Deep reticulation: the long legacy of hybridization in vascular plant evolution

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SUMMARY

Hybridization has long been recognized as a fundamental evolutionary process in plants, but our understanding of its phylogenetic distribution and biological significance across deep evolutionary scales has been largely obscure—until recently. Over the past decade, genomic and phylogenomic datasets have revealed, perhaps not surprisingly, that hybridization, often associated with polyploidy, has been common throughout the evolutionary history of plants, particularly in various lineages of flowering plants. However, phylogenomic studies have also highlighted the challenges of disentangling signals of ancient hybridization from other sources of genomic conflict (in particular, incomplete lineage sorting). Here we provide a critical review of ancient hybridization in vascular plants, outlining well-documented cases of ancient hybridization across plant phylogeny as well as the challenges unique to documenting ancient vs. recent hybridization. We provide a definition for ancient hybridization, which, to our knowledge, has not been explicitly attempted before. Further documenting the extent of deep reticulation in plants should remain an important research focus, especially since published examples likely represent the tip of the iceberg in terms of the total extent of ancient hybridization. However, future research should increasingly explore the macroevolutionary significance of this process, in terms of its impact on evolutionary trajectories (e.g., how does hybridization influence trait evolution or the generation of biodiversity over long time scales?), as well as how life history and ecological factors shape, or have shaped, the frequency of hybridization across geologic time and plant phylogeny. Finally, we consider the implications of ubiquitous ancient hybridization for how we conceptualize, analyze, and classify plant phylogeny. Networks, as opposed to bifurcating trees, represent more accurate representations of evolutionary history in many cases, but our ability to infer, visualize, and use networks for comparative analyses is highly limited. Developing improved methods for the generation, visualization, and use of networks represents a critical future direction for plant biology. Current classification systems also do not generally allow for the recognition of reticulate lineages, and our classifications themselves are largely based on evidence from the chloroplast genome. Updating plant classification to better reflect nuclear phylogenies, as well as considering whether and how to recognize hybridization in classification systems, will represent an important challenge for the plant systematics community.

Keywords: Ancient hybridization, angiosperms, gene flow, phylogenomics, polyploidy, reticulation, vascular plants

INTRODUCTION

Hybridization—or crossing between individuals from different species or divergent populations (Stebbins 1959; Harrison 1990; Arnold 1997)—is a biological process that has fundamentally shaped the Tree of Life from its tips to some of its deepest branches. The circumstances that give rise to hybridization, control its frequency, and shape its evolutionary outcomes are complex and involve the interplay of ecological, genomic, population genetic, and life history factors. Hybridization can counter divergence by homogenizing previously isolated subpopulations. Intermittent hybridization can facilitate the sharing of adaptive alleles among divergent populations or separate species. Hybridization can also lead to the formation of new (reticulate) lineages through hybrid speciation, when an evolutionarily stable population of hybrid individuals is established in reproductive isolation from its parental species. However, in addition to these "creative" aspects of hybridization (sensu Arnold 1992), hybridization can also break apart co-adaptive gene groups, cause Dobzhansky-Muller incompatibilities, and even erode the genetic integrity of rare species (Todesco et al. 2016).

The study of hybridization has a long history in botany (Haartman 1751; Kölreuter 1766; Roberts 1929), and it was solidified as a fundamental process in plant evolution during the Modern Synthesis (Anderson and Hubricht 1938; Anderson 1948; Stebbins 1959). The study of hybridization, including hybridization in association with polyploidy (allopolyploidy), has continued to be a central focus of plant biology (e.g., Grant 1971; Soltis and Soltis 2009; Yakimowski and Rieseberg 2014; Alix et al. 2017). Recent advances in genomics and phylogenomics have greatly increased our ability to detect both recent and ancient hybridization and to understand its genetic and evolutionary consequences. It has been estimated that ~25 percent of plant species in the UK flora actively hybridize with relatives (Mallet 2005), and that more than 30% of living plant species are polyploids (Wood et al. 2009). It is also now clear that ancient polyploidy is prevalent across plant phylogeny and that all major vascular plant lineages have polyploidy in their past (Jiao et al. 2011; Amborella Genome Project 2013; Leebens-Mack et al. 2019; Stull et al. 2021). It is unclear how many of these ancient polyploidy events were associated with hybridization (i.e., allopolyploidy), but recent phylogenomic studies have dramatically increased the number of documented cases of ancient hybridization in plants (both homoploid and polyploid). This is perhaps not surprising–given the prevalence of recent hybridization and the appreciation that it can lead to evolutionarily stable lineages (Ungerer et al. 1998; Soltis and Soltis 2009; Katche et al. 2021), we might expect that hybridization has been an important process throughout the entirety of plant evolutionary history, and also that some major lineages of plants could be of hybrid origin (via hybrid speciation) or have experienced varying degrees of introgression with other lineages.

In light of mounting evidence for ancient hybridization, and the dramatic recent increase in research on the subject, a critical review of the topic is timely to synthesize current knowledge and guide future work. There are numerous extensive reviews on hybridization in plants (e.g., Mallet 2005; Soltis and Soltis 2009; Abbott et al. 2013; Yakimowski and Rieseberg 2014; Suarez-Gonzalez et al. 2018, Edelman and Mallet 2021), but these generally focus on the study of recent hybridization. Here we provide a critical review focused in particular on *ancient* hybridization (see definition below). We survey well-documented examples of ancient hybridization between major lineages of plants, ranging in temporal scale from thousands of years ago to over 100 million years ago, and outline what we view as compelling evidence for recognizing hybridization that occurred in deep time. We suggest that genomic data should, when possible, be paired with other lines of evidence (e.g., biogeographic and niche reconstructions) in order to evaluate the plausibility of historical hybridization scenarios, but that the feasibility of such approaches is perhaps limited for putative hybridization events predating the Cenozoic.

While we assume that hybridization in the distant past is a fundamentally similar process to that happening in recent time, it is clear that detecting and studying ancient hybridization involves unique challenges that deserve special attention.

The consideration of hybridization across deep time also raises macroevolutionary questions worthy of discussion. For example, has the prevalence of hybridization been relatively consistent through time, or have certain geologic periods witnessed, or even facilitated, increased frequencies? What is the relationship between hybridization and diversification? Do particular traits tend to facilitate hybridization or the persistence of hybrid populations? Is the propensity to hybridize itself an adaptive life history strategy? Our discussions here build on previous considerations of these questions (e.g., Grant 1971; Ellstrand et al. 1996; Whitney et al. 2010; Folk et al. 2018a; Mitchell et al. 2019; Mitchell and Whitney 2021). The prevalence of reticulation has also led some to question whether a bifurcating tree is the most appropriate way to model the diversification process and conceptualize evolutionary history more generally (e.g., Funk 1981; Linder and Rieseberg 2004; Bapteste et al. 2013). This has fundamental implications for the field of evolutionary biology, as well as for biological nomenclature and how we construct classifications. By reviewing current knowledge about ancient hybridization and discussing important issues related to its detection and association with broader patterns and processes of plant evolution, we hope to provide a roadmap for future research on the topic.

ANCIENT HYBRIDIZATION DEFINED

To our knowledge, there have been no *explicit* attempts to establish a definition for “ancient hybridization” as a phenomenon distinct from “recent hybridization”, despite the widespread use of the former term in the literature. To some extent, such a distinction is arbitrary since the same process is at play, but given that the population genetic and genomic consequences of hybridization tend to unfold in relatively discernable stages (Moran et al. 2021), there might be a point in the evolutionary trajectory following hybridization that we can refer to past hybridization events as “ancient” in a meaningful sense. We consider hybridization events to be “ancient” if (a) gene flow between the lineages in question has ceased, (b) the genomes in the hybrid populations have stabilized, and (c) the lineage of hybrid origin has subsequently diversified or undergone a prolonged period of anagenesis following genome stabilization (e.g., Fig. 1). Regarding (b), genome stabilization can occur through the rapid purging of much of one parent’s ancestry (the minority parent) in cases of introgression (historically called introgressive hybridization), thus reducing

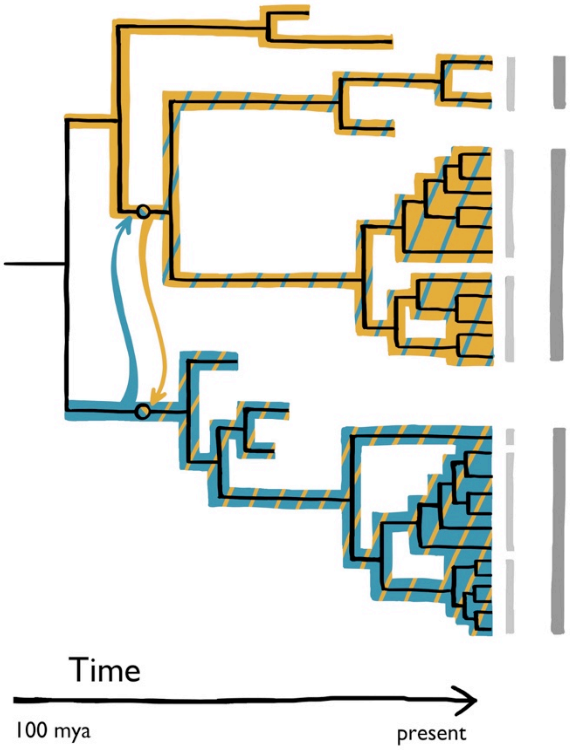

Figure 1. A chronogram of a fictional exemplar clade, with both extinct and extant taxa, in which ancient hybridization occurred between members of sister clades (highlighted in cobalt and gold) 80 million years ago; the reticulation event is marked by circles.

heterozygosity; through recombinational hybrid speciation, where chromosomal arrangements are established that render the hybrids interfertile but incompatible with either parent; and/or through polyploidization, which results in fixed heterosis. We can call this a “genomic” definition for ancient hybridization, and we follow this definition here.

