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Assessment of Mating System in *Helianthus annuus* and *H. petiolaris* (Asteraceae) Populations

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Abstract: *Helianthus annuus* subsp. *annuus* and *H. petiolaris* are wild North American species that have been naturalized in central Argentina. They have a sporophytic self-incompatibility genetic system that prevent self-fertilization but the occurrence of self-compatible plants in Argentina was observed in both species and could in part explain their highly invasive ability. Their geographical distribution coincides with the major crop area. The domestic sunflower is self-compatible, can hybridize with both species and presents a considerable amount of gene flow. The aim of this study is to understand the self-incompatibility mechanism in both wild *Helianthus* species. Reciprocal crossing and seed production were used to identify self-compatible genotypes, the number and distribution of self-incompatibility alleles within populations and the type and extent of allelic interactions in the pollen and pistil. The behaviour of S alleles within each population was explained by five functional S alleles and one non-functional allele in each species, differing in their presence and frequency within accessions. In both species, the allelic interactions were of dominance/recessiveness and codominance in pollen, whereas it was only codominance in the pistil. Inbreeding effects in wild materials appeared in the third generation of self-pollination, with lethal effects in most plants. The number of S alleles is low and they behave in a similar way of other Asteraceae species. The self-compatibility was addressed to non-functional S alleles introgressed in wild *Helianthus* plants through gene flow from self-compatible sunflower.

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Introduction

Self-incompatibility (SI) in angiosperms is the inability of a hermaphroditic plant to produce viable seeds through self-pollination; however, it produces viable gametes (Castric and Vekemans, 2004). Pollen grains reaching the stigmata of the same plant are unable to fertilize its ovules because pollen tube growth is eventually interrupted. Consequently, no seeds are produced via self-pollination. This arrests growth or development of pollen tubes within the pistils involves the recognition by the pistil of its own pollen or foreign. Most SI systems have a simple genetic control based on a single multiallelic S locus. The S locus usually consists of a gene that expresses in the pistil (female determinant) and in pollen (male determinant) that forms a recombinant S haplotype (S allele). The products of pistil and pollen of the same S allele recognize each other and interact specifically to start the inhibitory response of pollen that prevents fertilization (Hiscock and McInnis, 2003). Self-incompatibility systems comprise two groups: gametophytic self-incompatibility (GSI), in which the pollen phenotype is determined by its own haploid genotype, that being the genetic constitution of the gametophyte; and sporophytic self-incompatibility (SSI), in which the phenotype of the pollen grains is determined by the genotype of the anther (diploid sporophyte) where they originated (De Nettancourt, 2001). Functional GSI requires strict codominance among alleles, while SSI systems usually have some dominance hierarchy. In the later, homozygous plants for recessive S alleles can arise because of dominant interactions, being more permissive than GSI systems where homozygosis is not possible (Brennan *et al.*, 2002). The number and distribution of S alleles and dominant/codominance interactions in SSI systems include different ranks (Brennan *et al.*, 2011; Geleta and Bryngelsson, 2010). The presence of functional S alleles (self-incompatibility alleles) and non-functional alleles (self-compatibility alleles) have been recorded in some species (Geleta and Bryngelsson, 2010). In *Helianthus*, loci that govern self-incompatibility and self-pollination have been discovered and it was demonstrated that wild alleles diminish self-pollination. This constitutes the most common problem in the maintenance of wild sunflower collections (Atlagic and Terzic, 2016). However, the number and distribution of S-locus variants is unknown in sunflower (Gandhi *et al.*, 2005).

Helianthus annuus L. subsp *annuus* and *H. petiolaris* Nutt. (Asteraceae) are wild North American species that have been naturalized in central Argentina (Cabrera, 1963; Covas, 1966). They both have SSI genetic systems that prevent self-fertilization while domesticated sunflower (*Helianthus annuus* L. var. *macrocarpus* (DC.) Cockerell) can self-fertilize due to self-compatibility originated from artificial selection (Gandhi *et al.*, 2005). The geographical distribution in Argentina of both wild species coincides with the major crop area. The domestic sunflower can hybridize with both wild species and presents a considerable amount of gene flow (Gutierrez *et al.*, 2010; Linder *et al.*, 1998; Rieseberg *et al.*, 1999; Ureta *et al.*, 2008). Consequently, crop genes are likely transferred to wild plants (Gutierrez *et al.*, 2010).

The eco-geographic distribution of wild *Helianthus* populations in Argentina was described by Poverene *et al.* (2008). *H. annuus* populations are widespread from 31°58′ to 37°31´S, and 60°33′ to 68°14′W. Plants grow in patches in disturbed habitats such as roadsides, ditches, and field margins in the sunflower production areas. Population size vary from a few dozen to more than 100,000 plants, with mean densities between 0.25 and 6 plants.m^{-1} but some populations reach 80 plants.m^{-1}. *H. petiolaris* range from 35°08′ to 38°08'S, and 62°16′ and 65°56′W, with dense populations reaching 18,000 plants with a mean density of 0.25–6 plants.m^{-2}, and up to 40 plants.m^{-2}. Self-compatible plants were occasionally found in different accessions of both species when cultivated in the experimental field, and the trait was inherited to their progeny (Gutierrez *et al.*, 2014). Self-incompatibility is usually a quantitative and phenotypically plastic trait. Many species exhibit marked phenotypic variation in the expression of SI that is often influenced by environmental conditions (Stephenson *et al.*, 2000). Self-compatible populations could originate by natural selection during the invasion process of a species, derived from self-incompatible populations. Therefore, individuals of varying self-compatibility could originate in self-incompatible populations through mate system selection (Petanidou *et al.*, 2012). The occurrence of self-compatible plants in Argentina was demonstrated in both species (Gutierrez *et al.*, 2014) that could partially explain the high invasive ability of those annual *Helianthus* species. Moreover, the breakdown of self-incompatibility provides a useful opportunity to study and understand the SSI mechanism. The aim of this work was (1) to verify self-compatibility in progenies of different naturalized populations of the two annual *Helianthus* species, (2) to assess the number and distribution of S alleles within populations, and (3) to determine the type of allelic interactions in the pollen and pistil.

