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Genomic variation in *Helianthus*: learning from the past and looking to the future

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Abstract

Helianthus is an economically important and genetically diverse genus, containing both evolutionary model species and cultivated species. Genetic variation within this genus has been examined at many different scales, from genome size changes to chromosomal structure to nucleotide variation. The growing amount of genomic resources within the genus has yielded insights into the importance of paleopolyploid events, and how transposable elements can cause rapid genome size increases. The rapidly evolving chromosomes in *Helianthus* have provided a system whereby it has been possible to study how chromosomal rearrangements impact speciation, adaptation and introgression. Population and quantitative genetic studies have used the abundant nucleotide variation to identify a number of candidate genes which may be involved in both local adaptation and domestication. The results from these investigations have provided basic knowledge about evolution and how to utilize genetic resources for both agriculture and conservation. Targeting *Helianthus* for further study as new technologies emerge will allow for a better understanding of how different types of genomic variation interact and contribute to phenotypic variation in a complex system that is ecologically and economically significant.

Keywords: *transposable elements; karyotype; nucleotide variation; hybridization; speciation*

INTRODUCTION

Genetic variation is the raw material that natural and artificial selection acts on. Characterizing the nature and extent of this variation, as well as how it is linked to phenotypic and ecological diversity has important implications for evolutionary biology, biodiversity conservation and plant breeding [1–3]. Until recently the full depth of genetic variation, which spans from gene and genome duplication to large structural rearrangements and to single nucleotide variation, was only being characterized in a limited number of model species (e.g. *Arabidopsis thaliana*; reviewed in [4]). Recent advances in technology, primarily sequencing technologies, have allowed researchers to significantly broaden the taxonomic scope of these efforts. By integrating mapping approaches, karyotyping methods and high-throughput DNA sequencing, it is now possible to assess genome-wide levels of genetic variation in almost any taxonomic group of interest [5–7].

Helianthus is an exemplar genus for the study of genetic variation in the wild. Wild sunflowers occupy a wide variety of habitats, which range from open plains to sand dunes and salt marshes [8]. Native to North America, *Helianthus* comprises 12 annual and 37 perennial species [9–11] that have been the subject of a number of intensive evolutionary genetic studies. Within many of these species there is ecological and genetic diversification. Motivated in part by the observation that hybridization happens rampantly among *Helianthus* taxa, this group has been used to dissect the genetic

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determinants of species cohesion [12, 13]. Work on two widespread annual species—*Helianthus annuus* and *Helianthus petiolaris*—and their three independent homoploid hybrid derivatives—*Helianthus deserticola*, *Helianthus paradoxus* and *Helianthus anomalus*—have helped clarify the role of chromosomal rearrangements and ecological divergence in the formation of hybrid taxa [14–16]. Use of various molecular markers with this system has yielded insights into rates of adaptive molecular evolution [17], genetic changes that accompany or facilitate adaptation to extreme environments [18–20] and the contribution of introgressive hybridization to local adaptation [21, 22].

The genus contains two economically important crops, the annual oilseed crop common sunflower (*H. annuus*) and the perennial tuber crop Jerusalem artichoke (*Helianthus tuberosus*). *Helianthus annuus* is the more widely grown, being cultivated on ~26 million hectares worldwide in 2011 [23]. Grown for its edible oil, *H. annuus* is favored among producers for its abiotic stress tolerance [24]. Cultivated *H. annuus* has the second largest hybrid seed market in the world with a substantial private investment in breeding efforts. Genetic analyses performed primarily on *H. annuus*, a species that exhibits a classic domestication syndrome, have shed light on genome-wide consequences of domestication [25, 26], and have facilitated the identification and functional validation of causal domestication genes [27, 28].

Here, we give an overview of research into genetic variation in the genus *Helianthus*. We provide examples of how our knowledge of fundamental questions in evolution and genome organization has been improved by the study of variation in genome size, chromosomal structure and nucleotide sequence in wild and domesticated sunflower species. We conclude by highlighting new research opportunities for genetic work in the genus.

