Population Genomics of Speciation and Adaptation in Sunflowers

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Abstract Sunflowers are well-established model organisms in evolutionary biology; studies of them have made important contributions to our understanding of hybridization as an evolutionarily constructive process. Here, after introducing earlier foundational work, we review recent population genomics studies in this group. We discuss the origin of sunflowers, and how genomic data has helped disentangle species relationships. We then review work on past and ongoing speciation, as well as adaptation in natural populations or during domestication and the evolution of invasiveness. Results from these studies have shed light on the nature of sunflower species, revealing that sunflower genomes are mosaics that retain evidence of past and ongoing hybridization with congeners. This occurs even in species for which multiple compounded isolating mechanisms prevent interbreeding. Studies of cultivated sunflowers have similarly clarified that a substantial fraction of the domesticated gene pool is derived from introgressions from as many as half a dozen different species, while also identifying cases of crop-wild gene flow. In invasive species, hybridization may occasionally spur highly competitive genotypes, including in perennial species where the beneficial effects of hybrid vigor can be maintained. Population genomics studies have shown that large chromosomal blocks of high linkage disequilibrium, many of which are chromosomal inversions, facilitate local adaptation of sunflower populations given widespread gene flow. These haploblocks were found to control multiple traits and are often themselves the result of hybridization and introgression. We conclude by considering future research challenges for the sunflower community. These include a thorough characterization of sunflower structural variation and the generation of new reference genomes, revisiting earlier studies based on non-genomic data, and the optimization of

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transformation methods that can be used to validate the function of ecologically or economically important genes.

1 Introduction

In evolutionary biology, a few species and clades have become sufficiently well studied so as to contribute to the advancement of multiple lines of research on the genetics of speciation and adaptation. Some of these model systems such as the fruit fly *Drosophila melanogaster* or the house mouse *Mus musculus* have been the focus of genetic research since the turn of the twentieth century, shortly after the rediscovery of Mendel's laws of inheritance (Markow 2015; Phifer-Rixey and Nachman 2015). The goal at this stage was to study classical genetics, often in connection with development and physiology (Barr 2003). Since the 1950s, many more model systems have been developed, mainly against a backdrop of molecular and sequencing technology improvements. These advancements have allowed evolutionists to choose study organisms not because they had short generation times and were amendable to laboratory experiments, but because their natural history and ecology permitted new questions to be asked about speciation and adaptation in the wild.

The genus *Helianthus* is a notable example of one such model system. Commonly known as sunflowers, these charismatic plants started to draw the attention of evolutionists beginning in the 1940s and 1950s. This was largely due to experiments performed by botanist and evolutionary biologist Charles B. Heiser Jr. Relying on data from morphology, cytology, and crossing experiments, Heiser meticulously documented the occurrence of hybridization between sunflower taxa (e.g., Heiser 1947, 1951). In a few years, he had amassed evidence for a sufficient number of species to note, in his 1969 monograph of sunflowers, that "the discovery of another interspecific hybrid combination in the genus would scarcely be noteworthy" (Heiser et al. 1969, p. 23). At the time, by combining such experimental evidence with ecological and geographical information, Heiser had contributed some of the most compelling examples of natural introgressive hybridization (Heiser 1949). As such, from the early years of the modern synthesis, sunflowers were part of the debate on whether hybridization is a constructive or destructive force (Dobzhansky 1937; Anderson and Stebbins 1954; Stebbins 1959).

Work performed since the 1990s has expanded Heiser's work significantly. This was achieved using a combination of approaches, including field experiments, genetic and association mapping, and population genetics and genomics in natural hybrid zones (e.g., Rieseberg et al. 1999a, 2003). As a result, sunflower research has considerably added to our understanding of hybrid and non-hybrid speciation, as well as evolution during domestication, evolution of invasiveness, and local

adaptation. Here, we review these contributions, focusing on studies that use population genomics.

2 *Helianthus* Diversity and Origin

Helianthus comprises 49 named species, all of which are of North American origin (Heiser et al. 1969; Schilling and Heiser 1981). The diversity captured by the genus is remarkable and can be partitioned along multiple axes. One of these is ecology. Members of the group can be found in environments as disparate as deserts, salt marshes, prairies, rock outcrops, woodland understories, and wetlands (Fig. 1; Heiser et al. 1969; Kantar et al. 2015). For a number of species, local adaptation to these environments has been confirmed by field experiments and population genomic analyses (see Sect. 4.3 below). A second axis of diversity involves reproductive strategy. Specifically, 12 members of the genus are annuals, while the remainder are perennials (Kantar et al. 2014). The annual/perennial distinction can have important bearing on the tempo of speciation (e.g., the speed of hybrid sterility evolution; Owens and Rieseberg 2014) as well as adaptation to local environments or domestication (e.g., Gaut et al. 2015). We will discuss some of these aspects below. Finally, a third major axis of diversity is ploidy level. While most species are diploid, 13 sunflower taxa are polyploids, including tetraploids and hexaploids (Kantar et al. 2014).

Efforts to understand how this diversity originated hinge upon accurate phylogenetic reconstruction. For *Helianthus*, recovering phylogenetic relationships has been notoriously difficult. In retrospect, this is not surprising. Many of the characteristics that make sunflowers an exceptional model system for evolutionary study are also known to negatively impact phylogenetic inference. Such characteristics include rapid speciation and recent origin, very large effective population sizes (Strasburg et al. 2011), a high propensity to hybridize (Sambatti et al. 2012), recent proliferation of repetitive elements (Staton et al. 2012), and multiple rounds of past polyploidization (Barker et al. 2008; Badouin et al. 2017). Under these conditions, it is easy to see why early attempts at phylogenetic reconstruction, drawing from morphology, crossing data, phytochemistry, isozymes, or molecular marker genetic data, were only partially informative.

