Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

The role of domestication and maternal effects on seed traits of crop–wild sunflower hybrids (*Helianthus annuus*)

F. Hernández[1,2], L.I. Lindström[1], E. Parodi[3,4], M. Poverene[1,2] & A. Presotto[1,2]

1 Dpto. Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina
2 CERZOS, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina
3 IADO, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina
4 Dpto. Biología, Bioquímica y Farmacia (UNS), Bahía Blanca, Argentina

Keywords
Crop–wild hybrids; domestication; maternal effects; pericarp anatomy; seed dormancy; seed morphology; sunflower.

Correspondence
F. Hernández, Departamento de Agronomía, Universidad Nacional del Sur, San Andrés 800, 8000 Bahía Blanca, Argentina.
Email: fhernandez@cerzos-conicet.gob.ar.

Received: 9 March 2017; revised version accepted: 2 May 2017.

doi:10.1111/aab.12368

Abstract

Hybridisation between crops and their wild relatives may promote the evolution of weeds. Seed germination and dormancy are the earliest life-history traits and are highly influenced by the maternal parent. However, the ecological role of the maternal effect on seed traits in the evolution of crop–wild hybrids has received little attention. In this study, we test the relative importance of maternal and hybridisation effects on seed traits of the first generation of crop–wild sunflower hybrids (*Helianthus annuus*). Seed germination was tested in two wild populations with contrasting dormancy, two cultivated materials and their reciprocal crosses at four different times after harvest and three different temperatures. Seed germination at each of the four times, after ripening response and secondary dormancy were recorded along with four morphological traits. Additionally, the pericarp anatomy was analysed with light and scanning electron microscopy. We observed strong maternal effects on all seed traits. Seed germination, morphology and pericarp anatomy differed largely between the crop and wild seeds and these traits in the crop–wild hybrids resembled their female parent. Slight but significant hybridisation effects were observed in germination, mainly in seeds produced on wild plants. Crop hybridisation changed seed germination, the after ripening response and secondary dormancy in the crop direction. Morphological and anatomical traits associated with domestication strongly correlated with the observed differences in seed germination and dormancy in crop–wild sunflower hybrids. The large maternal effects along with the evolutionary divergence in seed traits were responsible for the large phenotypic differences observed in crop–wild hybrids with the same genetic composition. Wild-like seed traits of hybrids suggest that there are no barriers to crop gene introgression at the seed level whereas crop-like seed traits could be strongly selected against, conditioning the selection of traits expressed later in the life cycle and in the next generations.

Introduction

Hybridisation followed by gene introgression (i.e. permanent allele incorporation from another gene pool) between crop species and their wild relatives has implications in natural population dynamics, such as a reduction in their genetic diversity, crop-gene assimilation and an increase in invasiveness or weediness (Ellstrand *et al.*, 1999; Ellstrand & Schierenbeck (2006); Mercer *et al.*, 2014). Similarly, hybridisation between crops and their wild relatives could hasten the evolution of feral forms, which may constitute self-maintained populations of noxious weeds from domesticated ancestors (Warwick & Stewart, 2005; Bagavathiannan & Van Acker, 2008; Ellstrand *et al.*, 2010). Here we define weeds as those populations that interfere with agriculture, competing

with the crop for resources, while wild populations are those that grow and reproduce away from the agriculture environments, whether natives or not (Ellstrand *et al.*, 1999, 2010). Therefore, increased weediness is the higher ability of a population to invade agricultural fields. Hybridisation occurs in sexually reproductive plants when the flowering of crop and wild plants coincides spatially and temporally, and in the case of insect-pollinated species, they share pollinators (Ellstrand *et al.*, 1999). Natural hybridisation between crops and their wild relatives has been documented in most of the world's important food crops and in several cases it has implications in weed evolution (reviewed in Ellstrand *et al.*, 1999, 2013). During the domestication process, conscious selection by early farmers as well as unconscious selection as a result of agricultural practices shaped crop phenotypes from their wild ancestors (Hancock, 2012; Meyer & Purugganan, 2013). Such change from wild to crop forms was typically driven by the selection on small number of quantitative trait loci (QTL), each one with large effect on the phenotype (Hancock, 2012; Meyer & Purugganan, 2013). Domesticated sunflower exhibits the typical domestication syndrome, that is, large seeds, loss of dormancy, determinate growth, monocephalic inflorescence, non-shattering and an increase in reproductive allocation and seed yield (Purugganan & Fuller, 2009; Presotto *et al.*, 2011; Meyer & Purugganan, 2013). However, genetic analysis in sunflower has demonstrated that a large number of QTLs, with minor effects, control these traits (Burke *et al.*, 2002; Wills & Burke, 2007).

The F1 crop–wild hybrids usually have intermediate values in most of the traits in which their parents differ (Mercer *et al.*, 2007; Presotto *et al.*, 2011). Following hybridisation, some early life-history traits in hybrid populations, like seed traits, strongly depend on the maternal parent (Roach, 1987; Snow *et al.*, 1998; Weiss *et al.*, 2013; Alexander *et al.*, 2014; Mercer *et al.*, 2014; Presotto *et al.*, 2014; Pace *et al.*, 2015). The maternal parent mainly affects seed traits through the tissues surrounding the developing embryo, especially the pericarp (Roach, 1987). These tissues begin their development regardless of the embryo fertilisation and may have great influence on seed size, seed morphology and finally on seed dormancy and germination. Seed dormancy is defined as an innate seed property, which impedes germination under environmental conditions that are otherwise favourable for germination (Finch-Savage & Leubner-Metzger, 2006). While seed requirements prevent germination in unfavourable environments, seed dormancy is a much more complex mechanism avoiding seed germination in environments only ephemerally favourable (Willis *et al.*, 2014). Thus, seed dormancy plays a crucial ecological role in natural populations of several plant species, mainly in spring annuals, contributing to persistent soil seed bank formation which is a key feature for dispersal over time, survival under unfavourable conditions, and the establishment of self-maintained populations (Alexander & Schrag, 2003; Montesinos-Navarro *et al.*, 2012). Additionally, as seed dormancy and germination are two of the earliest life history-traits expressed in the life cycle they may be strongly selected for during the colonisation of novel habitats, conditioning the selection of other traits expressed later in the life cycle (Huang *et al.*, 2010; Chiang *et al.*, 2013; Willis *et al.*, 2014). In crop cultivars, selection for more rapid and uniform germination and more effective seedling competition has decreased seed dormancy and seed-coat thickness and has increased seed size (Hancock, 2012). As well as other domestication traits, in sunflower, seed dormancy is controlled by several QTLs, with minor effects (Burke *et al.*, 2002; Gandhi *et al.*, 2005; Wills & Burke, 2007). In this sense, a comprehensive analysis of seed dormancy, including whole seed and embryo dormancy, found 43 seed dormancy QTLs across six linkage groups (Brunick, 2007) displaying the high complexity of seed dormancy in sunflower.

Cultivated sunflower was domesticated approximately 4000 years ago from wild sunflower (*Helianthus annuus* L.) in the current east-central United States (Harter *et al.*, 2004; Blackman *et al.*, 2011). *H. annuus* is a complex taxonomical group, made up of crop cultivars, wild and weedy biotypes, and volunteers that emerge from fallen crop seeds and may act as noxious weeds. These make sunflower an ideal model system for studying evolutionary aspects of domestication, weed evolution, crop–wild hybridisation and crop ferality. Cultivated sunflower is currently one of the most important oil-crops worldwide and in some countries its production area overlaps the distribution area of wild *Helianthus* species where spontaneous hybridisation has been well documented (Snow *et al.*, 1998; Ureta *et al.*, 2008; Muller *et al.*, 2011). *H. annuus* is the most widespread of the wild species. In Argentina, it was probably introduced as a forage crop approximately 70 years ago and it has become a non-native invader, spreading over the central region of the country (Poverene *et al.*, 2008; Cantamutto *et al.*, 2010; Presotto *et al.*, 2011). Sunflower exhibits non-deep physiological dormancy (PD; Oracz *et al.*, 2007). Seeds with PD are water-impermeable and have a physiological inhibiting mechanism in the embryo that prevents radicle emergence (Baskin & Baskin, 2004). Embryos excised from PD seeds produce normal seedlings and this kind of dormancy can be broken with gibberellic acid treatments, by scarification, after ripening in dry storage, and with cold or warm stratification (Baskin & Baskin, 2004; Finch-Savage & Leubner-Metzger, 2006; Presotto *et al.*, 2014). In addition, covering structures can prevent

germination by restricting the water and oxygen movement, avoid the leaching of inhibitors from the embryo, contain germination inhibitors or physically restrict the emergence of the radicle (Baskin & Baskin, 2004).

Despite the importance of seed traits in the colonisation of novel habitats, such as non-native or agricultural environments, few attempts have been made to explain the ecological role (i.e. out-season germination, ability to form soil seed banks and changes in the competitive ability) of the maternal effect on seed traits in crop–wild hybrids. Strong maternal effect on seed dormancy and germination along with slight and variable hybridisation effects have recently been reported in crop–wild sunflower hybrids (Weiss *et al.*, 2013; Alexander *et al.*, 2014; Presotto *et al.*, 2014; Pace *et al.*, 2015). However, most of the studies have focused on the crop to wild hybridisation and shallowly explored the morphological and anatomical traits possibly involved in seed germination differences.

