Reconsidering cytonuclear discordance in the genomic age

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Abstract

Historically, phylogenetic datasets had very few loci, but were overrepresented for cytoplasmic sequences (mitochondria and chloroplast) because of their ease of amplification and large numbers of informative sites. Under those circumstances it made sense to contrast individual gene tree topologies obtained from cytoplasmic loci and nuclear loci, with the goal of detecting differences between them—so-called cytonuclear discordance. However, in the current age of phylogenomics and ubiquitous gene tree discordance among thousands of loci, this contrast no longer presents a straightforward interpretation. Simply observing discordance between cytoplasmic trees and a species tree inferred from many nuclear loci does not reveal the cause of discordance. Here, we examine what inferences one can make from trees representing different genomic compartments. While topological discordance can be caused by multiple factors, the end goal of many studies is to determine whether the two compartments have different evolutionary histories: what we refer to as "cytonuclear dissonance." Answering this question is much harder than simply asking whether there is discordance, requiring additional analyses to determine whether introgression has affected only (or mostly) one compartment. Furthermore, even when these histories differ, expectations about which compartment is more likely to have introgressed are not always clear. We conclude by pointing to current research and future opportunities that may help to shed light on topological variation across the multiple genomes contained within a single eukaryotic cell.

Introduction

The field of phylogenetics has a long history of studying inconsistencies between tree topologies inferred from cytoplasmic (i.e. chloroplast and mitochondria) sequences and trees inferred from nuclear loci (reviewed in Toews & Brelsford 2012; Sloan et al., 2017). Early studies in both animals (Ferris et al., 1983; Gyllensten & Wilson, 1987; Powell, 1983) and plants (Doebley, 1989; Rieseberg et al., 1990a; Rieseberg et al., 1990b) presented the contrast between cytoplasmic and nuclear markers as an effective tool for revealing introgression between species. Many of these studies sampled multiple individuals across a geographic range, which often allowed them to infer introgression of cytoplasmic DNA (cytDNA) across species without an accompanying signal of introgression among a modest number of nuclear genes. These patterns invited numerous biological explanations, from selection against nuclear introgression, to selection for cytoplasmic introgression, to female-biased dispersal (Rieseberg & Wendel, 1993).

As the number of nuclear loci used to infer phylogenies has increased, researchers continue to be interested in using inconsistencies between trees from cytoplasmic and nuclear loci to determine whether these two genomic compartments have different histories. That is, they would like to know whether the cytoplasmic genomes have one history while the nuclear genome has another—what we will call "cytonuclear dissonance." Such dissonance of histories can only occur due to introgression of one compartment or the other, and it is important to differentiate between the evolutionary concept of cytonuclear dissonance and the empirical observation of mismatching patterns between nuclear and cytoplasmic loci. Therefore, we use the common term "cytonuclear discordance" strictly to describe mismatching topologies between the trees from these different DNA compartments, though usages of related terms in the scientific literature are diverse and possibly confounding (Box 1). While cytonuclear discordance is one signal of dissonance, it is neither necessary nor sufficient evidence of differing histories. Establishing cytonuclear dissonance requires further analyses to demonstrate the incompatibility of nuclear and cytoplasmic histories. Furthermore, even when dissonance can be statistically demonstrated, such a result still does not tell us which genome introgressed across species boundaries, only that they differ in their introgression histories.

In this paper, we first briefly review the causes of gene tree discordance, followed by a more in-depth discussion of analytical approaches that can be used to determine whether cytonuclear dissonance has occurred. We end with a discussion of the possible biological causes of this dissonance, as well as outstanding questions in the field.

Box 1. Discordant terminology

Cytonuclear discordance is the most common term for describing inconsistencies between phylogenetic trees inferred from nuclear and cytoplasmic loci. Observations of cytonuclear discordance are linked to multiple biological explanations (see main text), with a commensurate number of terms used to describe the patterns, processes, and outcomes.