Another approach to defining hybridization as “ancient” vs. “recent” is in relation to human history. For example, we can consider hybridization events to be “ancient” if they occurred before the Holocene (i.e., before 11,600 years ago). We call this a “historical” definition of ancient hybridization, and this roughly follows the logic of Welch and Rieseberg (2002). We chose the Holocene as the demarcation point as it has a concrete geologic definition and represents a general turning point in human history, when activities such as agriculture began to significantly impact Earth’s ecosystems (Larsen 2006); furthermore, the proliferation of agriculture both directly and indirectly promoted widespread hybridization in plants (Anderson 1948, 1949). In some respects, this definition is more arbitrary and less biologically meaningful than the “genomic” definition above. For example, we would expect hybrid genomes to stabilize much more quickly in lineages with short vs. long generation times, so a hybridization event that occurred 15,000 years ago would be, in an evolutionary/genomic sense, perhaps more “ancient” in an annual herbaceous lineage than it would be in a woody lineage with much longer generation times. However, this historical definition is perhaps more straightforward in its application, and it also brings into focus the significance of anthropogenic activities in facilitating gene flow and the formation and persistence of hybrids over the last 11,600 years (Ottenburghs 2021), and the importance of identifying “natural” hybrids that occurred prior to major phases of anthropogenic disturbance and stewardship (Welch and Rieseberg 2002).

HISTORICAL VIEWS ON ANCIENT HYBRIDIZATION

Although the role of hybridization in plant evolution has been recognized for nearly a century, hybridization has generally been considered on recent timescales, whether viewed via “genomic” or “historical” perspectives. Ancient hybridization has tended to be associated with polyploidy. For example, Stebbins (1950) identified several angiosperm families as having ancient polyploid roots, having arisen via allopolyploidy, i.e. the combined processes of hybridization and chromosome doubling. (Although Stebbins did not explicitly note allopolyploidy in the origins of these ancient polyploid clades, he considered autopolyploidy to be ‘rare and maladaptive’ (Stebbins 1950, 1971); thus, his inferences of ancient polyploidy must have involved hybridization.) The magnoliid families Magnoliaceae, Myristicaceae, Calycanthaceae, and Lauraceae were proposed as ancient polyploids, as was the rosid family Salicaceae (Stebbins 1950). These hypotheses have been supported by genetic and genomic data (e.g., Soltis and Soltis 1990; Cui et al. 2006; Tuskan et al. 2006; Chaw et al. 2019; Chen et al. 2019). In contrast to ancient polyploidy, explicit hypothesized instances of ancient homoploid hybridization are rare in the classic botanical literature, perhaps because the effects of hybridization per se seem to be greatly obscured through time.

The genetic expectations for a stabilized homoploid hybrid species, based on Mendelian principles, were described by Gallez and Gottlieb (1982). Additivity of parental alleles at a *single locus* would be expected in a hybrid derivative, for example, allele *a* from parental species A and allele *b* from parental species B. In addition, because generations of hybridization and backcrossing may lead to loss of one parental allele or the other at some loci, rendering these loci monomorphic, additivity should be apparent *across loci* within a population or hybrid species, such that allele *a* from parental species A would be detected at locus 1 and allele *b* from parental species B would be detected at locus 2, etc. These forms of additivity at specific genetic loci are valuable guides for detecting and documenting homoploid hybridization on recent timescales. However, through time, as alleles from one parent or the other are lost due to drift or selection and new alleles arise via mutation and accumulate, the genetic make-up of the hybrid derivative will deviate from these expectations, and the hybrid origins of the lineage will eventually be obscured. Thus, genetic comparisons, even at the level of genome sequences, may not be useful in identifying cases of ancient hybridization, and alternative approaches to detecting ancient hybridization are needed.

HYBRIDIZATION DETECTION

Hybridization is often detected via gene tree discordance, wherein the evolutionary histories of different genes conflict (e.g., Rieseberg et al. 1990; Wendel and Doyle 1998; Sang and Zhong 2000). In plants, this often manifests as cytonuclear discordance, where genes from the chloroplast genome show relationships distinct from those of the nuclear genome due to “chloroplast capture,” a phenomenon in which the chloroplast genome of one lineage becomes fixed in another following hybridization and backcrossing (Rieseberg and Soltis 1991; Soltis and Kuzoff 1995). This occurs because chloroplast genomes typically experience uniparental inheritance, lack recombination, and have small effective population sizes (Palmer 1985; Rieseberg and Soltis 1991), which prevents the sharing of genetic material between parental plastomes and can lead to rapid fixation of one parental genome in hybrid populations. Given that chloroplast genomes are typically maternally inherited (with some important exceptions such as paternal inheritance in gymnosperms and biparental inheritance in some angiosperms), chloroplast capture can be helpful for identifying maternal (or seed) contributions to gene flow (Asmussen and Schnabel 1991; McCauley 1994). Discordance among nuclear loci across the genome can also stem from hybridization and introgression, though these signals are generally more complicated to interpret than comparisons between summary nuclear and chloroplast topologies due to biparental inheritance and recombination/segregation in nuclear genomes. For either case, a topology that differs from the overall inferred species tree indicates that the gene in question has a different evolutionary history that may be explained by hybridization and introgression from a different species. However, there are other evolutionary processes that produce patterns of discordance similar to those resulting from gene flow, necessitating tests to differentiate between them.

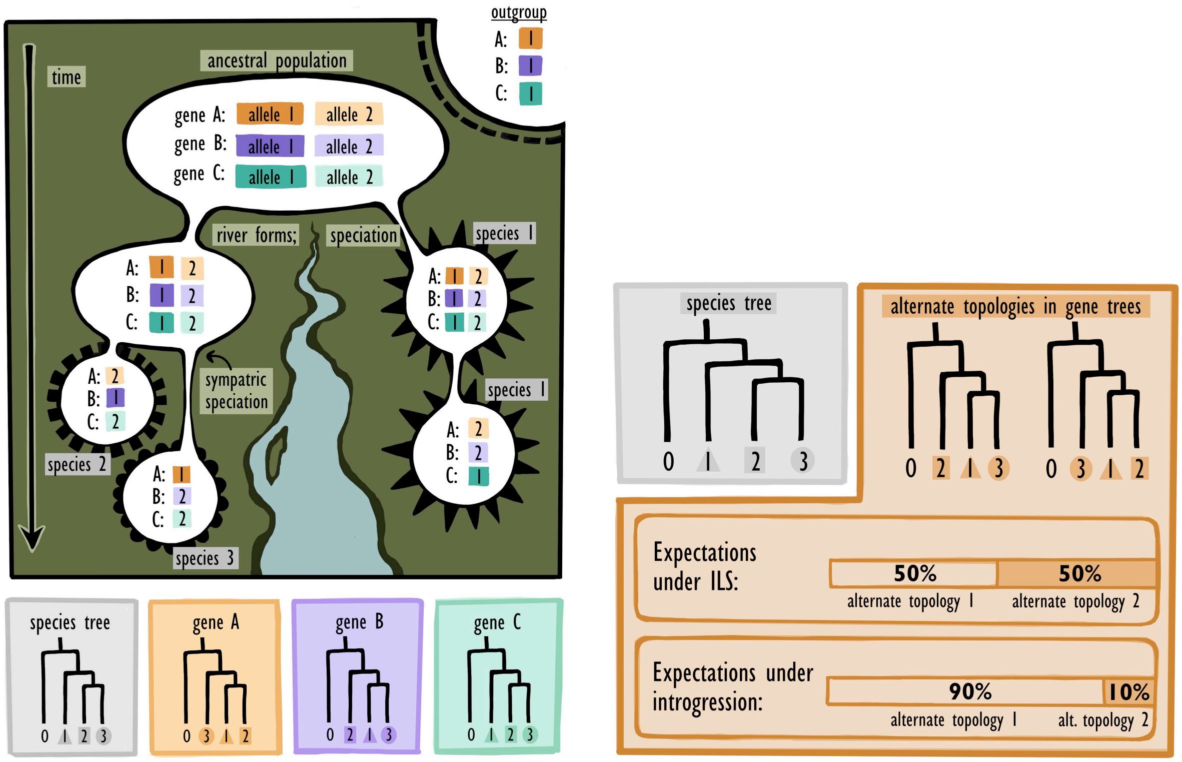

Figure 2. A) Diagram illustrating the process of incomplete lineage sorting, depicting an ancestral population where three genes have two alleles maintained. In subsequent speciation events, the two alleles are maintained in lineages and then randomly fixed, creating gene trees with topologies inconsistent with the species tree. B) Diagram illustrating expectations for the ABBA-BABA test under incomplete lineage sorting and introgression. Because incomplete lineage sorting occurs randomly across all genes, both alternative topologies are expected to occur in equal frequency. Because introgression occurs for specific taxa at specific loci, one alternative topology is expected to dominate in frequency.

The most common confounding factor for detecting hybridization is incomplete lineage sorting (ILS; Fig. 2), a process by which loci that are polymorphic at the time of species divergence randomly fix afterwards in a pattern that does not reflect the evolutionary history of the lineage, causing gene tree discordance (Nei 1986; Doyle 1992; Maddison 1997). When comparing chloroplast topologies to nuclear topologies, coalescent simulations can help identify when discordance is likely to arise under just ILS (Joly et al. 2009a; Folk et al. 2017). When comparing topologies of multiple loci across the nuclear genome, ILS can be distinguished from hybridization by comparing the ratio of sites or genes supporting topologies discordant with the inferred species tree for the whole genome or a subsection of the genome (ABBA-BABA test, Green et al. 2010; Patterson's D, Durand et al. 2011; $\widehat{f_d}$, Martin et al. 2015). Under ILS, an even ratio of all discordant topologies is typically expected, as ILS acts randomly across the whole genome. In cases of introgression, a bias towards a particular discordant topology is expected, as introgression typically only results in specific genomic regions displaying discordance. This framework has been extended to allow inference of not only the presence, but also the directionality, of gene flow in more complex phylogenetic scenarios (DFOIL; Pease and Hahn 2015). ILS and hybridization can also be distinguished by examining the distribution of branch lengths for discordant genes (QuIBL, Edelman et al. 2019), with an exponential distribution expected under ILS and an exponential distribution with additional modes expected under hybridization.