Schilling and Heiser (1981) used morphology, reproductive strategy, and crossability to infer the first phylogeny for the genus. Subsequent studies provided additional key information, including the identification of *Phoebanthus*, a genus of two perennial species that are narrowly distributed in Florida, as the sister group to *Helianthus* (Schilling 2001). Also notable is the clarification that widespread diploid annual species, including the important oilseed crop *H. annuus*, form a monophyletic clade (Schilling 2001; Timme et al. 2007). Finally, early phylogenetic studies based on genetic data from ribosomal genes were able to identify three putative instances of homoploid hybrid speciation (Rieseberg 1991), which will be discussed below (see Sect. 3.1).
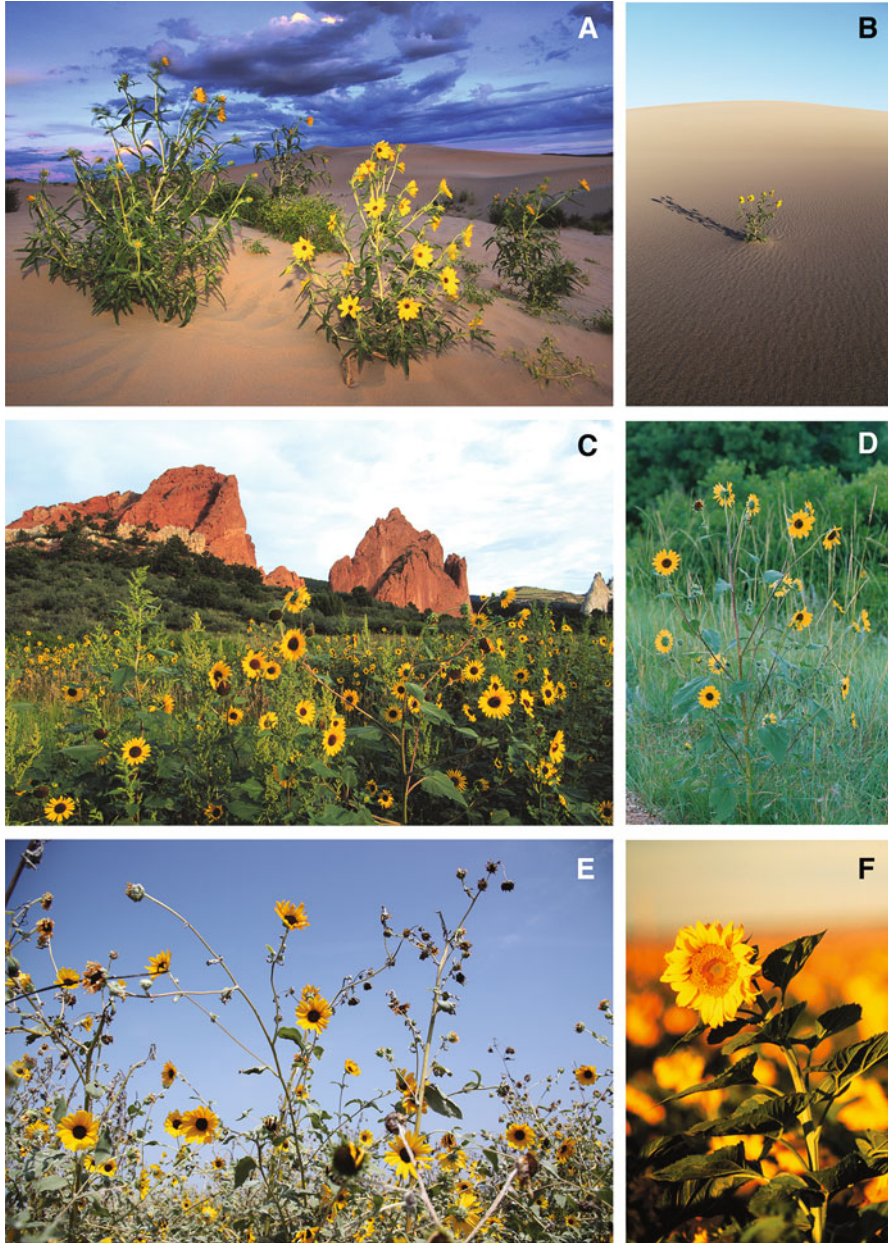
Fig. 1 Representative *Helianthus* diversity. (**a**) *H. anomalus*, (**b**) dune ecotype of *H. petiolaris* ssp. *fallax*. (**c, d**) wild *H. annuus*. (**e**) *H. argophyllus* coastal ecotype. (**f**) cultivar of *H. annuus*. Images courtesy of Nolan C. Kane, Mariana A. Pascual-Robles, and Jason Rick

The use of next-generation sequencing has improved, over the past five years, our understanding of infrageneric species relationships. Stephens et al. (2015) used capture probes and Illumina sequencing to simultaneously isolate 170 phylogenetically informative loci across 37 diploid *Helianthus*. Results showed that most taxa are part of three large clades, one of which contains annuals, and two of which contain perennials. Also, this study provided evidence that the ancestral sunflower was likely a perennial species, with annual life history evolving subsequently three times (Stephens et al. 2015). This is in agreement with observations from other plant taxa, which indicates annual species tend to evolve from perennial ancestors as an adaptation to harsh environmental conditions, such as aridity (Friedman and Rubin 2015). The phylogeny from Stephens et al. (2015) was further used to provide evidence that temperature seasonality constrains genome size expansion in *Helianthus* (Qiu et al. 2019).

Low-coverage whole genome sequencing (i.e., genome skimming) and reduced-representation sequencing have been used to disentangle any remaining ambiguous relationships and revisit previously suspected hybrid species (e.g., Baute et al. 2016; Owens et al. 2016; Zhang et al. 2019). Some of these studies focused on polyploid hybrids. Because these taxa are also perennials, and therefore, fewer generations removed from their progenitors, they have proven particularly difficult to resolve using traditional phylogenetic markers. Information from complete cytoplasmic genomes, as well as much of the rDNA nuclear segment was informative in two cases so far, the hexaploid tuber crop *H. tuberosus* (Bock et al. 2014a), and the critically endangered tetraploid *H. schweinitzii* (Anderson et al. 2019). Lastly, notable among studies using genome skimming is Lee-Yaw et al. (2019). The authors integrated phylogenetic methods and selection analyses to understand drivers of discordance between nuclear and cytoplasmic markers. Cytonuclear discordance has been a common finding in the plant literature and is usually ascribed to incomplete lineage sorting or hybridization. Aside from confirming the occurrence of hybridization and subsequent cytoplasm introgression, results pointed to the contribution of natural selection in driving patterns of plastid DNA variation. This possibility has previously been supported using field experiments in sunflowers (Sambatti et al. 2008) and is being considered increasingly often in other taxa as well (Bock et al. 2014b).

Given what we know so far on sunflower phylogeny, what can we say about the geography, timing, and tempo of *Helianthus* diversification? Considering that *Phoebanthus*, the sister group to *Helianthus*, as well as basal sunflowers such as *H. porteri* have a distribution that is restricted to the South-Eastern US (Schilling 2001; Stephens et al. 2015), it seems likely that the ancestor of *Helianthus* was a perennial species of Central American origin. Further supporting this possibility is the fact that sister-taxa to the clade formed by *Helianthus* and *Phoebanthus* occur in Mexico and South America (Schilling 2001). Molecular clock analyses, while inherently uncertain (Donoghue and Benton 2007), timed the split between the first sunflower species and this ancestor at ~3.6 million years ago (Mason 2018). This places the start of sunflower diversification within the Pliocene, a period characterized by cooling, drying, and considerable vegetation restructuring in

North America, including reductions of closed forest habitat (Mason 2018). This makes sense considering that sunflowers typically occur in open-vegetation environments (Heiser et al. 1969). Subsequent to range expansion from Central America, diversification within North America led to the formation of three major clades. One of these contains species that are more common in dry soils of the South-Western US. Adaptations to these challenging environments likely included an annual reproductive strategy, but also traits that enhance water use efficiency and promote fast growth (Mason and Donovan 2015). Subsequent radiation from the South-Western US occurred later for widespread members of this annual clade, such as *H. petiolaris* (Heiser 1961). The other two ancestral *Helianthus* clades consist of perennials occurring, respectively, in riparian habitats across the Central-Eastern US and water-rich environments of the South-Eastern US. Speciation within all three clades is recent (often <1–2 Myr; Stephens et al. 2015) and occurred in the context of substantial interspecific gene flow (Sambatti et al. 2012; Lee-Yaw et al. 2019) and genome rearrangements (Burke et al. 2004; Barb et al. 2014). We discuss studies investigating these speciation events below.

3 Population Genomics of Sunflower Speciation

Sunflowers are well-known for their contribution to speciation theory. Building on earlier research performed by Heiser (discussed above), species formation has been studied extensively in this group, facilitated in part by its recent origin. This provides the opportunity of studying speciation from taxa that are fully isolated to those that are just transitioning to the status of incipient species (e.g., Andrew and Rieseberg 2013; Ostevik et al. 2016). Here, we follow this speciation continuum. After introducing key previous results, we review population genomics studies of past and ongoing sunflower speciation.

3.1 Sunflowers as Models of Recombinational Speciation

Students of evolutionary biology not familiar with sunflowers are most likely to be introduced to this system in the context of homoploid hybrid or "recombinational" speciation. This is because three sunflower species, *H. anomalus*, *H. deserticola*, and *H. paradoxus* are thought to offer some of the strongest empirical support for this mode of speciation (Yakimowski and Rieseberg 2014). Phylogenetic evidence suggested that these taxa were of hybrid origin and that they likely share the same two progenitors, the widespread annuals *H. annuus* and *H. petiolaris* (Rieseberg 1991). Molecular data further indicate these speciation events occurred rapidly, likely within hundreds of generations (Ungerer et al. 1998; Buerkle and Rieseberg 2008). Despite their presumed hybrid origin, the hybrid neospecies are strongly isolated from their progenitors, as both intrinsic (in the form of almost complete F_1

hybrid sterility; Heiser 1958; Rieseberg 2000) and extrinsic (in the form of ecological differentiation; Rieseberg 1991; Lexer et al. 2003) barriers to gene flow are known. As predicted by the recombinational speciation model (Stebbins 1957; Grant 1958), the hybrids differ from parental taxa in multiple chromosomal rearrangements (Rieseberg et al. 1995a; Lai et al. 2005) that are associated with F_1 sterility (Lai et al. 2005).

