The Use of Crop Wild Relatives in Maize and Sunflower Breeding

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ABSTRACT

Conservation of crop wild relatives (CWR) has always been predicated on the promise of new and useful traits, and thus modern genetics and genomics tools must help fulfill the promise and continue to secure the conservation of these resources. However, the vast genetic potential present in CWR is often difficult to tap, as identification of superior alleles can be hampered by the effects of the environment on expression of these alleles and masked in different genetic backgrounds; transfer of superior alleles into breeding pools to create new crop varieties can be slow and expensive. Some crop species have been more amenable to introgression of traits from wild relatives than others. In some cases, these species may be less diverged from their wild ancestors, which become a good source of mono- to oligogenic traits, many of which are more qualitative in nature, and sometimes of quantitative traits. Sunflower (*Helianthus annuus* L.) is an introgression success story, and many traits, including cytoplasmic male sterility, herbicide tolerance, drought and biotic stress resistance, and modified fatty acid profiles, have been introgressed into the cultivated gene pool from wild relatives without depression of oil yield and quality. Others, including maize (*Zea mays* L.), have shown little progress in widening the cultivated gene pool using exotic sources due to temporary yield depression, potential for loss of quality, and disturbance of current logistical habits. Here, we review the breeding history of sunflower and maize and explore variables that have limited the use of CWR in some species and allowed success in others. Surprisingly, in both sunflower and maize, biological limitations are similar and smaller than expected and appear to be surmountable with sufficient determination. Possible new technologies and policies to allow a deeper mining of these genetic resources in all crop species are discussed.

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Abbreviations: CIMMYT, Centro Internacional de Mejoramiento de Maíz y Trigo; CMS, cytoplasmic male sterility; CWR, crop wild relatives; GEM, Germplasm Enhancement of Maize; GMO, genetically modified organism; GRIN, Germplasm Resources Information Network; GWAS, genomewide association study; INIFAP, Instituto Nacional de Investigaciones Forestales, Agricolas, y Pecuarias; NIL, near-isogenic introgression line; NPGS, US National Plant Germplasm System.

Genetic variation is the raw material for advances made by plant and animal breeders. Plant genetic resources include all the populations whose DNA sequence variation and epigenetic variation (those heritable changes in gene function that do not change DNA sequence) can be accessed naturally or aided by science in the improvement of a specific crop species (Brown et al., 1989). The loss of genetic variation in domesticated crop species was well documented by the work and writings of N.I. Vavilov and others starting as early as the 1920s, and more recently in both scientific literature (Esquinas-Alcázar, 2005; van de Wouw et al., 2010) and popular writing (*National Geographic*, May 2011 and July 2012; Fowler and Mooney, 1990). The extensive system of genebanks set up by the CGIAR, the USDA-ARS, and many other national agriculture programs in the 1950s and 1960s was a response to the realization that, as improved varieties were

introduced into many countries, traditional and variable landrace or farmer's varieties were being discarded by farmers in favor of the higher-yielding new varieties. The goals of the genebanks include ensuring the long-term safety and integrity of agriculturally valuable genetic resources and associated information, identification and use of novel traits and alleles, and reduction of crop genetic vulnerability to biotic and abiotic stresses.

Many wild species are safely stored in national and international genebanks, and the need for these collections of crop wild relatives (CWRs) continues to increase as expanded development and largescale agriculture destroy habitat suitable for the growth of wild and weedy relatives of the modern crops that feed the world. In addition to ex situ collections, a few in situ collections of wild relatives allow these species to continue to evolve to adapt to new environmental conditions and diseases as they appear. These in situ collections are rare, however, as the costs associated with them are higher than for ex situ collections. There are only two protection programs for teosinte, the wild relatives of maize (*Zea mays* L.): the Sierra de Manantlán Biosphere Reserve established in Jalisco, Mexico, in 1987, which preserves *Zea diploperennis* Iltis, Doebley & Guzman (Maxted et al., 2010) and the Reserva de Recursos Genéticos de Apacunca (RRGA) in Nicaragua, created in 1996 to protect *Zea nicaraguensis* Iltis & Benz (Galluzzi and Lopez, 2014). Despite new techniques to edit and engineer genes and genomes, the most economical and efficient solution to the need for new sequence variation to continue to improve crop species is to tap existing sequence variation that often already exists in expanded gene pools represented in the CWR collections (Michael and VanBuren, 2015; Brozynska et al., 2016) and allow new diversity to evolve under natural settings (Meilleur and Hodgkin, 2004).

Nevertheless, the use of CWR in plant improvement has had variable success in different species. In sunflowers (*Helianthus annuus* L.), there are many instances of variation introgressed into cultivated varieties from CWR, particularly from closely related species at the same ploidy level (Hajjar and Hodgkin, 2007). Other crops with rich reservoirs of diversity in closely related wild species and very few barriers to interspecific hybridization have had much lower success rates in the use of CWR in plant breeding. This notably includes maize, and the high levels of genetic diversity known to exist in the wild relatives have been essentially untapped for the improvement of elite maize germplasm. Despite the fact that many studies indicate useful genetic variation in wild species that can be easily introgressed and expressed in a maize background, no commercial varieties currently on the market in temperate growing areas contain sequence variation from recent introgressions from maize CWR. Some biological variables may increase both the need for expanded variation

and the ease with which the expanded variation may be tapped. In more recent allopolyploid species, which often suffer from a severe genetic bottleneck, synthetic populations that recreate the interspecific cross can be used to bring all the variation still present in the wild ancestral species (and there are generally several species) to the domesticated gene pool, as has been done with wheat (*Triticum aestivum* L.; Ogbonnaya et al., 2013) and peanut (*Arachis hypogaea* L.; Stalker et al., 2013). More recent domesticates may cross more easily with their ancestral species, and successful introgression from wild to domesticated species for tomatoes (*Solanum lycopersicum* L. = *Lycopersicon escuelentum* Mill), potatoes (*Solanum tuberosum* L.), and sunflowers is common. On the other hand, little introgression has been documented from other recent domesticates, such as strawberry (*Fragaria × ananassa* Duch.) and eggplant (*Solanum melongena* L.), perhaps due in part to smaller research budgets and longevity of projects for these specialty crops.