Several phrases have been used to suggest the magnitude and underlying biological basis of cytonuclear (but usually cytoplasmic) introgression. For example, **cytoplasmic capture** is often used as a synonym for cytoplasmic introgression (Rieseberg & Soltis, 1991; Tsitrone et al., 2003). This term—or a similar one, like **mitochondrial capture** or **chloroplast capture**—is also used to describe a species possessing the cytoplasmic haplotype of another species while lacking signal for nuclear introgression (Good et al., 2015; Secci-Petretto et al., 2023; Wielstra & Arntzen, 2020). Although the term "capture" could imply a benefit to the recipient species, these terms are often used without reference to adaptive introgression. A less suggestive alternative to "capture" is **cytonuclear mismatch** (Beresford et al., 2017; Lee-Yaw et al., 2014; Pritchard & Edmands, 2013), which itself may suggest functional consequences (see below).

Cytonuclear disequilibrium was first used to formalize models that describe associations between cytoplasmic and nuclear genes and to infer their biological basis (Arnold, 1993; Arnold et al., 1988; Asmussen et al., 1987; Asmussen & Arnold, 1991; Latta et al., 2001). In many papers, this term is also used as a synonym for cytonuclear discordance (Fields et al., 2014; Monsen et al., 2007; Won et al., 2003). In a more applied context, the introgression of foreign cytoplasmic genes can be referred to as **cytoplasmic rescue** when used as a tool for escaping the burden of genotypes with many deleterious mutations in threatened or endangered populations (Gemmell & Allendorf, 2001; Havird et al., 2016) or as a preventative mechanism against negative interactions between nuclear and organellar genomes (Barnard-Kubow et al., 2017).

When investigating the molecular interactions between cytoplasmic- and nuclear-encoded proteins, a different but sometimes confounding set of terms are used. **Cytonuclear integration** or **cytonuclear interactions** can describe how proteins that originate from cytoplasmic organelles and the nuclear genome interact to conduct important cellular functions (McDiarmid et al., 2024; Rand et al., 2004; Sloan et al., 2018). Differences in mutation rates, effective population sizes, and mode of inheritance between compartments may select for sequence changes in the other because of these interactions, generally described as **cytonuclear coevolution** (Rand et al., 2004; Wang et al., 2021; Weaver et al., 2022) or **cytonuclear coadaptation** (Edmands & Burton, 1999; Sackton et al., 2003). More specifically, it is proposed that the nuclear genome may undergo **cytonuclear**

compensation (Havird et al., 2015; Sloan et al., 2014; Zhang & Broughton, 2013) in response to deleterious alleles that appear in cytoplasmic genes (Hill, 2020).

As a result of coevolutionary processes, enzyme products from cytoplasmic genes that work well with their native nuclear genes may be reduced in function when present on another nuclear background. Cytonuclear interactions may be interrupted via hybridization and backcrossing, and many authors have documented corresponding reductions in metabolic performance and fitness (Klabacka et al., 2022). These situations can be referred to as **cytonuclear incompatibility** (Hoekstra et al., 2013; Meiklejohn et al., 2013; Moran et al., 2024; Sambatti et al., 2008) and are part of a broad class of postzygotic incompatibilities between cytoplasmic- and nuclear-encoded proteins (Burton et al., 2013; Burton & Barreto, 2012; Dobler et al., 2014; Sloan et al., 2017). In addition, **cytonuclear conflict** can describe the situation in which cytoplasmic and nuclear genomes are under opposing selection pressures. For instance, any process that benefits the transmission of mitochondria at the cost of reducing transmission of the nuclear genome (Havird et al., 2019).

The breadth of these terms is potentially confounding. Does a pattern of cytoplasmic introgression between two species imply cytonuclear coevolution? Does a lack of cytoplasmic introgression suggest cytonuclear incompatibility? Depending on which field authors are approaching the concept from, cytonuclear discordance may include pattern, process, consequence, or all of the these (Dong et al., 2014; Funk & Omland, 2003; Lee-Yaw et al., 2019; Rose et al., 2021; Toews & Brelsford, 2012).

