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Irrational exuberance for resolved species trees

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Phylogenomics has largely succeeded in its aim of accurately inferring species trees, even when there are high levels of discordance among individual gene trees. These resolved species trees can be used to ask many questions about trait evolution, including the direction of change and number of times traits have evolved. However, the mapping of traits onto trees generally uses only a single representation of the species tree, ignoring variation in the gene trees used to construct it. Recognizing that genes underlie traits, these results imply that many traits follow topologies that are discordant with the species topology. As a consequence, standard methods for character mapping will incorrectly infer the number of times a trait has evolved. This phenomenon, dubbed "hemiplasy," poses many problems in analyses of character evolution. Here we outline these problems, explaining where and when they are likely to occur. We offer several ways in which the possible presence of hemiplasy can be diagnosed, and discuss multiple approaches to dealing with the problems presented by underlying gene tree discordance when carrying out character mapping. Finally, we discuss the implications of hemiplasy for general phylogenetic inference, including the possible drawbacks of the widespread push for "resolved" species trees.

KEY WORDS: **Comparative methods, convergence, incomplete lineage sorting, introgression.**

The Role of Phylogenomics in Resolving Ambiguity

The major goal of phylogenetic systematics is to uncover evolutionary relationships among species (Edwards 2009). The trees describing these relationships are used for many purposes, including estimating divergence times (Arbogast et al. 2002; Tamura et al. 2012), understanding the dynamics of speciation and extinction (Pyron and Burbrink 2013; Stadler 2013), and ensuring that taxonomic classification matches evolutionary history (de Queiroz and Gauthier 1990, 1992). By far the most common use of species trees is to understand the evolution of phenotypic, behavioral, and genomic characters. Without a phylogeny, we cannot begin to understand the evolutionary history of traits (Felsenstein 1985). With a phylogeny, we can ask questions about the number of times a character has evolved, the direction of character evolution, the most likely state of ancestral species, the geographic origins of taxa and traits, and many other aspects of character-state evolution.

In many cases a small number of sequenced loci, or even morphological characters, are sufficient to infer phylogenetic relationships between organisms without any ambiguity. In a growing number of cases, however, different genes (or other genomic loci) produce different tree topologies (e.g., Pollard et al. 2006; Scally et al. 2012; Cui et al. 2013; Brawand et al. 2014; Jarvis et al. 2014; Jónsson et al. 2014; Lamichhaney et al. 2015). Multilocus datasets containing discordant (also called "incongruent") topologies among genes proffer ambiguous relationships among species, and therefore it has been of utmost importance both to understand the causes of discordance and to develop methods for resolving discordance. Disagreement among tree topologies can be due to multiple biological factors, including incomplete

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lineage sorting (ILS), introgression, horizontal gene transfer, and high levels of convergence, possibly driven by selection (reviewed in Maddison 1997; Degnan and Rosenberg 2009; Nakhleh 2013). Small numbers of genes, small numbers of informative sites, incorrect assignment of orthology after gene duplication and loss, and multiple sources of "non-phylogenetic signal" (Philippe et al. 2011) all also contribute to topological heterogeneity among gene trees.

Larger datasets and increasingly complex computational models have been deployed to infer species trees in the face of rampant discordance. Whole genomes or transcriptomes are now regularly sequenced so that phylogenies can be inferred from thousands of genes and millions of sites. These datasets have largely overcome variation due to small sample sizes, and yet topological discordance is still observed at a large fraction of loci. There are multiple computational approaches used in such cases, each of which aims to distill the species tree from the set of gene sequence data or trees inferred from these data (here we use "gene" in a broad sense that encompasses any locus, whether protein-coding or not). Some methods assume that the most commonly observed topology represents the species tree (concatenation and majority-rule methods), whereas some relax this assumption but assume that ILS is the only cause of discordance (so-called "coalescent" methods). It is not our intention to review these methods or their features here (see, e.g., Nakhleh 2013; Gatesy and Springer 2014), except to say that they all have as their goal the inference of the species topology, often including some measure of support for individual nodes.

Phylogenomic approaches to species tree inference have succeeded, sometimes in remarkable ways. To pick a recent example, Jarvis et al. (2014) sequenced 48 bird genomes to construct a tree of the major avian orders. Their final analysis included 41.8 million base pairs from 14,536 different loci, and produced a well-supported (high bootstrap support) species tree that was largely robust to the phylogenetic method used. Underlying this single topology was large-scale incongruence: *none* of the 14,536 trees from individual loci matched the inferred species tree, and many nodes with 100% bootstrap support appeared in $<10\%$ of the gene trees (Jarvis et al. 2014). In the case of the bird tree, phylogenomic methods worked exactly as intended. Tree-building methods overcame high levels of ILS, short internal branches, and massive levels of convergence at synonymous sites to produce a likely accurate set of species relationships.

However, in the rest of this essay we argue that the "resolution" of species trees in clades like this one will often lead to incorrect, but strongly statistically supported, inferences about character evolution. We explain how these incorrect inferences come about, when to expect them, and some possible ways for dealing with them. We also discuss several larger implications these ideas have for phylogenetics.