THE DYNAMICS OF GENOME EXPANSION AND CONTRACTION IN *HELIANTHUS*

It has long been known that genome size varies tremendously across plant species [29]. Duplication of DNA, both at large scales involving whole genomes and chromosomes and small scales where transposable elements (TEs) proliferate, is ultimately responsible for genome size increases. The multiplication of whole chromosome sets, known as whole genome duplication, or polyploidization, has been recognized as an integral part of plant biology for over a century [30, 31]. During the past decade, with the explosion of large-scale datasets, the study of polyploidization has seen increased interest (reviewed in [32]). Particularly productive research themes include the frequency at which polyploidization events have occurred and their role in biological diversification [33, 34], the genomic consequences of polyploidization [35, 36] and the genetic basis of adaptation to polyploidy [37, 38]. In contrast, much less is known about genome size variation originating from copy number differences of TEs (reviewed in [29]). Patterns of TE proliferation and deletion have been investigated in a number of systems [39–42]. However, the factors responsible for generating these patterns remain enigmatic. Here, we provide a synopsis of research on genome size variation in *Helianthus*, with an emphasis on TE dynamics. We provide examples of how sunflower research has advanced our understanding of this active area of study.

Similarly to the rest of the plant kingdom, *Helianthus* exhibits considerable variation in genome size (Table 1). Much of this variation is attributable to differences between ploidy levels. Indeed, *Helianthus* contains diploid ($2n = 2x = 34$), tetraploid ($2n = 4x = 68$) and hexaploid species ($2n = 6x = 102$) [8, 43] that formed during neopolyploidization events occurring since the radiation of the genus (i.e. 1.7–8.2 million years ago; [44]). Investigations using expressed sequence tag data have revealed that all *Helianthus* species have also undergone at least two rounds of older polyploidization events: one at the base of the tribe Heliantheae, dated at 26–31 million years ago and one at the base of the family Compositae, dated at 40–45 million years ago [33]. Beyond identifying a source of genomic redundancy in the sunflower genome, these findings revealed that retention of duplicated genes is paralleled across different tribes in the Compositae [33], despite 33–38 million years of divergence [45]. The categories of paleologs retained were, however, unlike those recovered in other plant families [46, 47] (structural components and cellular organization in Compositae versus transcription and signaling in other families [33]), suggesting that forces determining the fate of duplicates after whole-genome duplication, although conserved within lineages, diverge at higher taxonomic levels [33].

Much of the research on genome size variation in *Helianthus* has been directed toward understanding

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Table 1: Species, ploidy, chromosome number and genome size of all the species in *Helianthus* for which genome sizes are available and one interspecific hybrid that is not a species

Species	Ploidy	Chromosome number	1C genome size (pg)	1C genome size (Mb)	Source
Helianthus agrestis[a,b]	2	34	12.95	12 665	Sims and Price, 1985 [136]
Helianthus angustifolius[a,b]	2	34	6.1	5966	Sims and Price, 1985 [136]
H. annuus[b]	2	34	3.7	3619	Baack *et al.*, 2005 [48]
H. anomalus[b]	2	34	5.76	5633	Baack *et al.*, 2005 [48]
H. argophyllus[a,b]	2	34	4.43	4328	Sims and Price, 1985 [136]
Helianthus bolanderi[a,b]	2	34	4.4	4303	Sims and Price, 1985 [136]
H. debilis[a,b]	2	34	3.3	3227	Sims and Price, 1985 [136]
H. deserticola[b]	2	34	5.64	5516	Baack *et al.*, 2005 [48]
Helianthus divaricatus[a,b]	2	34	8.45	8264	Sims and Price, 1985 [136]
H. exilis[a,b]	2	34	4.8	4694	Sims and Price, 1985 [136]
H. giganteus[a,b]	2	34	4.83	4719	Sims and Price, 1985 [136]
Helianthus heterophyllus[a,b]	2	34	4.9	4792	Sims and Price, 1985 [136]
Helianthus microcephalus[a,b]	2	34	5.1	4988	Sims and Price, 1985 [136]
Helianthus neglectus[b]	2	34	3.2	3130	Sims and Price, 1985 [136]
Helianthus niveus	2	34	3.65	3570	Sims and Price, 1985 [136]
H. paradoxus[b]	2	34	5.48	5355	Baack *et al.*, 2005 [48]
H. petiolaris[b]	2	34	3.44	3364	Baack *et al.*, 2005 [48]
Helianthus praecox[b]	2	34	3.53	3447	Sims and Price, 1985 [136]
Helianthus radula[b]	2	34	5.88	5748	Sims and Price, 1985 [136]
H. tuberosus	6	102	14.55	14 200	Kantar *et al.*, 2014 [85]
H. annuus × *H. tuberosus*	4	68	9.98	9760	Kantar *et al.*, 2014 [85]
Helianthus winteri	2	34	3.55	3470	Moyers and Rieseberg, 2013 [137]