Within the majority of crop plants that do not represent fairly recent polyploidy events, or recent domestication events, the rate of use of CWR to expand elite gene pools varies greatly. This paper explores some of the possible reasons for differences between species, namely the factors that encourage the use of CWR in crop breeding in one species (sunflower) and discourage it in another (maize), and if possible, offers suggestions to mitigate the latter.

CASE 1: SUNFLOWERS

Sunflowers belong to the genus *Helianthus*, which is native to North America, with species ranging from central Mexico across all of the United States and into and across southern Canada. Although many of the taxa are endemic to very specific ecogeographic areas delineated by soil characteristics, the entire genus contains vast genetic and phenotypic diversity. Cultivated sunflower was domesticated some 4000 to 5000 yr ago in North America (Smith, 2006). Although most of the wild-type sunflower species have multiple stalks tipped by relatively small flowers (Fig. 1), cultivated sunflowers are nonbranching plants with determinate-type reproductive growth and a single, large-sized head with numerous achenes. Cultivated sunflower has been characterized into three types on the basis of achene size: oil-type sunflowers have smaller achenes with high oil content (ranging 40–54%), whereas confectionary and bird food sunflowers have large achene size, lower oil content (≤30%), and high hull contents. The genus *Helianthus* is composed of 52 species and 19 subspecies, of which 14 are annual and 38 are perennial (Schilling and Heiser, 1981; Schilling, 1993). The cultivated species is diploid ($2n = 2x = 34$; Tahara, 1915), as are all annual wild species. Perennials are generally tetraploid ($2n = 4x = 68$) and hexaploid ($2n = 6x = 102$). Some species occur in dual ploidy series, such as

Fig. 1. *Helianthus argophyllus* in bloom, showing a typical wild sunflower phenotype including multiple stalks tipped by relatively small flowers.

H. ciliaris L., which displays both teraploid and hexaploid states, and *H. decapetalus* L., which exists in diploid and tetraploid forms (Atlagić, 2004).

Modern elite sunflower varieties were selected via recurrent half-sib selection, in which half-sib progenies were evaluated and reserve seed of selected superior progenies were intermated to constitute the base population by V.S. Pustovit in the former Soviet Union during the early 19th century, when sunflower seed oil content increased from 20 to >40%. Discovery of cytoplasmic male sterility (CMS) and restorer genes from *H. petiolaris* Nutt. paved the way for the development of single-cross hybrids but also resulted in a genetic bottleneck in cultivated germplasm, as all now incorporate the same source of male sterility and restorer genes. Diversifying the sources of male sterility is one objective of modern sunflower breeders. Selection for high oil content and dominance of a few hybrids for global cultivation has also raised concerns regarding reduced diversity in the cultivated elite gene pool, as cultivated varieties only retain 50 to 60% of the genetic diversity present in wild sunflower populations (Kolkman et al., 2007; Liu and Burke, 2006; Mandel et al., 2011). The sequence diversity present in the CWR of sunflower has diversified the genome of the cultivated species and provided new alleles to benefit multiple traits (see section

below). However, introgression of negative alleles for oil content, yield, and plant architecture could also slow breeding gain, as new and beneficial recombinants would need to be created and selected over several generations.

Abundant genetic variation exists in sunflower CWR, and details of the related species as potential sources of genetic improvement of sunflower are reviewed in Table 1. In addition, DNA sequence diversity has been measured in cultivated and wild sunflower species using molecular markers (Mandel et al., 2011). Work has been done to develop and characterize new genetic resources to expand the diversity available to breeders for traits of interest. Individuals of the species *H. argophyllus* Torr. & A. Gray, *H. annuus*, *H. petiolaris* and *H. debilis* Nutt. were crossed with cultivated sunflower, leading in some cases to increased molecular marker diversity in derived lines and in other cases to chromosomal structural rearrangements, which could be difficult to breed with in the future (Sujatha et al., 2008). These results suggest that the wild diploid species can be a source of introgression of novel variation, especially from the more distantly related *H. petiolaris*, if chromosomal pairing can be achieved. Both horizontal and vertical disease resistance is known to exist in sunflower wild relatives. Resistance to all races of rust (*Puccinia helianthi* Schw.) is high in wild annuals, whereas resistance for all races of powdery mildew (*Erysiphe cichoracearum* DC.) is only present in two populations of *H. argophyllus* and *H. debilis* (Chandler et al., 1986). *Helianthus tuberosus* L. contains resistance to stem–infecting disease including *Phomopsis* spp. stem canker, Phoma black stem (*Phoma macdonaldii* Boerema), and charcoal rot [*Macrophomina phaseolina* (Tassi) Goidanich], whereas many perennial and annual species show resistance to broomrape (*Orobanche aegyptiaca* Pers.) (Fernández-Martínez et al., 2000); Christov et al., 2004; Jan et al., 2014 (Table 1).

