

# Broadening the Genetic Basis for Crop Improvement: Interspecific Hybridization within and Between ploidy Levels in *Helianthus*

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## ABSTRACT

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The genus *Helianthus* is both economically important and genetically diverse. It contains two important crop species *H. annuus* and *H. tuberosus* in addition to being a model for evolutionary studies. The large number of species within the genus and the ability to hybridize makes the use of crop wild relatives as sources of novel phenotypes particularly promising as a way to introduce novel variation. Additionally, the promiscuity of genus allows for an understanding of the genome dynamics within and between ploidy levels at many different evolutionary distances. *Helianthus* is an excellent system to study how hybridization can be used to explore the utilization of wild germplasm in crop improvement and how it led to the creation of hybrid sunflower industry. New mating designs and technology combined with the need to develop crops resilient to changing environments will increase the value of wild germplasm.

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## Introduction

### ***Plant Genetic Resources: Crop Wild Relatives***

Crop plants often are less resistant to biotic and abiotic stresses than their wild relatives. The loss of resistance accompanying crop domestication and improvement is hypothesized to be a by-product of selection for yield under ideal conditions. This hypothesis is based on reports of trade-offs

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between plant productivity and stress resistance (e.g. Mayrose et al. 2011). In addition, population bottlenecks during domestication and improvement have likely lead to the stochastic loss of resistance alleles in crop germplasm (Tanksley and McCouch 1997). The loss of stress resistance in crops, coupled with reduced diversity of crop gene pools, has become especially worrying recently as we attempt to increase crop productivity in the face of climate change, rapid population growth, and heightened competition for land and water (McCouch et al. 2013; Dempewolf et al. 2014; Gentzbittel et al. 2015).

One approach to minimize yield losses caused by environmental stress is to breed cultivars that combine high yield with resistance to biotic and abiotic stress. However, such breeding efforts require access to resistant germplasm, which may not exist in the cultivated gene pool. On the other hand, such resistance often resides in the wild relatives of crop plants, which often thrive under stressful conditions (Ricklefs and Jenkins, 2011; Thormann et al. 2012). While the genes and mutations underlying stress resistance can be obtained through genetic engineering, it is typically cheaper and less technologically challenging to obtain them through sexual hybridization and introgression. For hybridization to be useful to humans, crops must be cross-compatible with their wild relatives, hybrids must have non-zero fitness, and agronomically valuable alleles must be present in the wild background (Burke and Arnold, 2001; Rieseberg, 1997; Arias and Rieseberg, 1995). Indeed, many different crop wild relative species meet these criteria, and both intra- and interspecific hybridization has been employed to transfer useful traits into many different crop plants (reviewed in Hajjar and Hodgkin, 2007). In this chapter, we discuss how hybridization has been (or could be) employed to aid crop improvement in the genus *Helianthus*, which contains two crops: the diploid sunflower (*Helianthus annuus* L.) and the hexaploid *Helianthus tuberosus* L. ( $2n = 6x = 102$ ).

### **Definitions**

The term hybridization can be restricted to offspring formed by matings between species, or defined more broadly as the offspring of individuals from genetically differentiated populations. We prefer the second, broader definition for two reasons. First, it avoids issues with species definitions. Second, it is consistent with the plant breeding literature, in which hybridization is typically employed to describe crosses within the species, while wide hybridization is generally used to refer to crosses between different species. Likewise, introgression can be narrowly defined as the transfer of genes or traits between species via backcrossing, or more broadly as the sexual transfer of genetic material between genetically distinguishable populations. Again, we prefer the broader definition since it provides greater flexibility in usage.

Traditionally wild plant species related to cultivated species, i.e. crop wild relatives (CWR), have been placed in groups based on their crossing

relationship with the crop. The primary germplasm have no crossing barriers with the crop producing fully fertile progeny, the secondary germplasm produce hybrids with some meiotic abnormalities (due to chromosome translocations etc. that influence  $F_1$  meiosis but not viability or hybridization), the tertiary germplasm requires special techniques (e.g. embryo rescue, protoplast fusion) to produce hybrids, and within the quaternary germplasm hybrids cannot be produced via sexual or somatic means, although genetic introgressions can be achieved using recombinant DNA technology (Harlan and De Wet, 1971; Harlan, 1976; Gepts 2000).

### *Helianthus*

*Helianthus* is native to North America and contains 52 species (67 total taxa) (Marek and Seiler, 2011; Kane et al. 2013), occupying diverse ecological niches across much of North America including deserts, marshes and open plains. *Helianthus* is a promiscuous genus with many species readily hybridizing within and between ploidy levels. Hybridization and introgression appear to have facilitated colonization of diverse environments (Whitney et al. 2006, 2010; Thompson et al. 1981; Rogers et al. 1982). The two *Helianthus* crops originated from different sections within the genus. Sunflower arose within the annual section *Helianthus*, centered in central and western North America, while *H. tuberosus* is an autoallohexaploid from the perennial section *Divaracatus*, centered in northeastern North America.