Hybridization can also be detected against a background signal of ILS using phylogenetic network inference–specifically, programs that infer phylogenetic networks using multilocus data and the multispecies coalescent. One such program, PhyloNet (Wen et al. 2018), can use maximum parsimony or maximum likelihood methods on a set of gene trees to estimate a phylogenetic network or use a Bayesian framework directly on a multiple sequence alignment. However, the calculations to infer an entire network can be computationally costly, especially as the number of taxa and hybridization events increases, so the authors suggest subsetting to taxa and putative hybridization events of interest when applying this program (Wen et al. 2018). The other popular program for this type of analysis, SNaQ (Solis-Lemus et al. 2017), takes a different approach. It infers a phylogenetic network from a set of genes or quartet concordance factors (Larget et al. 2010). The implemented algorithm increases scalability of the analysis to larger datasets, but does not have a formal method for evaluating the true number of hybridization events and cannot infer hybridization between sister species (Solis-Lemus et al. 2016).

Population genetics approaches offer another window onto hybridization/introgression. Genome-wide markers such as SNPs from RAD-seq, genotyping-by-sequencing, or whole-genome resequencing can be used to identify hybrid individuals and, in some cases, to delimit regions of the genome that have been introgressed. Putative hybrids are genotyped, along with individuals from the parent species thought not to be admixed. Admixture analysis (i.e., in STRUCTURE, Pritchard et al. 2000) is then used to characterize the genetic composition of putative hybrids, estimating the genetic contribution of K source populations to individuals. This admixture analysis can then be used to calculate a hybrid index and estimate the type of hybrid (F1, F2, advanced generation). When marker coverage of the genome is sufficiently dense and genetic distances between markers are known, admixture mapping analysis can be conducted for smaller genomic regions to delimit which are likely of hybrid origin (Chakraborty and Weiss 1988). Because ILS also affects this type of data, Patterson's D (and related statistics) and QuIBL tests are used as well. As a caveat, admixture analysis assigns variants to populations based on existing variation in the dataset and specified K and is therefore sensitive to biased sampling and misspecified K (Lawson et al. 2018), which could lead to misidentification of hybrid individuals and genomic regions. Other metrics that can be used to delimit introgressed genomic regions are relative and absolute divergence (FST and dXY), with the assumption that regions of the genome of hybrid origin will have a higher divergence value compared to the background rate for the entire genome. Like the other metrics mentioned here, divergence is affected by factors other than hybridization such as local recombination rate (Noor and Bennett 2009). Therefore, researchers usually use many different metrics together to build an overall case for hybrid origin of individuals and genomic regions.

CHALLENGES OF DETECTING DEEP RETICULATION

Detecting hybridization becomes an increasing challenge when considering possible events that occurred deeper in evolutionary history. Sources of anecdotal evidence useful for detecting recent or contemporary hybridization such as intermediate morphologies, overlapping ranges of putative parents, and increases in chromosome number (in the case of allopolyploids) are typically no longer available given thousands to millions of years of evolutionary change in phenotype, geographic range, and genome structure. The inference of ancient hybridization therefore relies largely on phylogenomic signal, which also tends to erode with the passage of evolutionary time.

On the one hand, with hybridization and subsequent backcrossing, purging of introgressed alleles via recombination can quickly reduce the extent of information available for reconstructing past hybridization (Moran et al. 2021). On the other hand, signal for gene flow (especially when limited) can be very challenging to tease apart from the noise of ILS, a more or less constant backdrop to phylogenetic signal that varies in intensity with factors such as time between divergences and population size. In general, we might expect the circumstances that give rise to extensive ILS (e.g., rapid diversification across heterogeneous landscapes) to also create ample opportunities for hybridization (due to close proximity of closely related species, which may be brought into repeated contact with climatic fluctuations). Rapid diversification will generally leave little signal in gene trees for later inference of discrete evolutionary processes, and when that signal is eroded over time due to saturation or selection, most individual gene trees might offer limited useful information. Taken together, in many cases, we might be left with a limited ability to identify cases of ancient hybridization with certitude. Because signal for past hybridization from the nuclear genome can be lost or obscured over time, well-supported cytonuclear discordance might represent the best remaining evidence for ancient hybridization (Sun et al. 2015; Stull et al. 2015, 2020). However, often both nuclear and chloroplast data show uncertainty or conflicting signals for particular deep relationships (e.g., Gitzendanner et al. 2018; Leebens-Mack et al. 2019), in which case cytonuclear discordance itself is no longer itself a reliable indicator. Furthermore, many instances of ancient hybridization will not be reflected by cytonuclear discordance given that chloroplast capture does not always accompany introgression.

Recent studies on Amaranthaceae s.l. (Morales-Briones et al. 2021) and Malpighiales (Cai et al. 2021) represent excellent examples of the challenges of detecting ancient hybridization in the context of ancient, rapid radiations. In both cases, there are extreme levels of gene tree discordance along the backbone, to the extent that it seems impossible to resolve clear bifurcating relationships among major lineages, or even clear patterns of alternative conflicting relationships. While signal for gene flow does appear to be present (e.g., imbalanced frequencies of alternative topologies), this occurs alongside extensive ILS and gene-tree estimation error. Inferring discrete hybridization events from these complex phylogenomic contexts is beyond the abilities of current methods, but reframing how we think about such phylogenomic challenges might nevertheless offer meaningful biological insights (e.g., Parins-Fukuchi et al. 2021).

Extinction represents another roadblock to the detection of ancient hybridization, a point that has received relatively little attention until recently (e.g., Thomas et al. 2017; Tricou et al. 2022). Extinction can obscure the parental lineages involved in ancient hybridization events, and in some cases might lead to incorrect inferences of the parental lineages involved. For example, relatives of the extinct parental lineages might be incorrectly inferred as participating in gene flow in the absence of the parental lineages themselves (Lawson et al. 2018). In the case of polyploidy, instances of allopolyploidy could be incorrectly reconstructed as autopolyploidy in gene-tree reconciliation approaches if both parental lineages have gone extinct (Thomas et al. 2017). Similar problems with inference can also emerge from incomplete sampling of extant lineages, but such problems should be remedied with the addition of more complete data. Extinction, however, will likely represent an intractable barrier to a more complete understanding of ancient hybridization in many cases. While extinction should generally represent a more common confounding factor in the reconstruction of ancient hybridization events, we suggest that it is an important issue to consider even when examining more shallow hybridization events given the extent of recent and ongoing extinction in the Anthropocene.

GENETIC CONSEQUENCES OF HYBRIDIZATION

Although much of this review focuses on the positive ("creative") potential of hybridization in evolution, it is important to stress that hybridization often, and perhaps predominantly, has negative outcomes, ranging from ecological selection against hybrids to more fundamental genetic and/or genomic obstacles. In this section we outline the more immediate genetic consequences of hybridization, both positive and negative, and the different means by which populations and genomes can stabilize after hybridization.

Hybridization can result in distinct evolutionary outcomes depending on the parental contributors and the processes involved in the stabilization of hybrid populations (Arnold 1992). A fundamental initial filter for hybrid success and subsequent genetic outcomes concerns whether or not the F1 and subsequent hybrid generations are viable and fertile, which is influenced by the genetic composition of the parental lineages and the environments in which they live (Yakimowski and Rieseberg 2014; Moran et al. 2021). Ecological selection against F1 hybrids may occur due to abiotic filtering of unsuitable intermediate phenotypes from either parent's niche (e.g., in Encelia, DiVittorio et al. 2020; in Mimulus, Kenney and Sweigart 2016). Pollinator and herbivore interactions may also be disrupted in F1 hybrids (e.g., in Mimulus, Schemske and Bradshaw 1999; in Eucalyptus, Whitham et al. 1994). Regarding innate reproductive isolation mechanisms, both genetic incompatibilities (reviewed in Bomblies 2010) and the accumulation of deleterious alleles (hybridization load) can negatively impact the viability and fitness of hybrid individuals. Fertility in particular is often compromised by incompatibilities (e.g., in Mimulus, Sweigart et al. 2006). However, the extent of hybrid sterility varies greatly, and even with highly limited fertility and rarely viable offspring, there is potential for hybrid success, especially since fertility can increase with subsequent generations and the process of polyploidization can "rescue" hybrids from sterility (Stebbins 1950). Assuming some level of fertility in hybrids, several processes can stabilize F_1 hybrids or subsequent hybrid generations and influence evolutionary outcomes. These include polyploidization, introgression, and recombinational speciation (Grant 1971).

In the context of hybridization, polyploidy is significant in several ways. With hybrids that are partially or largely sterile due to chromosomal incompatibilities, polyploidy can lead to the recovery of fertility. Polyploidy can also result in reproductive isolation from parental lineages, effectively serving as an "instantaneous" speciation mechanism. Polyploidy is also significant in that it results in fixed heterosis, while other forms of hybrid stabilization typically result in the rapid erosion of heterozygosity. The importance of allopolyploidy (i.e., polyploidy involving hybridization between different species, also termed amphiploidy) in plant evolution has long been emphasized. Stebbins (1959) remarked that "[a]mphiploidy, or the production of stable, true-breeding new species through the doubling of chromosome number of a sterile interspecific hybrid, is now generally recognized as one of the commonest ways in which plant species arise." A survey by Wood et al. (2009) found that, in angiosperms and ferns, 15% and 31% of speciation events, respectively, are accompanied by ploidy increases, generally underscoring the importance of polyploidy in plant speciation. Understanding the long-term evolutionary consequences of polyploidization is an area of active research, building off recent studies documenting the presence of ancient polyploidy in most major plant lineages (e.g., Jiao et al. 2011, Jiao et al. 2014; Li et al. 2015; Leebens-Mack et al. 2019; Stull et al. 2021).

Another means by which hybrid populations/generations can stabilize is through recombinational speciation, whereby segregation and recombination of chromosomal variants of the two parents result in hybrids that are both fertile and reproductively isolated from their parental populations. This is perhaps the primary mechanism of homoploid hybrid speciation (Coyne and Orr 2004). As noted by Yakimowski and Rieseberg (2014), this model of hybrid speciation was initially proposed by Stebbins (1957a, 1959) and then more formally developed and named by Grant (1958). While this mode of hybrid speciation appears to be much less common than hybrid polyploid speciation, there are multiple empirical examples (e.g., Stebbins 1957b; Grant 1971, 1981; Rieseberg 1991).