In this manuscript, we investigated the dynamics of seed dormancy, and further explored morphological and anatomical features of the seeds possibly involved in seed dormancy, using reciprocal crosses of wild and crop sunflower. The aims of this study were: (a) to quantify the maternal and hybridisation effects on seed traits at different times after harvest; (b) to explore seed morphological and anatomical differences between crop–wild hybrids and their parents; and (c) to detect morphological and anatomical traits that might regulate seed dormancy.

Materials and methods

Plant material

For evaluating the maternal effect on seed traits, reciprocal crosses between wild sunflower populations (wild) and cultivated materials (crop) were used. Wild populations were collected in central Argentina (Cantamutto *et al.*, 2010): Diamante (DIA; 32°03′S, 60°38′W) and Colonia Baron (BAR; 36°10′S, 63°53′W) which were selected for their contrasting dormancy levels (Presotto *et al.*, 2014). Cultivated materials were represented by two commercial cultivars: Cacique CL (CAC; Criadero El Cencerro) and Paraíso 104CL (PAR; Nidera Semillas) which were randomly selected. All the reciprocal crosses were made, except for the cultivated materials, which were not crossed between each other. So, 14 crosses (hereafter referred as biotypes) were characterised according to their female and male parents and grouped into four categories (cross types; Table 1): wild, wild–crop (wild maternal parent), crop–wild (crop maternal parent) and crop. In order to minimise the effects of the maternal environment, all the achenes (hereafter referred as seeds) were produced in a common garden at the Agronomy Department, Universidad Nacional del Sur, Bahia Blanca, Argentina (38°41′38″S, 62°14′53″W) during the 2012–2013 growing season. The seeds from all the biotypes were produced under controlled pollination of the heads of 20–30 plants covered with paper bags at the pre-flowering stage. At the flowering stage, heads were emasculated in the morning and pollinated at late afternoon with the corresponding pollen source to produce wild, wild–crop and crop–wild seeds. Despite the natural self-incompatibility of wild sunflower, variable selfing rates were found in invasive populations from Argentina. In BAR population, no self-compatible plants were found but approximately 20% of the DIA plants were partially self-compatible (Gutierrez *et al.*, 2014), thus all the crosses were made on emasculated plants. Crop seeds were produced by self-pollination, covering the heads with paper bags at the pre-flowering stage.

Table 1 Female and male parents used in the four different cross types. BAR: Colonia Baron; CAC: Cacique CL; DIA: Diamante; PAR: Paraíso 104 CL. BAR and DIA are wild populations from Argentina chosen by their contrasting seed dormancy (BAR with high dormancy and DIA with low dormancy; Presotto *et al.*, 2014) while CAC and PAR are commercial cultivars randomly chosen

Biotype	Cross Type	Female	Male
BAR	Wild	BAR	BAR
BAR × DIA	Wild	BAR	DIA
DIA	Wild	DIA	DIA
DIA × BAR	Wild	DIA	BAR
BAR × CAC	Wild–crop	BAR	CAC
BAR × PAR	Wild–crop	BAR	PAR
DIA × CAC	Wild–crop	DIA	CAC
DIA × PAR	Wild–crop	DIA	PAR
CAC × BAR	Crop–wild	CAC	BAR
CAC × DIA	Crop–wild	CAC	DIA
PAR × BAR	Crop–wild	PAR	BAR
PAR × DIA	Crop–wild	PAR	DIA
CAC	Crop	CAC	CAC
PAR	Crop	PAR	PAR

Germination experiments

Germination experiments were carried out in a completely randomised experimental design at different times: immediately (t1); at 2 weeks (t2); 6 months (t3); and 18 months (t4) after harvest and different temperatures at a single time point (10°C, 20°C and 30°C). To simulate winter conditions naturally occurring at the field, the seeds used in t3 were placed in tri-laminar aluminium bags, to protect seeds from humidity, in a growth chamber at 5°C during 6 months. For dry inducing secondary dormancy, seeds released from the growth chamber were dry conditioned for 1 year at room temperature (t4). During all four times the seeds were

placed on filter paper and moistened with distilled water at constant 20°C with a 12 h photoperiod; they were counted periodically (2–3 days intervals) during 16 days. The incubation temperature was chosen according to a previous study where the effect of hybridisation on seed germination was tested at different times after harvest with temperatures from 5°C to 30°C (Presotto *et al.*, 2014). In such study, hybridisation effects were only detected at 20°C and 25°C. In addition, at the end of the current experiment, germination of seeds from 3 years after harvest, dry stored during this period, was tested at three incubation temperatures (10°C, 20°C and 30°C) with a 12 h photoperiod for testing the effect of temperature on the seed germination at a single time point. Light was provided by fluorescent lamps (60 μmol m^{-2} s^{-1}). For each experiment, the proportion of germinated seeds was calculated as the ratio between the total germinated seeds at the end of the experiment (day 16) and the total viable seeds, while the proportion of seed dormancy was calculated as the difference between the total viable seeds and the germinated ones. Seed viability was obtained using a tetrazolium test on non-germinated seeds for all the replicates at the end of each experiment (ISTA, 2004).

Morphological and anatomical experiments

Ten dried seeds of each of the 14 biotypes were used to determine seed size and form. Length, width and the length/width ratio were recorded. Width was measured on the basal third of the seed. In addition, seed weight was recorded for each of the 14 biotypes by weighing five independent samples of 50 seeds. In order to evaluate the anatomical features of the seed pericarp that was possibly involved in seed dormancy, cross sections of pericarps from the two wild populations (DIA and BAR), one cultivar (PAR) and their reciprocal crosses were observed under a scanning electron microscope (SEM). The cross sections were obtained by cutting half entire seeds by hand under a stereo microscope. For pericarp softening, seeds were previously imbibed (12 and 3 h for wild and crop maternal plants, respectively). Cuts were fixed following Croce & Parodi (2013). Then, cross sections were fixed in 2.5% glutaraldehyde-water in cacodylate buffer at 5°C for 2 h immediately after cutting and they were dehydrated in a graded acetone series up to 80%. Samples were finally critical point dried during 1 h, coated with gold and observed with a Jeol 35 CF SEM. Additionally, pericarp cross sections of one wild population (BAR), one cultivar (PAR) and their reciprocal crosses were observed under a light microscope. Cross sections of the pericarps were obtained following Andrade *et al.* (2015). Entire seeds were then fixed in formalin/acetic acid/alcohol (FAA) solution, embedded in paraffin wax and processed using conventional techniques for cutting (10 μm) and staining (safranin–fast green). All sections were mounted in glycerine/water (1:1). Photographic observations and recordings were made using a Nikon Labophot-2 microscope (Nikon Corp., Tokio, Japan) with an attached Nikon Coolpix 4500 camera and ocular micrometer (Nikon Corp., Tokio, Japan).

Statistical analysis

Analysis of variances (ANOVAs) were performed with generalised linear mixed models (GLMM) using PROC GLIMMIX (SAS University edition; SAS Institute Inc., Cary, NC), unless otherwise specified. GLMM were chosen because they are the recommended tool for analysing non-normal data with random effects (Bolker *et al.*, 2009). Because of the natural non-normal distribution of the proportion data, all the models were adjusted using Beta distribution instead the normal one (Bolker *et al.*, 2009; Montesinos-Navarro *et al.*, 2012). Significance of fixed effects was tested using quasi-Newton pseudo-likelihood estimation and reported by the F and P values. Meanwhile, significance of random effects was tested removing each random factor one at a time and comparing −2 Log pseudo-likelihood of the nested models with χ^2 tests (Bolker *et al.*, 2009; Montesinos-Navarro *et al.*, 2012) using the COVTEST GLM option (SAS University edition). Non-significant random effects were removed from the model while significance of each random effect of the final model was tested using Wald tests and reported by Z and P values. When the main fixed effect was significant, least square means were compared using orthogonal contrast. All the means in the text are reported with their standard error.

Maternal effects on seed germination traits

One ANOVA was fitted for each germination time. Female, male and female by male interaction effects were considered as fixed effects. The after ripening effect on seed germination was calculated as the difference in seed germination between t2 and t1. The maternal effect was calculated by using the female and male effect ratio from the ANOVA's output (F_{female}/F_{male}), values close to 1 indicate no maternal effect while the larger the ratio, the larger the maternal effect.

Cross type and crop hybridisation effects on seed traits

Due to the greater importance of cross type than individual combinations, the biotypes were pooled in four different cross types: wild, wild–crop, crop–wild and crop (Table 1). For seed germination, all the times were

analysed jointly. In the ANOVA, the cross type effect was considered as fixed, whereas the biotype within the cross type, time and cross type by time interaction effects were considered as random. The interaction term was broken down using orthogonal contrasts. The cross type effect on the after ripening response, calculated as above, was evaluated. The cross type effect was considered as fixed, whereas the biotype within the cross types was considered as random. Similarly, to evaluate whether crop hybridisation affects seed traits, the cross type (including wild and wild–crop hybrids) was included as fixed effect, whereas the biotype within the cross type, time and cross type by time interaction effects were considered as random.