### **Sunflower Domestication and Improvement**

The process of domestication involves both the elimination of unwanted traits, and the development of traits that facilitate cultivation and improve yield. These two overlapping aspects of domestication transform plants from their natural forms to new and distinguishable types. Improvement refers to the phenotypic changes wrought by modern breeding, which may overlap with those sought by early farmers. Modern breeders typically target yield and quality traits, traits that confer resistance to biotic or abiotic stress, and traits that confer adaptation to local environments or that optimize crops for particular uses.

While it is clear that artificial selection (both conscious and unconscious) drives phenotypic evolution during domestication and improvement, the role of controlled crosses (both within and between species) in domestication is less clear. However, the use of controlled crosses is a critical component of modern breeding programs. Sunflower (*H. annuus*) offers an especially compelling example of the value of wide hybridization in crop improvement.

Sunflower ( $2n = 2x = 34$ ) was domesticated in eastern North America approximately 4000 years ago (Harter et al. 2004, Blackman et al. 2011) in present day states of Arkansas, Kentucky, Illinois and Tennessee (Smith, 2006; Smith 2013). 'Wild' traits that were eliminated during sunflower

domestication include branching, seed shattering, self-incompatibility and extended dormancy. Traits bred by early farmers to facilitate harvesting and enhance yield include increased seed size, increased oil content and adjustment of flowering time (Harlan et al. 1973; Burke et al. 2002a; Harter et al. 2004; Seiler and Jan, 2010; Blackman et al. 2011).

At the time of European contact, sunflower played a significant role in North American agriculture and was associated with many different Native American tribal nations (Sturtevant 1885; Jenks 1916; Jones et al. 1933; Thone 1936; Heiser, 1951; Heiser 1955; Kaplan 1963; Wasley 1962; Fritz 1990). Sunflower is often present in North American folklore (Heiser, 1951), and was of particular importance to the tribal nations of the American Southwest (Navajo, Apache, Pueblo and Hopi), figuring prominently in their folklore (Wallis 1936; Yarnell 1965; Minnis 1989). There were many regional and use-specific landraces (Nabhan and Richhardt, 1983); for example, the desert dwelling Hopi tribe bred sunflowers that produced specific dyes (Heiser 1951; Willis et al. 2010). Additionally, sunflower was part of traditional polycultures in the American Southeast (Scarry and Scarry 2005), with its complex agricultural systems continuing in modern times, particularly in organic farming systems (Jones and Gillett 2005).

Sunflower was introduced to Europe in the sixteenth century as an ornamental plant (Heiser 1955) and was further improved for oil content during the nineteenth century in Russia. Sunflower robustness in adverse environments was recognized, which enhanced its cultivation in wide range of environments around the globe (Hanna 1924, Shantz 1940). However, as alluded to above, domestication and improvement is typically accompanied by a reduction in genetic variation due to intensive inbreeding and selection, leading to increased vulnerability of cultivated varieties to environmental stresses, diseases and pests (Rieseberg et al. 1995; Harter et al. 2004). In sunflower, the cultivated genepool is estimated to include approximately 67% of the diversity present in wild populations of its progenitor, common sunflower (also *H. annuus*) (Lui and Burke 2006; Kolkman et al. 2007; Mandel et al. 2011). The relatively high proportion of genetic variation remaining in the cultivated gene pool is due in part to purposeful introgressions from the wild (Seiler, 1991a; Seiler, 1991b; Seiler, 1991c; Seiler and Marek, 2011; Baute et al. 2015).

Sunflower is currently cultivated on ~26 million hectares worldwide (FAO Stat), ranking second among hybrid crops in area harvested (Singh et al. 2007) and 13th among all crops. Commercial oilseed varieties dominate cultivation (75-90% of production) due to high demand for low trans-fat, high oleic vegetable oil (Berglund 2007). The biodiesel and direct consumption markets are smaller but still of economic value. Sunflower production in 2013 was 44.75 million metric tons worldwide and 0.92 million metric tons in U.S. (FAO stat). Over the last twenty years there has been an increase in sunflower production worldwide (Khoury et al. 2014) due to increases in both production area and productivity per hectare (Berglund 2007).

### **Domestication and Improvement of Jerusalem Artichoke**

The second *Helianthus* crop is the hexaploid Jerusalem artichoke (*H. tuberosus*). *Helianthus tuberosus* displays a domestication syndrome consistent with tuber crops, where tuber number is reduced, individual tuber size increases and there is a more synchronous transition to reproduction. *Helianthus tuberosus* is native to central North America (Kays and Nottingham, 2008; Rogers et al. 1982) and was domesticated in the eastern United States prior to European contact. *Helianthus tuberosus* is an autoallohexaploid whose diploid progenitors are *H. divaricatus* and *H. grosseserratus* (Bock et al. 2014; Kostoff, 1934; Kostoff, 1939; Scibria, 1938). Levels of genetic diversity in the cultivated gene pool relative to that found in its wild progenitor (also *H. tuberosus*) are unknown.