Introgression is another primary means by which the products of hybridization can become established and effectively contribute to evolution. Introgression occurs when hybrids, after F_1 formation, subsequently backcross with one or both parental species, with demographic processes and selection on recombinant types quickly winnowing heterozygosity. This process can result in the transfer of a few genes or chromosomal segments from one species to another. It has long been appreciated that introgression could serve as a major source of variation for evolution (Anderson 1949)—a means of generating considerable variation and perhaps novel features more rapidly than could other processes such as mutation (Abbott et al. 2013; Soltis 2013; Suarez-Gonzalez et al. 2018). While adaptive introgression (i.e., the transfer of fitness-increasing alleles from one population to another) is widely recognized as an important process (Edelman and Mallet 2021), we note that introgression can also break up adaptive allele combinations (segregation load) and result in the transfer of deleterious mutations (hybridization load), resulting in fitness decreases (Moran et al. 2021).

Introgression can manifest in different ways. In some cases, introgression might result in one widespread species effectively swamping into extinction a second, rare species (Todesco et al. 2016). Hybrid populations could be relatively ephemeral, with a relatively brief phase of backcrossing with one or both parents, such that both parental lineages largely retain evolutionary independence. With hybrid swarms, however, persistent hybrid populations can contribute to ongoing backcrossing and gene flow between the two parental species, perhaps resulting in more blurred species boundaries, at least at geographic points of contact between hybrid and parental populations. Syngameons, groups of related species that engage in frequent hybridization, represent another context where introgression is common and potentially a significant source of adaptive variation (Lotsy 1925; Grant 1971), a topic that will be discussed further below.

EXAMPLES OF ANCIENT HYBRIDIZATION

Below we review examples of well-documented cases of ancient hybridization (in the sense of our genomic definition above) across vascular plant phylogeny that occurred at different points in geologic history. Several clades (e.g., Fagales and Rosaceae) are discussed in greater detail because they have experienced extensive hybridization throughout their evolutionary history. The examples discussed here are not meant to be comprehensive but instead highlight that ancient hybridization spans the phylogenetic breadth of vascular plants and underscore major themes concerning the evolutionary significance of deep reticulation and the challenges of detecting it. We also note that one way to bolster inferences of ancient hybridization is through reconstructions of ancestral ecological niches and paleo-distributions, which can aid in evaluating the plausibility of ancient hybridization events suggested by phylogenomic evidence. This type of approach is exemplified by Folk et al. (2018b), examining ancient hybridization in Saxifragaceae, which we discuss further below. While most studies of this type have focused on putative hybridization events during the Pleistocene (e.g., Ma et al. 2019), there is clearly potential to apply such approaches to deeper time scales as paleoclimatic models permit (Folk et al. 2022).

Rosids

The large rosid clade of angiosperms (~90,000 species; Sun et al. 2020) represents one of the first documented cases of ancient reticulation involving major angiosperm lineages. The placement of the rosid subclade of Celastrales, Oxalidales, and Malpighiales (known as the COM clade, including ~19,000 species) has been highly contentious, with different data sets supporting very different placements (Sun et al. 2015; reviewed in Soltis et al. 2018). Chloroplast genes support a relationship between the COM clade and members of Fabidae, one of the two large rosid subclades. In contrast, mtDNA and nuclear genes both support a relationship between the COM clade and members of Malvidae, the other large rosid subclade (e.g., Duarte et al. 2010; Soltis et al., 2011; Zhang et al. 2012). Several morphological features, including an unusual type of ovule and contorted petals, also support the placement of the COM clade with malvids (Endress and Matthews 2006). Signals for both conflicting topologies (i.e., COM with fabids vs. COM with malvids) are present in nuclear data and were likely not caused by sampling or systematic errors, supporting a biological source of conflict such as reticulation or ancient introgression during the early diversification and rapid radiation of the rosid clade (Sun et al. 2015).

Saxifragaceae

Multiple examples of deep hybridization have been documented in Saxifragaceae. One of the most noteworthy of these involves the well-supported capture of the chloroplast genome from the ancestor of two species of *Mitella* (currently with a circumboreal distribution) by the ancestor of a clade of five species of Heuchera from southern Californian (Folk et al. 2018b). Extant members of these two clades are currently separated by ca. 1,300 km, which, at face value, might suggest that hybridization between these two lineages is improbable. However, Folk et al. (2018b) used a multifaceted approach employing niche modeling and estimates of ancestral ranges to show that the two clades potentially shared suitable habitat during the Pleistocene, when the reconstructed geographic distributions of the clades overlapped in southern California (Fig. 3). During that time, the analyses revealed that the distribution of the *Mitella* clade ancestor was pushed well to the south, providing an opportunity for ancient hybridization and gene flow.

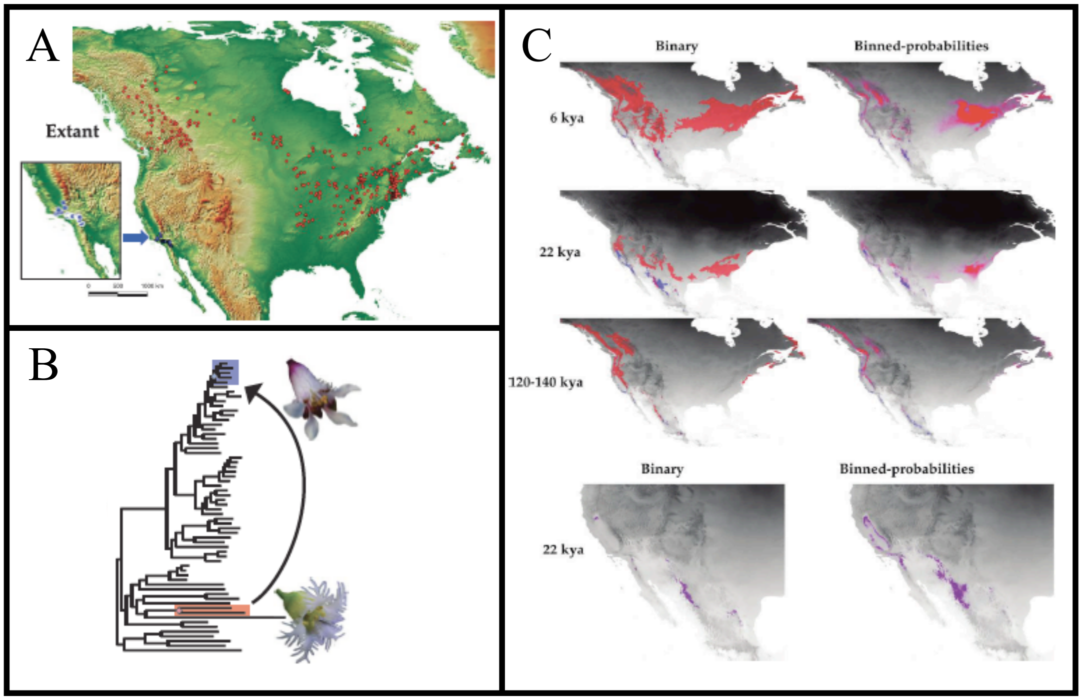

Figure 3. Adapted from figs. 1 and 5 of Folk et al. (2018). (A) Current distribution of *Heuchera* (blue) and *Mitella* (red). (B) Phylogenetic positions of the focal clades (*Heuchera*, blue; *Mitella*, red). (C) Projections of ancestral geographic ranges based on estimates of ancestral niche space for *Heuchera* (blue) and *Mitella* (red).

Fagales

Oaks (*Quercus*) have long been an exemplar for hybridization, with a reputation for rampant hybridization and phenotypic variability (Palmer 1948; Hardin 1975). Recent hybridization is thought to occur only among species within the same taxonomic section (Palmer 1948; Hardin 1975), but evidence also suggests that ancient hybridization events occurred between the ancestors of different sections of Quercus as well as between those of different genera of subfamily Quercoideae of Fagaceae. Almost every genus in Fagaceae has been described as sharing chloroplast haplotypes with another (Manos et al. 2008; Xiang et al. 2014; Simeone et al. 2016; Yang et al. 2021), belying nuclear and morphological data that resolve them as monophyletic (Manos et al. 2001; Oh and Manos 2008). This cytonuclear incongruence indicates that long-retained signals of either ILS or hybridization have persisted in these genomes for millions of years. Almost all studies found that haplotypes clustered by geography, with a North-Central American and a Eurasian clade, similar to chloroplast haplotype clustering by geography within Quercus itself (Whittemore and Schaal 1991; Petit et al. 1993; Dumolin-Lapegue et al. 1997; Simeone et al. 2016).

The extent of ancient hybridization in oaks and relatives in Quercoideae revealed within the nuclear genome has been the focus of several recent studies. Liu et al. (in review) tested for signals of hybridization using hundreds of nuclear loci and full chloroplast genomes for over 400 species in Fagaceae and recovered geographic structuring of chloroplast haplotypes and nuclear evidence of multiple deep reticulation events between oaks and other genera in Fagaceae. Similarly, Zhou et al. (2022; Fig. 4) used over 2000 nuclear loci and complete chloroplast genomes to investigate signals of hybridization for over 90 species across Fagaceae and also recovered geographic clustering of chloroplast genotypes and rampant cytonuclear discordance at the base of the clade containing *Castanea*, *Castanopsis*, *Chrysolepis*, *Lithocarpus*, *Notholithocarpus*, and *Quercus*. However, analyses of nuclear genes did not detect deep reticulation and found strong evidence of hybridization only within *Quercus*.

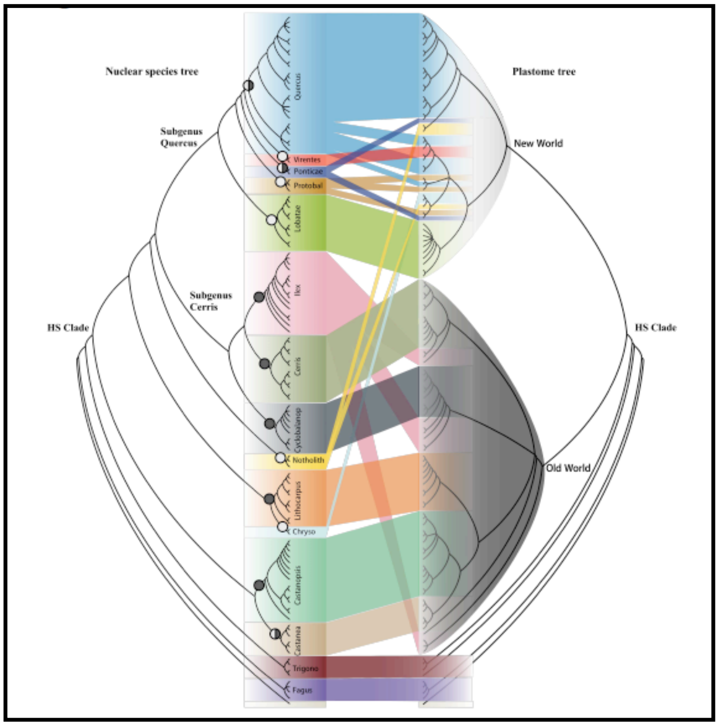

Figure 4. From Zhou et al. (2022; fig. 2) showing conflicts between nuclear and chloroplast phylogenies.