Maternal effect on seed morphological traits

We performed a multivariate analysis of variance (MANOVA) on the four traits (length and width of the seed, length/width ratio and seed weight) to evaluate the maternal effect on seed morphological traits. Female, male and female by male interaction effects were considered as fixed. MANOVA was evaluated using the Wilks' Lambda test criterion. Following any significant effect in MANOVA, we used principal component analysis (PCA) using a correlation matrix to avoid scale effects to produce principal components (PCs) that explained the multivariate variation in seed morphology. In addition, to evaluate the overall relationship between germination and morphological seed traits, we ran a PCA on six seed germination traits: germination at each of the four times, after ripening response and dry-induced secondary dormancy. PCA was run using PROC PRINCOMP (SAS University Edition) with mean values of each of the 14 biotypes as the input data set. Linear regression between seed morphological and germination traits was run using PROC REG (SAS University Edition). The anatomical traits considered as quantitative (middle layer thickness and number of cell layers) were recorded by eye in at least three independent samples from light microscopy and the means were compared using an unpaired *t*-test and reported with their standard error.

Results

Strong maternal effect on seed dormancy and after ripening response

There were significant female, male and female by male interaction effects on seed dormancy (Table 2). However, the female effects were much larger than male or female by male interaction effects at all of the four times evaluated (Table 2) and under the three temperatures tested (Fig. 1B). Immediately after harvest (t1), seed germination was relatively high in wild, low in crop and intermediate in their hybrids; but this trend was reversed at t2, and maintained at t3 and t4 (Fig. 1A). At t1, the female effect was 8.5-fold higher than the male effect showing a strong maternal effect. Crop hybridisation decreased seed germination on wild plants (0.25 ± 0.08 vs 0.12 ± 0.04, for wild and wild–crop seeds, respectively) whereas seeds produced on crop plants exhibited very low germination regardless of the pollen source (approximately 3% for both crop and crop–wild hybrids; Fig. 1A). At t2, the maternal effect was almost twice that at t1 (16.1; Table 2), seed germination of crop–wild hybrids, produced in both senses resembled their female parents (Fig. 1A). At t3 and t4, seeds produced on crop plants exhibited no dormancy, whereas in seeds produced on wild plants, the germination was higher in wild–crop hybrids than in the wild seeds (0.80 ± 0.08 vs 0.58 ± 0.12 at t3 and 0.45 ± 0.12 vs 0.35 ± 0.13 at t4, for wild–crop and wild, respectively; Fig. 1A). In addition, using seeds of 3 years after harvest, different incubation temperatures did not alter these results. Female effect was at least 15.6-fold higher than the male effect. Seeds produced on crop plants exhibited no dormancy at any of the incubation temperatures (Fig. 1B) whereas in seeds produced on wild plants, the germination was higher in wild–crop hybrids than in the wild seeds at all the three temperatures (0.77 ± 0.04 vs 0.53 ± 0.06 at 10°C, 0.48 ± 0.09 vs

Table 2 Analysis of variance (ANOVA) for seed germination at each of the four evaluated times. Different times are immediately (t1); 2 weeks (t2); 6 months (t3) and 18 months (t4) after harvest. Two wild populations (BAR and DIA) and two commercial cultivars (CAC and PAR) were used as both female and male parents in 14 of the 16 possible combinations (biotypes; Table 1). Data were analysed with generalised linear mixed models, models were adjusted using a Beta distribution. All the main effects (female, male and female by male interaction) were considered as fixed while residuals were considered as random

	Seed Germination		
Source of Variation	d.f.	*F*	*P*
Time 1			
Female	3	52.88	<0.0001
Male	3	6.19	0.0014
Female × male	7	4.01	0.0019
Time 2			
Female	3	107.77	<0.0001
Male	3	6.66	0.0006
Female × male	7	15.96	<0.0001
Time 3			
Female	3	83.26	<0.0001
Male	3	6	0.0017
Female × Male	7	2.46	0.033
Time 4			
Female	3	186.55	<0.0001
Male	3	5.64	0.0024
Female × Male	7	3.59	0.0041

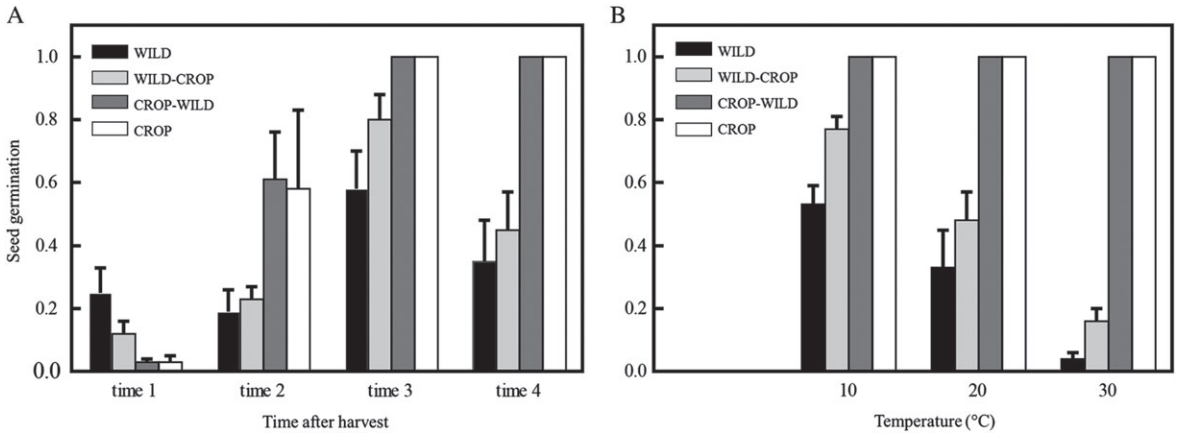

Figure 1 Seed germination across different times after harvest (A) and different temperatures (B). Crop–wild hybrids are denoted as crop–wild and wild–crop cross types to indicate the maternal parent. The bars represent mean proportion of germinated seeds of each cross type incubated at constant 20°C (A) and constant 10°C, 20°C and 30°C (B). All the seeds were incubated under neutral photoperiod. Standard error bars indicate variability within each cross type. Different times are immediately (t1); 2 weeks (t2); 6 months (t3) and 18 months (t4) after harvest. Seeds with 3 years after harvest, dry stored during this period at room temperature were incubated at constant temperatures of 10°C, 20°C and 30°C during 16 days.

0.33 ± 0.12 at 20°C and 0.16 ± 0.04 vs 0.04 ± 0.02 at 30°C, for wild–crop and wild, respectively; Fig. 1B).

In order to analyse the impact of short and long-lived dormancy, typical of crop and wild materials, respectively, we included the after ripening response as a variable (the difference of seed germination between t2 and t1). There were significant female and female by male interaction effects ($F = 135.17$; $P < 0.0001$ and $F = 12.68$; $P < 0.0001$) on the after ripening response, with no significant male effect ($F = 0.74$; $P = 0.5331$). The female effect was 182.7-fold higher than the male effect. In addition, there was a significant cross type effect ($F = 5.69$; $P = 0.0155$). The after ripening response was much higher in the crop than in wild seeds (contrast crop versus wild: $F = 6.56$; $P = 0.0283$), whereas the crop–wild hybrids response was similar to their female parents (contrasts: $F = 0.03$; $P = 0.8637$ and $F = 0.06$; $P = 0.811$ for crop versus crop–wild and wild versus wild–crop, respectively). This differential response confirmed the differences in the type of dormancy rather than the level between seeds of crop and wild plants.

Strong maternal effect on the seed morphological and anatomical traits

In the MANOVA, we found significant female, male and female by male interaction effects on seed morphological traits (Table 3). In the ANOVA, the female effect was significant and much stronger than the male and female by male interaction effects in each of the four variables. Female effect was 14.9-fold higher than the male effect in the MANOVA (Table 3). There was a significant cross type effect for length ($F = 197.06$; $P < 0.0001$), width ($F = 164.22$; $P < 0.0001$) and seed weight ($F = 101.25$; $P < 0.0001$), while no significant length by width ratio was found ($F = 3.04$; $P = 0.0795$). The biotype within the cross type effect improved the null model in all of the four variables (Table S1, Supporting information). Crop seeds were larger (0.81 ± 0.04 vs 0.59 ± 0.10 cm), wider (0.39 ± 0.04 vs 0.26 ± 0.04 cm) and heavier (43.8 ± 2.9 vs 18.5 ± 7.1 mg seed^{-1}) than the wild seeds whereas the wild seeds exhibited a greater length by width ratio than the crop ones (2.28 ± 0.06 vs 2.12 ± 0.09). Although slight changes in length, width and weight of the seeds of crop–wild hybrids were produced in the male parent direction, no significant contrasts were detected between the wild and wild–crop seeds, nor between the crop and crop–wild seeds for any of the four variables (Table S1).