The crop was first introduced to Europe in the early 17th century, where it was an immediate success among the royal court of France, quickly becoming an important food source among the European aristocracy: traveling in quick succession from France to Italy, to the Netherlands and then to England (Kays and Nottingham, 2008). In fact, extensive cultivation guides were published in the mid-18th century (Brookes, 1763). Jerusalem artichoke production continued to increase for ~200 years until it was largely replaced by potato production in the mid-19th century. However, the crop retained an important place in many European culinary traditions, with spikes in cultivation occurring at different times during different periods of history, for example during World War II (Kays and Nottingham, 2008).

The tubers have excellent nutritional properties and are a favorite in gourmet cooking, with some of the first recorded recipes emerging in England in the seventeenth century (Kays and Nottingham, 2008). In addition to food, proposed uses of Jerusalem Artichoke include industrial (i.e. for rubber; Seiler et al. 1991), biofuel (Seiler and Campbell, 2006; Rodrigues et al. 2007), medicinal (i.e. inulin from tubers can be used treating diabetes; Kays and Nottingham, 2008), and as a forage crop (Seiler and Campbell, 2004). However, many of the desirable compounds are present at very low concentrations, making production economically unfeasible (Seiler et al. 1991).

When commercially produced, the crop is grown as a winter or summer annual. Production has fluctuated between food and forage production, with recent interest developing in biofuel production. Despite its widespread use there has been little information obtained on the genetics of wild *H. tuberosus*. Additionally, as far as we are aware, there has been little intentional interspecific introgression into domesticated *H. tuberosus*.

### **Gene Flow Between Wild and Cultivated Populations**

Gene flow between wild and cultivated sunflowers occurs frequently as crop fields and wild *Helianthus* are often adjacent to one another (Burke et al. 2002b; Arias and Rieseberg, 1994). Most of the gene flow is with wild

populations of *H. annuus*, but limited introgression has been observed with a related species, *H. petiolaris*, as well (Rieseberg and Kim, 1998). Gene flow is mainly from cultivated into wild populations, and cultivated alleles can persist for decades in wild or weedy populations (Snow et al. 1998; Whitton et al. 1997; Cummings et al. 2002). The mixed growth forms resulting from such admixture are sometimes observed in cultivated fields and can hurt production (Lu et al. 2013). With that said, significant care is taken to eliminate wild populations from areas of seed production. Thus, the influence of this unintended crop-wild gene flow on the genomic composition of the cultivar appears to be limited (Baute et al. 2015). Indeed, all domesticated sunflowers form a distinct lineage compared to wild relatives, with wild *H. annuus* making a fairly small direct contribution to domestic genomes (Mercer et al. 2006; Snow et al. 2003; Harter et al. 2004; Wills and Burke, 2006; Mandel et al. 2011; Baute et al. 2015).

### **How Can We Utilize Hybridization Within *Helianthus*?**

Hybridization can be used in several ways. First, as discussed above, it offers a means for accessing agronomically valuable genetic variation, especially disease resistance alleles. Second, hybridization can reveal useful cryptic variation present in wild or cultivated germplasm. For many traits, individuals carry alleles with opposing effects (Tanksley, 1993). The existence of these alleles can be exposed by creating hybrid populations, potentially leading to extreme phenotypes, in a phenomenon referred to as transgressive segregation (Rieseberg et al. 2003; Nolte and Tautz, 2010; Mao et al. 2011; Dittrich-Reed and Fitzpatrick, 2013). Lastly, alleles derived through hybridization can mask deleterious alleles in cultivated lines, potentially leading to heterotic effects (Springer and Stupar, 2007; Birchler et al. 2010; Mezouk and Ross-Ibarra, 2014). Below we describe some of these potential uses in *Helianthus*, drawing on examples from both natural and artificial hybridization experiments. We also describe potential barriers to introgression such as chromosomal rearrangements, and potential ways to overcome these barriers.

#### **Hybridization in Nature**

Interspecific hybridization and introgression permits large portions of the genome to change simultaneously, potentially facilitating rapid divergence or adaptation. Interspecific gene flow within *Helianthus* is common (Heiser 1947, 1951; Stebbins and Daly, 1961; Heiser, 1978), and the transfer of alleles between species has been shown to affect phenotype and fitness (Whitney et al. 2006, 2010). Studies of natural hybrids in the genus have provided insights into the genetics of adaptation, especially with respect to the roles of transgressive segregation and chromosomal rearrangements in ecological divergence (Strasburg et al. 2011; Sambatti and Rice, 2006; Kane and Rieseberg, 2007; Andrew et al. 2012; Scascitelli et al. 2010; Whitney et al. 2010).