The primary gene pool of sunflower contains cultivated and wild species of *H. annuus* and winter sunflower (*H. winteri* J. C. Stebbins), all of which readily hybridize (Stebbins et al., 2013). The secondary gene pool (e.g., *H. anomalus* S. F. Blake, *H. paradoxus* Heiser, *H. petiolaris*, and *H. deserticola* Heiser) consists of species that have undergone some degree of differentiation with respect to the genome of the cultivated species, creating potential meiotic difficulties during hybridization. The tertiary germplasm pool (e.g., *H. hirsutus* Raf., *H. tuberosus*, and *H. divaricatus* L.) has a high degree of differentiation and requires specialized techniques such as embryo rescue for the recovery of interspecific hybrids. Differentiation among the species can be measured through molecular, cytological, and morphological bases, and the extent of wild species use decreases from primary through tertiary pools due to differences in ploidy level and growth habit, as well as reproductive barriers. To introgress a single or a few genes between species, the fastest transfer occurs

Table 1. Some examples of traits for which potentially useful genetic variation has been reported in the wild *Helianthus* species, along with their natural habitat.

Trait	Species	Habitat	Reference
Oil concentration and fatty acid profile	*H. anomalus* and *H. deserticola*	Utah, Arizona, and Nevada	Seiler (2007b)
Increased α-linolenic acid	*H. porter*, *H. atrorubens*, and *H. schweinitzii*		Seiler et al. (2010)
Broomrape resistance	*H. anomalus*, *H. agrestis*, *H. debilis* subsp. *Cucumerifolius*, and *H. exilis*	Desert southwest USA (*H. agrestis* only grows in Florida)	Fernández-Martínez et al. (2000)
Sclerotinia sclerotiorum resistance	*H. maximiliani*	Central USA	Rönicke et al. (2004)
Sclerotinia stalk rot, Phomopsis stem canker, Phoma black stem, and charcoal rot	*H. tuberosus*	Eastern Canada and North Dakota	Seiler (2010)
Drought and heat resistant genes (low cell membrane injury, high epicuticular waxes and high leaf hair density)	*H. argophyllus*	Coastal region of Texas	Hussain et al. (2016)
Single dominant gene resistant to race G broomrape (*Orobanche cumana* Wallr.)	*H. debilis* subsp. *tardiflorus*	Gulf coast and some inland areas of Florida, Georgia and Alabama	Jan et al. (2014)
Head and stalk rot resistance	*H. californicus*, *H. schweinitzii*, *H. maximiliani*, *H. giganteus*, and *H. grosseserratus*	California, North and South Carolina, Great Plains in central North America, eastern USA and eastern and central Canada, eastern and central parts of Canada and the USA	Liu et al. (2010)
Salt resistance	*H. paradoxus*	West Texas, and New Mexico salt marshes	Lexer et al. (2003)
Drought resistance and high water use efficiency	*H. anomalus*	Southwestern USA	Ludwig et al. (2004)
Abiotic stress tolerance	*H. deblis* Nutt., *H. anomalus* Blake, and *H. divaricatus* L	Atlantic and Gulf Coasts of USA, Southwestern USA, North America, Ontario, Quebec, Illinois, Florida, and Louisiana	Kantar et al. (2015)
Sunflower moth resistance	Wild sunflower (*H. annuus* L).	Albany, California	Beard (1977)
Herbicide tolerance (sulfonyl urea imazethapyr)	*H. praecox* accession 1823 and *H. nuttallii* NUT05	USA	Jacob et al. (2016)

within a ploidy level and is easiest between diploids. The slowest introgression occurs in crosses between diploids and hexaploids, with diploids × tetraploids at intermediate speeds, due to the time-consuming removal of extra chromosomes through backcrossing (Jan et al., 2014). Faster restoration of chromosomes to $2n = 34$ may occur through the use of the polyploid species as the male parent to overcome negative interactions of wild cytoplasm with the cultivated species genome.

Cytoplasm from wild species (when used as the female parent) can impart CMS, disease susceptibility, and other undesirable effects to hybrids with domesticated *H. annuus*. Wild sunflower species are still not well characterized at the cytological level (Vanzela et al., 2002), which generally involves chromosome banding and fluorescence in situ hybridization (FISH) techniques revealing differences in chromosome size, number, and morphology due to hetrochromatin rearrangement and ribosomal DNA sites. Difficulties may be overcome using more similar species, and many *Helianthus* species originated from multiple homoploid hybrid speciation events due to differential chromosome sorting and rearrangement in different interspecific hybrids from common ancestral parental species (Lai et al., 2005). For example, hybridization between *H. annuus* and *H. petiolaris*, followed by chromosome

doubling, led to the evolution of three different diploid hybrid species: *H. anomalus*, *H. paradoxus*, and *H. deserticola* (Gross et al., 2003). These three hybrid species are divergent in karyotype and gene order and linkage group differences due to chromosome sorting, de novo breakage, and fusion cycles (Lai et al., 2005). They have also undergone significant differentiation and adaptation to particular ecological conditions and now grow in a diverse range of challenging conditions (Rosenthal et al., 2002). Thus, these CWR can be regarded as sanctuaries of adaptive alleles that are being explored to augment diverse breeding goals, including disease, drought, heat, and salinity resistance (Skorić, 2009) and diversification of cytoplasmic sterility sources (Seiler, 1992; 2007a; Kantar et al., 2015).