Materials and methods

Two experiments were performed in the field at the Agronomy Department in Bahia Blanca (S38.71°, W62.26°) between 2015 and 2017. During the first period (summer 2015–2016) controlled crosses were carried out between progenies of accessions that have previously shown self-compatibility (Gutierrez *et al.*, 2014). Those accessions belonged to five *H. annuus* subsp. *annuus* and five *H. petiolaris* populations from different geographical regions of Argentina (Table 1, Figure 1).

Table 1: Origin of the wild accessions used in the study.

Species	Locality (CODE)	Province	Location
Helianthus petiolaris	Catriló (CAT)	La Pampa	S 36.56° W 63.55°
	Colonia Baron (BAR)	La Pampa	S 36.17° W 63.62°
	Ataliva Roca (ROC)	La Pampa	S 36.89° W 64.28°
	Padre Buodo (BUO)	La Pampa	S 37.30° W 64.28°
	Alpachiri (ALP)	La Pampa	S 37.37° W 63.76°
Helianthus annuus	Rancul (RAN)	La Pampa	S 35.07° W 64.77°
	Colonia Barón (BAR)	La Pampa	S 36.17° W 63.87°
	Diamante (DIA)	Entre Ríos	S 36.05° W 60.64°
	Media Agua (MAG)	San Juan	S 31.95° W 63.45°
	Las Malvinas (LMA)	Mendoza	S 34.92° W 68.23°

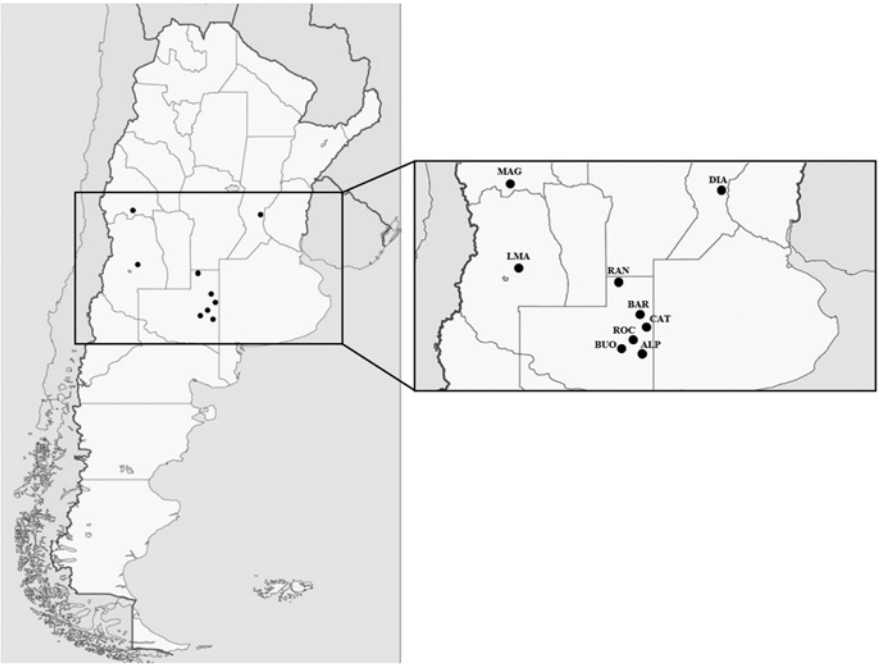


Figure 1: Geographic distribution of studied populations of *Helianthus annuus* and *H. petiolaris* in central Argentina. For labels see Table 1.

Wild seeds were stored at 4 °C for one week to break dormancy. Seeds were sown in multicell plastic trays in a greenhouse and were allowed to grow with adequate watering until the seedlings had 4–6 leaves. Then, they were transplanted to the experimental field into rows 0.70 m apart with a distance of 0.20 m between plants. A drip irrigation system was used and weed control was manual. Reciprocal crossing schemes for the first experimental field (summer 2015–2016) were made according to the number of plants within each population (Figure 2). At the emergence of the ray flowers or in the previous reproductive stage (R3, according to the growth stages of Schneiter and Miller, 1981), three heads per plant of each accession were bagged to prevent the access of foreign pollen: one head was allowed to self-fertilize and two were manually fertilized with pollen from the plants chosen as male parents (Figures 2a–d). All