A multispecies coalescent view of species tree and gene trees

Modern population genetics theory provides a view of the species tree wherein gene tree discordance is expected under a wide range of biological scenarios (Maddison, 1997). The multispecies coalescent model (MSC) describes the expected genealogical relationships between sampled species for many loci across the genome resulting from stochastic population processes (reviewed in Rannala et al., 2020). The genealogical history of each locus is represented by a gene tree, whereas the species tree represents the population history. Individual gene trees evolve within the species tree (or species network, when there is introgression). Therefore, the topology of a gene tree can be discordant with the species tree when coalescence at a locus does not occur in the most recent ancestral population, but instead occurs in a more distant ancestral population; this phenomenon is called incomplete lineage sorting (ILS; Figure 1)**.** Under the MSC, species tree branch lengths are often represented as time (t, measured in number of generations) divided by twice the effective population size (N_e). More ILS is expected when there are shorter branches in the species tree, either because the time between speciation events is short or population sizes are large. Population histories involving introgression can be accommodated by species

networks. Such networks are typically represented as a species tree with additional introgression edges, sometimes given weights according to the proportion of the genome inferred to have introgressed along a given edge. Recent empirical phylogenetic studies, no longer limited to sequencing a small number of genes, routinely observe high levels of gene tree discordance due to both ILS and introgression.

As is made clear by the multispecies coalescent model, a species tree or species network does not represent the same thing as a single cytoplasmic or nuclear gene tree. Species histories shape the many locus histories that exist among groups of organisms; locus histories are often investigated with the goal of inferring the species history. This fact is particularly explicit when using species tree methods (Edwards, 2009), which take a collection of individual gene trees and use them to infer a species history. Depending on the model assumed, these methods can infer divergence histories and sometimes introgression as well, resulting in the "nuclear tree" to which a cytoplasmic tree is often compared. While the inferred species tree from the nuclear loci is not exactly an average of the underlying marginal gene trees (because of some quirks of coalescent genealogies), neither does it have to match any of the individual gene trees, even without introgression. In other words, every gene tree in a dataset can be discordant with the species tree (e.g. Jarvis et al., 2014; Pease et al., 2016; Wu et al., 2018; Larson et al., 2024).

While methods to estimate a phylogenetic tree or network from a set of gene trees can rely on a solid foundation of established population genetic and mathematical theory, it is a different challenge to accurately infer individual locus trees in the nuclear genome. This is because there are many interacting biological processes such as recombination, homoplasy, and evolutionary rate heterogeneity that complicate both decisions about how to define loci and how to best estimate their histories. In practice, estimating gene trees is usually accomplished by selecting loci that are short enough that recombination is low within each locus, and then using maximum likelihood methods to infer the tree topology and branch lengths. Gene tree inference error can result when one or more assumptions of the model used to estimate the tree are violated. Methods that make use of site patterns, such as SVDquartets (Chifman & Kubatko, 2015), eliminate the need to delimit loci to infer a species tree, but these methods still require assumptions about the independence of individual genomic sites included in the analysis.