Transgressive segregation appears to be common in interspecific *Helianthus* hybrids in both greenhouse and natural environments (Schwarzbach et al. 2001; Welch and Rieseberg, 2002a; Ludwig et al. 2004; Rieseberg et al. 2003). The most studied hybrids are natural derivatives of the two most widespread annual sunflowers, *H. annuus* and *H. petiolaris* (Heiser, 1947), as this hybrid combination created three different species: *H. paradoxus* (Rieseberg et al. 1990; Welch and Rieseberg, 2002), *H. deserticola* (Rieseberg, 1991a; Rieseberg, 1991b), and *H. anomalus* (Rieseberg, 1991a; Rieseberg, 1991b). The hybrid species have the same chromosome number as the parental species, and so represent examples of homoploid hybrid speciation. However, each of the hybrid species is comprised of a different combination of parental chromosomal segments (including rearrangements), which has resulted in distinctive transgressive phenotypes, as well as strong chromosomal sterility barriers (see below) relative to the parental species and to each other (Rieseberg et al. 1993, 2003; Gross et al. 2003; Rieseberg, 2001; Rieseberg et al. 1995; Lai et al. 2005). The extreme adaptations found in the natural hybrid species suggest that hybridization could be useful in adapting cultivars to abiotic stress.

### **Chromosomal Compatibility Within the Genus**

Chromosomal rearrangements are commonly reported in progeny from both intra- and inter-specific crosses within *Helianthus*. Large-scale chromosomal translocations have been reported most frequently, mainly because the multivalent configurations they generate at meiosis are easily detected by conventional light microscopy. Large inversions have also been reported using the same approach. Both kinds of rearrangements were confirmed by initial low-density comparative genetic mapping studies (e.g., Rieseberg et al. 1995; Burke et al. 2004; Lai et al. 2005; Heesacker et al. 2009). Recent very high-density genetic maps have largely validated these initial mapping studies, and suggest that small-scale inversions and translocations are frequent as well (Barb et al. 2014).

Chromosomal translocations and inversions often cause reductions in the fertility of hybrids because recombinant gametes are frequently unbalanced (i.e., carry duplications or deletions). Because the unbalanced gametes are inviable, non-recombinant parental chromosomes will be over-represented in the gametes that survive, leading to an effective reduction of recombination rates in rearranged chromosomes. Hybrid fertility reduction is probably most important in preventing species' mergers following secondary contact (Noor et al. 2001; Rieseberg 2001). In some instances, mechanisms have evolved that suppress recombination in inversions prior to gamete development. In these situations, recombination suppression appears to be complete, although gene conversion rates can be surprisingly high (Gaut et al. 2007). Recombination suppression due to inversions has

been shown to facilitate the accumulation of hybrid incompatibilities, as well as local adaptation in the presence of gene flow (Kirkpatrick and Barton, 2006; Lowry and Willis 2010).

From a breeding perspective, chromosomal structure impacts the success of intentional introgression with different species, with collinear portions of genomes being easier to introgress than regions within or near rearrangements (Long, 1960; Whelan, 1978; Georgieva-Todorova, and Bohorova, 1980; Espinasse et al. 1995; Rieseberg et al. 1995; Rieseberg et al. 1996; Burke et al. 2004; Renaut et al. 2013; Barb et al. 2014). This is due both to the direct effects of the rearrangements and to linked hybrid incompatibilities (Orr et al. 1996; Lai et al. 2005). There is potential to utilize marker information to find rare recombinants through marker-assisted selection to eliminate these deleterious mutations (Robertson, 1960; Charlesworth, 2012).

*Helianthus* has a high rate of karyotypic evolution (Geisler, 1931; Seiler, 1992; Seiler and Rieseberg, 1997; Rieseberg et al. 1995; Fang et al. 2012; Feuk et al. 2006; Burke et al. 2004), estimated at 5.5-7.3 chromosomal rearrangements per million years (Chandler et al. 1986; Burke et al. 2004). This has led to the recognition of chromosomal subtypes among *Helianthus* species that predict crossing success (Schilling and Heiser, 1981; Chandler et al, 1986; Heiser et al. 1962; Sossey-Alaoui et al. 1998; Ceccarelli et al. 2007; Natali et al. 2008; Jan and Chandler, 1989). For example, the perennial polyploid species in *Helianthus* generally cross despite morphological differences (Long, 1955; Long 1960), different origins, and large variation in chromosome structure and pairing. Chromosomal subtypes have been identified; for example, homology has been reported between *H. ciliaris*, *H. tuberosus* and *H. annuus* as well as generally within the perennial diploid species' genomes (Espinasse et al. 1995). Different sections within the genus have shown differential abilities to hybridize with each other, with species within sections generally hybridizing better (Faure et al. 2002). The development of predictive chromosomal compatibility groups required that many different populations be tested, since there can be significant intraspecific variation in hybrid formation and vigor (Espinasse et al. 1995; Edmands, 2002). Understanding chromosomal structure provides an opportunity to better utilize wild populations in plant breeding, by providing insight into crossing success and what traits and genes are likely to be transferable. Chromosomal structure can often differ within species that are present in the primary, secondary and tertiary germplasm, and knowing which populations have structural variation could provide easier access to useful traits. While many crosses within *Helianthus* are possible, those involving more distant wild relatives sometimes require special techniques, such as embryo rescue and tissue culture or even the induction of an additional round of amphiploidy, with intraspecific variation in success (Jan and Chandler 1989, Feng and Jan 2008).