Ongoing work is using high-resolution linkage maps based on SNP data to study chromosomal evolution in the genus. Results have so far highlighted that karyotype changes in diploid sunflowers are dominated by inversions and interchromosomal translocations (Ostevik et al. 2020). Among these, inversions predominate (Ostevik et al. 2020). Also, when interpreting results across plant and animal groups, sunflowers appear to have exceptionally high rates of chromosomal evolution (Barb et al. 2014; Ostevik et al. 2020). Non-random patterns have further been identified across the genome, with some chromosomes involved in more translocations than others. Leading explanations for this involve ancestral homology retained after whole genome duplication, as well as repeat element content (Ostevik et al. 2020).

The relevance of chromosomal rearrangements is easy to see when one considers intrinsic isolation during hybrid speciation. Previous studies have shown that hybrid sterility is a result of either direct rearrangement effects or the effects of genic incompatibilities that cluster within rearrangements (Lai et al. 2005). However, this only addresses part of the barriers necessary for hybrid speciation. An important remaining question is how might ecological isolation be achieved at the same time? Theoretical studies indicate that, without niche divergence, hybrids can be outcompeted by numerically superior parental genotypes (Buerkle et al. 2000). As such, hybrid speciation requires the concomitant development of strong ecological divergence (Buerkle et al. 2000).

Luckily, in hybrid sunflower species, ecological isolation can be studied directly. This is because the putative parent taxa and hybrid derivatives are extant. As such, field experiments can be conducted to compare all taxa and/or artificial hybrids, therefore controlling for post-speciation divergence. This was the approach taken by Lexer et al. (2003) to study mechanisms of ecological isolation in *H. paradoxus*. The authors used interspecific BC_2 hybrids between *H. annuus* and *H. petiolaris* and performed QTL mapping for survivorship and elemental uptake traits in salt marshes, the environment typical of *H. paradoxus*. Results provided evidence for strong selection at QTLs with effects in opposing directions. This was early support for the possibility that rapid ecological divergence during hybrid speciation is achieved as a result of selection for recombinants with extreme (transgressive) phenotypes. The mechanistic basis for transgression in this case is complementary gene action (Rieseberg et al. 1999b). Under this model, extreme phenotypes are a product of the "stacking" of alleles from several QTLs that are fixed between parents, and that control the same trait (reviewed in Rieseberg et al. 1999b).

The complementation model provides a convincing explanation for the emergence of transgression and subsequent ecological isolation, but it is not the only mechanism at play. Specifically, gene expression changes likely contribute as well. Lai et al. (2006) used microarray analyses of *H. deserticola* and the two parental

taxa. Results showed evidence of transgressive expression in the putative hybrid species as compared to its parents, particularly involving transport-related genes (Lai et al. 2006). These changes are not observed in F_1 *H. annuus/H. petiolaris* hybrids, as estimated using RNA-seq (Rowe and Rieseberg 2013). Thus, current evidence points to gene expression divergence occurring in later generations. Explanations that have been put forth so far include mechanisms independent of the hybridization event such as post-speciation selection, as well as those that are related to the genome merger (Lai et al. 2006). Among this latter category, possibilities include genome rearrangements and transposable element activity (Lai et al. 2006), both of which are elevated in the hybrid sunflower taxa (Renaut et al. 2014a) and have been linked to gene expression divergence (Lai et al. 2006; Dion-Cote et al. 2014; Harewood and Fraser 2014).

3.2 Non-hybrid Speciation

While a substantial share of speciation research in sunflowers has focused on understanding mechanisms behind homoploid hybrid species formation, studies of non-hybrid taxa have been extremely insightful as well. These studies sought to understand patterns of introgression along the genome and to document the nature of sunflower species boundaries. These investigations have been facilitated by several characteristics of the sunflower system. For one, detailed information is available on reproductive isolating barriers for some members of the group (e.g., flowering time, pollen competition, hybrid sterility; Rieseberg et al. 1995b; Rieseberg 2000; Sambatti et al. 2012). Using knowledge of barrier strength, estimates can be obtained on the probability of hybridization, which can then be related to genome scans for differentiation between species (e.g., Sambatti et al. 2012). Also, exceptional resources for studying the population genomics of speciation are available in *Helianthus*. These include large EST databases, SNP and expression arrays, high-density genetic maps, and reference genomes (Heesacker et al. 2008; Kane et al. 2013; Badouin et al. 2017; Hübner et al. 2019). Finally, because of the widespread distribution of sunflowers across continental US, contrasts can be made among species pairs that differ in the potential for gene flow. This permits the study of the geographical context of speciation (e.g., Renaut et al. 2013).

An important finding emerging from these studies is that sunflower genomes often are mosaics, with a considerable genomic fraction having an interspecific origin (Kane et al. 2009; Scascitelli et al. 2010; Sambatti et al. 2012; Zhang et al. 2019). This occurs even in species for which interbreeding is rare and prevented by the compounded action of multiple isolating mechanisms. For example, studies of *H. annuus* and *H. petiolaris* have estimated a cumulative barrier close to 1 in both directions (Sambatti et al. 2012), placing these species at the upper end of barrier strength among flowering plants (Lowry et al. 2008). Using widely accepted criteria, these would be considered good species. The intuitive prediction in this case is that extremely rare hybridization translates into strong genetic differentiation between

taxa. To the contrary, population genomics has provided evidence of non-independence for the two gene pools. For example, Kane et al. (2009) used 26 microsatellites and 1420 EST-derived orthologs and identified long-term gene flow between nearby populations of *H. annuus* and *H. petiolaris*. This added to previous, more localized examples of genetic exchange between the two species (e.g., Buerkle and Rieseberg 2001; Yatabe et al. 2007).

To reconcile these apparently conflicting results, Sambatti et al. (2012) used coalescent modelling. Results pointed to the large effective population size of these two species as the primary reason for gene flow evidence, even when successful hybridization is extremely rare. Under this scenario, the traces of past introgression are preserved because of very limited genetic drift. Also notable is that the genomic mosaic observed in this case is not merely a result of the recent origin of *H. annuus* and *H. petiolaris*, as revealed by comparisons of even younger speciation events (Sambatti et al. 2012). Beyond documenting the lasting contribution of gene flow to genomic variation in sunflowers, these studies highlighted the important role of ecology in maintaining sunflower species cohesion. In spite of widespread genetic exchange, most species are known to maintain distinctive morphologies and habitat requirements (Kane et al. 2009). Conversely, in the absence of habitat differentiation and under human disturbance or biological invasion, hybridization can be rampant, and even result in the genetic assimilation of species (Kane et al. 2009; Todesco et al. 2016; but see Owens et al. 2016).

If, as discussed above, gene flow has been occurring between *H. annuus* and *H. petiolaris* throughout their evolutionary history, how has this shaped the genomic landscape of divergence? In sympatry and parapatry, the genomic landscape is predicted to be highly heterogenous (Nosil et al. 2009). Peaks of differentiation associated with loci under divergent natural selection are expected to be interspersed by valleys of differentiation corresponding to neutral regions that are being homogenized by gene flow. This contrasts with the expectation in allopatry, where the absence of gene flow should allow neutral and adaptive divergence to accumulate anywhere in the genome (Nosil et al. 2009). Identifying determinants of the genomic landscape of differentiation can contribute to our understanding of speciation. For example, some models of speciation with gene flow predict that initially narrow peaks of differentiation will progressively expand and facilitate divergence of other linked genes (Via 2012). Referred to as "divergence hitchhiking" this process is thought to ultimately lead to the formation of the so-called speciation islands during speciation with gene flow (Via 2012).