The Procrustean Bed of the Species Tree

With a well-resolved, well-supported species tree, researchers can test ideas about the origins of evolutionary novelties, convergent evolution, the homology of traits, and many other questions. In this way, a resolved species tree is viewed as a "comprehensive reliable scaffold for future comparative analyses" (Misof et al. 2014) and a "complete evolutionary framework for future comparative studies" (Wiegmann et al. 2009). A resolved species tree is therefore seen as the *sine qua non* of comparative analyses, and is the goal of much of phylogenomics.

Although researchers want a resolved species tree, in using these resolved trees we are ignoring the variation in phylogenetic relationships that was present in each of the individual gene trees used to infer it in the first place. Even when coalescent methods are explicitly used to account for massive amounts of discordance, comparative analyses are carried out on a phylogeny that has collapsed all discordance to a single point-estimate of phylogenetic relationships. Of course it has long been recognized that comparative analyses should take into account phylogenetic uncertainty (e.g., Huelsenbeck et al. 2000; Pagel et al. 2004). However, the uncertainty addressed by these methods is usually a lack of resolution—the seeming ease with which phylogenomics "resolves" species trees makes it appear as though such methods are no longer necessary (see below for more discussion of their possible uses). But as was eloquently articulated by Maddison (1997): "Phylogeny has a variance as well, represented by the diversity of trees of different genes. This variance does not represent uncertainty due to ignorance or measurement error; it is an intrinsic part of phylogeny's nature."

The problems caused by ignoring variation in gene tree topologies are manifest because these same genes underlie variation in the traits we are studying. That is, when mapping characters onto a species tree, we must recognize that sometimes these characters are determined by genes whose topologies do not match the species topology. In mapping all sorts of characters—behaviors, morphologies, nucleotide substitutions—onto a single tree, we are in effect making the assumption that the constituent gene trees match the species tree. When they do not, we are implicitly forcing the gene trees to fit into the species tree, and consequently are forcing character-state transitions to occur on the species tree. This procedure can lead to incorrect inferences about both the number of transitions and their timing (Fig. 1). Single changes on a discordant gene tree will only be reconciled with the species tree by proposing multiple changes, either multiple independent transitions to the same state or a combination of gains and losses. As the incorrect inferences often include finding convergent evolution when none has occurred, Avise and Robinson (2008) referred to this phenomenon as "hemiplasy." Although they originally used

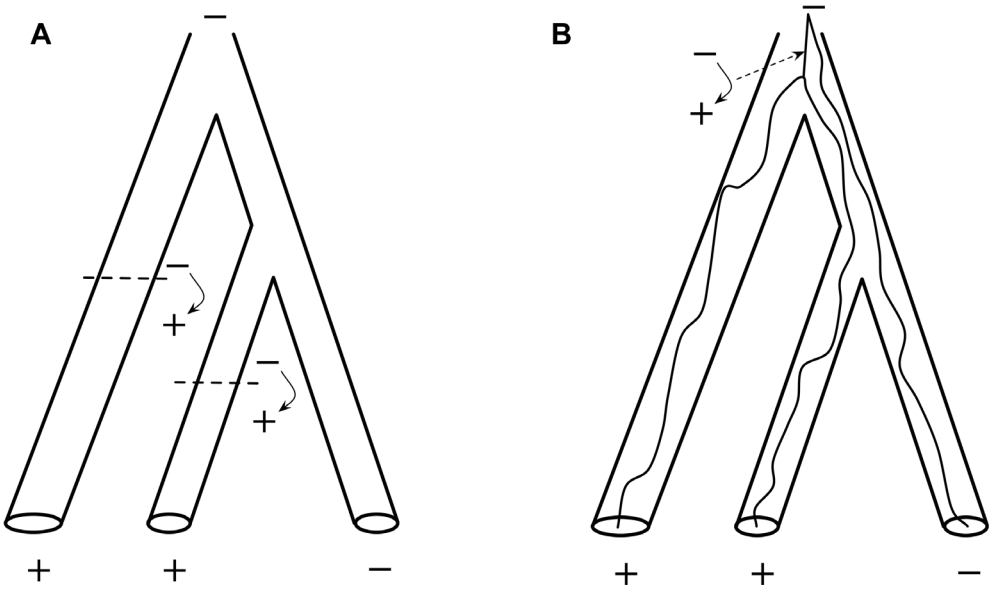

Figure 1. How hemiplasy affects inferences about character-state evolution. (A) An example species tree with character states (+ or –) labeled for the tip species. We assume that the "–" is accurately inferred to be the ancestral state of the clade based on other taxa not shown. Given these tip states and this species tree, standard methods would infer two changes: either two independent gains (pictured) or a gain and a loss. (B) If the gene underlying the trait of interest has a discordant gene tree (thin line inside species tree), then only a single change on this topology (on the branch indicated by the dashed arrow) can lead to the observed distribution of character states.

this term to refer only to cases in which discordance was due to ILS, here we use hemiplasy to mean any incorrect inference about character-state evolution caused by gene-tree discordance, regardless of the cause of discordance. The purpose of this expanded definition is to stress that there are multiple processes that lead to the incorrect inference that alleles identical-by-descent in different species have multiple origins, and that all of these processes lead to similar problems.