### **General Utility of *Helianthus* Crop Wild Relatives**

The ability of the cultivated sunflower to readily hybridize with many other *Helianthus* species has been exploited extensively through the intentional introduction of genetic material from wild relatives (both annual and perennial) into the cultivated gene pool (Table 1). Wild relatives are sources of disease resistance genes (Feng et al. 2006), cytoplasmic male sterility (Seiler and Jan, 1994), quality traits, and yield traits. Many of these traits have been the target of mapping efforts (Bert et al. 2004; Qi et al. 2012; Yue et al. 2008; Yue et al. 2010), which have indicated that large chromosomal segments sometimes introgress with the traits of interest. Indeed, different interspecific hybridization events cover approximately 10% of the cultivated sunflower genome (Baute et al. 2015). Interspecific introgressions can be difficult to eliminate (or reduce in size if linked to a trait under selection) because of limited recombination between different chromosomal types (Livaja et al. 2013). As genotyping becomes cheaper and more efficient, the ability to utilize marker assisted selection to introgress precise genomic regions from crop wild relatives is an increasingly feasible option to limit the extent of donor parent contributions to cultivated material.

### **Cytoplasmic Male Sterility and the Formation of a Hybrid Seed Industry**

Hybrid breeding has been used to improve performance in many crops, thereby making a fundamental contribution to the green revolution (Borlaug 2000). Hybrid vigor (heterosis) is formed by crossing different strains, varieties or species to produce offspring that outperform their parents in terms of biomass, growth rate, and fertility. This phenomenon was first described by Darwin in both natural and domesticated species (Darwin 1859). Hybrid production in crop species has been central to increasing crop production. The genetic basis of heterosis has been debated for over a century; however, a general consensus has been reached on three main models: dominance (Bruce 1910, Jones 1917), over-dominance (Shull 1908, East 1936, Crow 1948), and pseudo-overdominance (Crow 1952). The outcome of all three models is the same: increased performance in hybrid lines over their parents. Unfortunately, most crop plants bear anthers and stigmas in the same flower, or at closely associated flowers, so emasculation is required. In many plant species emasculation is tedious and requires a relatively high degree of technical training, reducing the economic potential of hybrid seed production (Kaya 2014).

In nature, several mechanisms have evolved to reduce self-fertilization and enhance outcrossing. One common mechanism is cytoplasmic male sterility (CMS), in which plants fail to produce functional pollen while maintaining female fertility. CMS is a maternally inherited trait and is thought to arise from an incompatibility between the nucleus and cytoplasm

**Table 1.** Interspecific hybridization within *Helianthus* with germplasm position defined by crossing relationships to the crop: the primary germplasm contains no crossing barriers, the secondary germplasm can be crossed but hybrids show meiotic abnormalities, and the tertiary germplasm requires special techniques such as embryo rescue to make the cross possible (Hartan, 1976).

Taxon	Position in Germplasm	Ploidy	Trait	Reference
<i>Helianthus annuus</i>	Primary	Diploid	Herbicide tolerance	Al-Khatib and Miller, 2000; Miller and Al-Khatib, 2002
<i>Helianthus anomalus</i>	Secondary	Diploid	Fertility restoration	Seiler, 1991a
<i>Helianthus argophyllus</i>	Secondary	Diploid	Downy mildew resistance, disease resistance, fertility restoration, salt tolerance, drought tolerance	Sieler, 1991a; Miller & Gulya, 1988; Jan et al. 2004; Hulke et al. 2010; Seiler, 1994
<i>Helianthus arizonensis</i>	Tertiary	Diploid	High linoleic acid concentrations in seed (potential)	Seiler, 1984
<i>Helianthus atrorubens</i>	Tertiary	Diploid	High linoleic acid concentrations in seed (potential)	Seiler, 1984
<i>Helianthus bolanderi</i>	Secondary	Diploid	Fertility restoration	Seiler, 1991a; Jan, 1992
<i>Helianthus debilis</i>	Secondary	Diploid	Powdery mildew resistance; fertility restoration	Jan & Chandler, 1988; Seiler, 1991a
<i>Helianthus debilis</i> subsp. <i>tariflorus</i>	Secondary	Diploid	Resistance to broomrape	Velasco et al. 2012
<i>Helianthus deserticola</i>	Secondary	Diploid	Downy mildew resistance	Seiler, 1991b
<i>Helianthus divaricatus</i>	Tertiary	Diploid	Broomrape resistance	Jan et al. 2002
<i>Helianthus giganteus</i>	Tertiary	Diploid	Fertility restoration; cytoplasmic male sterility	Whelan & Dedio, 1980; Seiler, 2000
<i>Helianthus grosseserratus</i>	Tertiary	Diploid	Broomrape resistance	Jan et al. 2002
<i>Helianthus hirsutus</i>	Secondary	Tetraploid	Fertility restoration	Seiler, 1991c; Seiler, 2000
<i>Helianthus maximiliani</i>	Tertiary	Diploid	Broomrape resistance; Cytoplasmic male sterility	Whelan & Dedio, 1980; Jan et al. 2002

cont.