To test these predictions in sunflowers, Renaut et al. (2013) used transcriptome data and pairs of taxa that differ in the geography of speciation. These consisted of *H. annuus* and *H. petiolaris* as representatives of sympatric divergence with gene flow. By considering two other taxa, *H. debilis* and *H. argophyllus*, additional comparisons could be made, representing parapatry with and without gene flow, and allopatry (Renaut et al. 2013). Contrary to expectations, the genomic landscape of divergence was not affected by interspecific gene flow. Regardless of sympatric, parapatric, or allopatric categorization, species were found to diverge at numerous independent genomic regions (Renaut et al. 2013). These results notwithstanding,
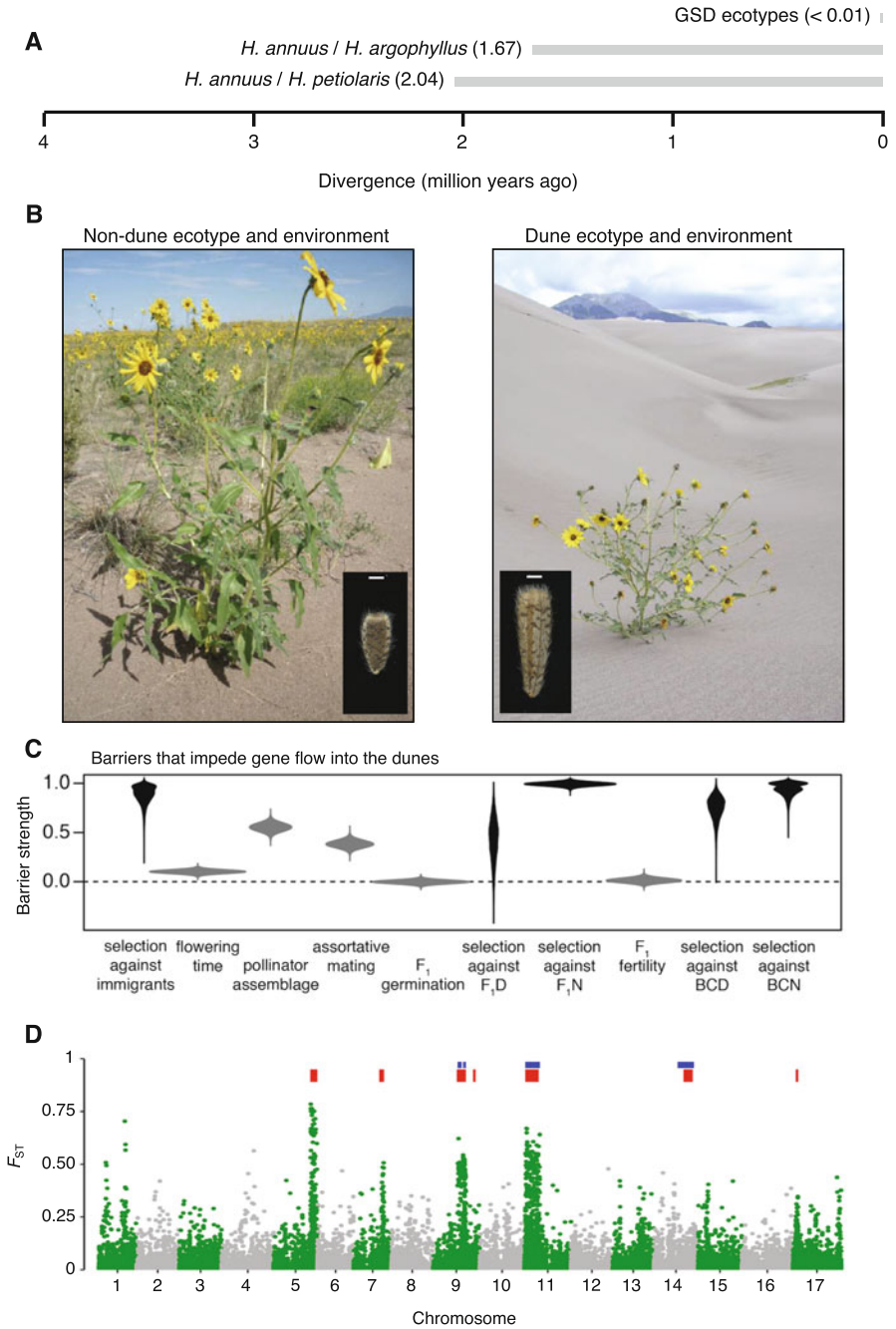
Fig. 2 Population genomics of incipient speciation in Great Sand Dunes (GSD) *H. petiolaris*. (**a**) Divergence time among GSD *H. petiolaris* dune and non-dune ecotypes, as compared to divergence times among two sunflower species pairs for which reproductive isolation has previously been

there was a strong association between islands of divergence and recombination rate. Thus, the study by Renaut et al. (2013) highlighted that, in sunflower species, the functional architecture of the genome, as opposed to gene flow, is a strong predictor of genome divergence (Renaut et al. 2013).

3.3 Sunflower Speciation in Action

Studies of past speciation such as those highlighted above help us understand the process retrospectively: we look back in time and make inferences based on the best available evidence (Via 2009). Ideally, however, we would also observe speciation in action. This is because studies of incipient species can provide valuable information on how gene flow is initially suppressed. Combining the two approaches is possible in sunflowers, since genotypes can be obtained that span the continuum from well-isolated taxa (above) to young ecotypes just transitioning to species status. A well-established system for the study of incipient speciation in *Helianthus* are the dune and non-dune ecotypes of *H. petiolaris* at Great Sand Dunes (GSD) National Park in Colorado (Fig. 2). This is due to a succession of studies that provided detailed information on the evolutionary history of the two ecotypes, reproductive barriers and genomic divergence.

Population genomic data indicate that dune-adapted *H. petiolaris* diverged from an ancestral non-dune population at GSD less than 10,000 years ago (Fig. 2a; Andrew et al. 2013). While gene flow is still occurring (Andrew et al. 2012, 2013), dune and non-dune ecotypes are locally adapted and maintain large divergence at key traits including seed size (Fig. 2b; Ostevik et al. 2016). In line with these observations, analyses of reproductive isolation identified several reproductive barriers that are already active in this system (Fig. 2c; Ostevik et al. 2016). As expected under local adaptation and recent divergence, many barriers were extrinsic. These included both prezygotic (selection against immigrants; divergence in pollinator assemblages) and postzygotic (selection against hybrids; Ostevik et al. 2016) barriers. One intrinsic barrier was identified as well (postpollination assortative mating, Ostevik et al. 2016). Comparisons of barrier strength revealed that, in the

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Fig. 2 (continued) investigated (e.g., using F_1 pollen sterility; see Owens and Rieseberg 2014). Divergence estimates are obtained from Andrew et al. (2013) for GSD and from Mason (2018) for the other two comparisons. (**b**) Representative plants of the non-dune and dune *H. petiolaris* ecotypes, in their respective habitats at GSD, Colorado. Scale bar for seed size indicates 1 mm. Photo credits Kate L. Ostevik (left GSD image), Rose L. Andrew (right GSD image), and Marco Todesco (seed images). (**c**) Barrier strength estimates considering gene flow from non-dune to dune plants, modified from Ostevik et al. (2016). Barriers for which seed size is likely to contribute are highlighted in black. (**d**) F_{ST} comparison between dune and non-dune ecotypes (from Huang et al. 2020). Blue bars are used to indicate the genomic location of seed-size QTLs (as identified in Todesco et al. 2020). Red bars indicate putative inversions

case of *H. petiolaris* at GSD, postzygotic barriers are more effective than prezygotic barriers. As well, extrinsic barriers were shown to be more effective than intrinsic barriers.

How do these barriers impact genome divergence, and how did they evolve? Between-ecotype genome scans identified three major "islands of divergence" against a backdrop of otherwise reduced genomic differentiation (Andrew and Rieseberg 2013). This result further confirmed that the two ecotypes are at the very early stages of speciation, when only a small fraction of the genome is contributing to isolation (Andrew and Rieseberg 2013). While few, the islands of divergence were wide, likely encompassing a large number of genes (Andrew and Rieseberg 2013). Thus, as compared to the substantially older *H. annuus/ H. petiolaris* species pair discussed above, the GSD ecotypes better correspond to expectations under "divergence hitchhiking."