We are not arguing that there is no species tree, or that current methods fail to give the correct species tree. Instead, we are cautioning against a singular focus on resolved species trees when large-scale gene tree discordance is present. The use of a single tree for carrying out comparative analyses will lead to incorrect inferences about character evolution because this fixed-tree representation is only an average of all the relationships for each gene and character. Returning to the study of birds as an exemplar, recall that Jarvis et al. (2014) found both a highly resolved species tree and a set of gene trees that did not match the species tree; but in every accompanying paper the single, fixed species tree was used, even when the study was explicitly focused on the evolution of genes (Zhang et al. 2014). This implies that many characters may have been mismapped onto the tree, leading to incorrect inferences about the tempo and mode of evolution (see, e.g., Mendes and Hahn 2015). To further demonstrate how studies of character evolution may be misled when there is gene tree discordance but a single species tree is used, we turn now to a specific example from bats.

An Example from Bats

Bats are fascinating mammals, uniquely capable of self-powered flight, with variation in the ability to echolocate and the type of echolocation call used (reviewed in Jones and Teeling 2006). Based on morphological characters, echolocating bats have traditionally been placed in one suborder, Microchiroptera (microbats), with nonecholocating bats placed in another, Megachiroptera (megabats). This grouping has recently been supported by much larger morphological datasets (O'Leary et al. 2013), and has been supported by molecular data from a small number of loci and species (Liu et al. 2001; Murphy et al. 2001).

In contrast, a growing number of studies with data from multiple loci, often sequenced across more bat species, have consistently found microbats to be a paraphyletic grouping (Teeling et al. 2000, 2002, 2005; Meredith et al. 2011; Tsagkogeorga et al. 2013). These studies strongly support (i.e., with high bootstrap support) sister relationships between one echolocating clade of bats and the nonecholocating Old World fruit bats, placing them in a new suborder, Yinpterochiroptera. The other clade of echolocating bats represents a second new suborder, Yangochiroptera, sister to the first (Fig. 2A). These results have important consequences for the evolution of echolocation. Nonsister relationships between the echolocating bats implies either convergent gains of echolocation in these two groups, or a gain of echolocation in the ancestor of all bats and then a loss in Old World fruit bats (Teeling 2009; Springer 2013). In either scenario, instead of a single character-state transition, mapping echolocation onto the

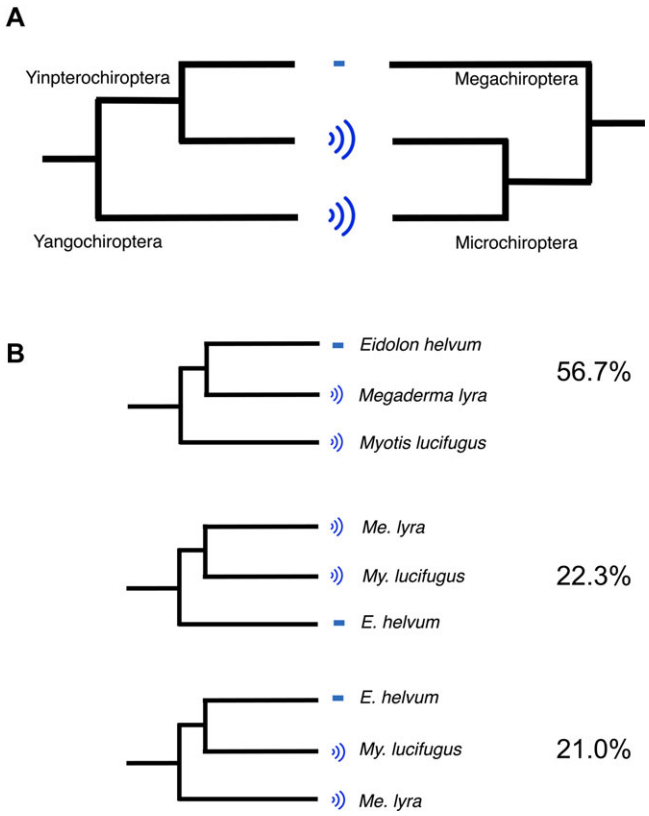

Figure 2. The evolution of echolocation in bats. (A) Species relationships between echolocating and nonecholocating bats (after Teeling 2009). The left-hand species tree shows the relationships inferred from DNA sequence data, putting both echolocating and nonecholocating taxa in the suborder Yinpterochiroptera, with the other echolocating bats in the suborder Yangochiroptera. The right-hand tree shows the traditional species relationships inferred from morphological characters (and limited sequence data). (B) Tree topologies inferred from 2083 genes using alignments from Tsagkogeorga et al. (2013) and Parker et al. (2013). Proportions shown are for the subset of genes (out of $n = 2320$ total) for which orthologs from the three bat species listed and at least nine other mammals were present. Note that the tree for *Prestin* used a different nonecholocating species (*Pteropus vampyrus*) because no ortholog was present in *Eidolon helvum*. Trees from the whole dataset as well all subsets were generated following the methods in Thomas and Hahn (2015). Briefly, alignments were masked with Gblocks (Castresana 2000) and topologies were inferred using RAxML (Stamatakis 2014).

newly resolved species tree requires that two transitions must have occurred.