[a]Indicates the measurement was made with Feuglen densitometry which may underestimate genome size in *Helianthus* due to secondary metabolites [138].
[b]Estimates were retrieved from the Kew Garden C-value database [139].

genome expansion and restructuring caused by TE proliferation within ploidy levels. Early studies revealed that nuclear DNA content is more than 50% larger in the homoploid hybrids *H. anomalus*, *H. deserticola* and *H. paradoxus* compared with their parental species *H. annuus* and *H. petiolaris* despite the fact that all five taxa have the same haploid chromosome number ($n = 17$; [48]). In all three independently derived hybrids, genome size increases have been associated with the expansion of *Ty3/gypsy*-like superfamily of long terminal repeat retrotransposons (LTR-RTs; [49]) and, to a lesser extent, of the *Ty1/copia*-like LTR-RT elements [50]. In addition, expansion appears to have occurred similarly in the homoploid hybrids with respect to the particular LTR-RT sub-lineages that proliferated within each superfamily [51]. These findings suggest TE proliferation events in homoploid hybrid *Helianthus* were associated with factors shaping the evolution of all three species (discussed below; [51]). The proliferations of the *Ty3/gypsy*-like and the *Ty1/copia*-like LTR-RTs were also confined, respectively, to pericentromeric and telomeric regions, further indicating that there may be negative selection associated with insertion of TEs in euchromatic regions [52].

Since the discovery of massive TE expansion in the genomes of *H. anomalus*, *H. deserticola* and *H. paradoxus*, two major factors known to have played an important role in the evolutionary history of all three hybrids, namely genome merger and abiotic stress, have been proposed and investigated as causative agents of retrotransposon proliferation [49]. However, recent results have, so far, failed to support these earlier assertions, and could not assign a causative role in TE proliferation to either factor. Specifically, a recent analysis of TE abundance in contemporary hybrid populations between *H. annuus* and *H. petiolaris* did not find evidence for genome expansion [53]. As well, hybridization with or without concomitant salt and wound stress did not induce TE expression in greenhouse synthesized *H. annuus* × *H. petiolaris* genotypes [54].

Following TE proliferation, host-encoded mechanisms are thought to act toward disrupting repetitive element proliferation, limiting genome obesity [29]. Recent *Helianthus* research has sought to clarify

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the identity of such molecular mechanisms. Staton *et al*. [55] performed a survey of sequence reads representing 25% of the genome of the cultivated sunflower *H. annuus* to investigate the relative efficiency of two major TE deletion mechanisms, unequal homologous recombination (UR) and illegitimate recombination (IR). This analysis revealed a high proportion of small deletions in repetitive regions, suggesting that IR may have a relatively greater impact than UR in counteracting genome expansion in sunflower [55], as appears to be the case in *A. thaliana* [40] and *Medicago truncatula* [56]. Investigations of wild populations of *H. annuus* and *H. petiolaris*, as well as recently formed hybrids between the two species also reported widespread transcriptional activity of LTR-RTs [53], although the level of transcription, as well as the identity of expressed elements is different among species [57]. Given the absence of contemporary genome expansion in most early generation *H. annuus* × *H. petiolaris* hybrids [53], these results suggest that post-transcriptional mechanisms of repression of LTR-RT proliferation play an important role in this system over microevolutionary timescales [53], and that these mechanisms are not necessarily element-specific [57]. On the other hand, several early generation hybrids were detected with genome sizes that exceed either parent. While these sizes increases are relatively small, such increases extrapolated over a fairly short time period (<100 generations) could account for the large genome sizes of the ancient hybrid species. Finally, RNAseq surveys in homoploid hybrid *Helianthus* have started to pave the way in identifying specific candidate genes involved in regulating and restraining TE expansions [57].