In 2010, FAO estimated that ~40,000 accessions of sunflower were preserved in 92 countries, with the largest collections held by Serbia, the United States, China, France, Russia, and India (FAO, 2010). The collection in the United States is managed and maintained through the US National Plant Germplasm System (NPGS) at the North Central Regional Plant Introduction Station in Ames, IA, with long-term backup storage at the NPGS location in Fort Collins, CO. The NPGS collection includes ~2500 cultivated *H. annuus* accessions and 2500 wild accessions, of which roughly 60% are annual species (L. Marek, personal

communication, 2016). There have been >30 explorations to collect wild sunflower seeds across the United States, Canada, and Australia, resulting in one of the most comprehensive wild sunflower collections in the world. Roughly half of the explorations were in collaboration with visiting researchers from other international genebanks, notably Serbia, Russia, and India. The NPGS collection is the only publicly accessible collection, and seed of the NPGS sunflower accessions are available to breeders throughout the world without charge and generally without restriction through the Germplasm Resources Information Network Global (GRIN Global) database.

The Sunflower Biological Resources Center maintained by the French National Institute for Agricultural Research in Toulouse, France, contains 5576 cultivated accessions, including mapping populations and an ethyl methanesulfonate (EMS)-mutant population, and >500 wild sunflower ecotypes. The Indian Institute of Oilseeds Research maintains 3273 accessions composed of 1200 exotic lines, 97 genetic stocks, 360 inbred lines, 42 wild species, and 154 wild species derivatives (Dudhe and Sujatha, 2016). The Oil Crop Research Institute of the Chinese Academy of Agriculture Science in Wuhan, China, maintains 2813 sunflower accessions, predominantly cultivated (Gao et al., 2001). The Vavilov Research Institute of Plant Industry in Russia maintains 2230 cultivated sunflowers and 550 wild accessions, encompassing 5 annual and 19 perennial species (Gavrilova et al., 2014). The Institute of Field and Vegetable Crops in Novi Sad, Serbia, has a cultivated collection of several thousand lines (S. Terzic, personal communication, 2016) and a wild sunflower collection of just over 1000 accessions of 47 wild species, although they do not have seed backup of 40% of the species (Atlagić and Terzić, 2015).

The economic benefits to the sunflower industry due to CWR contributions have been estimated to be more than US$ 1 billion (Seiler and Fredrick, 2011). The trait of most economical value to date is the CMS (*PET1*) introgressed from the wild species *H. petiolaris*, which, along with restorer genes also incorporated from wild species, is responsible for the success of the sunflower hybrid seed industry. Dozens of examples of introgression of important traits have been published, and these include herbicide tolerance (Jacob et al., 2016) and disease and insect resistance, including disease resistance genes for rust (Chandler et al., 1986), downy mildew [*Plasmopara halstedii* (Farl.) Berl. & De Toni in Sacc.], Verticillium wilt (*Verticillium albo-atrum* Reinke & Berthier), powdery mildew, *Phomopsis* stem canker, Sclerotinia wilt [*Sclerotinia sclerotiorum* (Lib.) de Bary], charcoal rot, Phoma black stem, and the parasitic weed broomrape (reviewed in Seiler, 2010). Other disease resistance has been introgressed from the wild perennial species *H. maximiliani* Schrad. and *H. giganteus* L., (Liu et al., 2010), as well as alleles for achene

yield and modification of fatty acids and other industrial and nutritional products (Gavrilova et al., 2014). Some of the breeding lines developed from introgressions from CWR have been listed in Table 2.

Challenges to accessing useful variation from CWR for sunflower improvement include the previously mentioned presence of overly divergent genomes in different species. Some annual wild species have shown karyotypic differences from cultivated sunflower (and each other) due to translocations and inversions. In the case where fertile hybrids cannot be generated directly, techniques such as in vitro fertilization or embryo rescue have been exploited to obtain viable hybrids. Embryo rescue techniques (3–4 d after fertilization) for otherwise abortive interspecific hybrids were found effective in transferring quantitative major genes and helped in generating interspecific crosses. Improvements in tissue culture techniques, including direct organogenesis from explants (Encheva et al., 2003), have also increased efficiency of interspecific hybrid recovery. Fertility of interspecific hybrids has been improved via genome duplication by the use of the chemical colchicine (Prabakaran and Sujhatha, 2004).

Another challenge limiting use of CWR is linkage drag during introgression, which can introduce undesirable traits in the process. Many diploid annual sunflowers are susceptible to diseases and thus have been less exploited by breeders for introgression into cultivated germplasm. Highly branched growth habits, lower fertility and achene or oil yield, and other unfavorable morphological and agronomic traits may also result from crosses with CWR (Hussain et al., 2016). Thus, one of the major challenges for the plant breeder is to isolate beneficial transgressive segregates having targeted alleles from the donor wild species with minimum linkage drag in the offspring of interspecific crosses. In complex crosses or species differing for ploidy level, hybrids can be backcrossed several times to the recipient species to increase the intensity of the genome of the recipient species. Molecular marker-assisted backcrossing using genome-specific markers may reduce linkage drag, number of backcrosses, and number of generations required to reach homozygosity, as has been done with the HA-DM1 line resistant to all known races of downy mildew (Qi et al., 2016; Qi and Seiler 2016).