The radiation of the major bat lineages took place in a very short period of time, with all modern bat lineages originating 50–60 million years ago (Teeling et al. 2005). This compressed series of lineage-splitting events is reflected in the very short internal branches separating these groups (e.g., Tsagkogeorga et al. 2013), and the conflicting results obtained using different loci. The opportunity for incomplete lineage sorting (or hybridization) to occur at the base of the bat phylogeny suggests the possibility that inferences about the evolution of echolocation suffer from hemiplasy. However, to our knowledge no study has explicitly reported levels of discordance among the most likely common topologies. We therefore used alignments from 2320 genes in 22 mammals from Tsagkogeorga et al. (2013) and Parker et al. (2013) to build individual gene trees and to quantify discordance. This dataset contains two species from each of the three major clades of bats: Yinpterochiroptera (echolocating), Yinpterochiroptera (nonecholocating), and Yangochiroptera (all of whom are echolocating).

There was major discordance among the trees we constructed. For instance, in many trees the three clades of bats are not individually monophyletic—25% of trees do not support the monophyly of the two species in Yangochiroptera, whereas 37% do not support the monophyly of the echolocating Yinpterochiroptera. The bats also do not always form a monophyletic group as a whole, with only 65.8% of trees supporting monophyly of the order Chiroptera. To examine discordance among the three possible topologies relating the three major clades of bats (Fig. 2B), we chose one species from each clade (the one with the largest number of genes present in alignments). Of the 2083 genes with data from all three species, this comparison revealed that 56.7% of trees support the grouping of the echolocating and nonecholocating clades within the Yinpterochiroptera, consistent with the inferred species tree. However, as expected given the relatively slight majority of trees supporting the species tree, 22.3% of genes group the two echolocating clades, whereas 21% of genes group Yangochiroptera with the nonecholocating Yinpterochiroptera (Fig. 2B). Therefore, although there is clear support for the species tree as the major topology, both minor topologies occur at high frequencies among the individual gene trees. Although the underlying disagreement among sites is clear from the original analysis (see Fig. 3 in Tsagkogeorga et al. 2013 and Fig. 1 in Zou and Zhang 2015), it was simply not expressed in terms of gene tree discordance.

One issue that should be addressed at this point is the role of bootstrap support in phylogenomics, especially as high bootstrap support is often used to justify the statement that species trees are well resolved. In short, bootstrap values are almost completely uninformative when dealing with genome-scale data, especially about the presence of discordance in a dataset (Salichos and Rokas 2013). With enough data any node supported by a plurality of sites can have 100% bootstrap support in concatenation analyses, and this is likely true of coalescent-based methods that bootstrap across gene trees as well. This is why the bat subordinal relationships can have 100% bootstrap support even though the most common topology is only present in ~57% of gene trees. With a large amount of data, each random sample in the bootstrap will

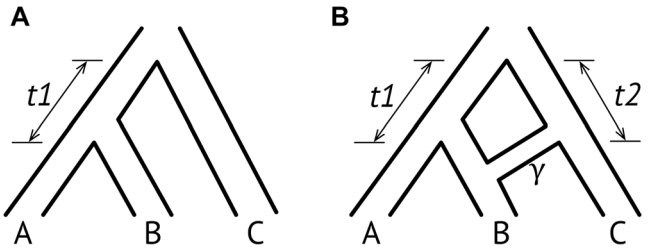

Figure 3. **Probabilities of discordance due to incomplete lineage sorting and introgression. Both panels show relationships among species A, B, and C. Assuming that one sequence is sampled from each of the three species, there are three possible gene genealogies in both panels: $g1 = ((A,B),C)$, $g2 = (A,(B,C))$, and $g3 = ((A,C),B)$. Branch lengths are in coalescent units, in which one unit equals $2N_e$ generations. (A) Under the multispecies coalescent (MSC) process with no introgression, the probabilities of the gene trees $g1$, $g2$, and $g3$ are $1 - (2/3)e^{-t1}$, $(1/3)e^{-t1}$, and $(1/3)e^{-t1}$, respectively. (B) When the MSC process is viewed within the branches of a phylogenetic network, the probabilities of the gene trees $g1$, $g2$, and $g3$ are $(1 - \gamma)(1 - (2/3)e^{-t1}) + \gamma((1/3)e^{-t2})$, $(1 - \gamma)((1/3)e^{-t1}) + \gamma((1/3)e^{-t2})$, and $(1 - \gamma)((1/3)e^{-t1}) + \gamma(1 - (2/3)e^{-t2})$, respectively. The parameter γ represents the probability of following the introgression branch connecting lineages B and C.**

still have the tree grouping the echolocating and nonecholocating clades within the Yinpterochiroptera as the most common tree, and the species tree will therefore consistently be inferred. So although bootstrap support does well at measuring consistency in small datasets and individual genes, it is not informative about discordance in large datasets; for this purpose measures such as concordance factors (Ané et al. 2007) or internode certainty (Salichos and Rokas 2013) are more informative and should be used.