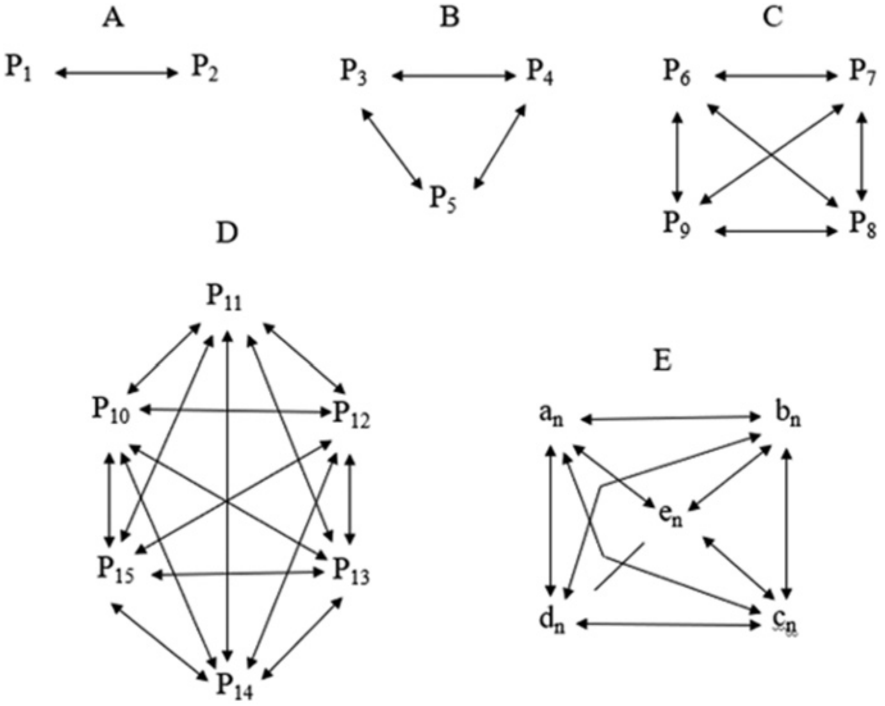


Figure 2: Schematic representation of the 2015–2016 crossing experiment in *H. petiolaris* and *H. annuus* populations according to the number of available plants (P). (A) ROC, BUO and BAR populations; (B) ALP, BAR, RAN and MAG populations; (C) CAT and DIA populations; (D) LMA population. Also, every plant in each scheme was self-pollinated. (E) a, b, c, d, e represents the five populations and n represent the random chosen plants per population in each species.

flowers were emasculated before RCs. In order to pollinate all the disk flowers, pollinations were made 3–4 times during the flowering period. For each species, 10 additional reciprocal crosses were carried out among individuals chosen at random within each population, in order to create relationships between the populations (Figure 2e).

From a total of 32 individuals (14 for *H. petiolaris* + 18 for *H. annuus*), 136 reciprocal crosses (RC) were made: 42 intra-population RCs + 10 inter-population RCs in *H. petiolaris*, and 74 intra-population RCs + 10 inter-population RCs in *H. annuus*. The progeny of each crosses and self-pollinations was classified according to seed production as incompatible, compatible or indeterminate, and as self-incompatible or self-compatible, according to Brennan *et al.* (2002) (Table 2).

Table 2: Offspring classification in self-compatible or self-incompatible and compatible or incompatible according to seed set in both crosses (selfed and crossed). Last column shows the symbols that were used in the diallel table.

Number of seeds per head	Classification when selfed	Classification when crossed	Symbol in diallel table
0–2	self-incompatible	incompatible	–
> 2–< 10	self-compatible	indeterminate	±
≥ 10	self-compatible	compatible	+

In the next generation (summer 2016–2017), the RCs involved plants produced from compatible crosses in the previous generation (summer 2015–2016). Likewise, during this second experiment, progenies of two populations of *H. annuus* (LMA and MAG) and three populations of *H. petiolaris* (CAT, BUO and ROC) were used for RCs (Tables 3 and 6, asterisks). Seed germination and seedling transplanting to the field were conducted as before. In the second experiment, the evaluated plants were progenies of two consecutive generations of self-pollination, a condition that favoured inbreeding effects and the loss of several individuals within each accession. Therefore, RCs schemes were made according to the number of surviving plants within each accession (Figure 3). Bagged heads, pollinations, and offspring classification were accomplished as in the previous experiment.

Based on previous studies in Asteraceae (Brennan *et al.*, 2002, 2011, 2013; Gandhi *et al.*, 2005; Geleta and Bryngelsson, 2010) and following the hypothesis of a single S-locus with multiple alleles being responsible for SSI in wild *Helianthus*, we proposed a scheme for the assignment of S genotypes to each plant. S-allelle interactions (dominant, recessive, or codominant) in pollen and pistil were assessed according to seed set within each accession, and the

Table 3: Crosses within *Helianthus petiolaris* accessions. Number of plants, seed set and S-genotypes of crosses in the first experiment (2015–2016). + denotes fully compatible cross; ± partially compatible cross; – incompatible cross (See Table 2). Bold crosses show self-compatibility and asterisk show the seeds used to perform controlled crosses in the second experiment. ♀, pollen recipient; ♂, pollen donor. > indicates dominance.