As evidenced by these studies, the chloroplast genome has retained a much stronger signal of ancient hybridization than the nuclear genome. The geographic clustering evident at even deep relationships implies that hybridization between the ancestors of *Quercus* and other genera was common enough to be preserved millions of years later. In contrast, recombination and continued divergence of introgressed haploblocks in the nuclear genome have made it much harder to identify which regions of the genome may have been exchanged by the ancestors of these genera. At the shallower scale, it is thought that the geographic clustering of chloroplast haplotypes between oak species is a signal of the confinement of many species to glacial refugia during the Pleistocene, which provided an opportunity for hybridization, and their subsequent northward recolonization when climates warmed afterwards (Petit et al. 2002). Given the association of hybridization with rapid changes in climate (e.g., Buggs 2007; Ryan et al. 2018), it is possible that the deeper events detected in these studies arose from similar circumstances of environmental upheaval. Oaks and their ancestors seem to undergo periodic cycles of hybridization and diversification corresponding to fluctuations in their ranges; Cannon and Petit (2020) suggest that these cycles and the group's ability to hybridize is actually adaptive in itself, allowing for species in the group to incorporate genetic variation from relatives which could help them to respond to changes in climate. Given recent evidence for introgression of genes associated with drought, waterlogging, and budbreak in several species of Quercus (Fitz-Gibbon et al. 2017; Leroy et al. 2020), the adaptive function of many introgressed alleles seems at the very least plausible. However, considering the difficulties of even identifying signals of ancient hybridization in the nuclear genome, verification of adaptive introgression of nuclear genes during ancient hybridization may be infeasible in most cases.

Ancient hybridization has been reported in other clades of Fagales (Zhang et al. 2019; Cardoni et al. 2021), with Betulaceae being a salient example. Wang et al. (2022), using genome resequencing to investigate ancient hybridization in Coryloideae (Betulaceae), found that Carpinus sect. Distegocarpus likely originated via homoploid hybrid speciation between the parental lineages Carpinus sect. Carpinus and Ostrya. Given that similar patterns of genomic admixture are shared between all three species of sect. Distegocarpus and the putative parental lineages, this reticulation event (dated at 17-33 mya) likely corresponds closely to the origin of the section, which subsequently diversified into three species (*Carpinus cordata*, *C. fangiana*, and *C. japonica*).

Rosaceae

Rosaceae are another excellent example of a clade with extensive hybridization throughout its evolutionary history, with numerous documented cases between closely related extant species (e.g., in *Rubus*, *Fragaria*, *Potentilla*, *Rosa*, Spiraea; Šarhanová et al. 2017; Debray et al. 2021; Qiao et al. 2021) as well as more ancient cases between the ancestors of small to large clades within the family (e.g., Morales-Briones et al. 2018; Hodel et al. 2021, 2022). The many examples of hybridization in Rosaceae illustrate well how the process can function as an evolutionary force, contributing to speciation and the origins of phenotypic and phylogenetic diversity.

Hybridization has long been considered common in *Prunus*, and molecular data reveal that it is indeed rampant. Cultivated species frequently hybridize with each other as well as with wild species. For example, the rare Eurasian shrub *Prunus fruticosa* (ground cherry) has hybridized with cultivated *P.* cerasus (Macková et al. 2018), while genomic data suggest extensive natural interspecific hybridization among sympatric species of Prunus (Baek et al. 2018). At a deeper level in Prunus, Hodel et al. (2021) documented a hybridization event involving subgenus Amygdalus and subgenus Prunus using a phylogenomic approach. In Rubus, hybridization is not only common among close relatives, but is also one of the driving forces in the formation of naturally occurring apomictic lineages in subgenus Rubus in Europe (Šarhanová et al. 2017). In the Pacific Northwest of the U.S., hybridization between native and introduced species of Rubus is generating novel genotypes that may represent the early stages of newly formed invasive species. Alice et al. (2001) documented hybridization between distant relatives (i.e., *R. caesius* from subgenus *Rubus* and *R. idaeus* from subgenus *Idaeobatus*); most of the detected hybrids appeared to be later-generation hybrids, suggesting that hybridization between these distantly related species has the potential for generating new species, or at least genetic diversity. *Alchemilla* also shows signatures of hybridization at both shallow and deep phylogenetic levels (Morales-Briones et al. 2018).

The apple tribe (Maleae) has also long been suspected to have a hybrid and/or allopolyploid origin given its high base chromosome number (x = 17) relative to other Rosaceae lineages (with x = 5, 7, 8, or 9), and several alternative hybridization scenarios have been hypothesized to explain Maleae origins, generally involving ancestors of amygdaloid or spiraeoid lineages (Evans and Campbell 2002). Recent phylogenomic analyses (Hodel et al. 2022) suggest that the ancestor of the Maleae (x = 17) + Gillenieae (x = 9) arose via hybridization between two distantly related Amygdaloideae lineages: (1) an ancestor of tribe Spiraeeae (x = 9) and (2) an ancestor of the clade Sorbarieae (x = 9) + Exochordeae (x = 8) + Kerrieae (x = 9); this was then followed by polyploidy (x = 18) and aneuploidy (x = 17) in the Maleae lineage, resulting in the apple tribe's current base chromosome number.

Asterids

Phylogenetic analyses of asterids based primarily on the chloroplast genome have consistently recovered Cornales and Ericales as successive sisters to the core asterids (e.g., Albach et al. 2001; Soltis et al. 2011; Stull et al. 2015), while data from the nuclear genome has supported Cornales and Ericales as sister (e.g., Zhang et al. 2012). In an in-depth comparison of asterid phylogeny based on chloroplast and nuclear datasets, Stull et al. (2020) determined that these conflicting relationships likely stem from hybridization during the early diversification of asterids (Fig. 5). The predominant nuclear signal (⅔ of well-supported nuclear genes) supports a sister relationship between Cornales and Ericales, but ⅓ of the nuclear genes support a sister relationship between Ericales and *Gentianidae* (the chloroplast topology). Along with network analyses and coalescent simulations, these results suggest the possibility that Ericales is a reticulate lineage resulting from hybridization between ancestors of Cornales and *Gentianidae*.

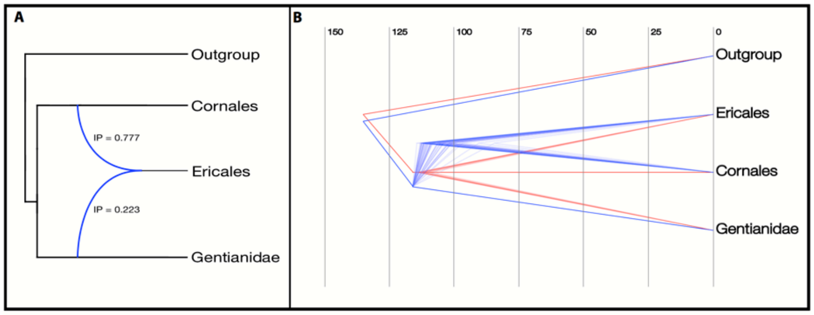

Figure 5. From Stull et al. (2020; fig. 5) showing (A) the optimal inferred phylogenetic network for asterids, with Ericales as a reticulate lineage, and (b) dated nuclear gene trees supporting alternative resolutions of asterid phylogeny.

Caryophyllales

Most studies examining ancient whole-genome duplication (WGD) do not specifically address whether inferred WGD events were caused by auto- or allopolyploidy, but this distinction is critically important for a better understanding of how polyploidy has shaped plant evolution. Yang et al. (2018), in a phylotranscriptomic study of Caryophyllales, devised an approach for inferring auto- vs. allopolyploidy from orthogroup trees, finding strong support for two ancient allopolyploidy events, one in Amaranthaceae and another in the ancestor of *Schiedea* (Caryophyllaceae). The type of polyploidy (auto- vs. allo-) responsible for the remaining 24 WGD events, however, appeared to be unclear. The question of whether most ancient WGD events represent auto- or allopolyploidy is one that deserves greater attention. It is also worth noting that some cases of presumed ancient homoploid hybridization could represent allopolyploidy obscured by subsequent diploidization.

Amaryllidaceae

Tribe Hippeastreae (Amaryllidaceae) provides an example of deep reticulation in the monocots. The tribe consists of 10–13 genera and ca. 180 species. Phylogenetic analyses of nuclear and chloroplast regions revealed two well-supported clades within the tribe (Garcia et al. 2014). Within one of these clades, the mainly Neotropical Hippeastrinae, Garcia et al. (2014, 2017) uncovered widespread cytonuclear discordance and rampant non-monophyly of genera. Closer examination of the data revealed that the discordance is likely due to numerous ancient hybridization events (some associated with polyploidy) preceding the radiation of this major clade (Garcia et al. 2017). In contrast, phylogenetic analysis of the second clade of Hippeastreae, subtribe Traubiinae, shows a tree-like pattern, consistent with the apparent absence of hybridization and allopolyploidy in this clade (Garcia et al. 2017).