What does the high level of gene tree discordance imply about the evolution of echolocation, and about the possible presence of hemiplasy? We think that an equally parsimonious scenario to those proposed previously involves a single origin of echolocation, with no losses. This scenario is possible under two different biological models. In the first, incomplete lineage sorting is the sole source of discordant topologies, and some rudimentary form of echolocation was present in at least some ancestral individuals. Indeed, the fact that the two discordant trees appear at approximately the same frequency (22.3 and 21%) is consistent with a history of ILS alone (cf. the "*D* test"; Huson et al. 2005; Green et al. 2010). Depending on the genetic complexity of the initial ability to echolocate, it may seem unlikely that all of the underlying genes contributing to the trait were polymorphic at the same time (see next section for more discussion of this issue); but the initial origin of echolocation may have had quite a simple genetic basis, and was certainly further embellished in the two major echolocating lineages—there are important differences in how each has evolved to echolocate, and even some nonecholocators use tongue clicks (Jones and Teeling 2006). It is also possible that there was structure in the ancestral species, with only a subset of populations having the ability to echolocate. Such structure might make polymorphic echolocation in the ancestor more likely, even with a complex genetic basis. The second scenario that could explain a single origin and no losses of echolocation involves hybridization and introgression between two lineages as the source of discordance. In this model echolocation originates in one clade (it should not matter which), and then hybridization between ancestral Yinpterochiroptera and ancestral Yangochiroptera results in the introgression of the underlying genes from one to the other. Given the rapid radiation of bat lineages, there is clearly the possibility for hybridization after speciation. A model involving introgression would also remove or minimize any requirements that multiple genes controlling echolocation had to have been polymorphic at the same time in the ancestral population.

Without further information about the genetic basis for a trait, distinguishing among the various scenarios for character-state evolution in the presence of genealogical discordance may seem impossible. Here, too, echolocation turns out to be an ideal example to consider, although it still may not be resolvable. Much is known about the genetics of echolocation, largely because much is known about the genes underlying both hearing and vocalization. One outstanding candidate gene that contributes to hearing is *Prestin*, which encodes a protein involved in amplifying acoustic signals in the cochlea (Zheng et al. 2000). *Prestin* shows unequivocal patterns of convergent evolution between echolocating cetaceans (dolphins and whales) and echolocating bats (Li et al. 2010; Liu et al. 2010), and therefore represents a clear candidate for convergence between the different echolocating bat lineages. Li et al. (2008) report evidence for such convergence, in the form of a "convergent" topology made from the *Prestin* gene tree, one that unites the echolocating bat clades. Finding this topology has been interpreted as supporting the convergent origin of echolocation (Li et al. 2008; Parker et al. 2013), although not by all authors (e.g., Teeling 2009; Springer 2013). As should be obvious from the discussion here, however, the inference of convergence is completely dependent on a view that forces the alternative topology of *Prestin* into the species tree topology. Given that 22% of genes share this discordant topology (and 21% share the other discordant topology) it seems much more likely that *Prestin* is affected by ILS and the accompanying problems of hemiplasy. Supporting this conclusion further is the fact that even within *Prestin* not all variable sites support the topology grouping the echolocators: 36% of substitutions agree with the species tree. In addition, 28% of synonymous substitutions support the topology grouping the echolocators together, which is not expected if the topology is solely due to convergent evolution of function. Given recombination within a gene, however, these patterns are exactly what one would expect under incomplete lineage sorting. Therefore,

the evidence from *Prestin* may in fact be good evidence against convergence and instead for ILS.

When Will Hemiplasy Be Important?

The example from bats used above was a simple one by design. There were only three possible topologies, the internal branch between speciation events was short (making both ILS and introgression more likely; see expectations below), and candidate genes involved in echolocation are known. However, the problem of accurately inferring character-state transitions in the presence of genealogical discordance may extend to much more complicated scenarios. In this section we discuss several factors that can affect the likelihood that hemiplasy occurs.

The following toy equation for the probability of hemiplasy will help as a starting point for discussion, and highlights the three major factors to consider:

$$\Pr(hemiplasy) \sim \frac{\#\ Discordant\ trees}{\#\ Total\ trees} \times \frac{1}{Branchlengths\ leading\ to\ clades\ with\ shared\ phenotypes} \times \frac{1}{Prior\ belief\ in\ probabililty\ of\ convergence}$$

The first term makes hemiplasy proportional to the fraction of gene trees (or sites) that are discordant with the species tree. This relationship should be relatively clear: the more discordant gene trees there are, the greater the probability that a character being mapped on a species tree is underlain by one of the discordant trees. It is important to note that only some processes produce discordant trees that imply hemiplasy—namely, ILS and introgression. We would not infer hemiplasy when discordance is caused by either convergence or nonphylogenetic factors, because in the former case homoplasy could be the true character history and in the latter we have not accurately inferred the gene tree relationships. All of this means that the fraction of trees that are found to be discordant for any dataset will only be an approximation of this term, though likely a very good one.

A rich body of theory can inform us about the fraction of trees expected to be discordant due to ILS, hybridization, and their joint action. If a single sequence is sampled from each of multiple species, incomplete lineage sorting occurs when sister lineages fail to coalesce in their direct common ancestor, instead coalescing in a more distant ancestor. The likelihood of this occurring can be quantified under the multispecies coalescent process (Hudson 1983; Tajima 1983; Pamilo and Nei 1988), which we illustrate with the history of three species in Figure 3A. Tracing the evolution of a single locus in these three species backward from the leaves toward the root, coalescence events can occur in the ancestral population of (A,B) or the ancestral population of all three species. If the A and B lineages coalesce in the ancestral population of (A,B), which happens with probability $1 - e^{-t1}$, the result is a gene tree that is congruent with the species tree. If no coalescence event occurs in the ancestral population of (A,B), which happens with probability e^{-t1}, then all three lineages coexist in the ancestral population at the root of the species tree and all three possible gene trees have equal probability of 1/3. Therefore, the gene tree that is congruent with the species tree (g_1) has probability $1 - (2/3)e^{-t1}$, whereas each of the other two gene trees (g_2 and g_3) have probability $(1/3)e^{-t1}$. For this three-taxon scenario, then, when only ILS is occurring the first term in the equation above is $(2/3)e^{-t1}$.