An alternative that can explain the large islands of divergence between these ecotypes is the occurrence of chromosomal inversions. Recent studies have increasingly emphasized that structural polymorphism is an important component of adaptive genetic variation (Mérot et al. 2020; Todesco et al. 2020). A follow-up study of the GSD system provided strong evidence for precisely this scenario. Using RADseq and analytical methods that facilitate inversion detection, Huang et al. (2020) demonstrated that islands of divergence between *H. petiolaris* ecotypes do indeed correspond to chromosomal inversions (Fig. 2d). Moreover, these inversions were shown to co-localize with seed-size QTLs (Fig. 2d), as well as environmental variables that differ between dune and non-dune sites (Huang et al. 2020). Thus, in GSD *H. petiolaris*, inversions contribute to divergent adaptation by preventing recombination between co-adapted alleles. Ongoing work is aiming to clarify whether postpollination assortative mating also maps to these inversions (Huang et al. 2020). This is particularly relevant because models of speciation with gene flow emphasize the importance of linkage between loci involved in local adaptation and those involved in assortative mating (Ortiz-Barrientos et al. 2016; Huang and Rieseberg 2020).

4   Population Genomics of Adaptation

The spectacular diversity of *Helianthus* has motivated a number of studies on the genetics of adaptation. Understanding how sunflowers cope with the local environment is relevant for both basic and applied reasons. First, ecological divergence is a major component of sunflower speciation. Thus, understanding local adaptation will provide a window into microevolutionary dynamics at the foundation of species diversity in the genus. Second, *Helianthus* contains two crops, the oilseed *H. annuus* and the tuber crop *H. tuberosus*. Thus, studies can be conducted to understand adaptation during domestication. Even more so, studies of adaptation can help identify genetic resources for breeding stress-resistant cultivars, which is relevant from the perspective of food security under climate change (e.g., Gao et al. 2019).

Lastly, *H. annuus* and *H. tuberosus* contain widespread invasive ecotypes. Thus, these species also allow us to understand evolutionary mechanisms of invasion success.

4.1 Evolution During Domestication

Not only have sunflowers been a model for understanding speciation, they have also been instrumental for understanding evolution under domestication, largely due to work on the oilseed crop *H. annuus*. Key topics that have been addressed include the geographic origin(s) of crops in the Americas, the speed of evolutionary change under domestication, and the genetic mechanisms that drive these changes (Burke et al. 2002; Harter et al. 2004). Despite this, there was still controversy about the origin of the domesticated sunflower as recently as the early twenty-first century (Lentz et al. 2008; Rieseberg and Burke 2008). However, this debate has been settled using analyses of candidate domestication genes (Blackman et al. 2011a). More recently, shotgun sequencing of archeological DNA contributed as well (Wales et al. 2019). These studies have clarified that extant domestic sunflower has a single origin, which occurred around 4,000 years ago in Eastern North America.

Genetics and genomics research over the past two decades has made clear that phenotypic transitions during domestication can be the result of a wide range of mutations (Purugganan 2019). The most common type is a non-synonymous single nucleotide polymorphism. In sunflower, a prime example of this are the flowering time genes. For example, the sunflower *HaFT1* locus, a homolog of a known flowering time regulator, contains a frameshift single nucleotide polymorphism that differentiates wild and domestic *H. annuus* (Blackman et al. 2010). Sequence analyses of the exon containing this frameshift have confirmed that *HaFT1* has been under selection in landrace and elite lines, while evolving neutrally in wild populations (Blackman et al. 2010). Other domestication traits mapped in sunflower include plant architecture and fatty acid synthesis (Wills and Burke 2007; Chapman and Burke 2012). These studies relied on linkage mapping, association mapping, and F_{ST} outlier scans to identify well over 100 candidate domestication genes (Burke et al. 2002; Wills and Burke 2007; Chapman et al. 2008; Mandel et al. 2013; Baute et al. 2015). While this contrasts with observations from other crops in terms of the number of inferred domestication loci, a polygenic signature of domestication is becoming more commonly observed with more widespread use of genomic data (Chen et al. 2020).

Not only were specific genes altered during sunflower domestication, but genome-wide patterns of polymorphism were changed as well. For example, comparisons between wild and domesticated *H. annuus* revealed differences in the content of transposable elements (Mascagni et al. 2015). Also, there have been significant changes in RNA-splicing (Smith et al. 2018). Another transition with genome-wide consequences was the incorporation of a hybrid breeding system, which occurred in the 1970s (Fick and Swallers 1972). This resulted in gene content

changes among heterotic groups, including genes involved in pathogen defense (Owens et al. 2019). Heterosis likely resulting from complementation contributed to large yield increases (Owens et al. 2019). This is relevant because, like many other domesticated species (Gaut et al. 2015), sunflower has gone through a bottleneck with genetic diversity being significantly reduced in cultivated lines (Liu and Burke 2006; Wales et al. 2019; Park and Burke 2020). In agreement with this observation, cultivated sunflower genomes were shown to contain more deleterious mutations than wild genomes (Renaut and Rieseberg 2015). However, deleterious polymorphisms currently present in the domestic sunflower gene pool will be challenging to remove because they have accumulated predominately in low-recombination regions (Renaut and Rieseberg 2015).

Following the development of the hybrid breeding system, improvement of domestic sunflower lines also relied on the introduction of genetic material from wild congeners (Baute et al. 2015). Population genomic analyses have helped reveal the genomic extent and the phenotypic consequences of these introgressions. Baute et al. (2015), for example, used transcriptome sequencing to show that introgressed regions account for ~10% of the genome in cultivated sunflower. All modern cultivars examined were found to contain one or more introgression-derived genomic regions (Baute et al. 2015). Hübner et al. (2019) further expanded these results in an analysis of the sunflower pangenome. Results reiterated that approximately 10% of the genome, and 1.5% of genes, originated *via* introgression. Genes involved in biotic resistance were over-represented among those found in introgressed regions. This was confirmed using a GWAS analysis for downy mildew (*Plasmopara halstedii*) resistance, which identified several strong associations that overlap with introgressions from wild species (Hübner et al. 2019).

Understanding the way evolution has changed species under domestication provides new ways to quickly select and develop additional domesticates. For example, known domestication genes represent excellent targets of selection and can help define expectations for breeding programs. A new area of interest in domestication research involves comparative analyses of annual and perennial crops (Gaut et al. 2015). *Helianthus* provides research opportunities in this direction, given that it contains both an annual domesticate (*H. annuus*) and a less well-studied perennial domesticate (*H. tuberosus*; Kantar et al. 2014). Further, there is much interest in domesticating new species to make agricultural systems more sustainable. The *Helianthus* genus provides key species in this endeavor as well (Asselin et al. 2020). We, therefore, anticipate that sunflowers will continue to be important to our understanding of how plants interact with human society, and how this relationship can be improved as more genomic knowledge is gained.

## 4.2 Evolution of Invasiveness

When considering problematic plants, a distinction can be made between agricultural weeds and invasive species. While admittedly blurry and rarely used, this

classification is based on the environment in which the plants typically occur. At one extreme, agricultural weeds are found in highly managed environments, such as cultivated croplands. At the other extreme, invasive species often spread in more natural communities that are minimally altered by human activity (Ellstrand et al. 2010). Aside from this distinction, agricultural weeds and invasive species are similar in many respects. For example, both are known to adapt rapidly in response to novel challenges they encounter. Agricultural weeds rely on adaptations, such as rapid growth, herbicide resistance (Baucom 2019), or crop mimicry (Barrett 1983) to thrive under the unique conditions resulting from cultivation. These include high availability of water and fertilizer, but also targeted removal. Invasive species as well have been shown to respond adaptively to a host of conditions including novel climates (Colautti and Barrett 2013) and reduced competition in empty niches (Dlugosch et al. 2015). Other shared commonalities include the occurrence of hybridization, reduced population size, and serial founder events (Bock et al. 2015; Hodgins et al. 2018). *Helianthus* contains two well-known representatives for both categories, the *H. annuus* agricultural weed and the *H. tuberosus* invasive species. We discuss below how population genomics studies in these taxa have contributed to our understanding of invasiveness in agricultural and natural settings.

