

## INVITED SPECIAL ARTICLE

For the Special Issue: Using and Navigating the Plant Tree of Life

# New prospects in the detection and comparative analysis of hybridization in the tree of life

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## Abstract

Assessing the relative importance of the various pathways to diversification is a central goal of biodiversity researchers. For plant biologists, and increasingly across the spectrum of biological sciences, among these pathways of interest is hybridization. New methodological developments are moving the field away from questions of whether natural hybridization occurs or hybrids can persist and toward more direct assessments of the long-term impact of hybridization on diversification and genome organization. Advances in theory and new data, especially phylogenomic data, have changed the face of this field, revealing extensive occurrences of hybridization at both shallow and deep levels, but lacking is a synthesis of these advancements. Here we provide an overview of methods that have been proposed for detecting hybridization with molecular data and advocate a time-extended, comparative view of reticulate evolution. In particular, we pose three overarching questions, newly placed within reach, that are critical for advancing our understanding of hybridization pattern and process: (1) How often is introgression biased toward certain genomes and loci, and is this bias selectively neutral? (2) What are the relative rates of formation of hybrid species and introgressants, and how does this compare to their subsequent fates? (3) Has the frequency of hybridization increased under historical periods of greater dynamism in climate and geographic range, such as the Pleistocene?

**KEY WORDS** coalescent; comparative methods; hybridization; introgression; reticulation.

Hybridization is increasingly recognized as an important process that can generate biological diversity (a topic that has been reviewed numerous times, for instance: Anderson, 1949; Anderson and Stebbins, 1954; Mallet, 2005, 2007; Soltis and Soltis, 2009; Abbott et al., 2013; Mallet et al., 2016; Feliner et al., 2017). While this viewpoint has long been held among botanists, fewer clear examples were known among other branches of the tree of life, particularly tetrapods and insects, studies of which largely inspired the intersterility-focused outlook of the modern synthesis (Mallet, 2007). Yet molecular data continue to reveal hybridization outside of land plants, even in organisms in which sterility barriers are well established (Garrigan et al., 2015). Hybridization is also implicated in the origin or subsequent population dynamics of many

economically important crops, invasive species, disease vectors, and other beneficial and pestilential species (e.g., Fontaine et al., 2015; Liu et al., 2015). Renewed recognition of the importance of hybridization—both recent and ancient—has magnified research efforts, stimulating a set of next-generation theoretical and empirical questions that provides the basis for this contribution.

Extensive research in the 20th century under the broad umbrella of “biosystematics” revealed that hybridization is actually a general heading for a collection of evolutionary dynamics observed in nature. Although such processes can co-occur and some have argued they exist on a continuum (Feliner et al., 2017), they can be conceptually divided into two groups. Perhaps the more familiar situation is hybrid speciation, in which a hybrid or population of

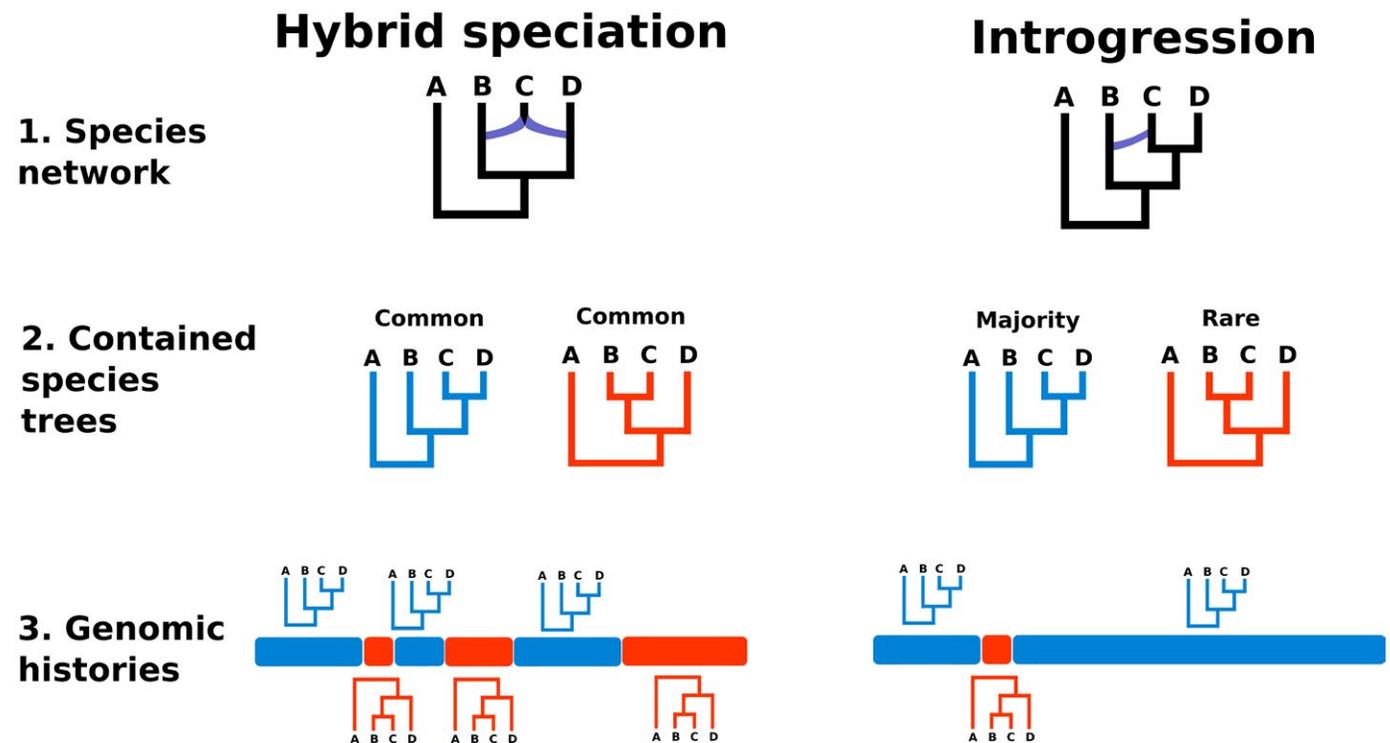
hybrid individuals stabilizes as an evolutionary lineage with a trajectory distinct from its parents (sensu Simpson, 1951). The term hybrid speciation contains all pathways that result in a net increase in lineages and in which the hybridization event is coincident with a speciation event. Yet hybridization can also be a mechanism that generates genetic, morphological, physiological, and other variation important for survivorship and reproduction; in this situation (termed introgressive hybridization or introgression sensu Anderson 1949), ephemeral hybrid intermediates undergo backcrossing to one of the parents without formation of a permanent new lineage, resulting in migration of genetic material from one lineage to another. A hallmark of this introgression scenario is the lack of a speciation event coincident with the gene flow event. For a summary of these concepts, see the networks in Fig. 1. Hybridization can overlap with a number of other biological processes such as ecological niche evolution and geographic range assembly, and its intersection with chromosome doubling as allopolyploidy is particularly prominent. The focus on this paper hereafter will primarily be on hybridization in the absence of polyploidy, often termed homoploid hybridization.