If hybridization is also involved, then the species phylogeny takes the shape of a phylogenetic network (Fig. 3B). In addition to the network's branch lengths, hybridization edges have an "inheritance probability" associated with them, denoted γ (Fig. 3B; Yu et al. 2012, 2014). This parameter represents the probability that a locus follows the hybridization edge, and can be thought of as proportional to the fraction of the genome that is inherited via introgression. For example, as the history of a locus sampled from species B is traced backward in time, that locus is inherited from lineage C with probability γ, and is inherited vertically from lineage B's ancestor with probability $1 - \gamma$. Accounting for the multiplicity of paths from leaves to the root in the network, the probabilities of all three gene tree topologies can be derived; in this case, the first term in the equation above equals $(1 - \gamma)((2/3)e^{-t1}) + \gamma(1 - (1/3)e^{-t2})$ (Fig. 3B). Observe that if the inheritance probability, γ, is very high, then the branch length *t1* plays less of a role in explaining incongruence, and character states shared between species B and C are likely due to introgression, even when *t1* is large.

The second term in our equation represents the amount of time available for convergence to occur once species relationships are set. Conditional on a topology, longer branches leading to each clade sharing a trait make it more likely that convergence has occurred (Fig. 4). Due to this, longer branches should be negatively correlated with the probability of hemiplasy. One reason the scenario in bats described above is such a good example of likely hemiplasy is that all three bat clades radiated just after their split, leaving relatively little time for the convergent evolution of echolocation in the two echolocating clades (similar to the left-hand side of Fig. 4). Of course longer branches also likely mean more intervening speciation events, each of which might lead to a lineage without the shared character. As the probability of having the relevant variants stay segregating across multiple speciation events becomes quite low (Suh et al. 2015), this makes it less and less likely that hemiplasy can explain the distribution of characters. For nucleotide substitutions, we can also directly include these branch lengths in our calculations (see Rannala and Yang 2003 for the case of ILS alone, and Yu et al. 2014 for the case of ILS and introgression), allowing us to directly evaluate the probability of hemiplasy.

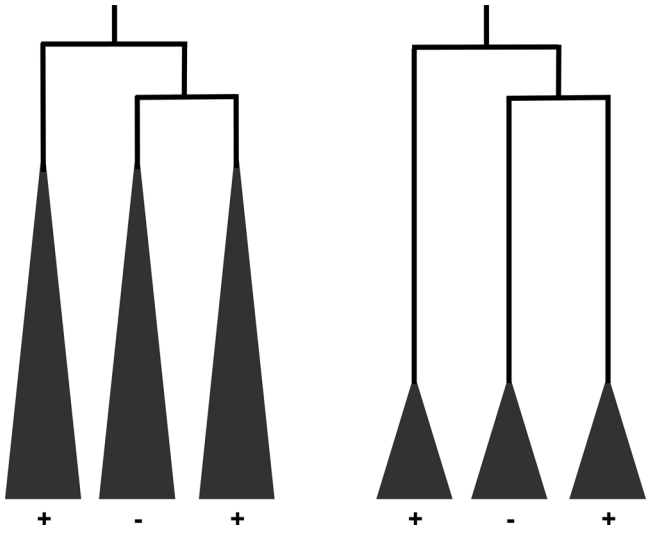

Figure 4. Effect of branch lengths on probability of convergence. Given equal probabilities of discordance in the two trees shown, we propose that convergence would be more likely in the tree on the right. This is because there is little time for convergent substitutions to occur in the tree on the left before each of the three clades independently radiates with the shared character of interest (these clades are denoted by triangles, as the exact relationships within them are not relevant). In the tree on the right the probability of convergent substitutions occurring is higher, whereas there seems to be no greater probability of hemiplasy.

The third term in our equation is the hardest to quantify, but also possibly the most important: what types of traits are more likely to be due to hemiplasy versus convergence? The more likely we believe a trait can be evolved by convergence, the less likely hemiplasy becomes. A naïve expectation is that the probability of convergence is associated with the genetic architecture of the trait under consideration; but thinking about specific traits, it actually seems quite hard to us to justify favoring one explanation over another, regardless of the genetic architecture.

The most obvious contrast should be between genetically complex and genetically simple traits. Consider the simplest trait, due only to a single nucleotide change: the probability of maintaining such a variant through successive speciation events could be quite high even if ILS (or introgression) is rare, giving hemiplasy a reasonable prior probability. But simple traits must also be the easiest to evolve convergently, being dependent only on the occurrence of two independent nucleotide substitutions in this example. It is therefore not clear which explanation to favor for simple traits.

What about for genetically complex, quantitative traits with different means among species? If traits are underlain by many loci of individually small effect, it initially appears quite hard to explain a paraphyletic distribution by hemiplasy. All of the relevant genes would have to be discordant, and under ILS all would have to be polymorphic in the ancestral population. But it is not clear whether all such genes would have to be discordant, or whether only a majority might have to be discordant. For threshold traits, only the tree describing species relationships at the locus that crossed the threshold would matter, regardless of the overall number of loci involved. And for many traits our determination of their quantitative basis is based on the evaluation of current genetic architectures. These traits almost certainly had a simple initial architecture that was elaborated on after their earliest appearance. As mentioned above, the shared echolocation trait differs in many respects between echolocating lineages, and might have had quite a simple beginning involving only one or a few loci. And it actually seems much less likely that genetically complex traits could evolve convergently: as the number of loci involved goes up, the probability of this same number of individual convergent substitutions goes down rapidly. Any argument for a reduction in the number of genes requiring convergent substitutions would of course also lower the barrier to hemiplasy due to ILS as an explanation.