The *H. annuus* agricultural weed is common in croplands throughout North America and Europe, where it can have a large economic impact (e.g., Deines et al. 2004). It achieves a patchier distribution in Australia and in Argentina, where it is more often found as a ruderal plant (Presotto et al. 2017). Population genetic and genomic analyses have indicated that weedy *H. annuus* has a diverse origin. This includes multiple derivations from nearby wild populations in North America (Kane and Rieseberg 2008), intraspecific crop-wild hybrid origins in Europe (Muller et al. 2011), and a mixture of wild and interspecific F_1 hybrid origins involving crosses with *H. petiolaris* in Argentina (Mondon et al. 2018). Common garden and drought experiments conducted using North American, Australian, and Argentinian genotypes have highlighted that, relative to wild *H. annuus*, weedy forms have evolved faster growth at the expense of reduced drought tolerance (Mayrose et al. 2011; Koziol et al. 2012; Presotto et al. 2017).

The genetic basis of wild to weedy transition has been investigated for North American *H. annuus*. This was done using genome scans for selection based on 106 EST-derived microsatellites (Kane and Rieseberg 2008) and using microarray-based gene expression analyses (Lai et al. 2008). These studies have highlighted that weediness in this system was likely achieved *via* changes at a small genomic fraction. Kane and Rieseberg (2008) reported evidence of selection at 0.9–5.6% of loci examined. Likewise, Lai et al. (2008) found evidence for significant gene expression differences at 5% of the genes investigated. Moreover, both studies identified limited overlap in outlier loci among weedy populations, thus indicating weediness is easy to evolve in sunflowers, and can have a diverse genetic basis (Kane and Rieseberg 2008). Ongoing work is attempting to expand on these results using whole genome resequencing (Drummond 2018). While comparisons of wild *H. annuus* with independently derived weedy genotypes did reveal some evidence of parallel genetic differentiation, the relative contribution of idiosyncratic changes
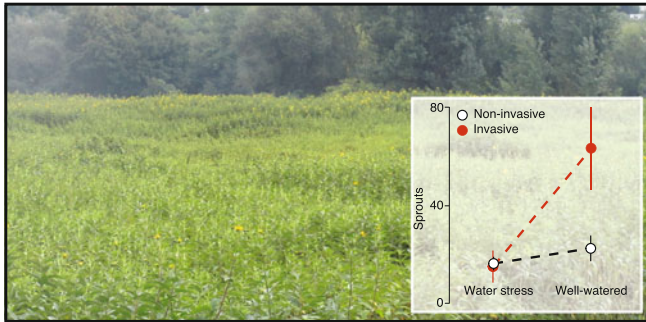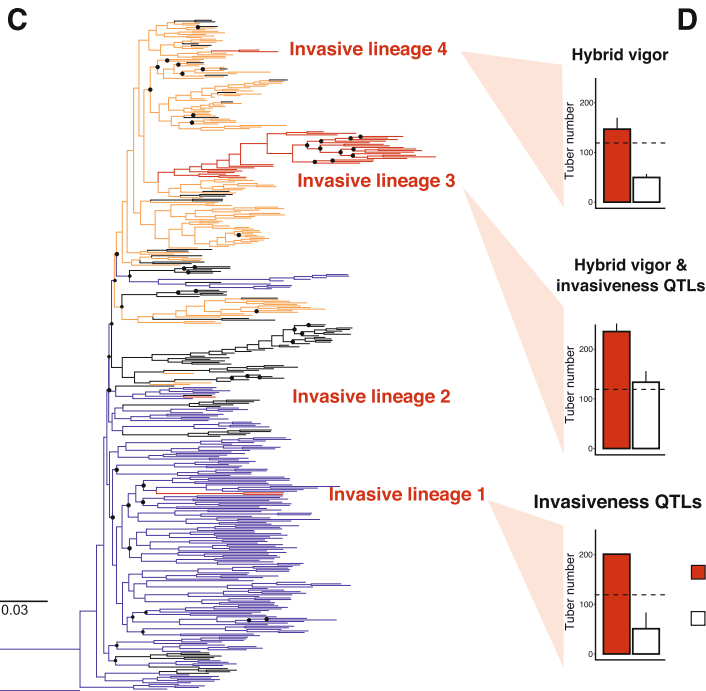
Fig. 3 Population genomics of invasiveness in *H. tuberosus* (modified from Bock et al. 2018). (**a**) Invasive *H. tuberosus* stand (Czech Republic, 2013; photo credit Dan G. Bock). Inset shows the number of sprouts (means ± s.e.m.) produced by invasive and non-invasive genotypes

could not be assessed at this point. Even so, preliminary results have reiterated that the transition to weediness likely involved a small fraction of the genome (<1%; Drummond 2018). Also, outlier regions contained genes associated with a variety of functions, as expected given that wild and weedy sunflowers diverge in multiple traits. These candidate weediness genes are associated with abiotic and biotic stress response, as well as herbicide tolerance (Drummond 2018).

In contrast to *H. annuus*, invasive genotypes of *H. tuberosus* are more often found in natural habitats (Fig. 3a). In Europe, where this perennial sunflower is considered a highly invasive species, populations frequently occur in nutrient- and water-rich soils along river courses (Descombes et al. 2016). A recent study investigated evolution of invasiveness in this system (Bock et al. 2018). Genome-wide SNP data showed that invasive genotypes have a diverse origin: some derive directly from wild accessions, whereas others are wild-domesticated hybrids (Fig. 3c). Common garden results further showed that invasive genotypes invest more in growth. Specifically, invasive plants were found to produce roughly twice the number of vegetative propagules (tubers) that wild and domesticated plants would typically make (Fig. 3b). Moreover, vegetative propagation was shown to be the primary driver of invasive spread in this species. A drought experiment further revealed that increased investment in vegetative propagation is manifested only when water resources are not limiting (Fig. 3a). This result has two implications. First, it shows that invasiveness in *H. tuberosus* evolved by genetic accommodation, *via* adaptive evolution of phenotypic plasticity. Second, it highlights that invasive *H. tuberosus*, similar to weedy *H. annuus*, has adapted to exploit a resource-rich environment in areas of spread. Lastly, association mapping analyses showed that invasive lineages differ in the genetic basis of invasiveness. Namely, two non-exclusive genetic mechanisms were shown to contribute to invasive spread: hybrid vigor and two large-effect QTLs (Fig. 3d; Bock et al. 2018). This result, thus, further strengthens the conclusion that several genetic routes to invasiveness are available in weedy and invasive sunflower populations.

Are results from sunflowers indicative of mechanisms for invasion success likely to be at play in other systems? Recent years have seen an acceleration of research on evolutionary drivers of invasiveness, implementing genomics and field experiments. As such, some preliminary generalizations can be made. For one, it seems likely that increased virulence of introduced genotypes can evolve easily and will have a diverse genetic basis. This is supported by genomic analyses in other taxonomic

Fig. 3 (continued) under water-stress and well-watered conditions. (**b**) Tuber yield from wild, invasive, and cultivated genotypes. (**c**) Maximum-likelihood phylogeny of *H. tuberosus*, including wild (blue), invasive (red), and cultivated (orange) genotypes. Invasive populations have at least four distinct origins. (**d**) For three origins with available phenotype data, means (± s.e.m.) are given for tuber number, the main invasiveness trait in this system, in invasive genotypes (red) and closely related non-invasive samples (white). Dotted line shows the mean across the collection. For each origin, inferred genetic mechanisms of invasiveness (i.e., hybrid vigor and/or invasiveness QTLs) are given

groups. For example, Hodgins et al. (2015) traced the signature of natural selection in 35 Asteraceae species, including six major invasive species. Results provided limited evidence for parallel changes in orthologous genes (Hodgins et al. 2015). Concordant results have also been obtained with regard to phenotypic and physiological changes that occur during invasions. Specifically, invasive genotypes have frequently been found to display superior growth under high-resource conditions, but reduced tolerance to abiotic stress. Thus, invasiveness appears to frequently evolve in resource-rich habitats (Hodgins et al. 2018). Such invasion-prone environments may be a result of changes in community structure, including historical declines of competitors (e.g., Dlugosch et al. 2015).