Research performed during the past decade on genome size variation in *Helianthus* has been highly informative. Avenues of future investigation may include research into the contribution of hybridization, as well as different stress agents to TE proliferation [54]. In addition, epigenetic mechanisms generating differential TE expression, such as TE methylation, as well as the extent to which methylated TEs can affect the expression of nearby genes, should be pursued. Aside from informing our understanding of the mechanisms of TE suppression, these topics could shed light on why TE proliferations are frequently localized in heterochromatic regions, as has been observed in *Helianthus* [55]. Similar questions are being asked in other genome-enabled plant systems [58, 59]. Finally, it would be interesting to investigate the importance of outcrossing and large effective population sizes to TE proliferation in *Helianthus* as in other species this is important to TE proliferation because it enables the spread of selfish genetic elements, despite their deleterious effects on fitness [60]. The development of genomic resources for an increased number of sunflower species, as well as the assembly of the complete *H. annuus* genome are bound to pave the way for these and other developments in future genome size research in *Helianthus*.

CYTOLOGICAL VARIATION AND CHROMOSOME EVOLUTION WITHIN *HELIANTHUS*

Observations of the high frequency of chromosomal rearrangements in wild populations have led to a number of long standing questions about their role in speciation and adaption [61]. Chromosomal inversions and translocations have been examined in organisms spanning the tree of life and have been related to many divergent phenotypes [62, 63]. It is of particular interest why karyotypic variability persists within populations as rearrangements often appear to reduce fitness by interfering with meiosis [61]. *Helianthus* has been a key system in understanding the role of chromosomal evolution due to its high rates of chromosome structure evolution and economic value [10, 11, 14, 64, 65]. Here, we provide an overview of studies conducted over the past ~80 years defining the chromosomal relationships within *Helianthus*, then we focus on the parental species of well-known homoploid hybrids, *H. annuus* and *H. petiolaris*, whose association has been essential to the current understanding of recombinational speciation [16, 66].

Chromosome numbers began to be investigated in *Helianthus* early in the 20th century [43], with interest in chromosome behavior of interspecific hybridization between *H. annuus* and other members of the genus quickly following [67–69]. Among annual *Helianthus* species most interspecific hybridization events (~70%, [70]) produce viable offspring (Table 2), despite the presence of at least six large translocations and eight paracentric inversions in the group [70]. Sunflower has the highest rate of karyotypic evolution of any studied plant taxonomic group [65], with 5.5–7.3 chromosomal rearrangements per million years and numerous polyploidization events. Rapid divergence has led to chromosomal subtypes among *Helianthus* species

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Table 2: Crossing and chromosomal similarity within annual species in *Helianthus*

Species	*H. annuus*		*H. petiolaris*	
	Crossing success	**Chromosome pairing**	**Crossing success**	**Chromosome pairing**
H. anomalus	Successful	Eleven bivalents, two chains of four and one chain of six [70], six breakages/six fusions [97]	Successful	Thirteen bivalents, two chains of four [70]
H. argophyllus	Successful	Thirteen bivalents and two chains of four or 15 bivalents and one chain of four [140], 28 collinear segments 10 collinear chromosomes seven rearrangements [141]	Poor seed set (10%)	Seven collinear chromosomes [141]
H. bolanderi	Successful, poor fertility [70]	Two univalents, eight bivalents and two chains of four [70]	Successful but poor fertility [70]	Zero to four univalents [70]
H. debilis	Successful, poor fertility [142]	Two chains of six and a ring of four [70]	Successful	Zero to 15 univalents [70]
H. deserticola	Successful	Mixed bivalent and multivalent pairing [70], four breakages, three fusions [97]	Successful	Mixed bivalent and multivalent pairing [70]
H. neglectus	Successful, poor fertility [70]	Zero to two univalents [70]	Successful	Fifteen pairs, one chain of four [143]
H. niveus	Successful, but poor seed set	Ten bivalents, one ring of 10 and one chain of four [70]	Successful [143]	Had zero to two univalents [70]
H. paradoxus	Successful	Mixed bivalent and multivalent pairing [70], five breakages/five fusions [97]	Successful	Mixed bivalent and multivalent pairing [70]
H. petiolaris	Successful	Ten pairs, one ring and two chains [86], 11 rearrangements, eight translocations and three inversions. Twenty-seven collinear fragments [65], seven collinear chromosomes [141]	NA	NA
H. praecox	Successful, poor fertility [70]	Twelve bivalents, one chain of four and one chain of six [70]	Successful but sterile [70]	Multivalent formation [70]

corresponding to cross-ability [71, 72], with the largest division occurring between perennial and annual sunflowers [9]. The rapid karyotypic evolution has brought sunflower to the intellectual center of debates on the role of chromosomal rearrangements in adaptation and speciation.