Both forms of hybridization are now more readily detected due to advances in statistical methods and data collection strategies. Systematists have long advocated examining discordance among gene phylogenies (e.g., see an early review by Rieseberg and Soltis [1991]). In addition to such manual comparisons of gene topologies, the detection of hybridization has been greatly facilitated by

the coalescent model (Pamilo and Nei, 1988; Degnan and Salter, 2005; Meng and Kubatko, 2009), a population genetic model that can be used (1) to predict allelic histories or gene genealogies (hereafter, gene trees) under population parameters or (2) to infer population/phylogenetic scenarios given a set of observed gene genealogies. Unlike the traditional concatenation-based framework, where molecular characters are treated as a single super-allele, whose phylogeny is the species tree estimate, coalescent methods treat individual gene partitions as singular independent data points (“gene trees”) drawn from some unknown species tree or network. Hence, coalescent methods require moderate to large sets of sampled genetic loci. In parallel, while challenges remain, the advent of next-generation sequencing (NGS) methodologies has made it increasingly feasible to resolve potential hybridization events across the tree of life.

**OUTSTANDING QUESTIONS**

The study of hybridization is moving away from merely demonstrating the occurrence and persistence of nascent hybrids in nature to its genomic implications and long-term impact on the tree of life. These new developments bring a much stronger deep-time perspective to the study of hybridization. Hence, we begin by posing and extending three outstanding key questions that, when answered, would significantly move the field of hybridization studies forward,



**FIGURE 1.** Conceptual distinctions among hybrid speciation and introgression. These distinctions include the expected timing of lineage formation with respect to interspecific gene flow ([1]; simultaneous under hybrid speciation), and commonality of alternative contained species trees across the genome ([2] and [3]; highly asymmetric under introgression). Representative species networks are given in (1), with branches representing hybridization events shown in violet. Note that under introgression, there is no speciation event simultaneous with hybridization. The two species trees contained in these networks are given in (2), with expected gene-wise frequencies in the genome shown as headers. Chromosomes are represented in (3); red and blue trees represent c-genes genealogies (“gene trees”) drawn from species trees in (2); each contiguously colored chromosome zone is an h-gene.

especially in terms of understanding the impact of hybridization. These questions simultaneously allow us to delve into methodological and conceptual prerequisites for such a deep-time paradigm.

- (1) How often is introgression biased toward certain genomes and loci, and is this bias selectively neutral?
- (2) What are the relative rates of formation of hybrid species and introgressants, and are these frequencies modified by differences in their subsequent fates?
- (3) Has the frequency of hybridization increased under historical periods of greater dynamism in climate and geographic range, such as the Pleistocene?

Following an introduction to the phenomenon and detection of hybridization across both shallow and deep timescales on the tree of life from the genomic point of view, we develop a conceptual framework for answering these questions with methods and data sets achievable in the near future.

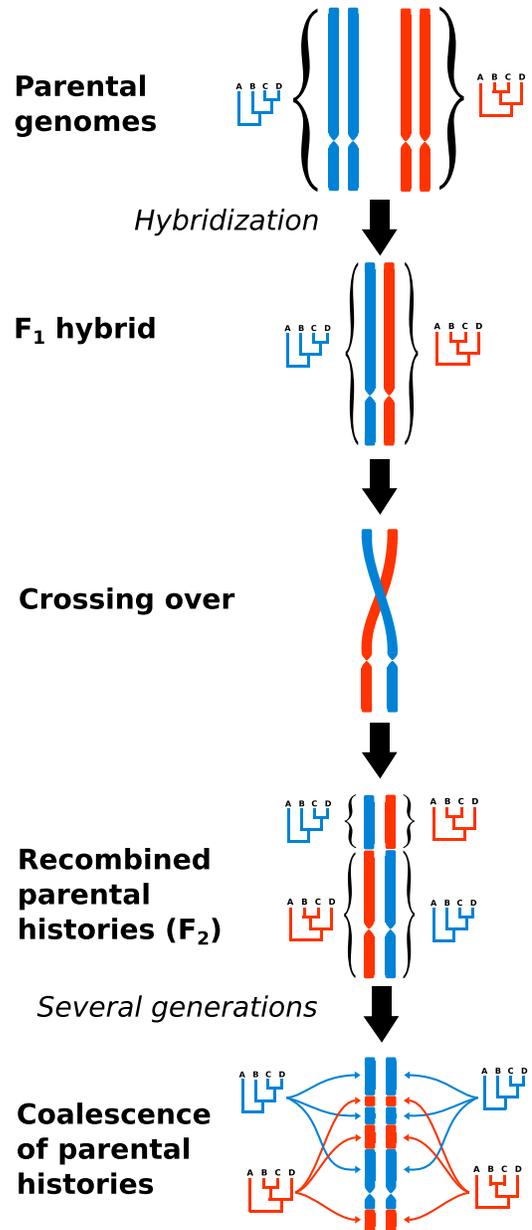
## GENOMIC CONSTITUTION OF HYBRIDS

### Historical mosaicism

The earliest moments of hybrid formation are typically thought to involve a population process rather than an individual founder (e.g., Mallet, 2007: Fig. 1). Resultant individuals are of “hybrid constitution” in one sense: they are heterozygous for parental alleles. On macroevolutionary timescales, these relatively unstable processes are expected to be ephemeral, at least in the absence of mechanisms that can maintain “heterozygosity” permanently, such as allopolyploidy or permanent translocation heterozygosity (Grant, 1981). This short period of heterozygosity, perhaps a single generation, eventually leads to a more stable status: after some degree of crossing-over, parental alleles sort out, and all parental heterozygosity is extinguished (alternatively, all alleles eventually coalesce after the hybridization event; Fig. 1). This results in a patchwork or mosaic of fixed genomic stretches (Fig. 2), each of which individually evolved under a single species tree and therefore is drawn from a different gene tree distribution (see Meng and Kubatko, 2009; Zhu and Degnan, 2016 for further particulars on modeling and testing hybridization in this way). The limits of this patchwork are defined by crossing over in the population time slice for which parental alleles maintain heterozygosity; after allele fixation, these limits are also fixed. This historical mosaicism is the second, less commonly used, sense of “hybrid constitution”. Thus, the genomic constitution of a hybrid at the homoploid level is expected to contain an ephemeral parental-heterozygosity phase, followed by the fixation of a mosaic of parental histories.