4.3 Local Adaptation with Gene Flow

How does local adaptation originate and persist when gene flow is occurring? Traditionally, gene flow has often been viewed as a disruptor of local adaptation (Tigano and Friesen 2016). This is because, when genetic exchange is rampant, alleles that confer an advantage in the local environment can be swamped by foreign variants. Under this scenario, even if locally suboptimal, alleles with the best fitness across populations will tend to become fixed, and local adaptation will be lost (Lenormand 2002). Modelling studies indicate that the likelihood of such swamping depends on the intensity of gene flow and selection (Tigano and Friesen 2016). Recently, examples of local adaptation with gene flow have become more common, facilitated by genomics tools that allow the topic to be studied in any organism (Tigano and Friesen 2016). As such, there has been a renewed interest in understanding the destructive as well as constructive roles of gene flow during local adaptation. This is particularly relevant in sunflowers, because of the high propensity for gene flow that is characteristic of the genus.

That sunflower populations are locally adapted has been supported by multiple studies using field experiments, population genomics, or a combination of these approaches. Field experiments have used reciprocal transplants, often highlighting photoperiod and soil characteristics such as water and nutrient content as important components of local adaptation in sunflowers (e.g., Sambatti et al. 2008; Whitney et al. 2010; Ostevik et al. 2016). Population genomic screens have searched for the signature of natural selection. The goal in this case was to characterize adaptive evolution from the perspective of types of genomic changes (Moyers and Rieseberg 2013), the number and types of genes (e.g., Kane et al. 2011; Renaut et al. 2012; McAssey et al. 2016), or the contribution of genomic landscape (e.g., Renaut et al. 2014b). Lastly, studies using a combination of the two have aimed to clarify the occurrence of local adaptation using comparisons of quantitative trait vs. neutral genetic differentiation (e.g., Blackman et al. 2011b; Moyers and Rieseberg 2016).

While local adaptation is frequent in sunflowers, how is it achieved given widespread gene flow? A recent study by Todesco et al. (2020) addressed this

question. Three species were considered, each containing pairs of locally adapted ecotypes that are well within gene flow contact. For *H. annuus*, sampling covered typical populations found throughout the US, often adapted to mesic soils. Additionally, populations of *H. annuus* subsp. *texanus* were used, which are adapted to sites with increased temperature and pathogen pressure in Texas (Whitney et al. 2010). For *H. petiolaris*, the study included dune and non-dune ecotypes that evolved independently in both Texas and Colorado (Ostevik et al. 2016). Finally, for *H. argophyllus*, sampling covered southern Texas early and late flowering ecotypes. These are differentially adapted to coastal barrier islands (early flowering ecotype) and mainland sites (late flowering ecotype; Fig. 4a; Moyers and Rieseberg 2016). In all, whole genome resequencing data for 1,506 genotypes was used in combination with an extensive developmental, morphological, and environmental dataset.

One of the most striking results was that many of the traits and environmental variables known to be involved in local adaptation in these ecotypes mapped to large chromosomal blocks of high linkage disequilibrium (Todesco et al. 2020). Moreover, many of these chromosomal blocks were found to control multiple traits. For example, in *H. argophyllus*, flowering time as well as leaf nitrogen and carbon content was associated with a 30 Mb region containing two main haplotypes (Fig. 4b–d). Plants with different haplotypes at this region flowered on average 77 days apart (Fig. 4f). Additional analyses confirmed that a large fraction of these plateaus of association correspond to structural variants, represented by inversions and more complex rearrangements. Analyses of the 1,506 genomes further revealed that structural variation is common across the three sunflower species, representing 4–16% of the genome (Todesco et al. 2020). Perhaps equally striking, the divergence times between haplotype blocks was found to exceed the inferred age of the species in which they currently segregate. This suggests some of the haplotype blocks currently contributing to local adaptation in sunflowers may be a result of introgression from older, currently extinct taxa (Todesco et al. 2020).

Aside from clarifying the genetic basis of several adaptive traits, results from this study contributed to our understanding of adaptation with gene flow in at least two important ways. For one, it supports previous theoretical work (Yeaman and Whitlock 2011) indicating that gene flow shapes the genetic architecture of local adaptation. In cases when adaptation occurs with interpopulation genetic exchange, selection will favor modular architectures such as those observed in sunflowers. This is because recombination modifiers including chromosomal inversions prevent the breakup of adaptive allele combinations. At the same time, Todesco et al. (2020) highlight that gene flow can facilitate local adaptation *via* adaptive introgression. This is supported by the finding that haplotype blocks acting as large-effect loci that control multiple adaptive traits can have an interspecific origin (Fig. 4g). This result thus further reinforces the constructive effect of hybridization for biodiversity, a finding that is becoming more and more common as new taxa are being investigated (Tigano and Friesen 2016).
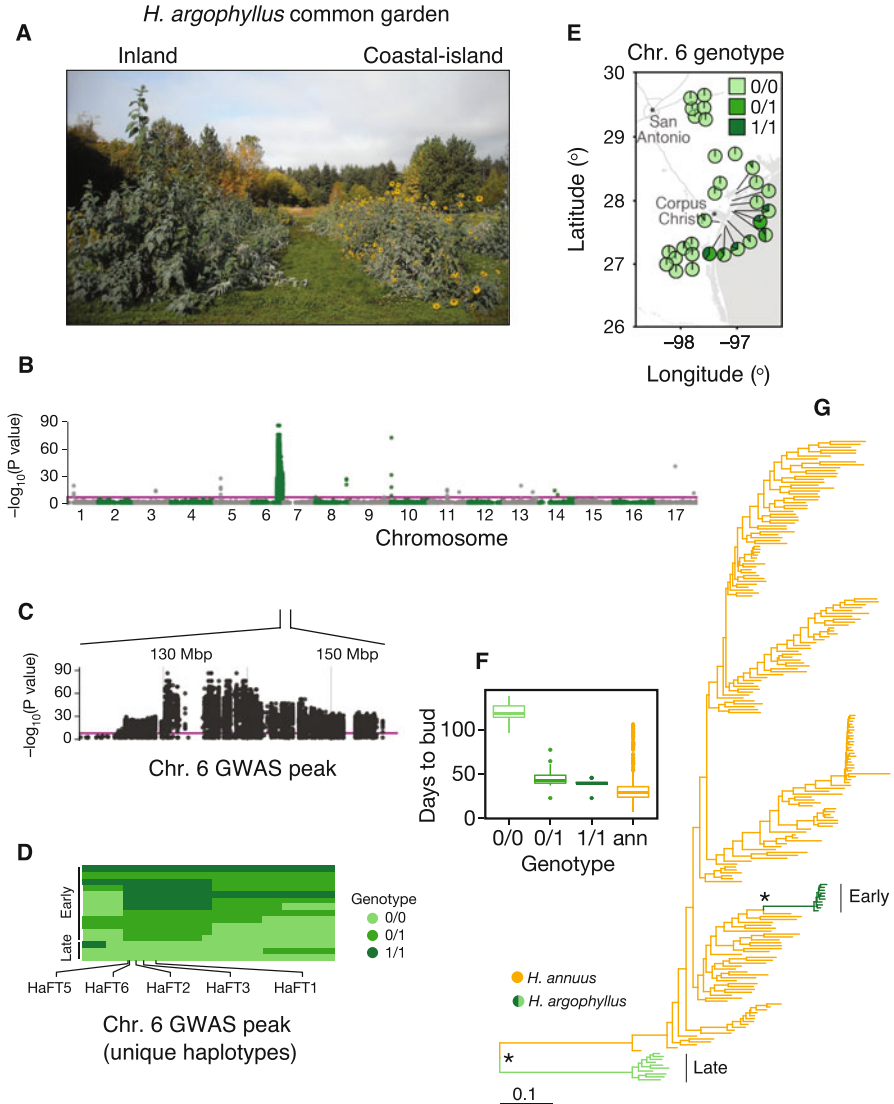
Fig. 4 Population genomics of local adaptation in *H. argophyllus* (modified from Todesco et al. 2020). (**a**) *H. argophyllus* inland and coastal-island plants grown in a common garden (photo credit Brook T. Moyers). (**b**) Flowering time GWAS results. (**c**) Close-up of the chromosome 6 region containing the main association peak. (**d**) Schematic of unique haplotypes contributing to the GWAS flowering time peak, with position of six sunflower homologues of the *FT* flowering-time regulator. (**e**) Geographical distribution of haplotypes identified under the GWAS peak on chromosome 6. Early-flowering genotypes identified using GWAS predominate in coastal populations. (**f**) Flowering time differences among genotypes at the chromosome 6 GWAS peak (ann = *H. annuus*). (**g**) Maximum-likelihood phylogeny of the chromosome 6 region, highlighting the interspecific origin (*H. annuus* – derived) of the early-flowering haplotype. Asterisks indicate well-supported major nodes