CASE 2: MAIZE

Maize (*Zea mays* subsp. *mays*) was domesticated in Mexico ~9000 yr ago from teosinte, *Zea mays* subsp. *parviglumis* Iltis & Doebley (Matsuoka et al., 2002; Doebley, 2004; Piperno et al., 2009). Teosinte is characterized by enormous genetic and phenotypic diversity (e.g., Fig. 2), and includes annual and perennial diploid ($2n = 20$) and tetraploid species ($2n = 40$). The distribution of teosinte extends from the state of Chihuahua, Mexico, to western Nicaragua and Costa Rica. Teosinte populations do not

Table 2. Breeding material developed from the introgression of wild traits into cultivated sunflower.

Source	Inbred line	Trait	Reference
Wild species	Interspecific Rf lines and mapping population	Restorer lines	Liu et al. (2013); Leclercq (1969)
H. annuus subsp. *texanus*	CMS-ANT, Rf-ANT1	Cytoplasmic male sterility and restorer fertility	Luoraş et al. (2002)
H. tuberosus	R101 and R104	Heterotic breeding	Encheva et al. (2003)
H. praecox and *H. argophyllus*	HA 339, RHA 340	Pl[7] Pl[8] downy mildew gene Pl[7], Pl[8]	Miller and Gulya (1991)
H. argophyllus	PS 1089	Rust resistance	Sujatha et al. (2008)
H. petiolaris	PS 2011 and PS 2032	Rust resistance	Sujatha et al. (2008)
H. bolanderi	PR-47/8, E-009	High oil contents (50.00–52.41%)	Christov (2012)
H. debilis, *H. pauciflor*, and *H. argophyllus*	Sc-2 L-611-6B, SC-3, Sc-5, Sc-8, Sc-9	Phomopsis, Phoma, gr. Alternaria, gr. Sclerotinia,	Christov (2012)
H. pauciflorus, *H. tuberosus*, *H. divaricatus*, *H. pauciflorus*, *H. hirsutus*, and *H. bolanderi*	PR-1/8, PR-9/8, PR-19/8, PR-25/8	Broomrape, downy mildew	Christov (2012)
H. ciliaris	1131/H, 1135/H, 1145/H, 1171/H, 1161/p and 1151/H.	Downy mildew and broomrape.	Nenova et al. (2014)
H. argophyllus- T, *H. annuus*- NM, and *H. annuus*-TX TX-16X	RHA 340, *H. argophyllus*- FL HA, 419/420, HA 428, and HA 458	Downy mildew	Seiler (2010)
H. paradoxus-1671 (NM) and *H. paradoxus*-1673 (TX)	HA 429 and HA 430	Salt tolerance	Seiler (2010)
Wild *H. annuus*	SURES-1, SURES-1, RHA-426, and RHA-427	Resistance to the sulfonylurea herbicide	Miller and Al-Khatib (2002; 2004)
H. californicus, *H. schweinitzii*, *H. maximiliani*, *H. giganteus*, and *H. grosseserratus*	163 and 313 progeny families; inbred lines close to release	Resistance against head and stalk rot (moderate to good head and stalk rot resistance).	L. Marek, personal communication (2016)
H. paradoxus	Prebreeding lines	Salt resistance	Ge Baute, personal communication (2016)

have uniform geographical distributions; rather, there are specific climate, soil, and human circumstances under which they can be found. Thus, taxonomy has been very difficult to establish, but according to ecogeographical characterization, cytological and morphological data, and DNA evidence, the genus *Zea* is reported to have nine taxa classified into two sections (*Zea* and *Luxuriantes*), which together encompass six species (Wilkes, 1967; Iltis and Doebley, 1980). Section *Luxuriantes* includes the perennial species *Z. diploperennis* Iltis, Doebley & Guzman and *Z. perennis* (Hitchc.) Reeves & Mangelsd. and the annuals *Z. luxurians* (Durieu & Asch.) Bird, *Z. nicaraguensis* Iltis & Benz, and *Z. vespertilio* Gómez–Laur. Section *Zea* includes only one species and four subspecies: the annual *Z. mays*

Fig. 2. Phenotypic diversity for plant type as seen in various teosinte and maize entries.

subsp. *mexicana* (Schrad.) Iltis (races Chalco, Central Plateau, Durango, and Nobogame), *Z. mays* subsp. *parviglumis* (race Balsas), *Z. mays* subsp. *huehuetenangensis* (Iltis and Doebley) Doebley (race Huehuetenango), and *Z. mays* subsp. *mays* for cultivated maize. Recently, three new taxa from Mexico were reported within the section *Luxuriantes* from Nayarit, Michoacan, and Oaxaca (Sánchez et al., 2011).

Sequence diversity within and among teosinte populations has been estimated using isozymes (Doebley and Goodman 1984) and simple sequence repeat markers (SSRs) (Fukunaga et al., 2005). *Zea* subsp. *parviglumis* and *mexicana* contain considerable and similar diversity, and races with narrow distribution and small population size show the least diversity (*Z. diploperennis*, *Z. perennis*, *Z. vespertilio*, *Z. luxurians*, and *Z. nicaraguensis* and races Nobogame and Huehuetenango). Despite differences in plant characteristics, *Zea* species are genetically very similar; maize and diploid teosinte are crossable and produce viable, fertile hybrids. However, hybrids between tetraploid perennial teosintes and maize have low fertility and produce few viable kernels. Incompatibility factors can disrupt hybridizations attempted using weedy types of teosinte as female. Three genetic systems conferring cross incompatibility have been described in *Zea*: Teosinte crossing barrier1-strong (*Tcb1-s*) found in teosinte, and gametophyte factor1-strong (*Ga1-s*) and *Ga2-s* found in maize and teosinte (Evans and Kermicle, 2001; Kermicle and Evans, 2010).