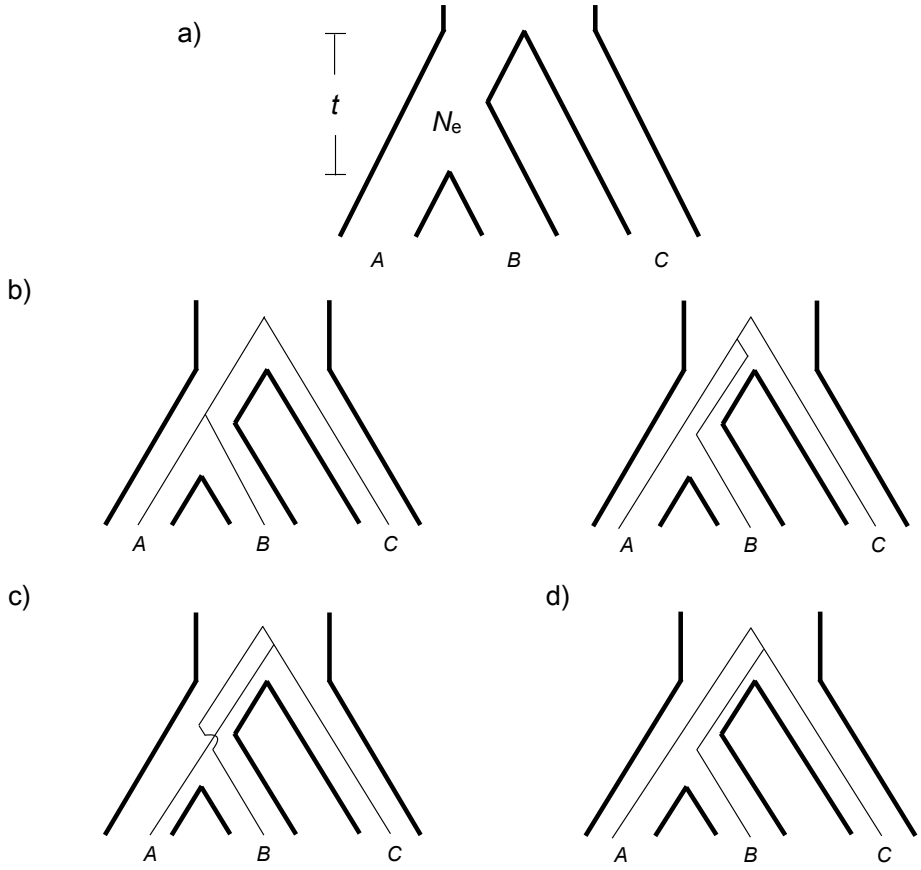

Figure 1. The multispecies coalescent model and incomplete lineage sorting. Under the MSC, many different gene trees can be produced by a single species tree due to incomplete lineage sorting (ILS). a) The species tree provides information about hierarchical relationships and divergence times among species. Here, we emphasize information about the time between the two speciation events (*t*) and the effective population size (N_e) of the ancestral population that exists between these two events. Together, *t* and N_e determine the amount of ILS that will occur in this population. b) The two concordant gene trees that are produced by this species tree. The one on the left coalesces in the ancestor of species *A* and *B* (i.e. lineage sorting), while the one on the right does not (i.e. incomplete lineage sorting). Panels c) and d) show the two discordant gene trees that can be produced by ILS in this species tree, one with species *A* and *C* more closely related (panel c) and one with species *B* and *C* (panel d).

A multispecies coalescent view of cytoplasmic discordance

Considering the description of species trees and gene trees given above, it is worthwhile asking what exactly cytonuclear discordance indicates. Recall that in some datasets every nuclear locus is discordant with the species tree, and that in almost all phylogenomic datasets there is biological discordance due to ILS, gene tree inference error, and/or introgression. In many ways, a cytDNA topology is essentially just one random draw from the multitude of genealogies that the species history comprises, just

as any particular nuclear locus would be (with a few differences discussed below). Thus, even without invoking introgression of the cytoplasm, finding a cytoplasmic gene tree that differs from the species tree (i.e. cytonuclear discordance) is to be expected in many biological scenarios.

To make this view clearer, imagine that instead of a cytoplasmic locus we had a high-confidence gene tree from the nuclear-encoded *alcohol dehydrogenase* (*Adh*) gene, a classic focus of many evolutionary studies. How would we interpret discordance between *Adh* and the species tree? This alco-nuclear discordance could of course signify introgression among species at the *Adh* locus, possibly even tied to coevolution between *Adh* and its interacting proteins. But the most parsimonious interpretation, absent additional evidence, is simply that it is discordant due to random genealogical processes and ILS. There is little reason to believe that inferences at cytoplasmic loci should be any different.

While cytDNA is subject to many of the same biological processes as nuclear DNA, there are important differences, including in the effective population size these genes experience. In most plants and animals, the mitochondrial and chloroplast genomes are haploid and uniparentally inherited, causing the cytDNA to have a lower effective population size than a typical nuclear locus. Under idealized conditions, N_e for cytDNA is expected to be four times lower than nuclear loci in a diploid species. However, this varies greatly depending on several biological factors and is difficult to measure empirically (Wright et al., 2008). The smaller N_e of cytoplasmic genomes means that coalescence will occur more quickly on average. Therefore, we might not expect the cytDNA tree to experience as much ILS as the average nuclear locus. As we discuss in the next section, some approaches are better able to account for this difference in N_e than others.