<b>Taxon</b>	<b>Position in Germplasm</b>	<b>Ploidy</b>	<b>Trait</b>	<b>Reference</b>
<i>Helianthus neglectus</i>	Secondary	Diploid	Fertility restoration	Seiler, 1991a
<i>Helianthus paradoxus</i>	Secondary	Diploid	Salt tolerance; fertility restoration	Seiler, 1991a; Lexer et al. 2003; Lexer et al. 2004;
<i>Helianthus pauciflorus</i>	Tertiary	Tetraploid	Cytoplasmic male sterility; sclerotinia resistance	Jan et al. 2006
<i>Helianthus petiolaris</i>	Secondary	Diploid	Verticillium resistance; disease resistance ; cytoplasmic male sterility ; sunflower moth resistance; fertility restoration	Hoes et al. 1973; Rogers et al. 1984; Seiler, 1991a; Jan et al. 2004
<i>Helianthus praecox</i>	Secondary	Diploid	Downy mildew, rust, verticilliumwilt and broomrape resistance; fertility restoration; downy mildew resistance	Seiler, 1991a; Seiler, 1991b
<i>Helianthus resinosus</i>	Tertiary	Hexaploid	Fertility restoration	Seiler, 1991c
<i>Helianthus salicifolius</i>	Tertiary		High crude protein concentrations in leaves (potential)	Seiler, 1983
<i>Helianthus silphioides</i>	Tertiary	Diploid	High oleic acid concentrations in seed (potential)	Seiler, 1984
<i>Helianthus strumosus</i>	Tertiary	Hexaploid	Fertility restoration	Seiler, 2000
<i>Helianthus tuberosus</i>	Secondary	Hexaploid	Broomrape resistance; sunflower moth resistance; fertility restoration	Putt, 1978; Rogers et al. 1984; Seiler, 2000

(Hanson and Conde 1985). Often, rearrangements in the mitochondrial DNA (mtDNA) have been associated with CMS, with collinear chloroplast DNA (cpDNA) in both male-fertile and male-sterile lines (Rieseberg and Seiler 1990). Although the molecular mechanism of CMS has been fully described in only a few species (Touzet and Meyer 2014), it provides an efficient mechanism to guide crossing in breeding programs and for production of hybrid seeds in many crop plants (Dewey et al. 1986, Makaroff et al. 1989, Bailey-Serres et al. 1986), including sunflower (Siculella and Palmer 1988). In parallel with the evolution of CMS in nature, a counteracting mechanism to this destructive mitochondrial effect has evolved to protect pollen functionality. This restoring mechanism is induced by nuclear genes that regulate the accumulation of transcripts or proteins associated with the CMS locus (Hanson and Bentolila 2004; Luo et al. 2013). Restorer genes typically belong to the penta-tricopeptide repeat (PPR) family (Brown et al. 2003, Wang et al. 2006), which is one of the largest gene families in plants. PPR genes occur in small clusters of closely related genes that have arisen through evolutionarily recent gene duplication and transposition, perhaps enabling them to respond quickly to the challenge posed by CMS (Schnable and Wise 1998).

Similar to in other species, CMS in sunflower is associated with genomic rearrangements in the mitochondria that lead to a chimeric open reading frame (ORF) (Leroy et al. 1985, Siculella and Palmer 1988, Köhler et al. 1991). The chimeric ORF in sunflower (ORF522) was shown to share sequence similarity with the ATP synthase subunit ORFB, which results in competition between the two proteins leading to decreased phosphorylation activity of the ATP synthase complex (Balk and Leaver 2001, Sabar et al. 2003). As the energetic demands increase during anther development, the expression of ORF522 compromises ATP complex activity and leads to developmental delay and pollen abortion. Although several nuclear restorers have been identified, the molecular mechanism of fertility restoration remains largely unknown. However, the most common locus (Rf-1) maps to a cluster of PPR genes on linkage group 13 (Baute 2015).

While many traits been introduced to cultivated sunflowers through interspecific hybridization, few have been as important as cytoplasmic male sterility (CMS). Today, commercial sunflower production is dominated by hybrid genotypes, made possible by the discovery of CMS in wild germplasm. However, commercial production of sunflower largely relies on a single cytoplasm, CMS PET-1, which originated from an interspecific cross of *Helianthus petiolaris* Nutt. With *H. annuus* L., and its corresponding fertility restoration gene, Rf-1 (Leclercq 1969, Gimenez and Fick 1975, Horn et al. 2003; Jan and Vick 2007). Hybrid production involves a female inbred parental line (CMS-HA), which carries the male-sterile cytoplasm (S-type cytoplasm) but not the fertility restorer allele (Rf) in the nucleus, and a male inbred parental line (RHA), which can carry a normal (N-type) or CMS (S-type) cytoplasm, but which carries the restorer allele (Rf). Therefore the female parent is a

male-sterile inbred line (S-rfrf) and the male parent is male-fertile inbred line (S-RfRf or N-RfRf). The resulting hybrid is male-fertile, containing both the CMS and the restorer allele (S-Rfrf). This complex system of production is utilized because the increase in performance seen in hybrids greatly increases the economic value of the crop. Hybrid breeding has been the impetus for much of the gains seen in worldwide sunflower production over the past 40 years (FAO Stat).