The importance of these chromosomal rearrangements can be seen with hybridization experiments. Interestingly, crossing different taxa of the same phylogenetic distance yields different results in terms of hybrid formation and fitness [73–75]. Results of hybridization experiments between *H. annuus* and a variety of perennial relatives were mixed with almost no successful hybridization for some, *Helianthus grosseseratus* [73], *Helianthus giganteus* or *Helianthus maximiliani* [76], triploidization with others *Helianthus hirsutus* [77] and successful offspring with unusual meiotic pairing in others (*Helianthus ciliaris*, [74]; Table 3). Early investigations of the perennial polyploid species in *Helianthus* found that they generally cross with each other despite morphological differences [78]. However, this is not always the case, for example, *H. tuberosus* will not form hybrids with one of its wild progenitors *H. grosseseratus* [73, 79]. Homology and crossing relationships have been exploited to introgress traits into cultivated *H. annuus* for improvement by targeting particular species as trait donors [10, 64, 80].

The chromosomal interactions between the two crops, *H. annuus* and *H. tuberosus*, have generated much interest [74, 81–83]. From cytological examinations, and the observation of moderate fertility and seed set of hybrids it was inferred that a subgenome of the hexaploid *H. tuberosus* pairs effectively with the *H. annuus* genome [67–69, 74]. *Helianthus annuus* × *H. tuberosus* hybrids are tetraploid ($2n = 4x = 68$), although there is mixed bivalent and multivalent pairing [70, 74, 81, 83]. Despite these difficulties, interspecific *H. tuberosus* × *H. annuus* hybrids have many uses from forage to a perennial grain and as bridge for trait introgression [10, 64, 84, 85].

Helianthus annuus and *H. petiolaris* have been important models for karyotype evolution ever since

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Table 3: Crossing similarity between *H. annuus* and perennial species in *Helianthus* for which information was available

Species	*H. annuus*	
	Crossing success	**Chromosome pairing**
H. ciliaris	Successful	Poor meiotic pairing (multivalents and univalent) [74], moderate homology [74]
Helianthus eggertii	Successful, moderate fertility [144]	Mostly bivalents with some quadrivalents [144], some homology
H. giganteus	Successful with embryo rescue, F_1s sterile [76]	Many univalents, F_1 hybrids were sterile, moderate homology [74]
H. grosseserratus	Not successful	NA
H. hirsutus	Crosses well but forms mostly sterile triploids [144]	Has multivalents, bivalents and univalents, moderate homology [77]
H. maximiliani	Successful, F_1s mostly sterile [76, 145]	Poor pairing, many univalents, F_1s mostly sterile, moderate homology [74]
Helianthus mollis	Successful, moderate fertility [145]	Mostly bivalents some univalent and multivalents [145], moderate homology
Helianthus rigidus	Successful, moderate fertility [144]	Mostly bivalents with some quadrivalents [144], some homology
Helianthus resinosus	Successful, moderate fertility [144]	Mostly bivalents with some quadrivalents [144], some homology
Helianthus salicifolius	Successful, moderate fertility [145]	Poor pairing, many univalent, F_1 [145], some homology
H. tuberosus	Successful, good seed set [85]	Mixed multivalent and bivalent pairing [74, 81, 83], good homology [67–69, 74]

Heiser [86] identified imperfect chromosome pairing in hybrids (Table 2) and later this species pair became of central importance in demonstrating recombinational speciation. Recombinational speciation occurs when sympatric species hybridize resulting in new species which colonize novel environments via transgressive segregation. *Helianthus annuus* and *H. petiolaris* have given rise to three different homoploid hybrid species, *H. paradoxus* [87, 88], *H. deserticola* [89,90] and *H. anomalus* [89, 90]. *Helianthus anomalus*, *H. deserticola* and *H. paradoxus* are mosaics of parental genomes [91, 92], with approximately one-third of chromosomal differences arising from existing chromosomal rearrangements between parental species, and the remainder arising within the new species (Table 2). The mosaic nature of hybrid species chromosomes indicates broad regulatory compatibility [93]. Different portions and proportions of parental genomes are retained in these hybrids indicating that multiple diploid species can evolve from a set of parents by selecting the genomic components best suited for the environment [93].