Mesangiosperms

Despite intensive study, phylogenetic relationships among the five clades (magnoliids, monocots, eudicots, Chloranthales, Ceratophyllales) of *Mesangiospermae* have remained uncertain due to their rapid radiation (Moore et al. 2007). Several phylogenetic analyses have recovered Chloranthaceae sister to magnoliids (reviewed in Soltis et al. 2019). However, incongruence has long been noted between nuclear and chloroplast DNA datasets regarding the placement of magnoliids + Chloranthaceae (or magnoliids alone in those studies in which Chloranthaceae were not included) relative to other mesangiosperms. Chloroplast trees have routinely recovered magnoliids and Chloranthaceae as sister to a clade of monocots plus eudicots; in contrast, phylogenetic analyses of nuclear genes typically placed monocots as the sister to a clade of magnoliids, Chloranthaceae, and eudicots (e.g., Moore et al. 2007; Leebens-Mack et al. 2019; Chaw et al. 2019; Chen et al. 2019; Li et al. 2019; Soltis et al. 2011). Using complete nuclear genome sequences for *Amborella* and representatives of all mesangiosperm lineages, Guo et al. (2021) conducted a variety of phylogenomic analyses to understand the conflict underlying these contentious relationships, finding that ancient hybridization is a plausible cause for the observed conflict concerning the placement of Chloranthales + magnoliids relative to eudicots and monocots.

Gymnosperms

Increasing evidence for WGD (both recent and ancient; Li et al. 2015; Wu et al. 2016; Leebens-Mack et al. 2019; Stull et al. 2021) and (relatively) recent hybridization (Worth et al. 2016; Sullivan et al. 2017; Ma et al. 2019) in various lineages of gymnosperms suggests a possible role for ancient hybridization in gymnosperm evolution, but well-documented examples are relatively few compared to those of flowering plants. However, one example comes from Cupressaceae: Liu et al. (2022) found evidence for ancient hybridization involving the ancestors of two clades of Cupressoideae, one including *Microbiota*, *Platycladus*, and *Tetraclinis* and the other including *Juniperus*, *Cupressus*, *Hesperocyparis*, *Callitropsis*, and *Xanthocyparis*. One intriguing incongruence that deserves further attention concerns the phylogenetic placement of *Ginkgo* (Stull et al. 2021). Most nuclear genes place *Ginkgo* sister to cycads, but an appreciable number of genes support *Ginkgo* sister to conifers, consistent with many morphological phylogenetic analyses of seed plants (e.g., Crane 1985). Phylogenomic analyses also support an ancient WGD in the common ancestor of extant gymnosperms (Stull et al. 2021; Liu et al. 2022), the branch subtending these alternative placements of Ginkgo.

Ferns

Hybridization and allopolyploidy are also common across ferns (Barrington et al. 1989; Otto and Whitton 2000; Sigel 2016; Leebens-Mack et al. 2019), and recent emphasis has been placed on examples of “deep hybridization” in ferns. However, these reports generally pertain to recently formed hybrids among distantly related lineages, rather than to instances of hybridization that occurred in the distant past. For example, xCystocarpium roskamianum is a recently formed inter-generic hybrid between Gymnocarpium and Cystopteris, which diverged ca. 60 mya (Rothfels et al. 2015). Similarly, xLindsaeosoria flynnii is another recently formed inter-generic hybrid involving Lindsaea and Odontosoria, whose divergence perhaps traces back to the Mesozoic (Lehtonen 2018).

Ranker and Sundue (2015) suggested that ferns exhibit slower evolution of reproductive barriers than angiosperms, which should facilitate hybridization in general, including among more distantly related lineages than is typical for other plant clades. If correct, hybridization among fern species should be widespread. But the extent of ancient hybridization in fern evolutionary history remains unclear. The best information from phylogenomics comes from the Leebens-Mack et al. (2019), which provides evidence for shallow as well as some deep polyploidy events, implicating ancient hybridization if allopolyploidy was at play. Additionally, recently published, high-quality fern genomes and transcriptomes provide strong evidence for a few ancient polyploidy events in ferns, with several polyploidy events inferred in the common ancestors of smaller orders and families (Huang et al. 2019, Pelosi et al. 2022), one associated with the origin of core leptosporangiate ferns (Li et al. 2018; Huang et al. 2019; Huang et al. 2022; Marchant et al. 2022) and possibly another associated with much of Polypodiales (Leebens-Mack et al. 2019; Pelosi et al. 2022). However, there remain few robust, large-scale comparisons of fern phylogeny based on comparably sampled nuclear vs. chloroplast datasets. Furthermore, because of their large and complex genomes, complete genome sequencing of ferns, which could reveal such ancient signatures, is in its infancy.

MACROEVOLUTIONARY SIGNIFICANCE OF HYBRIDIZATION

Hybridization can be viewed as a fundamentally microevolutionary process given that it essentially represents a form of gene flow that can either lead to the establishment of hybrid populations or genetic introgression from one species or population to another, and as a consequence, it influences the pool of variation on which selection can operate. However, it may have broader macroevolutionary significance in the sense that it may either influence or accompany other broader patterns in plant evolution. In particular, the reality that hybridization can occur among divergent populations as well as lineages considered taxonomically to be separate species underscores that hybridization is a phenomenon that in some senses transcends evolutionary scales. Given the capacity of hybridization to reconnect diverging lineages and potentially impart lasting influence on evolutionary trajectories, evaluation of the significance of hybridization events whose signals appear across broader evolutionary scales is warranted. It is also worth considering both biotic and abiotic circumstances that might influence the frequency of hybridization among different lineages and at different times in Earth history. In this section, we consider the macroevolutionary significance of hybridization. We do not use the term macroevolution in any specific mechanistic sense (e.g., in reference to species selection), but instead as a general umbrella for considering how hybridization might influence broader patterns of plant evolution, and how different circumstances or traits might influence the frequency of hybridization across space, time, and phylogeny.

The ecological, geographic, and geologic context of hybridization

One of the significant themes to come out of the last 70 years of hybridization work is the association of hybridization with ecological and range change. Many classic cases of hybridization date to the Pleistocene, as ranges of different species were brought into secondary contact in glacial refugia or subsequently following range expansion during interglacial periods (e.g., Anderson and Stebbins 1954; Petit et al. 1997, Edwards et al. 2006; Joly et al. 2009b). Other periods of significant geologic and environmental change might also have corresponded with increased levels of hybridization. For example, the availability of land bridges in the Northern Hemisphere during the early-mid Eocene may have facilitated oak migrations between Eurasia and the Americas, bringing lineages into contact and resulting in hybridization (Liu et al. in review). Similarly, climate change in the late Miocene to Pliocene might have brought various oak lineages into contact due to range shifts, again resulting in widespread hybridization (Zhou et al. 2022). The Cretaceous-Paleogene (K-Pg) mass extinction may have coincided with (or have been followed by or possibly even driven) a wave of polyploidization (Fawcett et al. 2009; Van de Peer et al. 2021), although further research is needed to evaluate the strength of this possible connection. This suggested wave has been explained both by the enhanced persistence of the polyploids themselves, as well as a possible increase in unreduced gamete production and diploid hybridization caused by the extreme environmental and disturbed ecological conditions present in the wake of the asteroid impact (Levin and Soltis, 2018; Levin 2020). Of course, responses to climate change and ecological disturbance can be lineage-specific and idiosyncratic, and so different geologic periods and environmental contexts might impact lineages very differently with regard to range shifts and patterns of gene flow. But nevertheless, periods of Earth history (and regions of the globe) that experienced bouts of ecological disturbance, climate change, and/or intermittent land connections (with consequent community-level range fluctuations) might have experienced increased frequencies of hybridization.

During remarkable phases of diversification of major lineages (e.g., angiosperms), there may have been generally increased opportunities for hybridization as well, simply as a byproduct of the presence of numerous closely related lineages (lacking pre- or postzygotic barriers) in close proximity. For example, the large-scale ecological shift from gymno- to angiosperm dominance in the mid-Cretaceous (at a global scale) might represent a unique period in angiosperm history where diversification rates were elevated, ranges were shifting relatively rapidly, and as a result hybridization was particularly common (Boulter et al. 1988); this is not inconsistent with the numerous angiosperm phylogenies showing considerable genomic conflict at nodes dating to the early-mid Cretaceous. Of course, rapid diversification itself can be a source of conflict (via ILS), and angiosperms have undergone repeated bouts of remarkable diversification (Magallón and Castillo 2009; Tank et al. 2015; Landis et al. 2018), so if there is any merit to the notion that the initial emergence and radiation of major lineages is often accompanied by increased levels of hybridization, we would expect to see periodic bursts of hybridization throughout geologic history at different locations in plant phylogeny.

It is important to note that the association of hybridization with large-scale geographic patterns is not new; it could be viewed as an extension of classic theory about ecological control of hybridization. Anderson's "hybridization of the habitat" (Anderson 1948) centered the importance of ecological circumstances in the promotion of hybrid formation and establishment. Visible early-generation hybrids were often spotted in anthropogenically disturbed places and in the transition zones between two types of habitats ("hybrid habitats"), leading Anderson to conclude that hybrids were probably excluded from parental ranges via ecological filtering or competitive exclusion with parents. Rapid environmental changes might in some cases result in "hybrid habitats" due to the establishment of large areas of unique habitat or a transition zone where no parental species is well-adapted, allowing for the persistence of hybrids. Another interpretation is that these "hybrid habitats" are simply points of contact between different species ranges and habitats, and as climates change, these areas of contact will naturally shift as well, creating more opportunities for hybridization.

Trait and phylogenetic correlations with hybridization frequency

Several attempts have been made to characterize traits commonly associated with hybridization, as well as to identify lineages in which hybridization is more frequent, perhaps as a consequence of the traits they possess (e.g., Grant 1971; Ellstrand et al. 1996; Whitney et al. 2010; Mitchell et al. 2019). While some traits might generally facilitate hybrid formation, others seem potentially important for facilitating hybrid persistence. Grant (1971) described several different life history paradigms associated with different hybridization patterns, observing that homoploid syngameons (e.g., Ceanothus) were usually long-lived woody species with stable chromosome structure, large population size, and often (but not always) wind pollination. Ellstrand et al. (1996) found strong taxonomic clustering of hybridizing taxa, as well as a tendency for hybrids to be outcrossing perennials with the ability to reproduce clonally; however, they did not differentiate between polyploid and homoploid hybrids in their study. We expect chromosomes of homoploid hybrid taxa to be comparatively conserved with regards to structure and sequence; otherwise viable hybrid offspring would be unlikely (without clonality to allow the persistence of hybrids with odd chromosome pairs). This corresponds with Grant's (1971) observation that trees often form syngameons; their long generation times result in slower rates of evolution, increasing the chance that diverged species will still maintain enough genetic similarity to cross successfully.