Plant	♀	**CAT$_1$**	**CAT$_2$**	**CAT$_3$**	**CAT$_4$**
♂	genotype	S_2S_4	S_3S_4	S_0S_0	S_5S_4
CAT$_1$	$S_2S_4(S_2>S_4)$	−0	+10	+17	+18
CAT$_2$	$S_4S_3(S_4>S_3)$	−2	−2	+43	−1
CAT$_3$	S_0S_0	+12*	+11*	**+47***	+63*
CAT$_4$	$S_5S_4(S_4>S_5)$	−0	−1	+31	−0

Plant	♀	**BUO$_1$**	**BUO$_2$**
♂	genotype	S_0S_0	S_0S_5
BUO$_1$	S_0S_0	**+25***	+47
BUO$_2$	S_0S_5	+10	+8*

Plant	♀	**ROC1**	**ROC$_2$**
♂	genotype	S_0S_5	S_3S_4
ROC$_1$	S_0S_5	+6*	±5*
ROC$_2$	S_1S_5	±5	−0

Plant	♀	**BAR$_1$**	**BAR$_2$**	**BAR$_3$**
♂	genotype	S_2S_3	S_2S_3	S_3S_4
BAR$_1$	S_2S_3	−0	−2	±3
BAR$_2$	$S_2S_3(S_2>S_3)$	−0	−0	+55
BAR$_3$	$S_3S_4(S_4>S_5)$	+12	+50	−0

Plant	♀	**ALP$_1$**	**ALP$_2$**	**ALP$_3$**
♂	genotype	S_1S_2	S_1S_5	S_3S_4
ALP$_1$	S_1S_2	−0	±6	±8
ALP$_2$	$S_1S_5(S_5>S_1)$	+10	−0	+30
ALP$_3$	S_3S_4	+23	+18	−0

S-genotype for each plant was assigned using seed set as the compatibility criterion. Then, crosses were made between some plants of different accessions to verify the relationships between accessions for each species.

To assign the S genotypes in each cross it was considered that in Asteraceae:
(a) The number of filled seeds determines if the cross is compatible or

Table 4: Crosses within *Helianthus annuus* accessions. Number of plants, seed set and S-genotypes of crosses in the first experiment (2015–2016).
+ denotes fully compatible cross; ± partially compatible cross; – incompatible cross (See Table 2). Bold crosses show self-compatibility and asterisk show the seeds used to perform controlled crosses in the second experiment. ♀, pollen recipient; ♂, pollen donor. > indicates dominance.

Plant	♀	**LMA_1**	**LMA_2**	**LMA_3**	**LMA_4**	**LMA_5**	**LMA_6**
♂	genotype	S_5S_3	S_0S_0	S_0S_0	S_0S_0	S_1S_3	S_4S_2
LMA_1	S_5S_3	−0	+ 13	+ 143	+ 35	± 3	+ 78*
LMA_2	S_0S_0	+ 10	**+ 90***	+ 122	+ 33	+ 10	+ 55
LMA_3	S_0S_0	+ 13	+ 176	**+ 15***	+ 26	+ 25	+ 12
LMA_4	S_0S_0	+ 14	+ 76	+ 107	**+ 18***	+ 13	+ 11
LMA_5	$S_1S_3(S_1>S_3)$	+ 67*	+ 169	+ 12	+ 28	−0	+ 52
LMA_6	S_4S_2	+ 20	+ 62	+ 43	+ 20	+ 89*	−0

Plant	♀	**MAG_1**	**MAG_2**	**MAG_3**
♂	genotype	S_0S_1	S_0S_0	S_1S_5
MAG_1	S_0S_1	**+ 9***	+ 79	± 3
MAG_2	S_0S_0	+ 41	**+ 36***	+ 10*
MAG_3	$S_1S_5(S_5>S_1)$	+ 21	+ 11	−0

Plant	♀	**DIA_1**	**DIA_2**	**DIA_3**	**DIA_4**
♂	genotype	S_1S_2	S_1S_2	S_1S_2	S_1S_3
DIA_1	S_1S_2	−0	−0	−1	± 9
DIA_2	S_1S_2	−2	−0	−0	± 3
DIA_3	**S_1S_2**	−0	−2	−0	± 7
DIA_4	$S_1S_3(S_1>S_3)$	−0	−0	−1	−0

Plant	♀	**RAN_1**	**RAN_2**	**RAN_3**
♂	genotype	S_2S_4	S_2S_3	S_5S_3
RAN_1	$S_2S_4(S_4>S_2)$	−0	+ 21	+ 22
RAN_2	$S_2S_3(S_3>S_2)$	+ 11	−0	−0
RAN_3	$S_5S_3(S_5>S_3)$	+ 19	+ 15	−0

Plant	♀	**BAR_1**	**BAR_2**
♂	genotype	S_1S_5	S_2S_5
BAR_1	S_1S_5	−0	± 3
BAR_2	S_2S_5	± 6	−0

incompatible, or it is self-compatible or self-incompatible in the case of self-fertilization (Brennan *et al.*, 2013); (b) The relationships between S alleles in the pistil are usually codominant, while in pollen they are usually dominant or

Table 5: Seed set and possible S-genotypes of reciprocal crosses for the second experiment (2016–2017) among surviving *Helianthus petiolaris* plants. + denotes fully compatible cross; ± partially compatible cross; − incompatible cross (table 2). $C_{3\text{-}3}$ is a progeny of $CAT_3 \times CAT_3$ and $C_{4\text{-}3}$ is a progeny of $CAT_4 \times CAT_3$. ♀, pollen recipient; ♂, pollen donor.