The major teosinte genebank collections are those of the Instituto Nacional de Investigaciones Forestales, Agricolas, y Pecuarias (INIFAP), the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), the NPGS, and the Universidad de Guadalajara. Only NPGS, CIMMYT, and INIFAP have long-term storage facilities. According to GRIN, NPGS reports 339 active accessions, of which ~75 have distributable seed (L. Marek, personal communication, 2016). CIMMYT holds ~300 accessions, INIFAP ~450, and the University of Guadalajara 515 accessions. Other institutions from Mexico and Central America preserve seed in different quantities and under varied conditions: the Universidad Autónoma Chapingo, Colegio de Postgraduados, Instituto de Ciencia y Tecnología Agrícolas (ICTA, Guatemala), and the Instiuto Nicaragüense de Tecnología Agropecuaria (INTA) (Nicaragua). Availability of seed samples is good at CIMMYT and uncertain for the rest of the listed institutions, at which only partial data are available for users, and only NPGS has online passport data, where the curator can provide inventory data. Most populations were collected as small seed samples during genetic and morphological studies, and larger samples for long-term conservation are still required.

Over the past 500 yr, human activities such as deforestation, urbanization (including road building), and cattle grazing have been identified as the major threats to teosinte. The biggest threat, however, is when the cultivation of maize landraces is abandoned, as several races of teosintes grow on the borders of or mixed within traditional Mexican farmer's fields (Wilkes 1967). Because of these threats, permanent monitoring programs and in situ conservation projects with participation of local farmer communities are critically needed. For the short term, collecting and ex situ conservation activities are urgently needed in Guatemala, Nicaragua, Costa Rica, and several sites in Mexico.

Maize, particularly elite temperate maize, has experienced a severe genetic bottleneck due to domestication, exacerbated by breeding practices over the past 100 yr (Tenaillon et al., 2004). These practices include the use of a limited number of tester lines from mainly three heterotic groups in the United States and limited numbers in other countries. Recycling of successful lines and pedigrees is a common practice that, to date, continues to result in yield increases. All new candidate inbred lines that do not form high-yielding hybrids with these testers are generally rejected; thus, even genetic resources possessing critical new traits may never be used, and the vast majority of allelic diversity present in the gene pools of maize has never been tapped. Thus, these lines must be considered an underused plant genetic resource (Goodman et al., 2014), and the teosintes may be sources of genetic variation for maize breeding, especially when genetic diversity is very low in breeding pools for economically important traits.

Teosinte is often found sympatric with maize and is thus subjected to the same biotic and abiotic stresses; in fact, teosinte has been under these stresses over a longer period of time. It can therefore be the source of resistance to these stresses, as has been reviewed by de Lange et al. (2014). Resistance to biotic stresses in teosinte has been well documented. De la Paz–Gutiérrez et al. (2010) found teosinte to be more resistant than maize to 66 genera of insects. Nault and Gordon (1982) found *Z. perennis* and *Z. diploperennis* teosintes to be resistant to several important viruses to which all other *Zea* are susceptible. Kling et al. (2000) and Rich and Ejeda (2008) found resistance to *Striga* spp. and root parasites from *Z. diploperennis*. In addition, abiotic stress resistance is also easy to find in teosinte. *Zea luxurians*, *Z. nicaraguensis*, and *Z. mays* subsp. *huehuetenangensis* all grow in areas that receive frequent rainfall and have been found to possess unique flooding and waterlogging resistance (Mano et al., 2005; Mano and Omori, 2007, 2013, 2015). Teosinte should possess useful functional variation to improve maize traits that are not immediately apparent (or easily measured in a teosinte background), including improved nutritional quality (Melhus, 1948; Swarup et al., 1995; Wang et al., 2008a; Flint-Garcia et al., 2009), productivity (Cohen and Galinat, 1984;

Magoja and Pischedda, 1994; Casas-Salas et al., 2001; Wang et al., 2008b), and cross-incompatibility factors, some of which are very strong and apparently unique to teosinte (Kermicle, 2006; Kermicle and Evans, 2010).

Useful variation has been identified in teosinte and incorporated into the domesticated gene pool via hybridization and backcrossing and/or selection, in a few cases. This has provided proof that useful phenotypic variation can be tapped from teosinte for maize improvement. Despite arguments to the contrary, it is also known that introgression occurs in maize via gene flow from teosinte and is an ongoing process in the center of origin (Warburton et al., 2011; Hufford et al., 2013). Tropical maize populations introgressed with various traits from teosinte have been created, including resistance to striga (*Striga hermonthica*; Menkir et al., 2006; Yallou et al., 2009), gray leaf spot (*Cercospora sorghi* Ellis & Everh.; Lennon et al., 2016), southern corn leaf blight [*Cochliobolus heterostrophus* (Drechs.) Drechs.], southern corn rust (*Puccinia polysora* Underw.), and maize streak virus (Menkir et al., 2006), and kernel composition traits (Liu et al., 2016b). In addition, various desirable characteristics have been transferred into maize by substituting three of the maize chromosomes with three chromosomes from *Z. perennis*, which was achieved by creating a BC_1F_3 generation of maize × perennial teosinte BC_1F_3 (Tang et al., 2005). However, finding documentation of even one trait present in a temperate maize hybrid currently on the market in the United States has been extremely elusive. It has been repeatedly cautioned that teosinte has been vastly underused for the improvement of maize because the time and uninterrupted effort needed is very high; however, the possibility of eventual discovery of unique and useful alleles is great (Goodman 1998; Goodman et al., 2014).