Table 3 Multivariate analysis of variance (MANOVA) for two sets of traits. Morphological set including seed length, width, weight and length by width ratio. Germination set including seed germination at each of the four times, the after ripening response and dry-induced secondary dormancy. Different times are immediately (t1); 2 weeks (t2); 6 months (t3) and 18 months (t4) after harvest. Two wild populations (BAR and DIA) and two commercial cultivars (CAC and PAR) were used as both female and male parents in 14 of the 16 possible combinations (biotypes; Table 1)

Trait Set	Effect	d.f.	Wilks' Lambda	*F*	*P*
Morphological	Female	12	0.0011	130.40	<0.0001
	Male	12	0.2040	8.74	<0.0001
	Female × male	28	0.0738	6.61	<0.0001
Germination	Female	18	0.0004	88.16	<0.0001
	Male	18	0.0755	8.72	<0.0001
	Female × male	42	0.0109	6.82	<0.0001

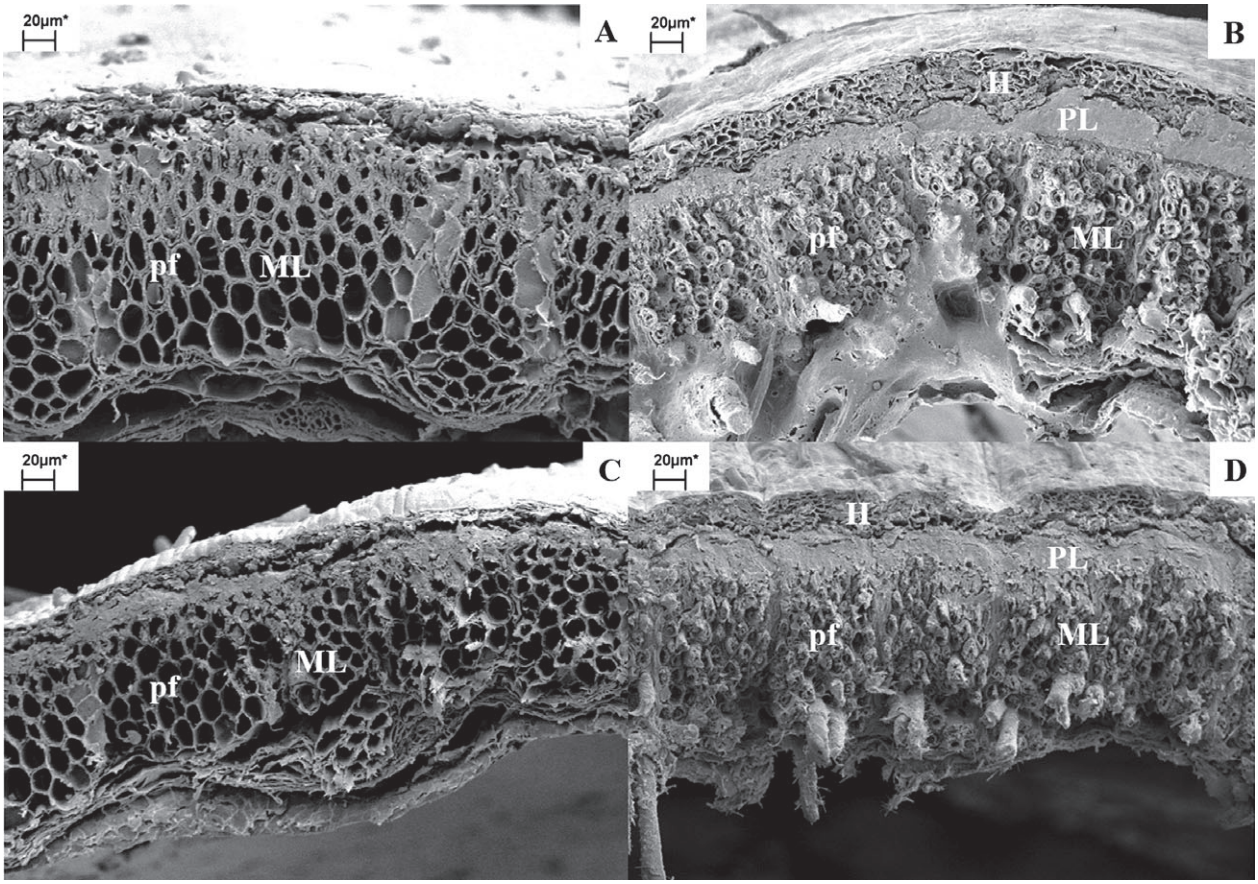

Figure 2 Pericarp anatomy observed with scanning electron microscopy of crop (A), wild (B) and crop–wild hybrids (C and D) seeds. Images shown correspond to Paraíso 104CL (PAR) as crop (A), Colonia Baron (BAR) as wild (B), and their reciprocal crosses (PAR × BAR as a crop–wild; C and BAR × PAR as a wild–crop; (D). H, hypodermis; ML, middle layer; pf, polygonal fibres; PL, phytomelanin layer. Lower case abbreviations indicate sub-structures.

In addition, clear differences in the anatomy of the crop and wild pericarps were observed. The greatest differences were observed in the middle layer. In wild pericarps, the middle layer consisted of 9.9 ± 0.7 cell layers with thick and strongly sclerified walls (Figs 2B and 3B), whereas the middle layer of crop pericarps consisted of 6.3 ± 0.7 cell layers with thin and much less sclerified walls (Figs 2A and 3A). In addition, parenchymatic rays and inner compressed cell layers were impregnated with phytomelanin in the wild pericarp but not in the crop pericarps (Fig. 3A and Fig. 3B).

Although the pericarp of crop–wild hybrids resembled their female parent (Figs 2 and 3), slight changes in the male parent direction were observed. Crop hybridisation affects the middle layer, decreasing the number of cell layers (7.3 ± 1.2 vs 9.9 ± 0.7 in wild–crop and wild, respectively; $t = 4.78$, $P = 0.0014$), the thickness (194.7 ± 4.6 µm vs 217.1 ± 18.0 µm for wild–crop and wild, respectively; $t = 2.07$, $P = 0.0725$) and the cell wall sclerification, mainly of the innermost cell layers (Fig. 3B and Fig. 3D). In the same way, wild to crop hybridisation affects the middle layer decreasing the thickness (183.3 ± 8.2 µm vs 232.0 ± 10.9 µm for crop–wild and crop, respectively; $t = 8.45$, $P = 0.0001$) and increasing the cell wall sclerification (Fig. 3A and Fig. 3C). In addition, in crop–wild hybrid pericarps, the parenchymatic rays and inner compressed cell layers were impregnated with phytomelanin as in the wild seeds (Fig. 3).

Crop hybridisation effects on seed traits

When wild–crop hybrids were compared with their wild counterparts, no significant differences were found in seed dormancy ($F = 0.05$; $P = 0.8285$). Similarly, when tested at the three incubation temperatures, no cross type ($F = 1.04$; $P = 0.4160$) nor cross type by temperature interaction effects ($F = 1.92$; $P = 0.1605$) were detected. Although the mean seed germination of wild–crop hybrids generally changed in the crop direction (Fig. 1A and Fig. 1B), such differences were explained by a large

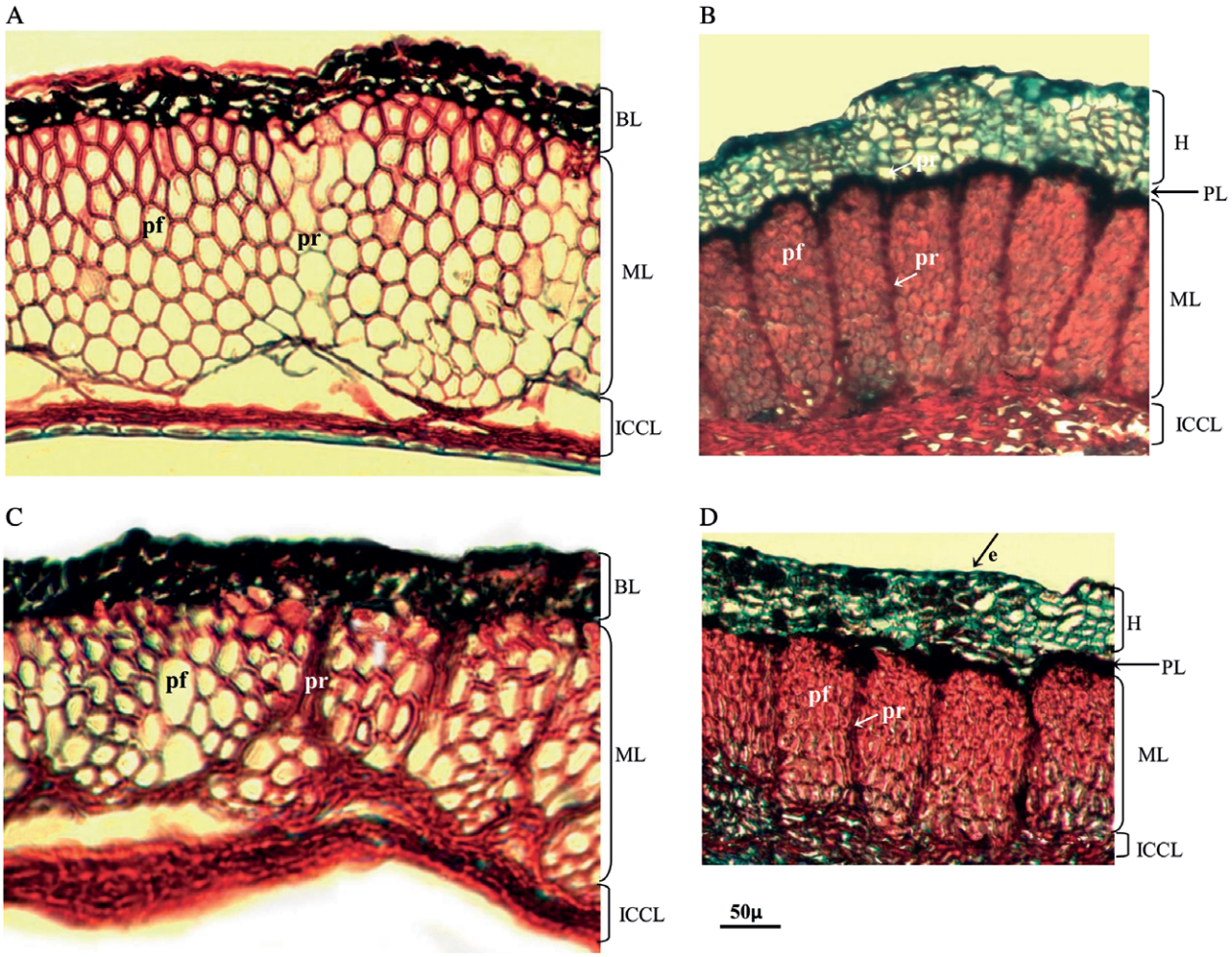

Figure 3 Pericarp anatomy observed with light microscopy. Images shown correspond to Paraíso 104CL (PAR) as crop (A), Colonia Baron (BAR) as wild (B), and their reciprocal crosses (PAR × BAR; C and BAR × PAR; (D). BL, black layer; H, hypodermis; ICCL, inner compressed cell layers; ML, middle layer; pf, polygonal fibres; PL, phytomelanin layer; pr, parenchymatic rays. Lower case abbreviations indicate sub-structures.