Recently, discussion of the c-gene, a concept introduced by Doyle (1997), has been revived to describe the minimal nonrecombining genetic segments that are the fundamental unit of phylogenetic inference based on gene trees. These segments are delimited by any sites of crossing over among genomes in the clade under consideration and are defined by the absence of any such sites within the segment. This distinction has proved useful for outlining the scope of detection and analysis of these minimal units (Gatesy and Springer, 2014).

We propose the term h-gene for the analogous concept in hybridization detection, which is the object of inference for understanding hybridization at the genomic level. An h-gene (Figs. 1 and 2, in



**FIGURE 2.** Origin of genomic historical mosaicism, beginning with an  $F_1$  hybrid and ending with fixation of parental histories, that is, coalescence of all alleles after the hybridization event. Populational polymorphism is lost between the penultimate and final steps. Each contiguously colored chromosome zone is an h-gene.

red and blue) denotes a single chromosomal stretch that (1) evolves under a single species tree (that is, one of the component bifurcating topologies in a hybridization network; cf. trees in Fig. 1) and (2) is punctuated by two crossing-over events that span differing parental alleles. The extent of an h-gene is fixed as soon as parental alleles are fixed by post-hybridization coalescence. In Fig. 1, part 3, each contiguous zone of one color on a chromosome represents a single h-gene. Commonly, an h-gene will contain many c-genes (cf. Allman et al., 2016: Fig. 10), because the cumulative span of lineages (time-extended metapopulations) in a phylogeny will typically be long in comparison to the brief temporal window of hybrid

heterozygosity, so that most recombination events will be outside this window. The reverse (a *c*-gene with several *h*-genes) is not possible because *h*-gene boundaries also delimit *c*-genes. It follows that *h*-genes are expected to be consistently larger than *c*-genes. Both the *h*-gene and *c*-gene concepts are related to local perfect phylogenies *sensu* Mailund et al. (2006; see also Thompson and Kubatko, 2013), phylogenies inferred from contiguous regions of the genome and to be free from combination and therefore predicted to have a single underlying gene genealogy. An *h*-gene in particular may not have a single local perfect phylogeny due to the fact that recombination events subsequent to hybridization and sorting of parental alleles could occur but would not delimit further *h*-genes.

The critical outcome of this *h*-gene model for hybrid genomes is that the expected gene tree distribution differs across the genome. Over this pattern lie other potential sources of gene tree variance such as incomplete lineage sorting (ILS), where such large-scale genomic structuring to gene tree expectations is not generally expected (Allman et al., 2016: Fig. 10). Until recently, it was impossible to identify, for a given case, the cause of phylogenetic discord, which could be explained by any of a long list of potential causes for differing histories (incomplete lineage sorting, introgression, duplication and loss, etc.). More recently, it has been appreciated that not all forms of observed gene tree discord are empirically equivalent and therefore, in principle, they can be distinguished (e.g., Reid et al., 2013). Motivated by the observation that gene tree expectations (topology, branch lengths, genomic structure) are different under these different sources of gene tree variance, much work has been devoted to developing coalescent-based approaches to detecting hybridization and inferring phylogenetic networks (that is, species trees that contain extra branches representing hybridization events, cf. networks in Fig. 1, part 1) in the presence of incomplete lineage sorting (e.g., Meng and Kubatko, 2009; Joly, 2011; Yu and Nakhleh, 2015; Solis-Lemus and Ané, 2016).

### Difficulties in the detection of hybridization

A useful concept in discussing the detection of hybridization is the parameter  $\gamma$  (introduced by Meng and Kubatko, 2009), which expresses the ratio of parental contributions. This parameter varies along (0, 1), where  $\gamma = 0$  and  $\gamma = 1$  would indicate a complete lack of hybridization; symmetrical hybridization is parameterized by  $\gamma = 0.5$ . Methods that explicitly infer hybrid networks depend on capturing alternative parental histories in gene tree data. These methods have consistently shown encouraging statistical power when hybridization events have  $\gamma$  near 0.5. This power decreases as  $\gamma$  becomes smaller and is commonly poor for  $\gamma \leq 0.1$  (e.g., Meng and Kubatko, 2009; Gerard et al., 2011), i.e., 10% of the genome from one of the parents, which is not particularly small under an introgression scenario; these simulation scenarios apply to locus numbers typical of modern massively parallel sequencing approaches. Such a pattern of statistical power is problematic when the goal is to detect introgressants. Few examples exist where workers have explicitly attempted to estimate  $\gamma$  from empirical data. Yet much of the traditional plant literature on hybridization has favored asymmetrical hybridization as the norm in nature where polyploidy is absent, a case most clearly made by Anderson (1949). Likewise, only rarely is the simulation of cytoplasmic loci attempted in benchmarking (e.g., absent in Yu and Nakhleh, 2015; Solis-Lemus and Ané, 2016; Kamneva and Rosenberg, 2017), so the performance of methods on the typical plastid introgression observed in plants, or

other locus-specific evolutionary dynamics, is not well characterized. Hence, in typical plant systems, the frequency of introgressants may be underestimated with these approaches.