Comparative mapping between *H. annuus* and *H. petiolaris* identified regions of collinearity and regions of structural divergence [65, 94]. In an experimental study it was found that 66% of the collinear portion of the genome introgressed in synthetic hybrids compared to only 19% of structurally divergent regions [95]. Studies of introgression between natural populations of these two species have corroborated these findings, with reduced interspecific gene flow observed near the breakpoints of the rearrangements [12, 13]. Likewise quantitative trait loci (QTL) for hybrid sterility largely map to rearrangement breakpoints, although Bateson–Dobzhansky–Muller incompatibilities [96] have also been identified [21, 97]. The correlation between chromosomal rearrangements and reduced gene flow supports the hypothesis that rearrangements contribute to adaptive divergence and speciation in face of gene flow [98]. In addition, this implies that rearrangements help maintain species integrity following secondary contact between formerly geographically isolated species (Figure 1; [13, 95]). Recreation of hybridization events showed a quick convergence (few generations) within the new hybrids in chromosomal structure on that of the homoploid hybrids, indicating that evolution may be repeatable [99, 100].

The study of chromosomal biology and evolution in *Helianthus* is sure to continue into the future. With cheaper denser genotyping, either with chip-based [101] or sequence-based approaches [13] high-density genetic maps will be feasible for many wild taxa and mapping populations. These maps may be used to more thoroughly examine the role of the rearrangements in local adaptation, an area of intense interest. Sunflower homoploid hybrids and species created by polyploid events in *Helianthus* (e.g.

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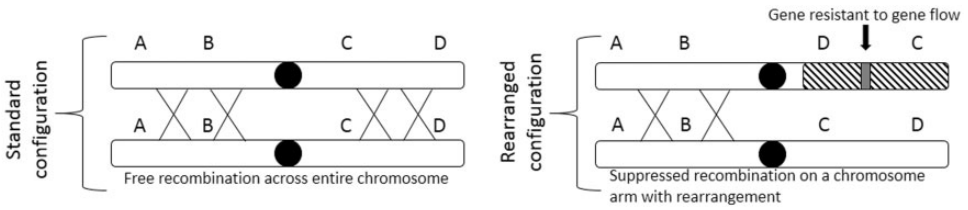

Figure 1: Hypothetical chromosomal rearrangement that reduces recombination protecting genomic regions from gene flow.

H. tuberosus), allow for testing hypothesis about chromosome structure changes over short time scales rather than over evolutionary time as well as providing an opportunity to examine the impacts of homologous versus homeologous pairing.

NUCLEOTIDE SEQUENCE DIVERSITY AND VARIATION IN *HELIANTHUS*

One of the goals of modern biology is to predict phenotypic variation from genotypic variation. This means that characterizing the functional role of genes and their various alleles is a fundamental component of genetic research. Functional genetics in model species such as *A. thaliana* is steadily unraveling the molecular workings of plant genes. Methods for identifying candidate genes can be empowered or directed by an understanding of the history of the gene or genes in question. Therefore, an essential aspect of this process is investigating the evolution of candidate genes and their movement between taxa. *Helianthus* with its myriad locally adapted taxa and rampant gene flow has proven to be a useful model in this regard [102, 103]. Combining evolutionary information with functional and quantitative genetics may help identify causative nucleotide changes that control everything from morphological characteristics to response to biotic and abiotic stress.

To study the evolution of a group, the taxonomic relationships between component species must be clarified. To this end, phylogenetic analyses in *Helianthus* have been performed using a variety of methods [9, 104, 105], although a consensus is yet to emerge on the exact relationship of all taxa [105]. Some of this confusion is likely due to the rampant gene flow in the group [102, 106], as well as its recent origin. The main clades of *Helianthus* are well established, although some species and subspecies may be unduly divided or pooled [105]. Molecular approaches, including reduced representation sequencing and whole genome shotgun sequencing, even at low depth coverage [79], may help resolve these issues. These molecular approaches promise to yield more information than just the relationship of the members of *Helianthus*.