The reduced use of wild *Zea* and related species for crop improvement is reflected by an underutilization of exotic maize landraces, the majority of which are adapted to tropical and subtropical growing environments. Although they have been found to contain much more sequence diversity than elite US temperate maize germplasm, the use of exotic maize parents in temperate breeding programs is very rare. The Germplasm Enhancement of Maize (GEM) project is one systematic and largescale effort to move useful sequence diversity from exotic to elite maize breeding populations (Salhuana and Pollak, 2006). The project has released >300 lines for public use since its inception (http://www.public.iastate.edu/~usda-gem/). Many other projects have also used exotic sources to create populations with higher levels of important traits, including drought stress resistance (Meseka et al., 2013), nutritional characteristics (Menkir et al., 2015), cell wall digestibility (Brenner et al., 2012), and aflatoxin accumulation resistance (Warburton et al., 2013). Increased sequence variation in tropical maize may be higher because a second bottleneck occurred when maize moved from Mexico into more northern climates in the United States, and also because gene flow between tropical maize and sympatric teosinte continues to bring in new variation from maize CWR (Warburton et al., 2011; Hufford et al., 2013). Thus, tropical maize could be used as a bridge between temperate breeding pools and maize CWR.

Several biological challenges have been given as reasons that wild *Zea* species or *Z. mays* landraces are not used more in elite temperate maize breeding. These include photoperiod sensitivity; division of tropical and temperate maize in their adaptation, and the fact that most landraces and all CRW are tropical; carefully balanced heterotic patterns into which most elite maize is assigned, and which introgression of exotic germplasm would disturb; and the very high yield demanded by growers, which is generally suppressed, if only for a few generations, by genetic drag during introgression. Crossa and Gardner (1987) found that exotic × elite populations yield less when they are composed of half the genetic variation from each source, as opposed to the majority of either, suggesting that desirable agronomic performance occurs in haplotypes selected as a whole, which can be disturbed by mixing preexisting germplasm pools. Introgression of a few genes, with a quick return to the background of the recurrent parent, avoids the problems associated with a complete mixing. However, since most agronomically important traits are under the control of many genes, this may not be a successful breeding option unless a few quantitative trait loci (QTLs) or genes have a larger effect on the phenotype. Gain from selection in the mixed germplasm pools have been demonstrated to continue for these quantitative traits (Albrecht and Dudley, 1987) and can be expected to eventually surpass the performance of the original exotic or elite populations. This would take many generations, however, which the need for a quick return on investment or research grant-imposed deadlines may not allow. Thus, large efforts in prebreeding using diverse germplasm and/or wild relatives while maintaining successful recurrent parents is likely an unappealing strategy in industry.

The identification of useful variation from teosinte can also be slowed by a lack of genetic resources in which to study this variation, particularly for quantitative traits that cannot be estimated for breeding purposes in a teosinte background (including most yield, ear, kernel, and plant morphology traits). However, the recent release of 928 near-isogenic introgression lines (NILs) from 10 *Z. mays* ssp. *parviglumis* accessions in a B73 background provides an opportunity to measure the phenotypic effect of teosinte sequence variation on cultivated maize (Liu et al., 2016a), which compliments older teosinte-maize introgression resources (Briggs et al., 2007). Linkage analysis of the newest NILs have already identified positive alleles from teosinte on traits including male flowering time, number of kernel rows, and 50-kernel weight in maize (Liu et al., 2016b).

Researchers have suggested methods to introgress useful traits from teosinte into maize breeding pools, including sequential backcrossing (Casas-Salas et al., 2001), joint multiple population analysis, genomewide association study (GWAS), and genomic selection via high throughput sequencing and genotyping technologies (Sood et al., 2014; Baute et al., 2015). A useful review on prebreeding maize × teosinte population crosses can be found in Ortiz (2015). Generating largescale genomic information from cereal CWR is now much more economical than ever, and much progress has already been made in sequencing and resequencing CWR to date, including studies published by Brozynska et al. (2016) and Khan and Budak (2015). Using sequence information to guide introgression for genomic regions known to be associated with useful traits will make this process very efficient, with negligible linkage drag from outside genomic regions of interest. This should allow minimal perturbation of heterotic groups and yield potential of the resulting backcrossed progeny.

It may also be possible to use maize wild relatives in a less direct manner to tap the allelic diversity necessary to incorporate new traits. If a beneficial allele can be found in an exotic source, including landraces or wild species, the sequence information itself may be sufficient to seek the same allele in a much more closely related temperate maize line and introgress it into the elite breeding pool via marker-assisted backcrossing, thus eliminating the potential for genetic drag from wide crosses. Alternatively, if this sequence diversity does not exist in elite maize breeding pools, it may still be possible to use the allele information from exotic sources to guide improvement in the elite temperate genome. Once the precise genomic region is identified via genetic mapping or other genomic, proteomic, or metabolomic studies of landraces or wild species, the causal mutation defining the beneficial allele from the exotic source can be characterized. If the sequence change is small, this information can be used to improve elite breeding lines via genome editing. Although the method is less straightforward, the resulting improved line may be more acceptable to large private companies who would also control the intellectual property of the line.