within cross type variability random effect ($Z = 1.69$; $P = 0.0454$). These results highlight the importance of including contrasting materials as random effects in overall comparisons in order to clarify the differences in arithmetic means. To unravel the biotype within cross type variability in seed dormancy we ran one ANOVA for each time and each temperature with the biotype as fixed effects, and we compared each wild population with their derived crosses to crop using orthogonal contrasts. The biotype effect was significant in all four times but the response was variable among times and between populations (Fig. 4A). Immediately after harvest, crop hybridisation affects seed dormancy in DIA but not in BAR populations, whereas the opposite effect was observed in all three remaining times (Fig. 4A). Except for DIA at t2, all the significant changes in seed germination were produced in the crop direction (Fig. 4A). Furthermore, with the exception of DIA at 20°C and BAR at 30°C, crop hybridisation significantly increased ($P < 0.05$) seed germination in both populations at each of the three temperatures (Fig. 4B). In addition, wild–crop hybrids exhibited a higher dry-induced secondary dormancy than wild seeds (0.35 ± 0.05 vs 0.24 ± 0.04 for wild–crop and wild, respectively).

Relationship between seed morphological and seed germination traits

Principal component analysis of the four morphological variables resulted in a composite variable designated as seed morphology PC1, which captured 79.65% of the total variation. Higher values of seed morphology PC1 represented higher values of length, width and seed weight, with loadings of 0.54, 0.55 and 0.51, respectively,

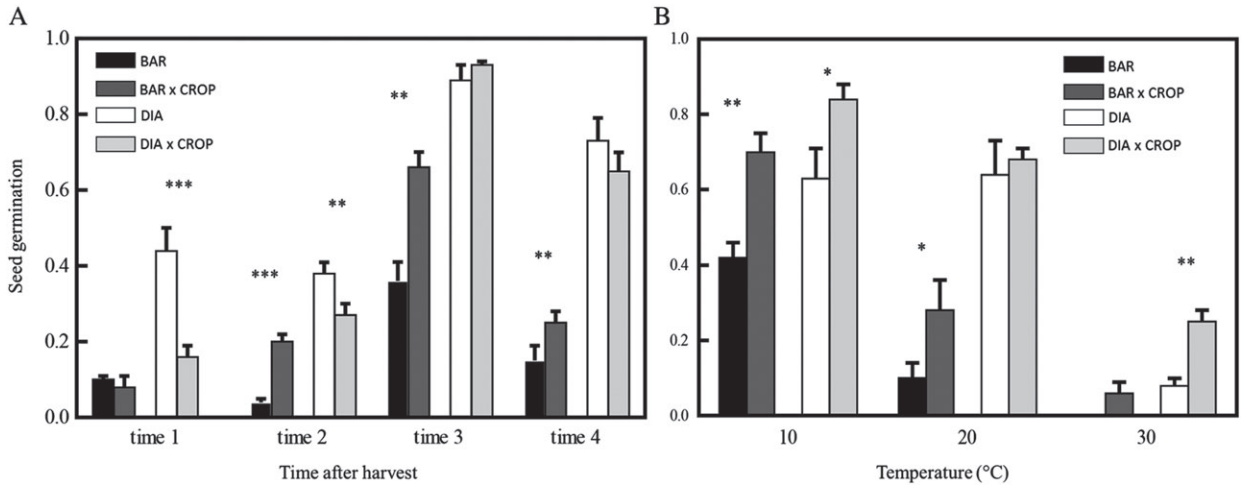

Figure 4 Crop hybridisation effect on seed germination at different times after harvest (A) and different temperatures (B). The bars represent mean proportion of germinated seeds of each biotype incubated at constant 20°C (A) and constant 10°C, 20°C and 30°C (B). All the seeds were incubated under neutral photoperiod. Standard error bars indicate variability within each biotype. Different times are immediately (t1); 2 weeks (t2); 6 months (t3) and 18 months (t4) after harvest. Seeds with 3 years after harvest, dry stored during this period at room temperature were incubated at constant temperatures of 10°C, 20°C and 30°C during 16 days. Within each time and temperature, each wild population [Colonia Baron (BAR) and Diamante (DIA)] is compared with their two wild–crop hybrids [(A) Paraíso 104CL (PAR) and Cacique CL (CAC) cultivars used as male parents] or with one wild–crop hybrid [(B) CAC cultivar used as male parent] using orthogonal contrasts. When significant the contrast: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$.

and lower values of length by width ratio, with a loading of −0.37. Higher and lower seed morphology values of PC1 represented crop-like seed morphology and wild-like seed morphology, respectively. Therefore, the seed morphology PC1 was used as a proxy of multivariate variation in seed morphology. In addition, the PCA of the six germination traits resulted in a composite variable designated as seed germination PC1, which captured 69.73% of the total variation. Higher values of seed germination PC1 represented higher values of germination at t2, t3, t4 and after ripening response, with loadings of 0.44, 0.41, 0.46 and 0.43, respectively and lower values of germination at t1 and dry-induced secondary dormancy, with loadings of −0.23 and −0.43, respectively. Except for the after ripening response, higher and lower values of seed germination PC1 represent exactly the crop-like and wild-like seed germination traits, respectively. The seed germination PC1 was then used as a proxy of multivariate variation in seed germination traits and was plotted against seed morphology PC1. There was a significant and strong correlation ($n = 14$, $r^2 = 0.92$, $P > 0.0001$; Fig. 5) between seed morphology and seed germination PC1s, suggesting that the maternal effects on seed germination traits are driven by the maternally inherited seed morphology.

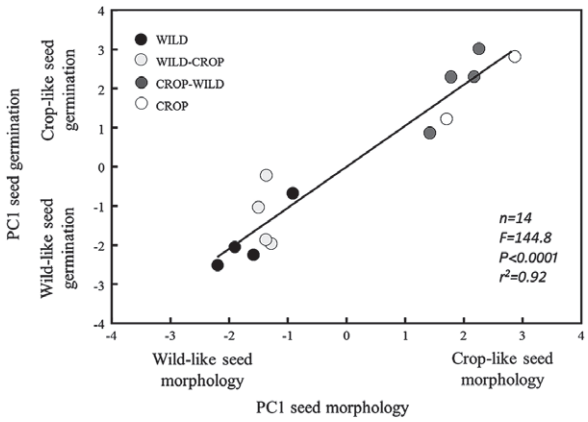

Figure 5 Relationship between seed morphological and seed germination traits. Both axes represent the first axes of the principal component analysis (see Materials and methods section for details). Crop–wild hybrids are denoted as crop–wild and wild–crop to indicate the maternal parent. Higher values of seed morphology PC1 represented higher values of length, width and seed weight, and lower values of length by width ratio. Higher values of seed germination PC1 represented higher values of germination at t2, t3, t4 and after ripening response, and lower values of germination at t1 and dry-induced secondary dormancy.

Discussion

In this study, we identified a seasonal pattern in seed germination and dormancy in wild seeds but not in the crop ones. Wild seed dormancy decreased after a simulated winter and increased when seeds were exposed to dry conditions, whereas crop seed dormancy was only strong immediately after harvest but it was rapidly removed with an after ripening period. In addition, neither seed dormancy nor the ability to develop secondary dormancy was

observed in crop seeds after the simulated winter (Fig. 1A and Fig. 1B). The selection pressure against seed dormancy during domestication may explain the observed differences in seed dormancy and germination. First generation hybrids between wild and crop parents resembled their female parents in seed germination traits with only slight effects from the male parents. Next, we analysed seed features inherited from the mother plant, such as seed size, form, weight and the pericarp anatomy. We found large differences between crop and wild in all seed morphological and anatomical traits (Figs 2 and 3), which strongly correlated with the observed differences in seed germination traits (Fig. 5). Thus, owing to the large maternal effect found in seed morphological, anatomical and germination traits, we focused the discussion on the evolutionary divergence between wild and crop seed traits and their implication in seed traits of crop–wild hybrids.