There are likely some additional scenarios that will remain recalcitrant to hybridization-detection techniques even with phylogenomic data sets. Simulation studies (e.g., Joly et al., 2009; Yu and Nakhleh, 2015) suggest that hybridization detection is sensitive to the length of sequences due to gene tree estimation error, with decreasing statistical power with use of shorter loci. Statistical power issues are not only methodological concerns relevant to gathering data, because with high recombination rates, it is possible in an introgression event to transfer very short segments of DNA for which introgressive histories will inherently be difficult to detect even where DNA sampling is complete. Simulation studies, even on fairly shallow species networks with few taxa, have also suggested an effect of time since hybridization, where low statistical power is seen when the time since hybridization is large compared to the time between speciation and subsequent hybridization (e.g., Joly et al., 2009; Yu et al., 2011); hence, more ancient hybridization events may be associated with lower statistical power. Although older gene genealogies are expected to have higher gene tree estimation error, even when simulations assume gene trees to be known without error, the problem persists (Yu et al., 2011), suggesting that gene tree distributions with and without hybridization are more difficult to distinguish in increasingly ancestral hybridization scenarios. It is likely that all of these factors (*viz.*, highly asymmetrical hybridization, short lengths of introgressed DNA, and ancient introgression) can interact to produce relatively recalcitrant scenarios for hybridization detection. As approaches in this field continue to develop, methods that can use as much information as possible in the data (e.g., both gene tree topology and branch length information; Meng and Kubatko, 2009; Yu et al., 2012) may improve prospects in these cases as much as accruing larger genetic datasets.

Another more practical limitation to these methods is the vast scale of computational resources required for explicitly testing networks. Not only is the elaborate model itself to blame, but the astronomical scale of ordinary tree search space with non-trivial numbers of taxa is also well-known. When extra hybridization edges are added to trees, this search space grows rapidly and becomes precisely infinite if no constraints are placed on the number and placement of extra edges in a hybridization network. Rapid progress has been made on this front, including quartet-based heuristics and parallelization (Solis-Lemus and Ané, 2016). Recent methods scale well with numbers of loci, such that methods are already available that are well suited for phylogenomic gene samples. However, typical runtimes still cause these methods to be unusable with the scale of taxon sampling now typical in phylogenetics without artificially reducing taxon sampling, often to fewer than a dozen operational taxonomic units (OTUs). More progress is needed in heuristics to reduce this search space to manageable sizes on large phylogenies; alternatively, gene tree simulations based on species trees already can scale well with currently available data scales (e.g., >60 taxa in Folk et al., 2017).

### Gene-centered vs. taxon-centered hypotheses

Many new hybrid-detection methods are conceptualized under a network inference paradigm—that is, estimation of a species network, a tree with extra edges representing hybridization events in the past. Typically, gene genealogies are treated as being sampled

from species trees contained in the species network (compare trees in Fig. 1, parts 1 and 2). The contained species trees, as well as their frequency derived from the  $\gamma$  statistic, are coestimated (cf. Gerard et al., 2011). The statistical problems associated with highly asymmetrical hybridization apply because these utilize taxon-centered hypotheses to genetic data, asking whether a particular OTU or ancestral branch in a phylogeny is of hybrid origin. It follows that taxa containing few horizontally transmitted genes will be difficult to detect.

There is another way to formulate and test a model for hybridization that may not directly suffer from highly asymmetrical hybridization: a gene-centered hypothesis, which asks whether a particular gene genealogy is expected under a given species tree. Typically, this approach involves an observation that an empirical gene tree distribution contains outliers and particularly that these outliers may have biologically significant distinctions (organellar, high-copy rDNA, etc.) relevant to gene genealogies. The aberrant topologies are set aside; if this is not done, aside from logical circularity, the coalescent branch lengths will potentially be underestimated by erroneously attributing these unusual topologies to ILS alone. The remaining gene genealogies, which putatively evolved under a single species tree in the absence of hybridization, are used to infer this species tree with associated coalescent branch lengths, parameterized in terms of effective population size and number of generations, which correspond to tree width and depth. This species tree is used to predict a gene tree distribution under ILS alone using the coalescent model. Finally, the outlier topologies can be compared in various ways to the observed gene genealogies, using, for instance, sequence distance (Joly, 2011), clade frequencies (Willis et al., 2013; Folk et al., 2017), topological distance (cf. Tian and Kubatko, 2017), or other metrics. For any gene histories found to be poorly explained by ILS alone, if other sources of gene tree incongruence can be ruled out, a posteriori hypotheses of hybridization scenarios can be inferred (Folk et al., 2017; García et al., 2017). Another important class of methods is based on summary statistics such as the *D*-statistic (Durand et al., 2011; see also Eriksson and Manica, 2012; Eaton and Ree, 2013; Martin et al., 2015; Pease and Hahn, 2015). These summary methods rely on counting patterns of discordant nucleotide sites across the genome, relying on the fact that hybridization and incomplete lineage sorting predict different gene tree distributions and therefore different nucleotide site patterns. While, importantly, these methods are typically limited to four-taxon trees or other simplified topologies (see Pease and Hahn, 2015), they have been frequently used with phylogenomic data scales to test hypotheses that can be reasonably represented as small or subsampled trees (e.g., Dasmahapatra et al., 2012; Pease et al., 2016; Roda et al., 2017). Although the test was originally intended to detect hybridization on a whole-genome basis (Green et al., 2010; Durand et al., 2011), related methods can be used to characterize which parts of the genome were involved in introgressive events (Martin et al., 2015).

A dichotomy between a tree-centered and a gene-centered viewpoint, while important for analytical purposes, is also relevant conceptually. It has been pointed out (Hahn and Nakhleh, 2016) that viewing completely resolved phylogenies as the single ultimate goal of phylogenomics has sometimes canalized viewpoints on the ways in which these data can be used and the kinds of analyses performed. It may be that an obsession with support values and resolution has created something of a distraction. As the one science that provides a consistent, unifying framework for understanding

biological diversity (Hennig, 1966), systematics contains more ambitious goals than maximizing phylogenetic support. Particularly when “unusual” evolutionary processes such as interspecific gene flow are under question, or the genome segments exhibiting discord contain functionally relevant genes, we would argue that in these cases the gene trees are more interesting and relevant than a single species tree or network. A corollary is that reporting single optimal trees in phylogenetic studies is becoming increasingly unsatisfactory. A series of new phylogenomics papers (Govindarajulu et al., 2015; Stephens et al., 2015; Sun et al., 2015; Morales et al., 2016; Crowl et al., 2017; Folk et al., 2017; García et al., 2017; McVay et al., 2017; Vargas et al., 2017) presents a promising change of heart, characterizing large samples of genomic histories and synthesizing these with species tree estimates in a holistic framework. Sun et al. (2015) found, among the most ancient examples known, evidence for genomic discord involving the COM clade, a deep-level clade in the rosids, which may represent an ancestral hybridization event. Results of this kind are simultaneously exciting and somewhat disturbing. The framework of angiosperm molecular systematics has traditionally been based primarily on plastid loci for methodological reasons (Doyle, 1993); the COM clade example may be only the first of many examples of deep-level, well-supported discord potentially representing reticulation that will be unmasked by retesting hypotheses that were based on plastid molecular markers.