DISCUSSION

Useful phenotypic variation and vast sequence variation exist in the CWR of both sunflower and maize. In both species, this variation has often been successfully transferred to experimental interspecific populations, but linkage drag has made the resulting hybrids not immediately useful. Rounds of intermating or backcrossing, followed by selection against traits brought in unintentionally, has been necessary to create acceptable breeding lines. This is simplified in the case of qualitative or oligogenic traits, which can be introgressed quickly and with a minimum of linkage drag via marker-assisted backcrossing. In the case of quantitative traits, population-level selection is generally required and is a slower process. The development and extensive characterization of genetic resources consisting of backcrossed interspecific populations can benefit many researchers simultaneously, and the sharing of all the characterization data will make the work more efficient. Taking this process through the research stage all the way to the release of new cultivars is slow but has been done successfully on many occasions in sunflowers and with significant economic impacts.

It would appear that the constraints on the use of maize CWR have been greater than those on sunflowers, as maize breeders have never successfully transferred allelic variation from CWR to a final commercial product for temperate growing areas. In fact, it would appear on the surface that insurmountable biological obstacles are preventing the use of the vast array of useful diversity from teosinte and other related *Zea* species in maize breeding, but that these obstacles are either much smaller, or have somehow been overcome, in sunflowers. It is true that many sunflower CWR evolved in temperate growing conditions, whereas all maize CWR are tropically adapted, and that cultivated sunflowers and maize grown in the United States and Europe are temperate. Nevertheless, on closer inspection, the real constraints in maize turn out to be logistical, economical, and habit driven. No biological constraint has been identified to date that would not be easily overcome with time and long-term financial support. Time and funding for such activities have been lacking in largescale temperate maize breeding, however, which values the fastest time to new cultivar release and profitability. To date, sufficient allelic diversity has been present to allow continued gain from selection in temperate maize breeding pools, although this may change at any time; given the evolution or introduction of a new disease agent or abiotic constraint, the risk has not yet been given sufficient weight in the private industry to change current breeding schemes.

Sunflowers may have benefited from the diversity available in CWR partly because the industry does not operate on the razor-thin profit margins that maize growers face, especially since maize is so heavily cultivated in the United States that supply is almost always equal to (and sometimes exceeds) demand, driving down prices and profits. Thus, tiny reductions in yield in new sunflower cultivars would not spell financial ruin for sunflower growers as it may in maize, especially if the new cultivars were substantially better in other respects. These possible yield reductions, caused by linkage drag from wild relatives and removable over time, will also be compensated in a bad growing year if varieties with robust biotic and abiotic stress resistance genes from CWR are able to withstand future epidemics of new diseases or environmental stresses from a changing environment. Genomics approaches may

help level the field between maize and sunflowers by more efficiently tapping diversity from CWR while reducing or eliminating linkage drag more quickly. Sharing of sequence and gene identification data is necessary for the most efficient transfer of sequence diversity or sequence information from maize CWR to elite populations, but this is not the norm in private industry. In sunflowers, a major portion of the breeding effort is still done in the public sector, and more open sharing of information may be a factor in the successful use of CWR for sunflower improvement in comparison with maize.

The difference in the use of transgenic technology in maize versus sunflower may also be causing a difference in the use of CWR in the two species. The first breeding efforts in sunflower began in Europe many years before genetically modified organism (GMO) technology existed, and the use of CWR for incorporation of useful traits has been practiced by sunflower breeders for >100 yr. To date, there has been little focus on transgenic work in sunflowers, because the primary centers of production and breeding are outside of the United States, in countries where there is strong resistance to the release of transgenic cultivars. The opposite is true of maize, where the United States is the leading global producer and breeder of maize, and the vast majority of the acreage in the United States is now planted to transgenic maize. Traits found in CWR and backcrossed into cultivated material may not lend themselves to intellectual protection as easily as traits inserted into a cultivar via transgenesis, which may encourage the use of GMOs by for-profit companies where possible. Another factor in the GMO discussion is that the primary products of sunflower, oil and confectionary seed, are consumed by humans, whereas 80% or more of maize production is for animal feed and ethanol; sweet corn, on the other hand, is almost entirely non-GMO.

The cost of funding a program to identify and introgress CWR–derived genetic variation into modern elite maize would be a tiny fraction of the cost of a single failed harvest season in the United States. Particularly for traits where sufficient sequence variation does not exist in the domesticated gene pool, investment in the identification and transfer of new sequence variation from CWR is long overdue. The consequence of a narrow genetic base in maize has been demonstrated on a large scale during the Southern Corn Leaf Blight of 1970 in the United States. The genetic base of US commercial maize has narrowed even further in the intervening 45 yr, leaving the nation even more vulnerable to a new epidemic and decreased yield due to more difficult and less predictable maize growing environments. The potential cost of increased genetic vulnerability is smaller than the cost of reducing this vulnerability. Sunflower CRW will continue to be sources of important traits, including diverse cytoplasmic male sterility, increased oil content,

and disease resistance; these efforts must continue. The creation of genetic resources to characterize and move traits from maize CRW to elite breeding lines, such as the GEM program, the teosinte NIL created by Liu et al. (2016a) and ongoing work by the CIMMYT Seeds of Discovery program, the teosinte GWAS studies being run by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and efforts to conserve the CWR of maize, sunflower, and all other important crop species as in situ collections must be funded, probably via public funding, and taken more into mainstream breeding.

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