Plant	♀	$C_{3\text{-}3}$	$C_{4\text{-}3}$
♂	genotype	S_0S_0	S_0S_4, S_0S_5
$C_{3\text{-}3}$	S_0S_0	− 0	+ 116
$C_{4\text{-}3}$	S_0S_4, S_0S_5	+ 155	− 0

Table 6: Seed set and possible S genotypes of reciprocal crosses for the second experiment (2016–2017) among surviving *Helianthus annuus* plants. + denotes fully compatible cross; ± partially compatible cross; − incompatible cross (Table 2). Plants were obtained from the following crosses: $L_{2\text{-}2}$ (LMA2 × LMA2), $L_{4\text{-}4}$ (LMA4 × LMA4), $L_{5\text{-}6}$ (LMA5 × LMA6), $L_{6\text{-}1}$ (LMA6 × LMA1). Bold crosses showed self-compatibility. ♀, pollen recipient; ♂, pollen donor.

Plant	♀	$L_{2\text{-}2}$	$L_{4\text{-}4}$	$L_{5\text{-}6}$	$L_{6\text{-}1}$
♂genotype	genotype	S_0S_0	S_0S_0	S_1S_2, S_1S_4, S_3S_2, S_3S_4	S_2S_3, S_2S_5, S_4S_3, S_4S_5
$L_{2\text{-}2}$	S_0S_0	+ 27	+ 87	+ 53	+ 188
$L_{4\text{-}4}$	S_0S_0	+ 47	+ 13	+ 215	+ 152
$L_{5\text{-}6}$	S_1S_2, S_1S_4, S_3S_2, S_3S_4	+ 144	+ 78	− 0	+ 217
$L_{6\text{-}1}$	S_2S_3, S_2S_5, S_4S_3, S_4S_5	+ 62	+ 88	+ 91	− 0

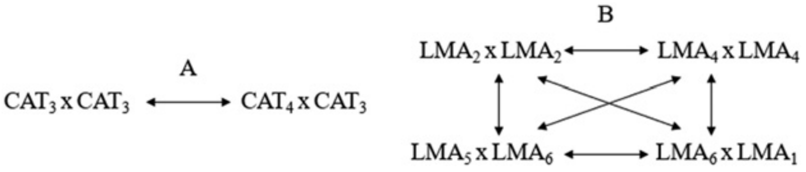


Figure 3: Schematic representation of the 2016–2017 crossing experiment in *H. petiolaris* (A) and *H. annuus* (B) populations. Also, every plant in each scheme was self-pollinated.

recessive (Llaurens *et al.*, 2009); (c) Within accessions, S genotypes should be explained with as few alleles as possible. Since in a reciprocal cross the maximum number of intervening S alleles is four, alleles were added if necessary, just to explain all the crosses within the same accession (Geleta and Bryngelsson, 2010).

The S-genotypes of the parental plants involved in the first generation crosses (summer 2015–2016) were the ones that determined the S-genotypes plants used as parental in the second experiments (summer 2016–2017). In each case, the assignment of the S-genotype for each plant was carried out independently within each accession. The S genotype in the parents was determined by the initial assignment of S alleles to a randomly chosen cross within the accession and then the different crosses were related to each one by assigning the respective S alleles to each plant. This way all the S genotypes were completed within each accession based on seed production and allelic interactions considering all possible scenarios (dominance, recessiveness or codominance).

Results

Self-compatibility in *Helianthus* plants

Nine out of 32 evaluated *Helianthus* plants (14 of *H. petiolaris* and 18 of *H. annuus*) during the first experiment (2015–2016) showed some degree of self-compatibility. In *H. petiolaris,* those plants belonged to CAT, ROC, and BUO populations, where seed production by self-fertilization varied from none to a few or several seeds per head. *H. annuus* plants belonged to MAG and LMA populations and their seed production varied from none to many seeds (Tables 3 and 4, in bold).

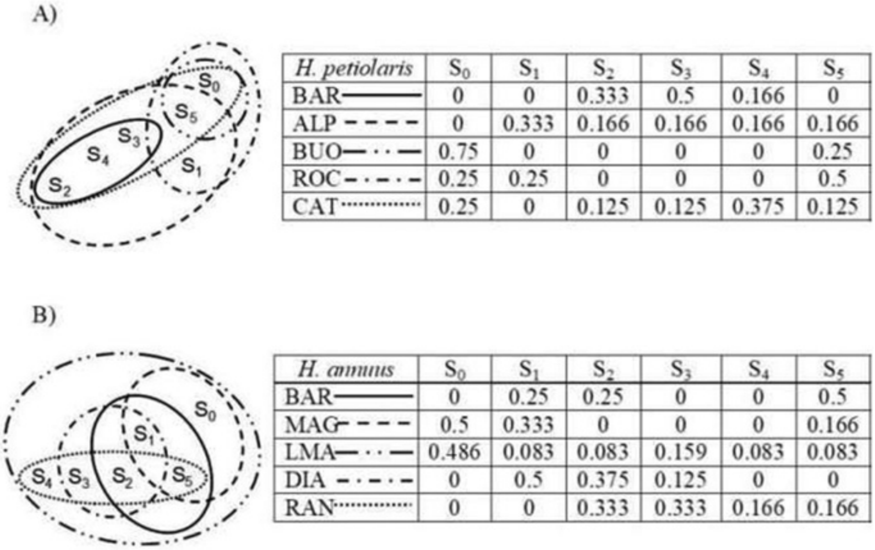
In the second experiment (2016–2017), the evaluated progenies presented marked inbreeding effects, such as hypocotyl breakage, seedlings lacking pigmentation, dwarfism, slow vegetative growth, late reproductive development and malformed floral buds. As a result, over 69 initial individuals of *H. petiolaris* and 83 of *H. annuus*, survival rate was of 2.9 % and 4.8 %, respectively. Inbreeding effects were apparent at different stages of plant development.