## GENOME BIAS: QUESTION ONE

### Differing trajectories among genomes

One of the most common and remarkable empirical outcomes of hybridization is the great contrast among the cell's genomic compartments in terms of their evolutionary fates during hybridization. At least at shallow phylogenetic levels, where such studies have most often been performed, researchers have repeatedly recovered estimates of nuclear phylogeny that largely match taxonomy, morphology, previous understandings of biogeography, and other biological data sets. However, comparing nuclear phylogenetic estimates to mitochondrial and/or plastid estimates has commonly revealed robust and dramatic contrast with nuclear data and with general biological knowledge of the studied groups (reviewed by Rieseberg and Soltis, 1991; some recent examples in Folk et al., 2017; García et al., 2017).

This is a surprising circumstance given that organellar genomes are expected to experience less ILS than nuclear loci are under the coalescent model (Moore, 1995); hence, hybridization has been the more common explanation for discordance. Aside from the frequent use of cytoplasmic markers for methodological reasons (Doyle, 1993), a possible explanation for the disproportionate frequency of cytoplasmic introgression is simply that introgressed organellar genomes are more likely to be fixed randomly due to the smaller effective population size ( $N_e$ ; Rieseberg and Soltis, 1991). Because organellar genomes typically evolve as a single nonrecombining unit (Doyle, 1993), it is straightforward to fix a single divergent history by chance alone. While whole-genome interrogation (cf. Arcila et al., 2017) has yet to be performed in this context, the availability of reduced genomic data has shown in at least two cases (Good et al., 2015; Folk et al., 2017) that while introgression is robustly detected in cytoplasmic genomes, it is not simply rare but truly absent from fairly large samples of loci from the nuclear

genome. This observation is difficult to explain by such a simple stochastic process as rapid random fixation, motivating a consideration of alternative explanations for the great difference in historical trajectories between nuclear and cytoplasmic genomes. Such a list would be long, and explicit tests of such alternative explanations in empirical systems have been few. Possible explanations include selection, fitness interactions among genomes, cytoplasmic male sterility, and sex-biased introgression (reviewed by Rieseberg and Soltis, 1991). Adaptive introgression of loci has commonly been invoked and tested to some extent (Bonnet et al., 2017). Another common class of explanations invokes the existence of genetic interactions between the nuclear and cytoplasmic genomes, such as fitness advantages of certain genomic combinations (Wendel et al., 1991; Tsitrone et al., 2003; Sehrish et al., 2015). One of the most interesting of these is the possibility of fixing the male genome in the female cytoplasm in a single generation, possible under various developmental scenarios including androgenesis (reviewed by Hedtke and Hillis, 2011; see also Wendel et al., 1991). However, testing such putative explanations is often difficult.

### Gene function and introgression

The idea that there might be underlying biases in the genomes or genic regions that would be transferred during introgression raises the question of precisely what genetic material is being transferred. Introgression in particular can be seen as a variation-producing mechanism (e.g., Mallet, 2007; Rieseberg and Willis, 2007; Arnold, 1997, 2007); this variation could be functionally relevant and perhaps the object of selection. It is in these cases where the discovery of h-genes, the genomic unit of hybridization, is most relevant. Delimiting these units opens up a series of hypotheses that can now be tested. For instance, the transferred gene content could be queried for known functions in homologs from model organisms; with population genetic data, the region could be screened for selective sweeps; large-scale population screens could be performed in well-documented ongoing introgression events to discover the demography associated with the spread and fixation of h-genes.

Characterization of genetic function in introgression events has been described in *Heliconius* butterflies, where Müllerian mimicry has been enabled by the exchange of wing patterning genes (Dasmahapatra et al., 2012), and in *Helianthus* sunflowers, where various adaptive responses to abiotic and biotic stressors have been introduced into *Helianthus annuus* (e.g., Whitney et al., 2010). Further cases include mosquitoes (Fontaine et al., 2015) and ancestral hominins (Racimo et al., 2015). These case studies represent a small fraction of the taxa in which introgression events have been documented. It remains poorly known whether adaptive introgression is the most common pathway; it is possible that demography (sex bias, small population size, etc.) and pre-existing species traits (cytoplasmic male sterility, etc.) could commonly result in non-adaptive introgression. A larger number of ancient reticulation events would serve as case studies for macroevolutionary approaches using comparative methods, enabling researchers to assess the selective relevance of hybridization in a larger number of taxa.

In a growing number of case studies, genome-scale data have been used to uncover the genomic structure of introgression, but these are still too few to draw general conclusions about patterns. Cytoplasmic–nuclear studies are being improved by the availability of plastid genomes and increasingly large nuclear locus samples,

demonstrating a clear bias towards cytoplasmic DNA introgression (e.g., Folk et al., 2017; Good et al., 2015) and not simply sampling bias due to historical methodological difficulties in working with nuclear loci (contra Sambatti et al., 2008, p. 1089). Much less work has been done to assess genomic bias and the genomic structure of introgression in nuclear data sets (above). As genomic data and chromosome-level genome assemblies become more common, more studies will be needed to understand genome-level dynamics (such as the presence of functionally relevant loci) involved in introgression and shed light on potential processes that may promote introgressive hybridization.

### MANDATE FOR A TIME-EXTENDED VIEW: QUESTION TWO

We find that much of the current hybridization literature, on both homoploid and polyploid systems, particularly for those systems most cited and best developed as evolutionary models (e.g., *Helianthus*, *Heliconius*, *Spartina*, *Tragopogon*, *Iris*, African cichlids, *Anopheles*), has maintained a focus primarily on fairly shallow timescales, lying on a continuum from population dynamics to incipient speciation and gene exchange in recent, rapid radiations (Arnold et al., 1990; Ainouche et al., 2004; Soltis et al., 2004; Rieseberg et al., 2007; Dasmahapatra et al., 2012; Fontaine et al., 2015; Meier et al., 2017). This work is important in clarifying the initial dynamics of hybrid establishment; yet, such a focus has also widened the disparity between study of these incipient units and knowledge of their subsequent fate. With this disparity in mind, while both macro- and microevolutionary perspectives stand to provide considerable and complementary insights, we see further development of more macroevolutionary approaches to interspecific gene flow as both necessary to close a major knowledge gap and likely to comprise a critical element of future growth in the field.