How can we identify cytonuclear dissonance?

Given discordance between a cytoplasmic locus and the species tree, one may want to know: is the observed discordance due to error, ILS, or introgression? There are several considerations to be made in determining whether the cytDNA has a history that is truly dissonant with that of the nuclear genome (Figure 2). By dissonant, we mean that the history reflected in the nuclear genome, including any history of introgression among nuclear loci, cannot explain the history of the cytDNA. That is, neither the species tree nor any species network inferred from nuclear data is likely to have produced the tree inferred from the cytDNA. Here, we discuss a series of tests that can help to determine whether the cytoplasm has a different history than the nucleus.

Testing for cytonuclear discordance

A primary consideration when evaluating cytonuclear dissonance should be the extent to which the nuclear and cytoplasmic trees truly differ. If there are no well-supported branches that differ between the cytDNA tree and an inferred species tree, then there is no reliable signal of cytonuclear discordance, and the observed differences are likely due to tree estimation error. Even with whole cytoplasmic genomes, one should not ignore gene tree inference error as a possible source of discordance (Kimball et al., 2021; Shen et al., 2017; Weisrock, 2012). Sequence alignments should be inspected to check whether taxa with particularly high levels of missing data are causing discordance or whether the gene tree shows signals of biologically unreasonable branch lengths, possibly due to misidentified orthologs or assembly errors. Species misidentification or taxonomic uncertainty can also be issues for some studies (Toews & Brelsford, 2012). It is important to note that any statistical assessment of cytonuclear discordance can only use a single tree per organellar genome—one cannot use each gene in the mitochondrial or chloroplast DNA separately, as doing so would require assumptions about recombination within cytoplasmic genomes that are not generally aligned with their biology (Edwards & Bensch, 2009). If there is support for cytonuclear discordance, the next step is to determine whether there is evidence of nuclear introgression, since this aspect of the species history will determine which are the most appropriate tests for cytonuclear dissonance.

Testing for nuclear introgression

The shape of the species tree or network determines the probability of observing any particular gene tree topology. Thus, characterizing any past introgression involving the nuclear genome is important to understanding whether the cytoplasm has a different history. There are currently many methods that can be used to detect introgression among nuclear loci, including *D*-statistics and *F*-statistics (reviewed in Hibbins & Hahn 2022); these tests generally rely on differences in the overall counts of gene tree topologies or site patterns across the genome, as introgression can cause some to occur more often than others. When testing for introgression, ILS is generally used as the null hypothesis and the absence of evidence for introgression is generally taken to be evidence for ILS. In reality, however, ILS is always occurring, even (or perhaps especially) in the same circumstances where introgression is likely to occur (i.e. closely related populations or species). Introgression inference at individual loci (such as the chloroplast or mitochondria) requires that a determination about introgression history be made about a single gene tree, which requires a different approach than testing for genome-wide introgression in the nuclear genome.

Testing for cytonuclear dissonance

Ultimately, establishing cytonuclear dissonance requires showing that the cytDNA and nuclear genomes have different introgression histories. In other words, one must show that the inferred nuclear species tree or network could not have produced a gene tree like that observed for the cytDNA. Only introgression of either the cytoplasmic genome or nuclear genome (to the near complete exclusion of the other) produces cytonuclear dissonance. Thus, the approach one takes to testing for cytonuclear dissonance depends on an understanding of the nuclear introgression history. If there is no evidence of nuclear introgression, and the species history can be reasonably modeled as a bifurcating tree, then establishing evidence of cytonuclear dissonance involves asking whether the inferred species tree could generate a tree like the cytDNA topology due to ILS. If nuclear introgression has occurred among species, then demonstrating cytonuclear dissonance involves determining whether the species network, including the hypothesized history of introgression, could generate the cytDNA tree. The approaches for identifying which gene trees are (realistically) possible, given the species tree or network, fall into two broad categories: examining the distribution of empirical nuclear gene trees estimated from sequence data (Method I below) and generating gene trees through simulation based on an inferred species tree or network (Method II below). We next discuss these two approaches in greater detail, as well as the strengths and drawbacks of each.