Sixty-nine *H. petiolaris* plants were seriously affected by inbreeding and almost all seedlings died during the vegetative stage in the greenhouse. A number of plants from CAT (38), BUO (18), and ROC (11) produced seedlings with hypocotyl breakage prior to transplantation in the experimental field and did not survive to the vegetative stage. Two surviving plants of the CAT accession that were transplanted to the field showed abnormalities in their reproductive stage. Plants had poor and slow vegetative and reproductive development in the experimental field. The *H. petiolaris* plants that survived (summer 2016–2017) did not self-fertilize (Table 5).

All the *H. annuus* plants overcame the greenhouse stage and were transplanted to the experimental field, although most of them (56 from LMA and 23 from MAG accessions) had a weak vegetative aspect and did not survive to the reproductive stage. Four plants of LMA population reached the flowering stage and showed some inbreeding effects, i. e. dwarfism, delayed phenological development, and floral abortion. However, two *H. annuus* plants ($L_{2\text{-}2}$ (and $L_{4\text{-}4}$) showed self-compatibility producing 27 and 13 seeds respectively (Table 6, in bold).

The S alleles in *Helianthus* and their interactions in the pollen and pistil

The evaluated crosses in two generations of *H. petiolaris* and *H. annuus* demonstrated the presence of five functional S alleles (S_1-S_5) and one non-functional allele (S_0) in each species. Presence and frequency of each S allele was different within the accessions (Figure 4). The inter-populations crosses within each species allowed confirmation of correct allele assignation within each accession. Thus, the initial assignment of the S-genotype for each plant independently within each accession (intra-population crosses) were the suitable S genotype for solving inter-population crosses (Tables 7 and 8). The non-functional allele (S_0) was present in accessions that had self-compatible plants. The parental S-genotype of these compatible plants was assigned according to



H. petiolaris	S_0	S_1	S_2	S_3	S_4	S_5
BAR———	0	0	0.333	0.5	0.166	0
ALP - - - -	0	0.333	0.166	0.166	0.166	0.166
BUO—··—	0.75	0	0	0	0	0.25
ROC-·-·-	0.25	0.25	0	0	0	0.5
CAT··········	0.25	0	0.125	0.125	0.375	0.125

H. annuus	S_0	S_1	S_2	S_3	S_4	S_5
BAR———	0	0.25	0.25	0	0	0.5
MAG- - -	0.5	0.333	0	0	0	0.166
LMA—··—	0.486	0.083	0.083	0.159	0.083	0.083
DIA -·-·-	0	0.5	0.375	0.125	0	0
RAN··········	0	0	0.333	0.333	0.166	0.166

Figure 4: Distribution and frequencies of the functional and non-functional S alleles within (A) five *H. petiolaris* and (B) five *H. annuus* accessions.

Table 7: Seed set and S genotypes of inter-population reciprocal crosses among *H. petiolaris* plants in the first experiment (2015–2016). + denotes fully compatible cross; ± partially compatible cross; – incompatible cross (Table 2). ♀, pollen recipient; ♂, pollen donor. > indicates dominance.

Plant	♀	**CAT$_3$**	**BUO$_1$**	**ROC$_1$**	**BAR$_2$**	**ALP$_1$**
♂	genotype	S_0S_0	S_0S_0	S_0S_5	S_2S_3	S_1S_2
CAT$_3$	S_0S_0	+ 106	+ 103	+ 43	+ 25	+ 53
BUO$_1$	S_0S_0	+ 61	+ 75	+ 82	+ 13	+ 60
ROC$_1$	S_0S_5	+ 33	+ 64	+ 25	+ 56	+ 25
BAR$_2$	$S_2S_3(S_2>S_3)$	+ 24	+ 36	+ 67	−0	−0
ALP$_1$	S_1S_2	+ 51	+ 49	+ 72	−1	−0

Table 8: Seed set and S-genotypes of inter-population reciprocal crosses among *Helianthus annuus* plants in the first experiment (2015–2016). + denotes fully compatible cross; ± denotes partially compatible cross; – denotes incompatible cross (Table 2). ♀, pollen recipient; ♂, pollen donor. > indicates dominance.