The diversity within each of the species is useful to understand the species histories. Population genetic statistics can yield estimations of diversity and population sizes and the likely effectiveness of selection in those populations. In general, many of the *Helianthus* species investigated to date have high diversity compared with other plant species in terms of both θ_w (population mutation rate based on the number of segregating sites; [107]) and average synonymous π (pairwise nucleotide diversity). Strasburg *et al.* [17] calculated the average synonymous θ_w and π for *H. petiolaris* ($\theta_w = 0.037$, $\pi = 0.034$), *H. paradoxus* ($\theta_w = 0.004$, $\pi = 0.005$), *Helianthus exilis* ($\theta_w = 0.023$, $\pi = 0.022$), *Helianthus argophyllus* ($\theta_w = 0.015$, $\pi = 0.014$) and wild *H. annuus* ($\theta_w = 0.024$, $\pi = 0.027$), whereas in other plant groups θ_w and π can be much lower (wild barley $\theta_w = 0.0081$ [108], rice $\theta_w = 0.0021$ [109]) (Figure 2; [17, 110]). Clearly, most *Helianthus* species have a high level of diversity and large effective population sizes [17] compared with other plant species (Figure 2). As expected the highest levels of diversity (and effective population size) are in the widespread species, *H. annuus* and *H. petiolaris*, with somewhat lower diversity found in more geographically restricted species such as *H. argophyllus*. The main outlier is *H. paradoxus*, which is thought to have undergone a severe genetic bottleneck during its hybrid origin from *H. annuus* and *H. petiolaris*. Although there have not been similar investigations into nucleotide diversity in the perennial *Helianthus* species, it has been estimated in *H. tuberosus*, which was found to have $\theta_w = 0.023$ and $\pi = 0.027$ [17]. Domestic *H. annuus* retains only 40–50% of the nucleotide diversity found in wild populations with domesticated *H. annuus* $\theta_w = 0.0094$ and wild lines $\theta_w = 0.0128$ [111, 112]. This large amount of nucleotide diversity both within *H. annuus* and other

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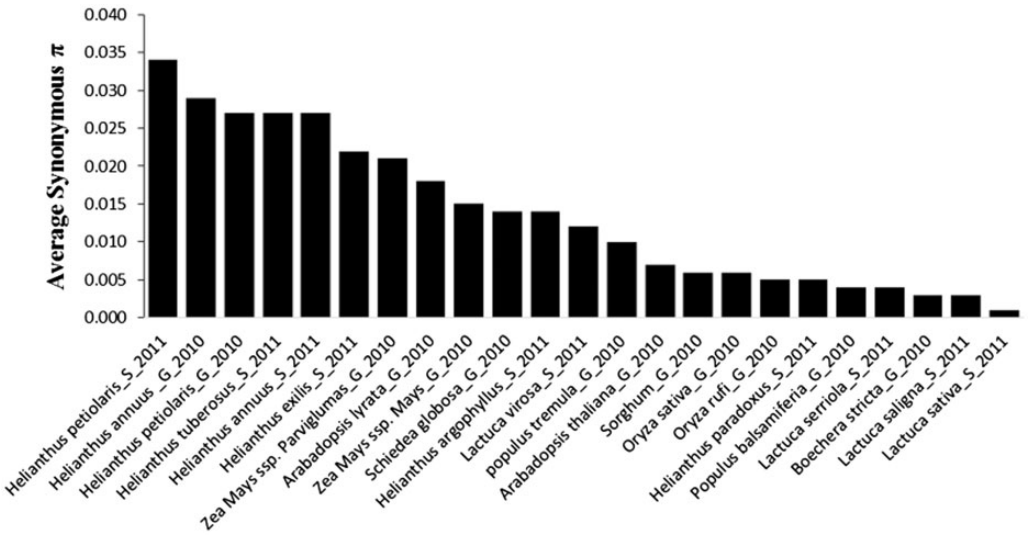

Figure 2: Pairwise nucleotide diversity (average synonymous π) for 21 species with data coming from Gossmann *et al.* (2010) when the suffix G.2010 is used and Strasburg *et al.* (2011) when S.2011 is used.

Helianthus species provides tremendous genetic resources for *H. annuus* breeding [10, 113].

Nucleotide sequence and molecular marker diversity varies across the genomes of these taxa and can be used for more than just understanding the relationship of species and the diversity within them, it can detect evidence for selection and quantify gene flow. Measurements of differentiation (e.g. Fst) can be used to identify loci that may have been targets of selection in during local adaptation or domestication. Such genome scans with Fst have identified differences in *H. petiolaris* between sand dune ecotypes and non-dune ecotypes, and the genes underlying these outliers may be involved in the local adaptation which is likely taking place [103]. Genes that may be involved in adaptation to drought in populations of *H. annuus* have also been identified in this way [114]. As domestication can be viewed as a special case of adaptation these methods can be applied to identify candidate genes for domestication traits as well [26, 27, 115, 116].