More than 70 CMS sources have been identified in sunflower wild relatives (Serieys 2005), but the corresponding restorer genes are known for only about half (Jan and Seiler 2006). Most CMS systems are from wild relatives and show complete male sterility: e.g. *H. petiolaris* (PET1), *H. resinosus* (RES), *H. rigidus* (RIG), *H. giganteus* (GIG1), *H. maximiliani* (MAX1), and *H. argophyllus* (ARG3) (Kaya 2014). For the most commonly used CMS (PET1), two genes (Rf1 and Rf2) were identified to restore fertility and have been used extensively in sunflower hybrid production (Fick and Miller 1997). These restoring genes were found to be effective also for the ARG1 and ARG3 derived sterility (Christov 1991; Jan and Seiler 2006). However, other CMS sources are still not widely used for hybrid production at commercial scale (Kaya 2014).

Use of alternative CMS/Rf systems is recommended for sunflower hybrid seed production to reduce genetic vulnerability (e.g., susceptibility to diseases, pests, environmental stresses) associated with cytoplasmic uniformity. Breeders tend to avoid exploring new CMS systems due to the substantial effort involved in producing new CMS lines and the introgression of the corresponding Rf genes. Different cytoplasms may significantly impact drought tolerance and other traits (Sambatti et al. 2008). Perhaps a better understanding of the benefits of different cytoplasms will encourage expansion of the cultivated cytoplasm diversity.

### *Branching*

One of the major domestication traits of sunflower is monocephaly (single head). A single head allows for more synchronous flowering and easier harvesting. However, after the initiation of hybrid sunflower production in the 1960's there was a need for male lines to flower for longer periods of time. This led to introgression of the recessive B-locus responsible for branching from wild *H. annuus* back into cultivated male lines (Mandel et al. 2013). This illustrates a difference in the needs of domestication and improvement phases of crop development and, like CMS (above), shows how wild diversity continues to contribute to sunflower improvement.

### ***Introgression of Disease Resistance***

Sunflower is one of a handful major crops that is widely cultivated at its center of origin, and is thereby exposed to the large number of pathogens that have coevolved with its wild progenitors. There are more than fifty

diseases of *Helianthus*, although very few are of economic importance (Gulya et al. 1997). Genetic resistance is often the most cost effective way to deal with disease pressure (Talukder et al. 2014); therefore understanding the extent of both intra- and interspecific genetic resistance in germplasm collections is of great value. The large number of species within *Helianthus* that are adapted to a wide number of different pests and pathogens are an important resource for breeding efforts. As described above, most *Helianthus* species hybridize (Long et al. 1960; Chandler et al. 1986; Espinasse et al. 1995), and this ability has been extensively exploited with respect to the introgression of disease resistance (Table 1). At least twelve different species have been utilized to introgress disease resistance for eight different major diseases of cultivated sunflower (Table 1). The donor species occur across most of North America with the exception of the southeastern USA, where native *Helianthus* species are mainly part of the tertiary germplasm. The species that have been utilized are also well distributed across the secondary (67%) and tertiary (33%) germplasm. For example, members of the secondary germplasm, *H. argophyllus* and *H. praecox*, have been utilized for resistance to downy mildew (*Plasmopara halstedii*), a common disease in the northern part of North America infecting seedlings and causing significant damage (up to 25% of a field). Resistance to the major fungal pathogen, *Sclerotinia sclerotiorum*, was identified in *Helianthus pauciflorus*; this disease has been a major disease in North America.

### **Crosses Between the Crops: Interspecific Hybridization Between *H. tuberosus* and *H. annuus***

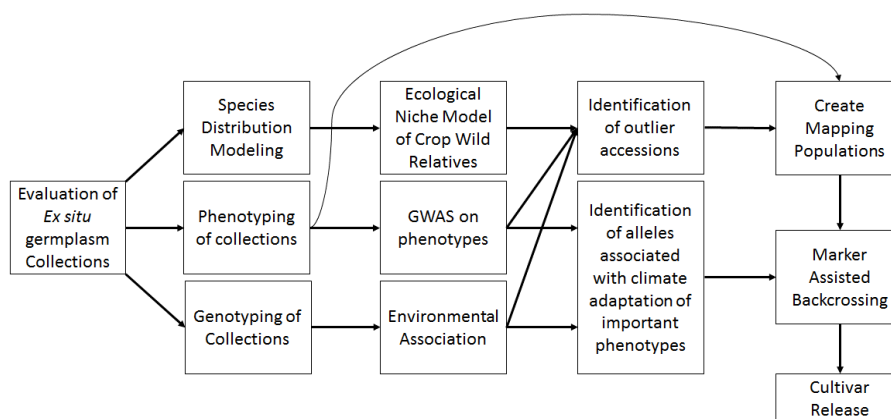
*Helianthus tuberosus* diverged from *H. annuus* approximately 1.7-8.2 million years ago (Schilling, 1997) and has been a major donor of useful genes for sunflower development (Hajjar and Hodgkin, 2007). The chromosomal interactions between the two crops have generated much interest and although the genomes are not the same, one of the sub-genomes of *H. tuberosus* pairs effectively with the *H. annuus* genome, such that strong viable hybrids are formed (Espinasse et al. 1995; Atlagic, 1993; Hulke and Wyse, 2008; Sujatha and Prabakaran, 2006). *Helianthus annuus* × *H. tuberosus* hybrids are tetraploid ( $2n = 4x = 68$ ), with mixed bivalent and multivalent pairing (Sujatha and Prabakaran, 2006; Atlagic et al. 1995). Chromosomes from different populations of *H. tuberosus* pair differently with *H. annuus* during meiosis due to translocations and inversions, resulting in variable fertility in hybrid plants (Kostoff, 1939; Atlagic et al. 1995; Atlagic et al. 1993; Natali et al. 1998; Chandler et al. 1986; Atlagic et al. 1995; Kantar et al. 2014).