	♀ genotype	**LMA$_3$**	**MAG$_1$**	**DIA$_3$**	**RAN$_2$**	**BAR$_1$**
♂genotype		S_0S_0	S_0S_1	S_1S_2	S_2S_3	S_1S_5
LMA$_3$	S_0S_0	+ 49	+ 41	+ 13	+ 55	+ 68
MAG$_1$	S_0S_1	+ 81	+ 15	+ 12	+ 23	+ 46
DIA$_3$	S_1S_2	+ 38	+ 4	−0	± 5	± 7
RAN$_2$	$S_2S_3(S_3>S_2)$	+ 54	+ 36	+ 14	−0	+ 50
BAR$_1$	S_1S_5	+ 43	−2	± 3	−11	−0

the offspring seed set (Table 2) as homozygous 1 S-genotype or heterozygous S-genotype. The parental S-genotype was considered homozygous (S_0S_0) when the self-compatible crosses had a seed production >10 and the intra-population reciprocal crosses were compatible; 2) The parental S genotype was considered heterozygous ($S_0S_{x(1,2,3,4,5)}$) when the self-compatible crosses had a seed production $>2<10$ and the intra-population reciprocal crosses were compatible, incompatible or indeterminate (Tables 3 and 4).

In *H. petiolaris* (Figure 4a) the frequency of functional S alleles ranged from 0.125 (S_2, S_3 and S_4 in CAT) to 0.5 (S_3 in BAR and S_5 in ROC). The non-functional S allele (S_0) showed the higher frequency in BUO accession (0.75). Table 3 shows the interaction of the S alleles in pollen and pistil observed in the crosses. In pollen, 50 % interactions behave as dominant or recessive and the remaining 50 % were codominant. This was demonstrated for a maximum of three alleles out of the five functional ones and the hierarchical dominance was S_2>S_4>S_3. In the pistil, the interactions were 100 % codominant.

In *H. annuus* frequencies of functional S alleles varied from 0.125 (S_3 in DIA) to 0.5 (S_5 in BAR) (Figure 4b). The non-functional S allele (S_0) was present in a high frequency (0.5). Table 4 shows the observed interactions among S alleles in pollen and pistil. The dominance/recessiveness and codominance interactions in pollen were 30 % and 70 %, respectively. Hierarchical dominance in *H. annuus* pollen was demonstrated for a maximum of three alleles out of the five functional ones, S_5>S_1>S_3. Pistils presented 100 % codominant interactions.

Discussion

The *Helianthus* genus exhibits a SSI system; wild species behave as self-incompatible and strictly cross-pollinated (Desrochers and Rieseberg, 1998; Gandhi *et al.*, 2005; Gutierrez *et al.*, 2014), whereas the domestic sunflower is highly self-compatible (Miller and Fick, 1997; See Radanovic *et al.*, 2018 for a review). The expression of SI varies with the action of specific S alleles (Stephenson *et al.*, 2000). Five functional S alleles were identified in the offspring of *H. petiolaris* and *H. annuus* studied plants. Low numbers of S alleles in populations may be due to bottlenecks in the introduction process of a species (Hiscock, 2000), but it could also be addressed to the small size of the investigated sample, since it was limited to the offspring of a few self-compatible plants found in the accessions under study (Gutierrez *et al.*, 2014). The results showed that in *H. petiolaris* there was a greater frequency of recessive S alleles. Theoretical models of SSI predict that the frequency of recessive alleles is high when the number of S alleles is low (Bateman, 1952; Byers and Meagher, 1992). Such a situation would be expected in small populations or where colonization has taken place recently. Wild *H. annuus* was introduced intentionally ca. 1948 in Río Cuarto, province of Córdoba (Bauer, 1991) and *H. petiolaris* presumptively entered as impurities in forage seed lots by the same time. In terms of diffusion of the species, the process is relatively new, less than 70 years old, and possibly not yet stabilized (Cantamutto *et al.*, 2010). The observed dominant/recessive allelic interactions confirmed the sporophytic nature of the SI system in wild *Helianthus* like in all Asteraceae (Castric and Vekemans, 2004; Hiscock, 2000; Hiscock and McInnis, 2003). The dominant/recessive allelic interactions present in pollen and absent in pistils explains the S-phenotypes of both *Helianthus* species agreeing with Gaude *et al.* (2006) who stated that S-haplotypes were generally codominant in stigmas and dominance interactions were present in anthers. Hierarchical dominance involved only three of the five S alleles in both *Helianthus* species.

Self-compatibility in wild *Helianthus* plants could be addressed to a a nonfunctional S allele (S_0) likely coming from sunflower crop by gene flow. There is a close relationship between sunflower crop and wild *Helianthus* relatives, in several studies of gene flow between both biotypes (Gutierrez *et al.*, 2010; Rieseberg *et al.*, 1999; Ureta *et al.*, 2008). Whitton *et al.* (1997) demonstrated the persistence of sunflower alleles in wild *H. annuus* populations for five generations at moderate frequencies. In Argentina hybridization between crop and wild *Helianthus* (Gutierrez *et al.*, 2010; Ureta *et al.*, 2008) and the persistence of crop alleles in wild populations has been demonstrated (Gutierrez *et al.*, 2011). Evidence of gene flow after the first hybrid generation confirmed the high probability of introgression between *H. petiolaris* and *H. annuus* (Heiser, 1947; Rieseberg *et al.*, 1999; Rogers *et al.*, 1982). Introgression of novel S alleles allowed the success of *Senecio squalidus* as a colonizer in Britain (Brennan *et al.*, 2002).

Self-incompatibility is known to vary with the composition of the pollen load (Desrochers and Rieseberg, 1998), with external environmental conditions such as temperature (Distefano *et al.*, 2017; Kawano *et al.*, 2016) and with internal stylar conditions such as the flower age (Stephenson *et al.*, 2000). In *H. annuus* and *H. petiolaris* the functional SI system can fail due to the induction of self-pollination by mixed loads of the own and heterospecific pollen in natural conditions such as in hybrid zones (Desrochers and Rieseberg, 1998). The presence of wild populations in the extended sunflower crop region in Argentina may create a similar condition.