Allowing introgression to explain the discordant appearance of traits on trees likely means that there are no barriers to the genetic complexity of quantitative traits, and hemiplasy becomes more likely. There is a long and growing list of examples of "adaptive introgression" between species (reviewed in Hedrick 2013), including of traits with quite complex genetic bases (e.g., host-seeking behavior in *Anopheles* mosquitoes; Fontaine et al. 2015). If many of the loci underlying a trait can be moved across species boundaries at the same time, one does not have to invoke convergence. In these cases it may be quite easy to explain trait distributions via hemiplasy.

It is important to note that the expectations laid out above are only general guidelines. For instance, even with a small proportion of discordant trees, hemiplasy may be the best explanation for paraphyletic distributions of character states. Cases exist in which only a very small number of loci introgress across species boundaries (e.g., Song et al. 2011; Heliconius Genome Consortium 2012; Brand et al. 2013), yet these loci control important adaptive characters. All of the calculations describing the expected proportion of discordant trees also assume no effects of selection. Most forms of selection will actually act to increase the fraction of concordant trees by reducing the time to coalescence (e.g., Scally et al. 2012; Pease and Hahn 2013), increasing the probability that the distribution of any particular character matches the species tree. Finally, we have not described specific biological scenarios in which hemiplasy is more likely to occur, but we would be remiss not to mention the high potential for misleading inferences in rapid species radiations. With extremely short times between speciation events, both ILS and introgression are likely to be occurring, and a large fraction of gene trees will be discordant (e.g., Brawand et al. 2014; Jarvis et al. 2014; Jónsson

et al. 2014; Lamichhaney et al. 2015; Suh et al. 2015). Even if a species tree can be inferred and ancestral states reconstructed (cf. Schluter et al. 1997), it seems highly likely that many traits will not follow this tree. In such cases an alternative approach to understanding trait evolution will be needed.

Dealing with Discordance

Even in the presence of discordance, researchers want to be able to make inferences about trait evolution. Here we discuss several approaches that may help to avoid the problem of hemiplasy. It is clear that more work in this area is needed, but the approaches outlined below represent important first steps.

There are many situations in which we believe strong inferences about convergent evolution can be made, despite generally high levels of gene tree discordance. Within any species tree, there are likely to be branches with varying levels of support (recognizing that support should be measured by concordance, not bootstrap values). Researchers can focus on branches and relationships with very high levels of concordance to make high-confidence inferences. For instance, if the appearance of similar phenotypes has occurred in distantly related species—too distant to be affected by ILS or introgression—it is likely that there is close to 100% concordance among gene trees for these relationships. In such cases we can be more confident in our inferences of convergence (e.g., Li et al. 2010; Liu et al. 2010; Zhen et al. 2012; Foote et al. 2015). In cases in which the genes responsible for convergence are known, one can also examine the precise nucleotide changes involved to distinguish between convergence and hemiplasy. Under hemiplasy the nucleotide changes will be the same in all lineages (because there is truly only one substitution), whereas under convergence the changes can be the same or different. Therefore, if distinct molecular changes are found, one can eliminate the possibility of hemiplasy. Conversely, if the same functional alleles are found together with the same nonfunctional (e.g., synonymous) alleles in separate lineages, the results are likely due to hemiplasy and not selection-driven convergence.

Approaches that take into account uncertainty in species relationships have also been used, albeit rarely (e.g., Richman and Price 1992; Huelsenbeck et al. 2000; Lutzoni et al. 2001; Pagel and Lutzoni 2002; Huelsenbeck and Rannala 2003; Pagel et al. 2004). Whereas these methods as originally applied considered the uncertainty to be due to a lack of resolution (usually due to a lack of data), they could be just as easily be applied to "resolved" species trees that have discordance—here the uncertainty is to which gene tree(s) the trait follows. These methods dealt with uncertainty by mapping characters on a set of trees from bootstrapped datasets (Richman and Price 1992; Ronquist and Liljeblad 2001) or across a series of trees inferred from the whole dataset via Markov chain Monte Carlo methods (Huelsenbeck et al. 2000; Lutzoni et al. 2001). In the context of gene tree discordance, it may be optimal to carry out character mapping across the range of tree topologies present in the individual gene trees, weighted by their frequency of occurrence (possibly using the individual gene tree branch lengths rather than the time-scaled lengths in the single species tree). One potential issue with all of these approaches is that the most common mapping of characters across trees is likely to be the same as the mapping done on the single species tree. So although the results would provide some measure of confidence in the inferences, the average result would likely still be one of convergence and not hemiplasy. As with all Bayesian approaches, we may be able to place a prior on the probability of convergence versus hemiplasy (as in the equation above). Given increasing amounts of data, we expect the signal in the data to overwhelm the prior, providing support for one of the phylogenetic hypotheses. However, even standard approaches for dealing with uncertainty can sometimes give biased results (Duchêne and Lanfear 2015), so there are clearly multiple issues with this approach that need to be followed up.