### Hybridization and diversification rates

Considering introgression and hybrid speciation, it might seem at first glance that primarily (or only) the latter is relevant to questions of diversification. The former may be considered inherently less consequential to diversification given that only a small part of the genome is involved and no new organismal lineages are generated. Of course, hybridization itself serves as a direct source of lineages in events that would be classified as hybrid speciation events, so at least where this process occurs, it increases the number of lineages. Yet asking whether hybridization drives diversification does not require the demonstration that hybridization is common, that it is a primary route to speciation, or even that it is ever a successful direct route to the creation of new lineages in the first place (contra Wagner, 1970). An alternative view is that either a hybrid (heterozygous) constitution itself, or a critical variant introduced by hybridization, is a force that can spur shifts in diversification rate. Since recombination of parental traits is not necessarily additive (Mallet, 2007), both hybrid speciation and introgression can in principle provide novel traits that have lasting impacts on speciation and extinction rates with or without the lasting relevance of a genome of hybrid constitution per se. Therefore, at least at the outset, both types of hybridization are relevant to questions of diversification.

Given the historical controversy about the evolutionary significance of hybridization (which could be quantified, for example, by its effect on speciation, net diversification, or impact on

evolutionary rates of species traits), applying phylogenetic comparative methods to ask whether diversification is related to hybridization is a seemingly obvious next step. Unfortunately, remarkably little is known on this front despite the growth in research using these methods in other fields (e.g., for testing the role of polyploidy in diversification; Tank et al., 2015). One of the problems with a focus on the temporally shallow systems now available is that few macrophylogenies are available to test hypotheses of hybridization in deep time; singular putative gene flow events, as that inferred by Sun et al. (2015), are equally challenging given that comparative methods typically rely on the presence of multiple state transitions. It has been methodologically challenging to find examples of clades with multiple hybridization events followed by cladogenic events. Estimation of diversification rates, squarely in the realm of macroevolutionary research and typically requiring fairly large phylogenies (Davis et al., 2013), is therefore hampered by the existing bias towards recent and incipient hybridization events.

One practical problem is that many models of diversification assume a tree-like structure to phylogeny in the first place. These methods use information about topology and branch length alone or together with trait data, often to ask questions about speciation/extinction rates, ancestral states, and other macroevolutionary dynamics. On first glance, it might appear that this limitation prevents possible uses of the comparative approach, but any limitations are complicated by the complexity of the hybridization process. In typical introgression scenarios, where the amount of gene material exchanged in each gene flow event is comparatively miniscule, the vast majority of phenotypic and genotypic features of hybridized lineages is expected to derive vertically rather than horizontally. In these cases, we would argue that as long as genes relating to any traits being analyzed are not transferred (which is a testable hypothesis), and for trait-naïve estimation of diversification, a tree-like model will be largely suitable. In these cases, hybridization reconstructions can be compared with diversification patterns, or preferably hybridization itself can be coded as a trait that represents underlying novel variation produced by the process. The network problem is more acute in instances where hybrid speciation is suspected, and many traits are indeed likely to be horizontally transmitted. Some interim solutions, such as breaking down hybrid networks into contained species trees and integrating results from analyses on these individual trees, are possible, although we are aware of no examples that explicitly handle comparative methods on networks. For example, if the percentage of contribution of each parent ( $\gamma$ ) is known or estimated (e.g., Meng and Kubatko, 2009), an inferred network could be deconstructed into its contained species trees for the purpose of downstream comparative analyses, and results inferred from each contained species tree could be weighted by  $\gamma$ .

### Scales of inference and directions in the field

Much recent work on hybridization has been framed in terms of incipient evolutionary units at the boundary of population-level and phylogenetic processes. This type of work is important in providing a window on the process in its first moments. Yet the other side of assessing evolutionary process, now nearly buried, is in the study of long-term trajectories after hybridization events, which when combined with microevolutionary studies would provide complementary and potentially novel insights. Although hybrids shortly after formation must achieve a certain frequency and/or overcome selectional pressure to establish and proliferate, many questions

about the fate of hybrids cannot entirely be captured by examining nascent systems in the present or recent time slice. Questions of the “importance” of hybridization (frequency, species richness, diversification rate, or other metrics) may receive different answers in different stages of lineage “ontogeny”.

If different pathways to hybrid formation have different implications in terms of population structure, genomic-historical structure, and phenotypic evolution, it follows that these processes may not be selectively neutral. Differential survival among different evolutionary pathways would act as a sieve to stratify hybrid and nonhybrid lineages, eventually shifting their frequency in the tree of life. Likewise, different expectations in terms of trait evolution may result in selective differences between different modes of hybridization. When viewed at macroevolutionary scales, a process that is more common at present but experiences less phylogenetic success may recede in frequency when looking back in time, whereas its complement might wax over long timescales but rarely form in the present. Such a temporal dependence means that the way in which the evolutionary prominence of hybridization has been typically measured—by rates of present-day hybridization in regional surveys (e.g., Mayr, 1992; Ellstrand et al., 1996; reviewed by Mallet, 2005)—fails directly to answer whether hybridization is a prominent force by ignoring the effect of timescales. In other words, much as in ecology, where questions and research programs are often framed by reference to geographic scale, here phylogenetic (relative or absolute temporal) scale comes to the fore. With all of these unaddressed qualifiers on the meaning of the “importance” of hybridization remaining, it is clear that we still know very little about hybrids in deep time. Such an observation is not entirely pessimistic. The difficulty of detecting ancient hybridization long imposed limits on testing such questions. Yet at the moment, given the new possibilities we have reviewed, it is increasingly want of effort and not of suitable approaches that has rendered deep-time hybrid systems as relatively untapped resources for comparative study.