4.4   Crop-Wild Gene Flow

In addition to genetic exchange among differentially adapted wild populations, gene flow has been documented between the domesticated sunflower, which is adapted to cultivation, and wild or weedy populations of *H. annuus* and *H. petiolaris* (Linder et al. 1998; Rieseberg et al. 1999c; Muller et al. 2011; Mondon et al. 2018). When the crop is abundant, such gene flow has the potential to reduce the fitness of adjacent wild populations (Ellstrand and Rieseberg 2016). Conversely, some crop traits such as resistance to herbicides or pests could enhance weediness (Snow et al. 2003).

While most studies of crop-wild gene flow in sunflowers have employed a relatively small number of molecular markers, two recent studies provide genome-wide estimates of hybridization and introgression (Corbi et al. 2017; Mondon et al. 2018). In the latter study, Mondon et al. (2018) confirmed the presence of hybridization and introgression between domesticated sunflower (*H. annuus*) and *H. petiolaris* populations in Argentina, despite the strong reproductive barrier between these species (Sambatti et al. 2012). However, it remains unclear whether hybridization is causative or incidental to the weedy life history traits found in Argentinian *H. petiolaris*. Corbi et al. (2017) tracked genome-wide allele frequency shifts in an experimentally synthesized crop × wild sunflower population, which was planted at two natural sites and allowed to evolve for two generations. As expected, most crop alleles were selected against, but a handful were favored. Allelic frequency changes were not closely correlated with shifts in phenotype, possibly suggesting that crop alleles that complemented deleterious alleles were favored rather than a particular phenotype *per se*.

5   Conclusions and Future Directions

As outlined in the introduction, sunflowers have emerged as a useful experimental model for addressing a wide range of evolutionary questions. Most importantly, studies of wild sunflower species have been key to establishing hybridization as a creative force in evolution, facilitating adaptation, and leading to the formation of new species (Rieseberg et al. 2007). Beyond hybridization, sunflowers are unusual in their high rate of chromosomal structural evolution, and studies of wild sunflowers have shown how large structural variants such as chromosomal inversions permit adaptive divergence and speciation in the presence of gene flow (Barb et al. 2014; Huang et al. 2020). Recently, these two previously disparate research themes have been merged with the discovery that polymorphic structural variants segregating in natural populations are often themselves the product of hybridization and introgression (Todesco et al. 2020).

While many of the large structural variants segregating in sunflower populations have been shown to be inversions, others are more complex, possibly representing

nested inversions and/or large deletions (Todesco et al. 2020). Also, the comparative genetic mapping and population genomics approaches used to detect structural variation are biased towards detecting large (>1 Mb) structural variants. An important goal of the sunflower community going forward should be to better characterize structural variation in cultivated and wild sunflowers. This will be most efficiently accomplished by generation of reference genomes for all wild sunflower species, focusing initially on the close relatives of the domesticated sunflower and then expanding to the perennials. Such an effort is currently underway, led by the International Consortium on Sunflower Genomics (https://www.heliagene.org/ ICSG/). Concomitantly, Hi-C sequencing and Bionano genome mapping of structural variants will be useful to fill in the gaps between reference sequences, as well to infer whether variants represent fixed differences or are segregating within populations or species. Of equal importance will be follow-up studies that attempt to link the structural variants with ecologically important phenotypic traits, climate variables, or soil characteristics.

Beyond generation of new reference sequences, the time has come to revisit well-studied examples of adaptive introgression and hybrid speciation in the sunflower system. Many of these examples were first developed based on cytogenetic and morphological data (e.g., Heiser 1947, 1949, 1951), followed by gene tree analyses (e.g., Rieseberg et al. 1988) and low-resolution comparative genetic mapping (e.g., Rieseberg et al. 1995a). Phylogenomic analyses offer a means to re-examine, enrich, and potentially re-interpret results from these earlier studies (e.g., Owens et al. 2016).

In addition to the wild species, the sunflower genus is blessed with two different crops, one annual (domesticated sunflower) and one perennial (Jerusalem artichoke). Thus, the genus can offer insights into the genetics of domestication and improvement, while permitting a contrast between annual and perennial crops. As with the wild species, arguably the most distinctive feature of the domestication and improvement process in *Helianthus* is hybridization. The cultivated sunflower genome is a mosaic, with introgressions from half a dozen different wild species (Hübner et al. 2019). These introgressions often lack genes that are present in wild *H. annuus*, potentially contributing to linkage drag (Owens et al. 2019). The Jerusalem artichoke is an allohexaploid, with its genome derived via hybridization between diploid Sawtooth Sunflower and tetraploid Hairy Sunflower (Bock et al. 2014a). Hybridization between domesticated and wild Jerusalem artichoke also appears to have played a non-exclusive role in the origin of invasive Jerusalem artichoke (Bock et al. 2018).

As with the wild species, a priority of research going forward should be a reference sequence for Jerusalem artichoke, as well as additional references for the domesticated sunflower. The reference sequences not only provide a means for detecting and characterizing structural variants, but they are also critical for identifying candidate genes underlying domestication or invasiveness traits. Given that >25% of sunflower genes exhibit presence/absence variation among domesticated lines (Hübner et al. 2019), the availability of reference genomes from diverse

cultivated lines increases the likelihood that a targeted gene will be present in at least one of them.

Despite successes from population genomic analyses of wild and cultivated *Helianthus*, there are some notable challenges to using sunflowers as an evolutionary or ecological study system. One such challenge is genome size. Sunflower's 3.6 Gb genome is 7.9x the size of the rice genome and 27x the size of the *Arabidopsis* genome. Thus, the recent study by Todesco et al. (2020), which reported on the sequencing of 1,506 sunflower genomes, would be equivalent to sequencing circa 12,000 rice genomes and 41,000 *Arabidopsis* genomes! The differential is even greater for Jerusalem artichoke's 10.9 Gb genome. Because the vast majority of the sunflower genome is made up of highly repetitive transposable elements, Todesco et al. (2020) employed a repeat depletion protocol to reduce the fraction of repetitive sequence by about 3-fold. Such an approach offers the advantage of reducing total sequencing costs while retaining all low copy regions of the genome.

An even greater challenge is sunflower's recalcitrance to transformation, which makes it difficult to validate the function of ecologically and/or agriculturally important candidate genes. Thus, sunflower biologists often rely on heterologous transformation in *Arabidopsis* to explore the phenotypic effects of sunflower alleles (Blackman et al. 2010; Todesco et al. 2020). While the approach works well for genes with phenotypes that can be assayed in *Arabidopsis*, it may not be reliable for traits which do not exist in *Arabidopsis* or whose expression is highly dependent on genetic background. Thus, a clear imperative for the sunflower community is to develop facile transformation and gene editing protocols. Until such methods exist, sunflowers will fail to achieve their potential as an ecological or evolutionary model.

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