Interspecific *H. tuberosus* × *H. annuus* hybrids have been suggested to have agronomic value in their own right, including use as a forage crop, trait introgression-bridge, trap crop for blackbirds, and as a perennial oil seed (Seiler 1992; Atlagic, 2004; Kays and Nottingham, 2008; Kantar et al. 2014). Hybrid cultivars have been released in Russia and Sweden as a forage crop.

Development of hybrids as a perennial oil seed crop has been suggested as a way to increase sustainable agricultural and ecosystem service production (Glover et al. 2010). Exploration of such a possibility has been underway for two decades (Hulke and Wyse, 2008; Cox et al. 2010), with experimental tetraploid populations having been extensively phenotyped (Kantar et al. 2014). Ongoing work is exploring both neo-domestication of interspecific hybrid populations and ecosystem services available from an emerging perennial crop.

### **New Tools for Easier Use of Wild Relatives**

Historically, exploration of crop wild relatives for use in plant breeding has utilized a biparental crossing approach, where diverse accessions (both intraspecific and interspecific) from *ex situ* collections were used to create populations in the search for useful phenotypic variation (Hajjar and Hodgkin, 2007). This method has been useful for the utilization of crop wild relatives (Khoury et al. 2015), but there are logistical limitations in the number of accessions that can be explored. Emerging sequencing technologies (2nd and 3rd generation), proteome data, metabolome data, and high-throughput phenotyping approaches provide a wealth of data for both basic and applied objectives and may help overcome logistical problems with conventional methods of exploring crop wild relative diversity (Mammadov et al. 2012; O'Driscoll et al. 2013). The cost, both per sample and per data point, of these emerging technologies is now affordable within standard laboratory budgets; this is particularly true for use of molecular markers (Edwards and Batley, 2010; Poland et al. 2012). Decreased costs have allowed for comprehensive assessments of genotypic diversity in crop wild relatives, and for implementation of marker-based selection schemes (e.g. marker assisted backcrossing, genomic selection) in many different species (Bernardo and Yu, 2007; Bernardo 2008; Storlie and Charmet, 2013). High resolution phenotyping also allows for increased precision and efficiency in trait dissection in both greenhouse and field settings (Araus and Cairns, 2014). This combination of technologies leads to a better understanding of local adaptation and domestication, as well as a more targeted use of crop wild relatives in plant breeding (Fig. 1). Finally, leveraging data from multiple species may broaden the utility of wild germplasm by identifying specific genes with conserved function across species (Du et al. 2010; Monaco et al. 2013; Bolger et al. 2014).



**Figure 1.** In order to fully utilize germplasm resources it is important to utilize both top-down and bottom-up approaches. These approaches are complementary, leveraging large databases (Germplasm, Genetic, Bioclimatic, and Biophysical), and local capacity to phenotype, to increase the use interspecific hybridization in plant breeding.

### **Next Generation Germplasm Resources**

To further enhance utilization there has been interest in creating populations that will allow more efficient deployment of wild alleles in breeding programs. Novel mating designs are being used to enhance the number of recombination events, increase the number of wild alleles segregating in populations, and to allow the effects of wild alleles to be simultaneously tested across numerous genetic backgrounds. With biparental mating designs, there is limited resolution to identify the specific genes underlying quantitative variation. New mapping designs that leverage increased molecular marker density and increased numbers of recombination events include association mapping (individuals sampled directly from breeding or wild populations), nested association mapping (populations based on a hub parent design) and multi-parent intercross populations, in which multiple parents are randomly mated to increase the number of recombination events (Morrel et al. 2012). These mating designs provide easier access to and better knowledge of the effects of alleles present in the primary, secondary and tertiary germplasm of crop species.

### **Conclusions**

Hybridization has been of central importance in the exploration and utilization of wild germplasm in sunflower improvement. It was central to the development of a hybrid sunflower industry, understanding chromosomal interactions among species, and the protection of yield through the provision of disease resistance. Hybridization is currently being used to develop new crops and to understand the physiological mechanisms and genetic basis of



resistance to abiotic stress. New mating designs and technology which enable precision breeding, combined with the need to develop environmentally resilient crops, will enhance the value of wild germplasm in years to come.

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