuus* and *H. petiolaris* in North America has originated three homoploid species (Rieseberg 1991; Rieseberg et al. 1990). Environmental variation and natural selection play an important role in this kind of speciation, and geographically isolated populations might speciate in parallel. Species recently introduced into a new habitat which quickly colonize extensive areas would probably spread on similar soils, occupy similar environments, and hybridize with closely related taxa. However, all sampled off-type plants were found in pure species stands and there were no evidences of both wild species growing together at the same sites. Thus hybridization between wild *Helianthus* species was discarded as a probable origin of intermediate plants.

Argentina is the second country following the US in GM crop production (over 18 million hectares) and 84 field trials for GM sunflower varieties have been approved. Our results provide enough evidence to show that sunflower wild–crop hybridization frequently takes place at several sites within the cultivated area in Argentina. Imidazolinone tolerance and eventually other novel traits will certainly be transmitted to wild *Helianthus* populations via pollen movement in the next few years, as reported by Al-Khatib et al. (1998) and Massinga et al. (2003). One concern about crop–wild gene flow is that intermediate plants are often found in western Buenos Aires

and La Pampa provinces, especially in those counties of high sunflower production. However, Burke et al. (2002) have pointed out that research should focus on the fitness consequences of the particular gene that is transferred to wild populations, rather than on the rate of hybridization.

The results demonstrate that hybridization occurs throughout the distribution range of wild *Helianthus* species within the sunflower cultivation area. Both first generation hybrids and advanced generation hybrids, or backcrosses could be found in the same site showing that crop–wild hybridization and introgression are recurrent processes.

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