### INTERSECTION WITH POLYPLOIDY: LIMITS AND POSSIBILITIES

The fact that hybridization and polyploidization often co-exist (under the concept of allopolyploidy) raises conceptual issues that have not always been appreciated. From the observation of frequent allopolyploidy alone, it could be argued that hybridization is an important macroevolutionary force. Much greater success has occurred to date in reconstructing ancient polyploidy (equivalently, ancient whole-genome duplication, WGD) than ancient hybridization (Jiao et al., 2011). While challenging to test, it has been speculated that many of these events are allopolyploid (Soltis et al., 2009); more generally, it is thought that allopolyploidy represents a significant fraction of polyploidization events (cf. Barker et al., 2016). Hence, many of the ancient WGD events detected to date may very well simultaneously represent hybridization events. Yet in a macroevolutionary question that is framed specifically in terms of hybridization, but tested using allopolyploids partly or in whole, polyploidy is clearly a lurking variable; the reverse, that hybridization is a potential lurking variable in testing the effects of WGD, is likewise true. The lurking variable problem is a particular instance of a general limitation of phylogenetic comparative methods—it is a challenge to determine whether trait-associated diversification is truly causative when hidden states (i.e., phenotypes responsible for

the diversification patterns observed, but where only their correlates were sampled) may be present and more proximally responsible for the observed patterns (Beaulieu and O'Meara, 2016; O'Meara and Beaulieu, 2016). Macroevolution is fundamentally a historical process; since the tree of life is not a repeatable experiment, controlling for variable effects is only indirectly possible. This issue is unusually acute in the case of allopolyploidy and hybridization because a rich historical literature attempts to argue extensively for and against the macroevolutionary significance of each individually or both in concert, so that the preliminary case for each is strong (to give a few well-known examples from the botanical literature for hybrids: Lotsy, 1916; Anderson, 1949; Anderson and Stebbins, 1954; Wagner, 1970; Mayr, 1992).

Although differentiating between homoploid hybridization and allopolyploidy has been successful at the genomic and transcriptional levels (Soltis et al., 2014), it is more challenging to do so in a macroevolutionary comparative framework. Such studies of hybridization have not been performed for want of suitable systems; more has been achieved in determining whether polyploidization events are dead-ends or otherwise are non-neutral with respect to diversification rates (Mayrose et al., 2011; Soltis et al., 2014; Tank et al., 2015).

Although there is no easy resolution of issues of lurking variables for polyploids in sight, work on the corresponding question concerning diversification and hybridization is nearly nonexistent. If polyploidy is itself not diversification-neutral, then the simple detection of allopolyploid-associated macroevolutionary shifts does not directly speak to the relevance of hybridization to lineage success; any impact could be due to polyploidy alone. Because distinguishing between polyploid- and hybrid-associated diversification is likely to be challenging (Beaulieu and O'Meara, 2016), significant progress will be made by studying hybridization in systems where it occurs in isolation from polyploidy (cf. Folk et al., 2017).

### ANCIENT HYBRIDIZATION AND HISTORICAL CLIMATE CHANGE: QUESTION THREE

Because hybridization is a process that involves biotic contact, geographic ranges of potentially sexually compatible lineages and the ecological niches that influenced these ranges are important to uncovering potential mechanisms promoting hybridization. We see a need for placing increasing emphasis on ecological and geographic context in modern molecular studies of naturally occurring hybridization, extending from studies of hybridization at shallow phylogenetic scales or primarily on contemporary dynamics (e.g., Rieseberg, 2003; Sambatti et al., 2008) to a more time-extended view. Such a contextualization may help elucidate poorly understood patterns, such as why hybridization is apparently more common in certain taxa than in others (Ellstrand et al., 1996; Dowling and Secor, 1997). A time-extended ecological context may also shed light on little-explored hypotheses, such as whether functional traits or geographic latitude are predictive of hybridization frequency.

Studies in the spirit of “biosystematics” (e.g., Wiegand, 1935) investigated how ecology and hybridization could interact, culminating in the hypotheses of Anderson (1949, chapter 2) that hybridization is enabled by the existence of “hybridized” (intermediate) habitat and that hybridization is expected to increase due to ecological disruption, often framed in terms of human-caused disturbed habitat (Arnold, 2016). The hypothesis that disturbance increases hybridization also applies

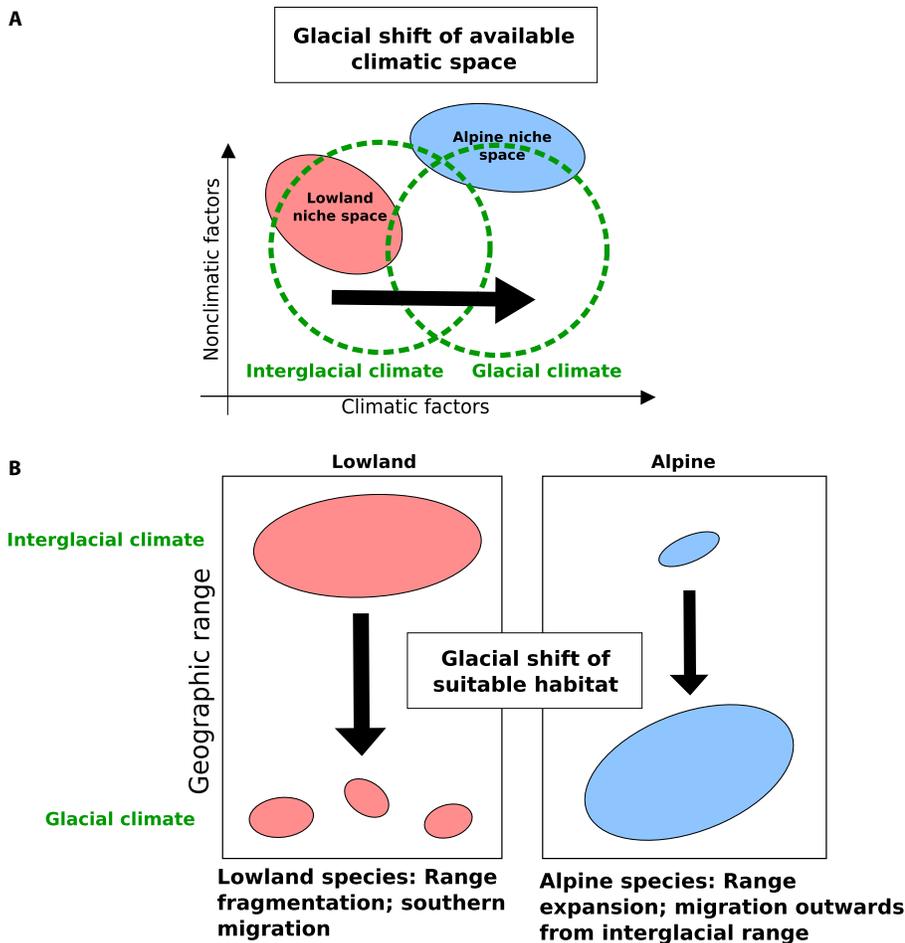
to natural sources of disturbance arising from climatological and geological processes in the Pleistocene and earlier periods (Anderson and Stebbins, 1954). We know from the fossil record that geographic ranges have been subject to remarkable degrees of dynamism, partly due to historical climate change (Roy et al., 1995; Graham et al., 1996; Lyons, 2003; Bush et al., 2004; deMenocal, 2004). Disturbance of habitat and organismal distributions is therefore far from unique to the present and recent past, raising the possibility that dramatic climatic shifts, among the strongest forms of habitat disturbance affecting organisms, may be responsible for many instances of historical hybridization. Ideas have been proposed for the role of hybridization in earlier periods of disturbance, including other types of climate change and orogeny events (Anderson, 1948; Anderson and Stebbins, 1954), although events hypothesized to date to earlier geological windows remain scarce.