A third general approach to dealing with discordance is to change the way that species trees are represented. The problem of hemiplasy has previously been recognized in studies of gene duplication and loss (Vernot et al. 2008; Rasmussen and Kellis 2012; Stolzer et al. 2012), in which the application of reconciliation algorithms to discordant gene trees leads to many misleading inferences (Hahn 2007). These methods have dealt with hemiplasy by representing the species tree as a polytomy at nodes with discordance, coupled with the notion of "conditional" and "required" events that either can or cannot be explained by hemiplasy (Vernot et al. 2008; Stolzer et al. 2012). It is not clear to us whether such approaches can be used for traits that are not directly associated with individual gene trees, but representing species trees as polytomies may be one future avenue for research. A separate approach is to represent the species phylogeny as a network rather than a bifurcating tree (e.g., Yu et al. 2012, 2014). Such approaches have the advantage of allowing for introgression in the mapping of traits (e.g., Jhwueng and O'Meara 2015). In fact, the network view of a phylogeny explicitly captures introgression, although gene trees may still disagree with the network due to ILS. One solution would couple the network representation of species relationships with the approaches for dealing with phylogenetic uncertainty discussed in the previous paragraph. We eagerly await the appearance of methods that can accomplish this task.

Implications for Phylogenetics

We think that the issues raised here, motivated by rapidly accumulating datasets containing high levels of discordance, have important implications for the types of inferences we draw from phylogenies. Most importantly, the problem of hemiplasy raises

multiple issues for the use of comparative methods to understand the evolution of traits on trees. It is clear that we will have to reconsider how we carry out and interpret character mapping when dealing with binary traits. It may be that we can never infer convergence with any certainty when analyzing discordant trios of taxa at any depth in a tree (as in the bat example), as both ILS and gene flow can easily explain discordant distributions of traits. As we become better at associating specific genes with individual traits, we will also have to address the question of how to represent changes that have occurred on discordant trees on the species tree. This is a problem because discordant trees contain branches that do not exist on species trees: how do we represent changes on these branches? Phylogenetic networks are one solution, although there is no single agreed-upon way to represent both ILS and introgression in such networks (see Huson and Bryant 2006; Nakhleh 2011). We also do not yet fully understand how the problems presented by hemiplasy will impact comparative methods that deal with continuous characters, especially when the questions concern the correlations between them (e.g., Huelsenbeck and Rannala 2003). These and other questions about the use of comparative methods will have to be addressed in the near future to enable strong inferences about evolutionary changes on trees.

Considering the effects of hemiplasy may also help to resolve longstanding questions in evolutionary biology. The debate over "morphology versus molecules" in building and interpreting phylogenetic relationships has a long history (e.g., Hillis 1987; Eernisse and Kluge 1993; O'Leary et al. 2013; Lee and Palci 2015). The debate has often centered around which type of data to give primacy to, or how to combine different types of data, especially when relationships from molecular and morphological characters appear to disagree. Recognizing that there is often a lot of discordance within molecular datasets—including in cases in which a single tree has high bootstrap support—and that genes underlie morphological traits, may help us to shift the argument from morphology versus molecules to "genes controlling some morphologies versus genes controlling other aspects of the organism." That is, the topologies implied by certain morphologies simply reflect discordant underlying gene trees, and no special treatment of such trees is needed. In fact, using morphological characters is no solution for avoiding hemiplasy, as they may show discordant patterns due to ILS or hybridization at least as often as molecular characters. We can also imagine how support for discordant gene trees from morphological characters could further inform evolutionary hypotheses, and could even implicate specific genes in the genetic basis of these characters (e.g., Pease et al., 2016).

Most importantly, the issues raised here should cause us to reconsider what it is we expect from our species trees. It may be that we do not want resolution of our phylogenetic relationships, as this will mislead as often as it helps. To be sure, many relationships among organisms are clear and incontrovertible, and inferences about convergence and character evolution from such relationships are undoubtedly strong (e.g., Li et al. 2010; Zhen et al. 2012). However, these are not the types of relationships that require the resolution offered by genome-scale data (recognizing that in some cases we may have simply lacked data for some clades to which genomics is applied). A major goal of recent phylogenomic studies specifically appears to be the resolution of tangled relationships: see, for instance, use of the words "resolve" or "resolution" in recent paper titles (e.g., Rokas et al. 2003; Dunn et al. 2008; Wiegmann et al. 2009; Meusemann et al. 2010; Smith et al. 2011; McCormack et al. 2012; Jarvis et al. 2014; Misof et al. 2014; Nater et al. 2015). We may need to reconsider this goal, or at least to always remember that not all resolved relationships are without incongruence. And when one genomic study reveals a slightly different resolution of relationships than another, we should not jump wholesale to new conclusions about the direction of evolution for key traits (e.g., Prum et al. 2015 vs. Jarvis et al. 2014). It seems just as likely that the topology of the gene trees underlying such traits remain the same in both cases, and that the preponderance of trees supporting one overall relationship or another has simply changed slightly. In the end, we must recognize that "phylogenetic incongruence [is] a signal, rather than a problem" (Nakhleh 2013), and certainly not a signal that we should conceal with a false sense of resolution.

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