Genome scans can act as an additional line of evidence in understanding the functional role of genes and alleles. Blackman *et al.* [116] used multiple lines of evidence, including metrics of selection, to identify candidate domestication genes. The importance of one, Flowering Locus T, has been supported with gene expression experiments, analysis of near isogenic lines and with transgenic work [27]. Genome scans may be used to help highlight specific genes within domestication QTL. *Helianthus annuus* provides a number of populations generated from wild, landrace and elite material [25, 111, 112] that can help identify causative mutations. Association mapping has also identified regions underlying domestication QTL [117].

Widespread interspecific gene flow in *Helianthus* has allowed adaptive alleles to move between species and has led to unique changes in habitat, growth form and genetic composition. For example, the subspecies *H. annuus* ssp. *texanus* has introgressed alleles from the local *Helianthus debilis* ssp. *cucumerifolius* [21, 118], with the introgressions hypothesized to have led to the divergence of *H. annuus* ssp. *texanus* from other *H. annuus* and led to its adaptation to the dry Texan environment [22, 118, 119]. The hybrid species, discussed in previous sections, are transgressive segregants created by combining the most useful alleles from their two parental species. The ability of *H. annuus* to readily hybridize with many other *Helianthus* species (Tables 2 and 3) has also been exploited extensively with the intentional introduction of genetic material from wild relatives (both annual and perennial) into the cultivated gene pool [120]. Wild relatives are being used in modern large scale breeding programs and serve as sources of disease resistance genes [121], cytoplasmic male sterility [122] and 'intrinsic yield' traits. Many of these traits have been the targets of mapping efforts [123–126]. The structural divergence between *H. annuus* and other species has often led to large chromosomal segments being introgressed with traits of interest

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(disease resistance and herbivory resistance) that are difficult to remove due to limited recombination between different chromosomal types despite breeding efforts [127]. There has been less intentional interspecific introgression into domesticated *H. tuberosus* than domesticated *H. annuus* [85].

Gene flow between wild and cultivated sunflowers can also occur in the opposite direction. In much of its growing area in North America crop sunflower fields are in close proximity to wild sunflower populations with gene flow being common among plants within 1 km of each other [106, 128] and wild populations maintaining cultivated alleles for many generations (up to decades; [129–131]). This can lead to mixed growth forms among wild and weedy (i.e. populations adapted to highly disturbed conditions, many of which have a crop × wild ancestry) annual sunflower that grow in close proximity to each other and may be problematic with respect to wild species conservation [132].

FUTURE DIRECTIONS

Here, we have reviewed research in *Helianthus* pertaining to variation in genome size, structure and sequence diversity. These types of variation overlap in *Helianthus* which will allow deep investigations into how these evolutionary phenomena interact and how they may shape diversity in *Helianthus* and elsewhere. Recent advances in sequencing have led to the development of genomic resources for an increasingly large number of sunflower species and will accelerate this process of discovery [133]. This in combination with the forthcoming assembly of the complete *H. annuus* genome makes this an exciting time to study genomic variation in *Helianthus* [134]. High-density sequence-based maps in *Helianthus* [13] will facilitate the study of chromosomal evolution across the genus. Combining these genetic maps with physical maps [135], will reveal the structure of repetitive elements in the genome as well as yield insights into the nature of recombination. We believe that the many unique populations that have been developed within (domestic, landraces and wild) and between many different (homoploid hybrids, annual and perennial) *Helianthus* species make this dynamic system well-suited for gaining further insight into genomic evolution and how genetic variation translates into phenotypes.

Key points

- *Helianthus* is a historically important genus for studying biological phenomena such as hybridization, chromosomal rearrangements and genome size evolution.
- Genome size varies in *Helianthus* by ~4.5-fold and has been shaped by paleopolyploidy, neopolyploidy and TE proliferation.
- *Helianthus* has the highest rate of chromosome structural evolution studied in plants.
- Widespread interspecific gene flow in *Helianthus* has facilitated local adaptation, lead to the formation of hybrid species and has been exploited extensively in sunflower improvement.

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