Evolutionary divergence between wild and crop seed traits

The loss of dormancy has been one of the earliest selected traits during domestication (Purugganan and Fuller 2009; Hancock 2012; Meyer and Purugganan 2013). Wild and weedy populations with non-deep PD exhibit seasonal patterns of seed dormancy (Baskin & Baskin, 2004; Gardarin & Colbach, 2015). Crops and their wild relatives with non-deep PD include most of the cereals, such as wheat, barley, sorghum, maize and rice, as well as model organisms, such as *Arabidopsis* and sunflower (Snow *et al.*, 1998; Baskin & Baskin, 2004; Finch-Savage & Leubner-Metzger, 2006; Li *et al.*, 2006; Vanhala & Stam, 2006; Montesinos-Navarro *et al.*, 2012; Shu *et al.*, 2015). In our study, the wild seeds exhibited variable levels of seed dormancy immediately after harvest, ranging between 57% and 91%. Differences in seed dormancy between these two wild populations were consistent across the times and the temperatures and the plausible causes were addressed in a previous study (Presotto *et al.*, 2014). The after ripening period did not increase seed germination in wild seeds (Fig. 1). After a simulated winter, seed dormancy was decreased up to 42% and after 1 year of dry storage, seed dormancy was increased up to 65% (Fig. 1A). In both populations and in all the four times and the three temperatures evaluated a proportion of seeds remained dormant, showing the ability of the wild populations to form persistent soil seed banks (Fig. 1A). This seasonal pattern observed in seed dormancy under controlled conditions has been previously reported in seed burial experiments using wild sunflower seeds (Snow *et al.*, 1998; Pace *et al.*, 2015) and reviewed in Gardarin & Colbach (2015) using seeds of weedy populations of several species. The seasonality of seed dormancy is the main adaptive trait of wild and weedy populations in spring annual species. This avoids germination under unfavourable conditions and contributes to the formation of a persistent soil seed bank.

In the crop, most of the seeds were dormant immediately after harvest (Fig. 1A). Additionally, crop seeds responded strongly to the after ripening treatment with up to 58% germination (Fig. 1A). After the simulated winter, no seed dormancy nor dry-induced secondary dormancy, in crop seeds was observed (Fig. 1A). In addition, when dormancy of crop seeds was lost, secondary dormancy was not expressed at any temperature (Fig. 1B) germinating up to 90% in the first 4 days (data not shown). A short-lived dormancy is a desirable trait in crop production because it prevents germination on the mother plant (pre-harvest sprouting) and allows a rapid and uniform germination in the next season (Shu *et al.*, 2015). A strong initial seed dormancy, totally or partially removed by an after ripening period was previously reported in cultivated sunflower (Brunick, 2007; Andrade *et al.*, 2015; Dominguez *et al.*, 2016; Roselló *et al.*, 2016) and results were similar to those reported in other crops, such as wheat, maize and rice (reviewed in Shu *et al.*, 2015). This short-lived dormancy in crop seeds may be the result of remnant dormancy from domestication (Dominguez *et al.*, 2016), of conscious selection against pre-harvest sprouting during modern improvement (Shu *et al.*, 2015) or a combination of both. As the loss of dormancy is associated with the earliest stage of plant domestication (Purugganan & Fuller, 2009; Hancock, 2012; Meyer & Purugganan, 2013) it seems unlikely that the sunflower crop was not fully domesticated for this trait.

Along with loss of seed dormancy, larger seeds with thinner and softer pericarps were selected during the earliest stages of domestication (Purugganan and Fuller 2009; Hancock 2012; Meyer & Purugganan, 2013). As expected, large differences in morphological traits between crop and wild seeds were observed. Crop seeds were 1.4-fold larger and 2.4-fold heavier on average than wild seeds. The increased seed size due to domestication is the rule in grain crops and this trait was probably selected for on account of the positive correlations between seed size and both uniform germination and early seedling performance (Purugganan & Fuller, 2009; Hancock, 2012; Meyer & Purugganan, 2013). On the other hand, thinner and softer pericarps were not only a byproduct of the selection for more uniform germination and reduced seed dormancy, but also, they were likely selected for the ease to shell the seeds for consumption. In addition to the expected reduction in pericarp thickness during domestication (Hancock, 2012), most of the genetic gain in oil concentration during modern breeding was reached by

decreasing the pericarp by embryo ratio (Mantese *et al.*, 2006; de la Vega *et al.*, 2007).

Next, we analysed the pericarp anatomy of one cultivated material, the two wild populations and their reciprocal crosses. The pericarp of the cultivated sunflower consists of an epidermis, hypodermis, a middle layer and, in some materials, a phytomelanin layer in between, with slight differences in the pericarp anatomy between dormant and non-dormant inbred lines (Andrade *et al.*, 2015). The phytomelanin layer is exclusive to a few tribes of the *Asteraceae* family (Pandey & Dhakal, 2001) and consists in a hard, black, resistant layer responsible for the black colour in the seeds and the resistance against sunflower moth in cultivated sunflower (Rogers & Kreitner, 1983; Pandey & Dhakal, 2001). In our study, the crop pericarp exhibited the typical structure previously reported for sunflower pericarp anatomy: a thin hypodermis and a middle layer of parenchymatic cells. The parenchyma cells are thin walled and loosely packed (Figs 2 and 3; Rogers & Kreitner, 1983; Mantese *et al.*, 2006; Andrade *et al.*, 2015). This structure may impose dormancy in cultivated materials in one or more of the following ways: decreasing the imbibition rate, delaying the entrance of oxygen to the embryo, containing germination inhibitors or preventing the leaching of germination inhibitors from the embryo (Baskin & Baskin, 2014). However, it seems unlikely that such a structure would act as a physical barrier. In contrast, the wild pericarp anatomy showed a thicker hypodermis, a thicker phytomelanin layer and smaller polygonal fibres with much thicker cell walls than the crop pericarp (Figs 2 and 3). The middle layer resembled the sclerenchyma more than the parenchyma cells. In addition to the much thicker and lignified walls of polygonal fibres, the phytomelanin layer fills the pericarp rays in the middle layer (Fig. 3). The phytomelanin layer adopts its morphology as it fills intercellular spaces in the pericarp, usually between the hypodermis and the sclerenchyma (Rogers & Kreitner, 1983) but also crossing the middle layer vertically and forming rectangular blocks (Fig. 3). These blocks may act as a physical barrier similar to those encountered in seeds with physical dormancy. This would partially explain the higher imbibition of scarified versus non-scarified wild seeds (Presotto *et al.*, 2014) but it does not fully explain the seasonal variation in seed dormancy. Seeds with physical dormancy are unable to germinate due to the presence of a water-impermeable seed or fruit coat, they commonly have non-dormant embryos and one or more layers of palisade cells, responsible for water-impermeability (Baskin & Baskin, 2004). Germination of seeds with physical dormancy usually occurs due to the formation of a specialised anatomical structure on the seed or fruit coat, acting as a water gap, through which water can move (Baskin & Baskin, 2004, 2014). Weiss *et al.* (2013) reported that the pericarp of the sunflower crop allows imbibition by the embryo through a pericarp gap present in most seeds from crop plants and absent in the most seeds from wild plants, suggesting a physical mechanism of seed dormancy. However, the seasonal pattern in seed dormancy observed in our study (Fig. 1A) and the absence of a palisade layer in the pericarp (Figs 2B and 3B) provide physiological and anatomical evidence of non-deep PD rather than physical dormancy in wild sunflower seeds.

The role of the maternal effect and domestication in seed traits of crop–wild hybrids

In our study, seed germination of wild–crop hybrids changed in the crop direction in all the four times and the three evaluated temperatures (Fig. 1A and Fig. 1B). The germination of wild–crop seeds immediately after harvest was lower than the germination of wild seeds, but in the three remaining times crop hybridisation increased seed germination (Fig. 1A). Crop seed dormancy is usually described as a short-lived dormancy mainly imposed by the embryo with slight and variable levels of seed-coat-imposed dormancy (Brunick, 2007; Andrade *et al.*, 2015; Dominguez *et al.*, 2016; Roselló *et al.*, 2016) whereas wild seed dormancy is a long-term dormancy mainly imposed by seed coats (Brunick, 2007). The increased dormancy of wild–crop seeds immediately after harvest may be explained by an additive effect of the dormant pericarp (from wild) plus the effect of crop and wild alleles in the embryo. After cold treatment, the hybrid embryo could be responsible for the increased germination of wild–crop hybrids because the surrounding tissues are shared between wild and wild–crop hybrids (Fig. 1A), although hybridisation effects on post-fertilisation events on seed pericarp, such as middle layer sclerification and/or phytomelanin layer deposition, cannot be discarded. The increased seed dormancy observed at higher incubation temperatures in both wild and wild–crop hybrids (Fig. 1A) has been previously reported (Presotto *et al.*, 2014) and may be result of the increased pericarp-imposed dormancy (Dominguez *et al.*, 2016). The increased seed germination with crop hybridisation was previously reported in sunflower crop–wild hybrids (Snow *et al.*, 1998; Mercer *et al.*, 2006) and other crop–wild complexes, such as oil-seed rape (Adler *et al.*, 1993) and rice (Dong *et al.*, 2011).