Wind pollination, as a more promiscuous form of pollen spread than animal-mediated pollination, is often thought to facilitate hybridization. Wind pollination is a feature of many taxa famous for homoploid hybridization (e.g., *Populus*, *Quercus*, *Nothofagus*; Smith and Sytsma 1990; Williams et al. 2001; Acosta and Premoli 2018), but many other groups in which hybridization is common are insect-pollinated (e.g., Helianthus, Eucalyptus; Rieseberg 1991; Van Diijk et al. 2020), indicating that pollinator choice can lead to porous species barriers. In fact, even in cases of tight pollinator specialization, such as figs, hybridization can still occur, perhaps in particular during pollinator-host switches (Wang et al. 2021). A study by Mitchell et al. (2021) examined the correlation of 11 different traits with hybridization and found weak positive associations between perenniality, woodiness, outcrossing, abiotic pollination, and larger genomes and increased frequencies of hybridization, but some of these results were scale dependent. Hybridization is a complex phenomenon that appears to require the confluence of several to multiple abiotic and biotic factors (and for populations to persist once formed), and so it is not surprising that particular traits generally show inconsistent relationships with hybridization frequency. For example, traits that facilitate hybrid persistence (e.g., perenniality) will not show a strong relationship with hybridization when paired with other traits that prevent hybrid formation in the first place (e.g., high-fidelity biotic pollination).

Through the lens of oak diversity, Cannon and Petit (2020) proposed that the ability to hybridize itself while maintaining species boundaries (i.e., the syngameon strategy) may be adaptive. This hypothesis is intriguing and will require future work to test fully, in particular, to establish which traits would need to be selected to maintain the ability to hybridize and whether these traits are consistent across the Tree of Life. Additional work from a speciation biology paradigm could test the plausibility of maintaining equilibrium between selecting for regions of the genome that preserve the ability to hybridize and other regions that contribute to species boundaries. Another line of investigation could be establishing the timing of diversification and hybridization events in conjunction with selective pressures for the ability to hybridize, which we may even be able to do in real time due to Anthropocene-driven environmental change and ecological disturbance. Of course, these are a few of many possible avenues of investigation regarding this question. Another important problem is obtaining a more complete understanding of how many lineages operate as syngameons. It has been suggested that many diverse tropical tree genera might represent syngameons, but these are more poorly understood compared to temperate systems (but see Larson et al. 2021).

Hybridization and species diversification

While perhaps more attention has been paid to hybridization as a vehicle for the evolution of novel phenotypes (Anderson and Stebbins 1954; Seehausen 2004; Suarez-Gonzalez et al. 2018), there has also been interest in whether hybridization might directly influence diversification rates in plants and other organisms. There are several ways that hybridization could influence diversification rates, either positively or negatively; hybridization could also in some cases impact standing levels of biodiversity without any influence on diversification rates per se. When hybridization (and the resulting rich genetic variation) is paired with the right ecological circumstances (e.g., the colonization of an island system by a hybrid population), this could lead to an adaptive radiation (Barrier et al. 1999; Choi et al. 2021) and, presumably, increased rates of speciation in the radiating lineage. Conversely, hybridization could lead to decreased speciation rates (or simply decreased levels of standing diversity) via the merging of recently divergent lineages upon secondary contact. However, there are also deeper theoretical concerns regarding the relationship between diversification and hybridization. Diversification itself might create more opportunities for hybridization—given close geographic proximity of multiple, minimally divergent lineages—and so the observation of species-rich lineages with rampant hybridization is not in itself sufficient evidence that hybridization is the driving force. In other words, disentangling the causal relationship of these two processes is not trivial (Mitchell and Whitney 2021), and perhaps in some cases there is a positive feedback of interrelated causality leading to increases in both. A recent study explicitly examining the relationship between hybridization and diversification (Mitchell and Whitney 2021) overall found a relatively limited relationship between these phenomena at a broad scale, although a stronger positive relationship was observed when taking life history traits (e.g., perenniality and woodiness) into consideration.

Diversification rates aside, hybridization (particularly in association with polyploidy) can hypothetically increase standing diversity via a ratchet mechanism (Scarpino et al. 2014), whereby allopolyploidy produces distinct lineages that are reproductively isolated from their parents. In clades where hybridization/allopolyploidy is ubiquitous, such as Rosaceae, this ratchet mechanism can perhaps lead to appreciable increases in species diversity without directly imparting any influence on inherent speciation rates in the conventional sense (Vamosi and Dickinson 2006). However, this raises the question of whether the resulting allopolyploidy lineages themselves might generally have elevated rates of diversification, but prevailing evidence suggests there is generally a limited relationship between polyploidization events and diversification (Tank et al. 2015; Landis et al. 2018; Smith et al. 2018; Stull et al. 2021), although this may, in part, be a data-deficient problem. Overall, the relationship between hybridization and diversification might best be characterized as idiosyncratic. We suggest that future research on this topic focus on understanding, for particular clades, the specific contexts in which (or circumstances by which) hybridization influences diversification rates (either positively or negatively) or standing diversity, instead of searching for a general positive or negative relationship between hybridization and diversification across major lineages. It seems likely that the relationship between hybridization and diversification/diversity is too complex to draw meaningful (simple) generalizations at a broad scale, but through focused case studies, more detailed general principles might emerge on how hybridization influences diversification (and vice versa).

NOMENCLATURAL IMPLICATIONS

The chloroplast genome has been the primary source of data in plant phylogenetics for over three decades given the relative ease of isolating, sequencing, and analyzing chloroplast DNA (e.g., Ritland and Clegg 1987; Palmer et al. 1988; Gitzendanner et al. 2018; Li et al. 2019). As a result, our current major classifications (e.g., APG IV 2016; PPG I 2016) are largely based on chloroplast phylogenies. But it was always made clear in the formulation of these classifications that nuclear data would ultimately be required to confirm their accuracy, especially in light of processes such as chloroplast capture that can mislead organismal phylogenies (Rieseberg and Soltis 1991). The surge in availability of nuclear sequence data from target capture, transcriptome, and whole-genome sequencing over the past decade has provided much-needed perspective on vascular plant phylogeny from the nuclear genome. Notably, as reviewed above, many nuclear phylogenomic studies have revealed deep incongruences with plastome phylogenies, in some cases involving the placements of clades recognized as families, orders, or more inclusive named clades (e.g., Folk et al. 2018b; Liu et al. 2022; Leebens-Mack et al. 2019; Stull et al. 2020). This raises the important question of how best to treat these chloroplast-nuclear incongruencies in classifications moving forward. One solution is to continue with the chloroplast placements, as in the APG treatments to date (APG IV 2016), for reasons of stability, with an asterisk or some other means used to indicate that a lineage stems from reticulation and might have an alternative nuclear phylogenetic placement. However, we argue this is an untenable approach given that the nuclear genome is more representative of organismal phylogeny as a whole, and therefore classifications should be updated to reflect the nuclear phylogeny. Then the issue still remains of how to treat or depict reticulate lineages in classification. One option, made possible by the PhyloCode (Cantino and De Queiroz 2020), is to place reticulate lineages in both parental clades. Thus, in the case of the COM clade (Sun et al. 2015), it would be placed in both Malvidae (the predominant nuclear signal) and Fabidae (the predominant chloroplast signal and the APG IV placement), as was done in the PhyloCode treatment of the COM clade (Judd et al. 2020a,b). This approach then raises the question: how admixed must a clade be to warrant this treatment? Arguably, the amount of admixture must be significant, but what counts as significant may differ based on the age and size of the clade. This issue is discussed further below.

In contrast to the PhyloCode, there currently is no readily available means for designating deep reticulation events in the *International Code of Nomenclature for Algae, Fungi, and Plants* (ICN; Turland et al. 2018). Under the ICN, hybrids between species and between species in different genera can be given names. For example, hybrids between *Magnolia denudata* × *Magnolia liliiflora* have been given the name *Magnolia* × *soulangeana*. However, when a person sees the name *Magnolia* × *soulangeana* in the literature, the parents would not be known to the reader without looking into the hybrid name in more detail. A hybrid can also be designated by using an × between the putative parents. For example, one could use *Magnolia denudata* × *Magnolia liliiflora* (to designate *Magnolia* × *soulangeana*). The code also permits the use of new generic names for hybrids between two species from different genera. The generic name *Sorbopyrus* has been used for hybrids between *Sorbus* and *Pyrus*, and ×*Sorbopyrus auricularis* is a hybrid between *Sorbus aria* and *Pyrus communis.* It is possible that the code of nomenclature could be extended to allow the application of these approaches to deep reticulation events involving named lineages above the generic rank. For example, ×Ericales would designate that the Ericales lineage has reticulation in its history or is of reticulate origin (Stull et al. 2020). However, this system of naming would not in itself indicate the putative parents and does not define the alternative clades, as the PhyloCode permits. This could be done by indicating the involved parental lineages in parentheses: ×Ericales (Cornales/*Gentianidae*).

Whether we are currently ready to overhaul (or update) angiosperm classification (or vascular plant classification more broadly) based on available nuclear phylogenies is another question. Recently generated large-scale nuclear datasets are incredibly valuable, but these can also be compromised by analytical challenges including paralogy, data quality, and low-information gene trees, which can result in spurious topologies, especially among major lineages. Many areas of angiosperm phylogeny display high levels of conflict between not only nuclear and chloroplast datasets, but within and among nuclear datasets themselves. Nuclear phylogenomic studies focused on particular problems in angiosperm phylogeny—e.g., asterids (Stull et al. 2020; Zhang et al. 2020), Caryophyllales (Walker et al. 2018; Yang et al. 2018), Caesalpinioideae (Leguminosae; Ringelberg et al. 2022), Commelinales (Zuntini et al. 2021), Gentianales (Antonelli et al. 2021), Orchidaceae (Pérez-Escobar et al. 2021), Piperales (Jost et al. 2021)—perhaps represent a more reliable basis for making specific classification or nomenclatural changes compared with broader analyses of plant phylogeny (Leebens-Mack et al. 2019; Baker et al. 2022).