### Climatic drivers

Change in the geographic distribution of suitable habitat (potential range) over time generally comprises (1) evolution of intrinsic traits underlying niche itself, which affects what types of conditions across the globe can support an organism, and (2) climate change, which affects the geographic distribution of these appropriate conditions. Understanding the circumstances behind hybridization events requires understanding these potential enablers of gene flow. While the impacts of both niche evolution and climate change on hybridization are poorly understood, more study has been devoted to the latter question, with many qualitative hypotheses that hybridization in various plant groups was enabled by increased geographic range contact under Pleistocene glacial conditions. One extreme situation where this situation arises is in putative ancestral hybridization events where the descendants are allopatric and highly disjunct, a phenomenon observed in several plant lineages (Klein and Kadereit, 2016; Marques et al., 2016; Folk et al., 2017).

Due to the dynamism of geographic ranges of species through history, modern-day distributions are not necessarily predictive of past potential for gene exchange. The potential for contact could be driven by the diversity of organismal responses to climate change resulting from trait differences (Guralnick, 2007). Under glacial conditions, increased geographic overlap of some species, particularly between those occupying lowland and alpine niches, is expected and does not require any shift in niche space for parents or hybrids. Figure 3 shows a simple model by which this range overlap might occur. The overall amount of possible niche space realized on the globe shifts dramatically during glaciation; while widespread lowland species occupy more of this space in the present, under glacial cool conditions, the situation reverses and alpine taxa occupy an increased proportion of this space. Stated equivalently, under glacial conditions, montane taxa possess increased suitable habitat. When niche space is translated into geographical range, we expect lowland taxa to experience southward migration and range contraction and fragmentation. This familiar situation has given rise to the paradigm of Pleistocene refugia (e.g., Tzedakis, 2002; Waltari et al., 2007; Provan and Bennett, 2008). Thus, many montane taxa do not experience refugia, but instead greatly expand their ranges; sky-island taxa experience the desert equivalent of land-bridges (e.g., in deserts of the American southwest; Wells and Berger, 1967; Lanner and Van Devender, 1981).

Alpine taxa that have been examined under Pleistocene cool conditions (e.g., Waltari and Guralnick, 2009; Espíndola et al., 2012; López-Alvarez et al., 2015; Marques et al., 2016) have typically shown a very distinct pattern from lowland taxa, greatly increasing



**FIGURE 3.** Impact of climatic shift on geographic distribution of potential hybridizers, considering accessible lowland and alpine regions at the same latitude. In (A), when niche space occupancy is held constant, lowland and alpine lineages have very different responses to glacial climatic conditions, resulting in a reversal in the portion of available niche space occupied by each. In (B), the translation of these niche expectations into geographic ranges predicts range contraction and fragmentation for lowland species; alpine species by contrast increase their range, providing opportunities for novel patterns of range contact.

geographic range due to more suitable habitat, not necessarily with any southern migration. These results, primarily based on ecological modeling and paleoclimate data, are consistent with the fossil record, which has long suggested the existence of forested dispersal corridors in what are currently desert regions (e.g., Gugger et al., 2011). These diverse range dynamics could add to the instability of geographic relationships among taxa, potentially creating some of the unusual distributions observed in ancestral hybridization events (Klein and Kadereit, 2016; Marques et al., 2016; Folk et al., 2017). Of course, these observations are not limited to Pleistocene scenarios; the accumulation of more ancient hybridization events will create opportunities for testing the association of hybridization with other periods of elevated disturbance.

#### An alternative paradigm for validation of hybridization

In recent decades, the detection of hybridization has largely been formulated as an exercise in molecular analysis. Gene tree data

offer substantial power for detecting genome historical mosaicism, the genetic hallmark of historical hybridization in the absence of allele-maintaining mechanisms. Nevertheless, for those taxa that have barriers weak enough to facilitate gene flow, the potential of overlap in geographic and ecological niche space is clearly relevant as an enabler of gene flow. Two ways in which knowledge of this overlap is useful are: (1) corroborating a posteriori hypotheses of hybridization inferred from other data partitions such as phylogenomics; and (2) identification of particular climatic drivers, such as historical climatic cooling, that may have facilitated lineage contact.

We see promise particularly in the prospect of validating a posteriori hybridization hypotheses that are initially formulated with analyses of molecular data. The detection of hybridization, particularly at the diploid level, is difficult (Mallet, 2007; Rieseberg and Willis, 2007; Soltis and Soltis, 2009); a combination of ecological contextualization with powerful model-based methods and robust phylogenomic data sets may provide the critical combination to lower the barrier for hybridization detection.

#### CONCLUSIONS

Biologists now seldom ask whether interspecific hybridization is possible or whether hybrids can persist; both are widely accepted as routes to diversification and novel phenotypic traits. The field can and should experience a shift toward asking more directly about the evolutionary impact of hybridization. Future growth is more likely to focus on strategies for detection and using instances of ancestral hybridization to develop suitable

hybridization models for key comparative questions. Widespread availability of genomic data has spurred further questions about the genomic constitution of hybrids, including those related to trait gain and genome-specific evolutionary dynamics. We see a further need for more time-extended and macroscale efforts on hybridization that would complement the dominant populational/incipient paradigm (cf. Payseur and Rieseberg, 2016); such a need is driven by a lack of appropriate evolutionary systems to deliver macroevolutionary perspectives. We therefore find urgency in developing more deep-time hybridization systems to pursue our three-pronged path forward, comprising (1) characterizing bias in parental contributors to a hybrid genome, (2) assessing relative rates of formation and subsequent diversification rates of hybrids, and (3) developing paleoclimatic-phylogenetic perspectives on potential enablers of hybridization. Answers to these questions will lead to a recognition of the diversity of patterns and processes that have led to present-day biodiversity, including hybridization at both recent and deep levels of the tree of life.

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