In addition to the increase of seed germination with crop hybridisation described above, a differential effect according to the wild recipient population was observed (Fig. 4A and Fig. 4B). Immediately after harvest, crop hybridisation only affects the less dormant wild population (DIA), however in the three remaining times crop

hybridisation increased seed germination in the more dormant population (BAR; Fig. 4A). Similar results were found in seed burial experiments by Snow *et al.* (1998) who reported increased seed germination with hybridisation in the two more dormant populations, but with no effects on the less dormant one. Such a differential response could have implications in crop gene introgression and weed evolution. Without any change in seed dormancy due to crop hybridisation, the relative fitness of F1 crop–wild hybrids will depend on the later life-history traits, such as seedling competition ability, flowering time, biomass and seeds production. Although relative fitness of F1 crop–wild hybrids is usually lower than in their wild counterparts (Presotto *et al.*, 2012), this advantage is reduced and may even disappear under stressful conditions (Mercer *et al.*, 2007, 2014) as crop gene introgression is highly environment-dependent. On the other hand, increased germination with crop hybridisation may apparently reduce the chance of crop gene introgression by increasing autumn maladaptive germination and by exposing F1 plants to intense competition with wild ones (Alexander *et al.*, 2014). However, maladaptive germination can be rapidly removed by selection (Huang *et al.*, 2010) and the increased spring germination may increase the aggressiveness of the population. Concerning the populations studied, they exhibit no more morphological evidence of crop gene introgression than populations from the native habitat (Cantamutto *et al.*, 2010). In the case of DIA, the absence of sympatry explains the absence of crop gene introgression. In contrast, the BAR population is sympatric with the crop, and natural crop–wild hybrids are produced (Ureta *et al.*, 2008). Interestingly hybridisation between the BAR population and the crop is the most likely origin of the first weedy sunflower biotype reported in Argentina (Casquero *et al.*, 2013).

The traits of seeds produced on crop plants did not change with wild hybridisation. In this study, no differences were detected between the crop and crop–wild hybrids in any of the evaluated traits. Immediately after harvest, the crop–wild hybrids showed a similar deep dormancy as their crop parent (Fig. 1A). Two weeks after harvest, the seed dormancy of crop and crop–wild hybrids was very similar but varied according to the cultivated material and the pollen donor, for example, CAC seeds germinated up to 33%, but seed germination increased with BAR hybridisation (66%) and decreased with DIA hybridisation (20%). The opposite occurred in PAR, as seeds germinated up to 82%, but in hybridisation with BAR and DIA it decreased by up to 66% and increased by up to 93%, respectively. So, although wild hybridisation affects seed germination, no clear pattern was evident. After the cold treatment, neither seed dormancy nor dry-induced secondary dormancy was observed in any of the crosses produced on crop plants (Fig. 1A). Moreover, no secondary dormancy was expressed at any of the evaluated temperatures (Fig. 1B). The rapid increase in seed germination along with the lack of dormancy after the cold treatment and the absence of secondary dormancy explain why seeds produced on crop plants did not usually persist for more than a year under field conditions (Warwick & Stewart, 2005; Bagavathiannan & Van Acker, 2008). The germination 2 weeks after harvest would be a maladaptive trait under field conditions (Pace *et al.*, 2015); that is, most of the seeds that germinate at autumn do not survive to winter, and they do not contribute to either the seed bank or the next generation. In addition to the morphological traits, the absence of wild hybridisation effects on seed traits may reside in the features of the crop seed pericarp. The anatomy observed on crop and crop–wild hybrid seeds showed that no apparent physical barriers exist at the pericarp level (Figs 2 and 3). Recently, Andrade *et al.* (2015), comparing one dormant and one non-dormant inbred lines with pericarp-imposed dormancy, noted that the hormonal profiles of the pericarp explained the differences in seed dormancy, not the pericarp anatomy. Therefore in crops with non-deep PD, even when the pericarp plays a role in seed dormancy, such an effect seems to be mediated by hormonal profiles, be of short-lived type and easily removed by an after ripening period (Andrade *et al.*, 2015; Shu *et al.*, 2015; Roselló *et al.*, 2016). Whether the increased pericarp-imposed dormancy observed at high incubation temperatures (Dominguez *et al.*, 2016) and warmer conditions during seed development (Bodrone *et al.*, 2017) still is mediated by hormonal profiles remains elusive.

In spite of the apparent obscure fate of the seeds produced on crop plants under field conditions, the survival of some seeds for only one season may be key in the evolution of feral forms. Early life-history traits, such as seed dormancy and germination timing, are key traits in feral populations (Warwick & Stewart, 2005; Bagavathiannan & Van Acker, 2008) and can be strongly selected for during the first stages of adaptation to new habitats (Huang *et al.*, 2010). Fallen seeds produced on crop plants under field conditions may exhibit an initial strong dormancy, regardless of whether they are crop–wild hybrids or not (Fig. 1A). The after ripening period will remove most of the seed dormancy in such seeds but germination will only occur if the germination requirements of moisture and temperature are met. In this case, seeds that germinated at autumn are unlikely to survive until the next spring, the autumn germination being a maladaptive trait (Alexander *et al.*, 2014). In our study, on average more than 50% of the seeds produced on the more dormant cultivated material (CAC) remained dormant.

Although this dormancy could have been reduced by a longer after ripening period or by lower incubation temperatures, higher temperatures during seed development (Bodrone *et al.*, 2017) and seed incubation (Dominguez *et al.*, 2016) might increase the seed dormancy expressed at the autumn, increasing the chance of seeds survival. On the other hand, a higher maladaptive germination of the seeds produced on crop plants may expose crop–wild hybrids to an intense selection for seed dormancy as the first adaptive trait, conditioning the selection for traits expressed later in the life cycle (Huang *et al.*, 2010). The rise of weedy sunflower biotypes with crop ancestry has been reported in Argentina (Casquero *et al.*, 2013) and in southern Europe (Muller *et al.*, 2011) in both cases facilitated by wild hybridisation. Those cases add to the weedy biotypes reported in sorghum, rice and radish of similar origin (Ellstrand *et al.*, 2010) and give a warning about the importance of crop–wild hybridisation in the rapid weed evolution.

In summary, we found evidence of large maternal effects on seed germination and dormancy traits imparted by maternally inherited tissues in crop–wild sunflower hybrids. Hybridisation between highly divergent taxa, such as crops and their wild relatives can exacerbate the importance of maternal effects on early life-history traits. Crop-like and wild-like seed traits imparted by the maternal parent will be likely to result in different behaviour in the field and thus there are different selection pressures on the first generation of crop–wild hybrids with the same genetic composition, affecting the selection of important traits expressed later in the life cycle and in following generations. Further studies using synthetic and natural crop–wild hybrids are needed to better understand which and how seed traits are being selected in the wild.

Acknowledgements

We thank the ANPCYT for a fellowship for F. H. We also thank to Emilia Acosta and Marcela Scarlatto for their valuable field and laboratory assistances and the anonymous reviewers for their helpful suggestions. This work was supported by grant 'Agencia Nacional de Promoción Científica y Tecnológica' (ANPCYT-PICT 2012-2854).

References

Adler L., Wikler K., Wyndham F., Linder C., Schmitt J. (1993) Potential for persistence of genes escaped from canola: germination cues in crop, wild, and crop-wild hybrid *Brassica rapa*. *Functional Ecology*, **7**, 736–745.

Alexander H.M., Schrag A.M. (2003) Role of soil seed banks and newly dispersed seeds in population dynamics of the annual sunflower, *Helianthus annuus*. *Journal of Ecology*, **91**, 987–998.

Alexander H.M., Emry D.J., Pace B.A., Kost M.A., Sparks K.A., Mercer K.L. (2014) Roles of maternal effects and nuclear genetic composition change across the life cycle of crop–wild hybrids. *American Journal of Botany*, **101**, 1176–1188.

Andrade A., Riera N., Lindstrom L., Alemano S., Alvarez D., Abdala G., Vigliocco A. (2015) Pericarp anatomy and hormone profiles of cypselas in dormant and non-dormant inbred sunflower lines. *Plant Biology*, **17**, 351–360.

Bagavathiannan M.V., Van Acker R.C. (2008) Crop ferality: implications for novel trait confinement. *Agriculture, Ecosystems and Environment*, **127**, 1–6.

Baskin J.M., Baskin C.C. (2004) A classification system for seed dormancy. *Seed Science Research*, **14**, 1–16.

Baskin C.C., Baskin J.M. (2014) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. 2nd edn. San Diego, CA, USA: Academic Press/Elsevier.

Blackman B.K., Scascitelli M., Kane N.C., Luton H.H., Rasmussen D.A., Bye R.A., Lentz D.L., Rieseberg L.H. (2011) Sunflower domestication alleles support single domestication center in eastern North America. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14360–14365.

Bodrone M.P., Rodríguez M.V., Arisnabarreta S., Batlla D. (2017) Maternal environment and dormancy in sunflower: the effect of temperature during fruit development. *European Journal of Agronomy*, **82**, 93–103.

Bolker B.M., Brooks M.E., Clark C.J., Geange S.W., Poulsen J.R., Stevens M.H.H., White J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, **24**, 127–135.

Brunick R.L. (2007) Seed dormancy in domesticated and wild sunflowers (*Helianthus annuus* L.): types, longevity and QTL discovery. PhD Dissertation. Oregon State University, Corvallis, Oregon.

Burke J.M., Tang S., Knapp S.J., Rieseberg L.H. (2002) Genetic analysis of sunflower domestication. *Genetics*, **161**, 1257–1267.

Cantamutto M., Presotto A., Moroni I.F., Alvarez D., Poverene M., Seiler G. (2010) High infraspecific diversity of wild sunflowers (*Helianthus annuus* L.) naturally developed in central Argentina. *Flora*, **205**, 306–312.

Casquero M., Presotto A., Cantamutto M. (2013) Exoferality in sunflower (*Helianthus annuus* L.): a case study of intraspecific/interbiotype interference promoted by human activity. *Field Crops Research*, **142**, 95–101.