Another fundamental question is what level of hybridization should we consider sufficiently substantial to be reflected in names and classifications. For example, if lineage A (sister to lineage B) shows a small proportion of nuclear genes (e.g., <5%) reflecting introgression from lineage C, should we seek to reflect this in our classifications? What if the proportion of introgressed genes is higher (e.g., 25%)? Does it matter what relationship the chloroplast genome reflects in such cases? With cytonuclear discordance, the chloroplast genome might support A and C as sister, even if the preponderance of nuclear signal supports A and B as sister. What about a case where 60 percent of the nuclear genome supports A and B as sister, while 40 percent of the nuclear genome (as well as the chloroplast genome) support A and C as sister? The issues raised above will represent important considerations for the plant systematics community moving forward.

DEPICTING AND ANALYZING A RETICULATE TREE OF LIFE

The concept of the Tree of Life is a wonderful metaphor for the evolutionary connectivity of all life but is also problematic biologically in that it assumes a bifurcating tree. We have known for a long time, in part based on rampant hybridization among the tips of the plant branch of the Tree of Life, that this assumption is unrealistic. With growing evidence for reticulation across the depth of green plant evolution, this raises the question of how best to depict plant phylogeny so that it is easy to view yet biologically realistic, while remaining a useful tool for teaching students and educating the public. Phylogenetic relationships in Bacteria and Archaea are generally depicted as a network, given the prevalence of reticulation via horizontal gene transfer (Dagan et al., 2008; Dagan and Martin 2009; Gontier, 2015; Kunin et al. 2005; Fig. 6), and perhaps the plant

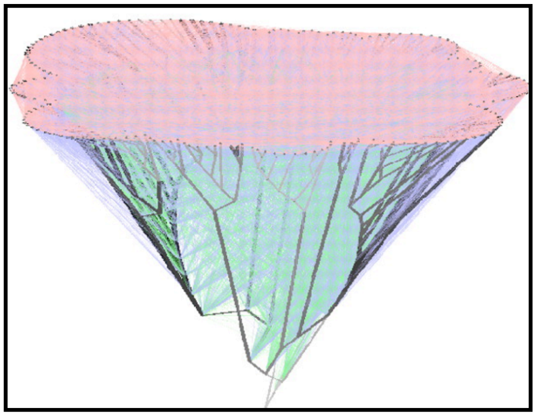

Figure 6. Network representation of prokaryote phylogeny including both vertical inheritance and lateral exchange (from Dagan and Martin 2009; fig. 2).

community should increasingly move toward these types of depictions (Fig. 7)—a Net of Life rather than a Tree of Life for plant evolutionary history. Of course, as noted above, network methods are increasingly being used in the phylogenetics community, but these are extremely limited in their scalability and are often wanting in effective communication of evolutionary patterns. Methods development in reconstructing, visualizing, and applying phylogenetic networks for evolutionary inferences is a critical direction in evolutionary biology that is currently in its infancy (e.g., Hibbins and Hahn 2021). It is important to note that the vast majority of comparative methods aimed at inferring divergence times, diversification rates, ancestral states and trait evolution, biogeographic history, and other aspects of evolutionary history typically require or assume a bifurcating tree. Of course, for many applications, the use of strictly bifurcating trees is likely a reasonable simplifying assumption. But in lineages with rampant hybridization, the reconstruction and analyses of phylogenetic networks will ultimately be essential for a more accurate understanding of evolutionary history and processes.

SUMMARY AND FUTURE DIRECTIONS

Hybridization has been recognized for nearly a century as an important force in plant evolution, but we are only now beginning to appreciate the extent of reticulation in shaping both genomes and their resulting phenomes across the plant branch of the Tree of Life. Not only does hybridization frequently tangle the tips of the Tree, it is also evident among the deeper branches, revealing past evidence of reticulation that renders plant evolutionary history as a potentially complex network, the extent of which is yet to be uncovered. This shift of perspective from a bifurcating tree to a network is not exactly a surprise, but the phylogenetic depth of the network paradigm as revealed by genomic and phylogenomic studies is reshaping our view of the structure of the Tree and requiring new developments in methods of reconstructing, visualizing, communicating, and computing over plant evolutionary history. The genetic consequences envisioned decades ago for recent hybridization are being reinterpreted to accommodate deep time. Moreover, genome sequences and nuclear phylogenies are revealing the complex, mosaic nature of plant genomes and the possible roles that hybridization (with or without WGD) may have played in catalyzing plant diversity, potentially blurring the boundary between microevolutionary processes and macroevolutionary patterns. We hope that this review will likewise catalyze new studies that cross disciplinary boundaries to clarify the legacy and fate of hybridization in plant evolution.

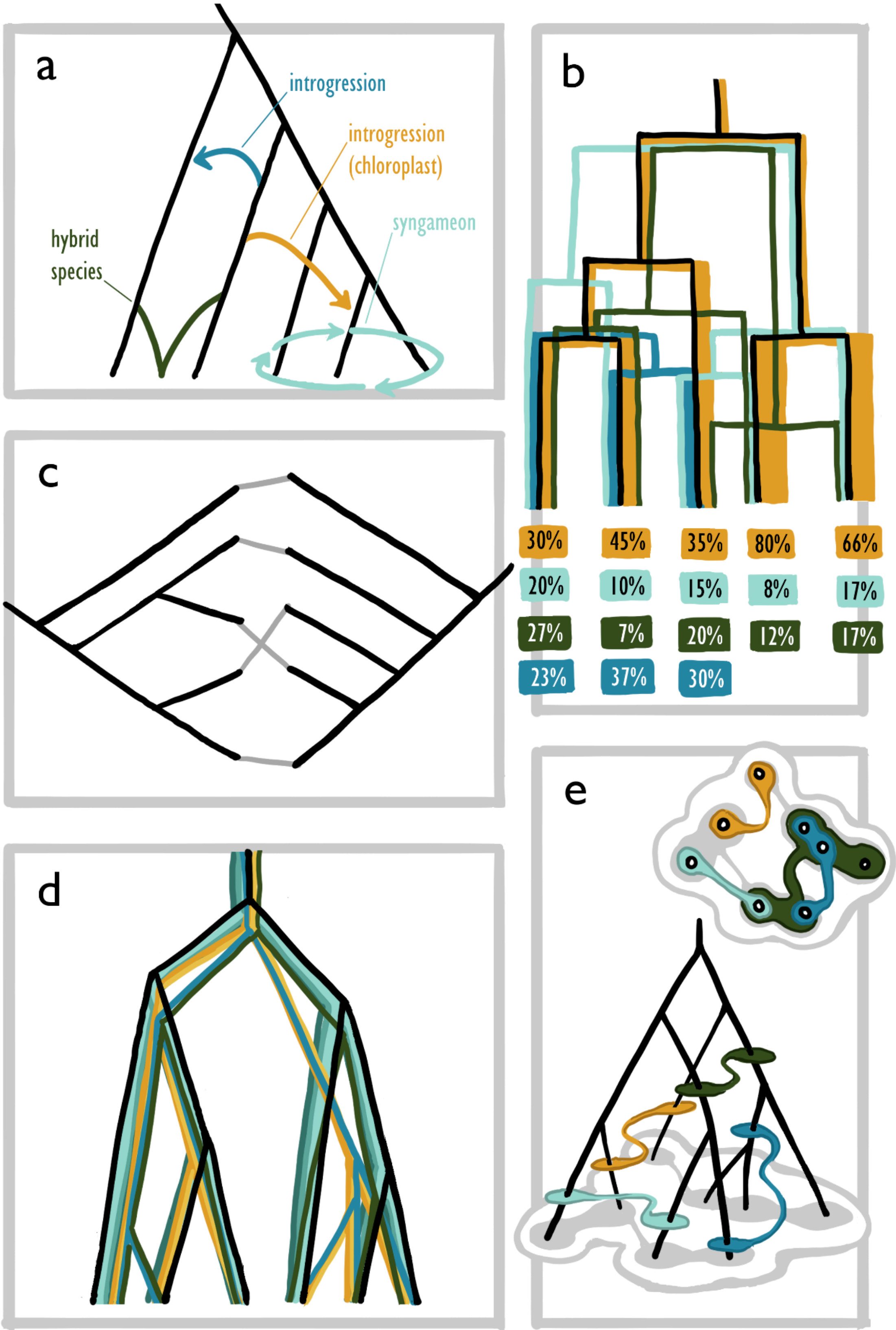
a
introgression
introgression
(chloroplast)
syngameon
hybrid
species
b
30%
45%
35%
80%
66%
20%
10%
15%
8%
17%
27%
7%
20%
12%
17%
23%
37%
30%
c
d
e

Figure 7. Different options for visual representations of reticulation. (a) Phylogeny with reticulation events depicted by arrows or lines between lineages connected by gene flow. This approach is commonly used to display results of phylogenetic network inference. This format is easy to read and can convey directionality (through arrows) and degree (through line weight), but does not directly show alternative topologies. (b) Main phylogeny in front with alternative topologies behind in different colors, with widths at tips proportional to the number of gene trees supporting the topology for each tip. This format directly conveys the degree of gene flow and alternative topologies and emphasizes as well the mosaic nature of genomes. However, it becomes hard to read when depicting too many alternative topologies or too many tips. (c) Tanglegram, two alternative topologies facing each other, with connections between corresponding tips. This method is commonly used to depict differences between two main topologies from different sources, such as nuclear and chloroplast trees. This format allows for easy visualization of group-wide patterns and alternative topologies, but can only accommodate two topologies at once, and thus may not be able to accommodate complicated multi-event reticulation scenarios or fully capture the extent of gene-tree discordance. (d) Main phylogeny in front with a cloud of alternative topologies plotted behind, one for every locus analyzed. Commonly used for large, genome-wide datasets. This format depicts each alternative topology and its amount of support, making it useful for comparing overall trends in the dataset. However, it can be hard to read, depending on the number and distribution of alternative topologies. (e) Main phylogeny plotted in three dimensions, with reticulation events depicted by lines between lineages connected by gene flow. Tips of the phylogeny are projected onto a two-dimensional cross-section with groupings of shared ancestry in gray and groupings based on reticulation events in colors corresponding to lines in three-dimensional phylogeny. Similar in concept to (a), but the three-dimensional rendering may allow for depictions of more reticulation events on one tree, and the two-dimensional projection quickly shows "main" and "reticulation" clusters of evolutionary similarity. Currently, to our knowledge, there are no tools for depicting phylogenies in three dimensions or tools

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