On the other hand, researchers have reported a relationship between self-incompatibility and temperature affecting both male and female functions and their interactions. Temperature has a clear effect on pollen germination, pollen tube growth (Kawano *et al.*, 2016), fruit development and absence of seeds (Distefano *et al.*, 2017). In some species the floral age produced variation in the expression of SI. Stephenson *et al.* (2000, 1992) showed that the self-pollination of young flowers (first day of the female phase) results in very low fruit and seed set than self-pollination of the old flowers (fourth day of the female phase) that results in a significant increase in fruit and seed set. Self-fertility and seed production are important traits that affect seed collection maintenance in wild *Helianthus* (Atlagic and Terzic, 2016). Therefore, there are several factors that produce variations in the SI, due to genotype, environment or genotype x environment interactions that can often be overlooked when evaluating the evolution of a mating system.

Conclusions

In this study we studied the SSI mechanism in wild *Helianthus* species (*H. annuus* and *H. petiolaris*). We were able to determine the low number and the distribution of S alleles within each population (five functional and one non-functional). Also, the frequency of each S allele varied within accessions. We assessed the type of allelic interactions in pollen and pistil, being dominance/recessiveness and codominance in pollen and only codominance in pistil in both species. The self-compatibility detected in this study is likely the result of non-functional alleles introgressed in wild *Helianthus* plants through gene flow from self-compatible cultivated sunflower, although inbreeding depression effects would prevent the persistence of these alleles in the wild populations. Inbreeding effects in wild materials appeared in the third generation of self-pollination, with lethal effects in most plants. As a consequence, self-compatibility could not explain the high invasive capacity of wild *Helianthus* species in Argentina.

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Resumen

EVALUACION DEL SISTEMA DE APAREAMIENTO EN POBLACIONES DE *Helianthus annuus Y H. petiolaris* (ASTERACEAE)

Helianthus annuus subsp. *annuus* y *H. petiolaris* son especies silvestres norteamericanas naturalizadas en el centro de Argentina. Tienen un sistema genético de autoincompatibilidad esporofítica que previene la autofertilización, aunque se demostró la aparición de plantas autocompatibles en ambas especies. Su distribución geográfica coincide mayormente con la del cultivo. El girasol doméstico es autocompatible y puede hibridar con ambas especies presentando considerable flujo génico. El objetivo de este estudio es dilucidar el mecanismo de autoincompatibilidad en ambas especies de *Helianthus* silvestres. Cruzas recíprocas y producción de semillas se utilizaron para identificar genotipos autocompatibles, número y distribución de alelos S dentro de las poblaciones y el tipo y extensión de interacciones alélicas en el polen y pistilo. El comportamiento de los alelos S dentro de cada accesión se explicó por cinco alelos S funcionales y un alelo no funcional en cada especie, diferenciándose en su presencia y frecuencia dentro de las accesiones. Las interacciones alélicas fueron de dominancia/recesividad y codominancia en el polen, mientras que sólo codominancia en el pistilo. Aparecieron efectos de endogamia en los materiales silvestres en la tercera generación de autopolinización, con importantes efectos letales. El número de alelos S fue bajo y se comportaron de manera similar al de otras especies de Asteraceae. La autocompatibilidad fue atribuida a los alelos S no funcionales introducidos en las plantas silvestres mediante flujo génico del girasol autocompatible.

Résumé

EVALUATION DU SYSTEME DE ACCOUPLEMENT DANS LES POPULATIONS DE *Helianthus annuus* et *H. petiolaris* (ASTERACEAE)

Helianthus annuus subsp. *annuus* et *H. petiolaris* sont des espèces sauvages d'Amérique du Nord qui se sont naturalisées dans le centre de l'Argentine. Ils ont un système génétique d'auto-incompatibilité sporophytique qui empêche l'autofécondation, mais la présence de plantes autocompatibles en Argentine a été démontrée dans les deux espèces. Leur distribution géographique coïncide avec la superficie cultivée. Le tournesol domestique est auto-compatible, peut s'hybrider avec ces espèces et présente une quantité considérable de flux de gènes. Notre but est de comprendre le mécanisme d'auto-incompatibilité chez les deux espèces sauvages d'*Helianthus*. Le croisement réciproque et la production de graines ont été utilisés pour identifier les génotypes auto-compatibles, le nombre et la distribution d'allèlesS, ainsi que le type et l'étendue des interactions alléliques dans le pollen et le pistil. Le comportement des allèles S dans chaque accesion a été expliqué par cinq allèles S fonctionnels et un allèle non fonctionnel chez chaque espèce, se différenciant par leur présence et leur fréquence au sein des accessions. Dans les deux espèces, les interactions alléliques étaient de dominance/récessivité et de codominance dans le pollen, mais uniquement de codominance dans le pistil. Des effets de consanguinité sont apparus sur des matières sauvages et le nombre d'allèles S était faible. L'autocompatibilité a été attribuée aux allèles S non fonctionnels introduits dans les plantes sauvages par le biais du flux génétique du tournesol auto-compatible.