Chiang G.C.K., Barua D., Dittmar E., Kramer E.M., de Casas R.R., Donohue K. (2013) Pleiotropy in the wild: the dormancy gene DOG1 exerts cascading control on life cycles. *Evolution*, **67**, 883–893.

Croce M., Parodi E. (2013). The turf-forming alga Gelidium crinale (Florideophyceae, Rhodophyta) on Atlantic Patagonian coasts. *Botanica Marina*, **56**, 131–141.

Dominguez C.P., Battla D., Rodríguez M.V., Windauer L.B., Gerbaldo M., Benech-Arnold R.L. (2016)

Pericarp-imposed dormancy in sunflower: physiological basis, impact on crop emergence, and removal at an industrial scale. *Crop Science*, **56**, 716–726.

Dong S.S., Xiao M.Q., Rong J., Liao H., Lu B.R., Chen J.K., Song Z.P. (2011) No effect of transgene and strong wild parent effects on seed dormancy in crop-wild hybrids of rice: implications for transgene persistence in wild populations. *Annals of Applied Biology*, **159**, 348–357.

Ellstrand N.C., Schierenbeck K.A. (2006) Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica*, **148**, 35–46.

Ellstrand N.C., Prentice H.C., Hancock J.F. (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics*, **30**, 539–563.

Ellstrand N.C., Heredia S.M., Leak-Garcia J.A., Heraty J.M., Burger J.C., Yao L., Nohzadeh-Malakshah S., Ridley C.E. (2010) Crops gone wild: evolution of weeds and invasives from domesticated ancestors. *Evolutionary Applications*, **3**, 494–504.

Ellstrand N.C., Meirmans P., Rong J., Bartsch D., Ghosh A., de Jong T.J., Haccou P., Lu B.-R., Snow A.A., Stewart C.N., Strasburg J.L., van Tienderen P.H., Vrieling K., Hooftman D. (2013) Introgression of crop alleles into wild or weedy populations. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 325–345.

Finch-Savage W.E., Leubner-Metzger G. (2006) Seed dormancy and the control of germination. *New Phytologist*, **171**, 501–523.

Gandhi S.D., Heesacker A.F., Freeman C.A., Argyris J., Bradford K., Knapp S. (2005) The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. *Theoretical and Applied Genetics*, **111**, 619–629.

Gardarin A., Colbach N. (2015) How much of seed dormancy in weeds can be related to seed traits? *Weed Research*, **55**, 14–25.

Gutierrez A., Rueda F., Cantamutto M., Poverene M. (2014) Self-pollination and its implication in invasiveness of *Helianthus annuus* ssp. *annuus* and *H. petiolaris*. *Journal of Basic and Applied Genetics*, **25**, 5–15.

Hancock J.F. (2012) *Plant Evolution and the Origin of Crop Species*. Wallingford, UK: CABI.

Harter A.V., Gardner K.A., Falush D., Lentz D.L., Bye R.A., Rieseberg L.H. (2004) Origin of extant domesticated sunflowers in eastern North America. *Nature*, **430**, 201–205.

Huang X., Schmitt J., Dorn L., Griffith C., Effgen S., Takao S., Koornneef M., Donohue K. (2010) The earliest stages of adaptation in an experimental plant population: strong selection on QTLS for seed dormancy. *Molecular Ecology*, **19**, 1335–1351.

ISTA-International Seed Testing Association. (2004). International Rules for Seed Testing Glattbrugg, Switzerland.

Li C., Zhou A., Sang T. (2006) Genetic analysis of rice domestication syndrome with the wild annual species, *Oryza nivara*. *New Phytologist*, **170**, 185–194.

Mantese A.I., Medan D., Hall A.J. (2006) Achene structure, development and lipid accumulation in sunflower cultivars differing in oil content at maturity. *Annals of Botany*, **97**, 999–1010.

Mercer K.L., Shaw R.G., Wyse D.L. (2006) Increased seed germination of diverse crop-wild hybrid sunflower seeds. *Ecological Applications*, **16**, 845–854.

Mercer K.L., Andow D.A., Wyse D.L., Shaw R.G. (2007) Stress and domestication traits increase the relative fitness of crop-wild hybrids in sunflower. *Ecology Letters*, **10**, 383–393.

Mercer K.L., Emry D.J., Snow A.A., Kost M.A., Pace B.A., Alexander H.M. (2014) Fitness of crop-wild hybrid sunflower under competitive conditions: implications for crop-to-wild introgression. *PloS One*, **9**, e109001.

Meyer R.S., Purugganan M.D. (2013) Evolution of crop species: genetics of domestication and diversification. *Nature Reviews. Genetics*, **14**, 840–852.

Montesinos-Navarro A., Picó F.X., Tonsor S.J. (2012) Clinal variation in seed traits influencing life cycle timing in *Arabidopsis thaliana*. *Evolution*, **66**, 3417–3431.

Muller M.-H., Latreille M., Tollon C. (2011) The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (*Helianthus annuus* L.) in Spain and France. *Evolutionary Applications*, **4**, 499–514.

Oracz K., Bouteau H.E.M., Farrant J.M., Cooper K., Belghazi M., Job C., Job D., Corbineau F., Bailly C. (2007) ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant Journal*, **50**, 452–465.

Pace B.A., Alexander H.M., Emry J.D., Mercer K.L. (2015) Seed fates in crop-wild hybrid sunflower: crop allele and maternal effects. *Evolutionary Applications*, **8**, 121–132.

Pandey A.K., Dhakal M.R. (2001) Phytomelanin in Compositae. *Current Science*, **80**, 933–940.

Poverene M., Cantamutto M., Seiler G.J. (2008) Ecological characterization of wild *Helianthus annuus* and *Helianthus petiolaris* germplasm in Argentina. *Plant Genetic Resources*, **7**, 42.

Presotto A., Fernández-Moroni I., Poverene M., Cantamutto M. (2011) Sunflower crop-wild hybrids: Identification and risks. *Crop Protection*, **30**, 611–616.

Presotto A., Ureta M.S., Cantamutto M., Poverene M. (2012) Effects of gene flow from IMI resistant sunflower crop to wild *Helianthus annuus* populations. *Agriculture, Ecosystems and Environment*, **146**, 153–161.

Presotto A., Poverene M., Cantamutto M. (2014) Seed dormancy and hybridization effect of the invasive species, *Helianthus annuus*. *Annals of Applied Biology*, **164**, 373–383.

Purugganan M.D., Fuller D.Q. (2009) The nature of selection during plant domestication. *Nature*, **457**, 843–848.

Roach D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, **18**, 209–235.

Rogers C.E., Kreitner G.L. (1983) Phytomelanin of sunflower achenes: a mechanism for pericarp resistance to abrasion by larvae of the sunflower moth (Lepidoptera: Pyralidae). *Environmental Entomology*, **12**, 277–285.

Roselló P.L., Vigliocco A.E., Andrade A.M., Riera N.V., Calafat M., Molas M.L., Alemano S.G. (2016) Differential hormonal and gene expression dynamics in two inbred sunflower lines with contrasting dormancy level. *Plant Physiology and Biochemistry*, **102**, 133–140.

Shu K., Meng Y.J., Shuai H.W., Liu W.G., Du J.B., Liu J., Yang W.Y. (2015) Dormancy and germination: how does the crop seed decide? *Plant Biology*, **17**, 1104–1112.

Snow A.A., Moran-Palma P., Rieseberg L.H., Wszelaki A., Seiler G.J. (1998) Fecundity, phenology, and seed dormancy of F1 wild-crop hybrids in sunflower (*Helianthus annuus*, Asteraceae). *American Journal of Botany*, **85**, 794–801.

Ureta M.S., Cantamutto M., Carrera A., Delucchi C., Poverene M. (2008) Natural hybrids between cultivated and wild sunflowers (*Helianthus* spp.) in Argentina. *Genetic Resources and Crop Evolution*, **55**, 1267–1277.

Vanhala T.K., Stam P. (2006) Quantitative trait loci for seed dormancy in wild barley (*Hordeum spontaneum* C. Koch). *Genetic Resources and Crop Evolution*, **53**, 1013–1019.

de la Vega A.J., DeLacy I.H., Chapman S.C. (2007) Changes in agronomic traits of sunflower hybrids over 20 years of breeding in central Argentina. *Field Crops Research*, **100**, 73–81.

Warwick S.I., Stewart C.N. (2005) Crops come from wild plants – how domestication, transgenes, and linkage together shape ferality. In *Crop Ferality and Volunteerism*, pp. 9–29. Ed. J. Gressel. Boca Raton, FL, USA: CRC Press.

Weiss A.N., Primer S.B., Pace B.A., Mercer K.L. (2013) Maternal effects and embryo genetics: germination and dormancy of crop–wild sunflower hybrids. *Seed Science Research*, **23**, 241–255.

Willis C.G., Baskin C.C., Baskin J.M., Auld J.R., Venable D.L., Cavender-Bares J., Donohue K., de Casas R.R., Bradford K., Burghardt L., Kalisz S., Meyer S., Schmitt J., Strauss S., Wilczek A. (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist*, **203**, 300–309.

Wills D.M., Burke J.M. (2007) Quantitative trait locus analysis of the early domestication of sunflower. *Genetics*, **176**, 2589–2599.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Analysis of variance (ANOVA) for four morphological traits