Method I: Compare cytoplasmic tree to nuclear gene trees

A straightforward approach to determining which gene trees a species tree or network is likely to produce is to examine the set of empirically estimated nuclear gene trees. We could of course compare the cytDNA tree topology to each estimated nuclear gene tree topology, but with any moderate number of tips we might not expect any to match completely, even under the same history. Instead, one can compare the nuclear gene trees to the species tree topology using a distance metric such as Robinson-Foulds distance (Robinson & Foulds, 1981) or “extra lineages” distance (Maddison, 1997; Than & Rosenberg, 2011) and then compare the cytDNA tree in the same way to ask whether the cytDNA tree is unusually distant (i.e. in the extreme tail of the distribution of distances). This approach provides a good empirical comparison because both sets of gene trees must be inferred from data and may therefore experience similar estimation error. If the cytDNA tree has a greater phylogenetic distance than is observed in any of the nuclear gene trees, this can be taken as evidence that the cytoplasmic genomes have a dissonant history. Several recent studies have taken such an approach (e.g. Kimball et al., 2021; Gardner et al., 2023).

An alternative approach asks whether there are specific branches that differ between sets of gene trees. If one has sampled a reasonably large number of nuclear loci, one can determine whether there are specific, well-supported branches in the cytDNA tree(s) that are not present among the nuclear gene trees. If they are not present in any nuclear trees, this is evidence that the cytoplasm has a different history.

Examples of this approach can be found in Buckley et al. (2006), Folk et al. (2017), and Gardner et al. (2023). Toews and Brelsford (2012) took a similar approach: "nuclear markers can be discordant among themselves, as a result of drift or of different patterns of dispersal, selection or demography. Only in cases where mtDNA was a clear outlier to the general pattern of other nuclear markers did we include it in our survey." However, the nuclear datasets considered in that study were very small, thus the breadth of nuclear trees observed were small as well, and likely did not reflect the full range of nuclear gene trees present. Approaches that focus on identifying branches that only occur in one tree or the other can help show which branches make the trees discordant. This, in turn, can provide information about where in the tree introgression has occurred, which is not possible using methods that only consider overall tree dissimilarity.

Method II: Simulate data and compare

A second general approach is to simulate gene trees with ILS using the species tree or network, but with tree branches lengthened to resemble those resulting from cytoplasmic inheritance. As discussed above, cytoplasmic loci have an effective population size that is smaller than an average nuclear locus and are therefore expected to experience less ILS. Species tree branch lengths can be estimated in coalescent units ($=t/2N_e$) experienced by the nuclear genome; however, one cannot estimate a tree in coalescent units from cytDNA directly. Instead, simulating the amount of ILS experienced by the cytDNA can be done by simply lengthening the branches of the nuclear-based tree by a factor of four. A variety of different coalescent simulators can then be used to generate a null set of gene trees expected under the nuclear history, but with the amount of ILS approximately experienced by the cytoplasm.

Once a set of cytDNA-like trees has been simulated, one can apply the same two approaches as described above for the empirical nuclear gene trees. That is, one can determine whether the total tree distance from the species tree is greater for the cytDNA trees than the simulated cytDNA-like trees (Gardner et al., 2023; Zhou et al., 2022) or investigate whether branches present in the cytDNA trees are also observed among simulated gene trees (Folk et al., 2017; García et al., 2017; Morales-Briones et al., 2018; Zhou et al., 2022). While simulation-based approaches are better at capturing the ILS experienced by the cytoplasm, they also have several disadvantages. First, the cytDNA tree is inferred from data, while the simulated trees are not. Therefore, the cytDNA tree might be more different from the species tree simply because it contains more error. Second, misspecification of the species tree or network used to simulate gene trees could lead to an incorrect distribution of gene trees.

Interpreting the evidence: what can we infer?

Thus far, we have introduced multiple biological processes that can lead to gene tree discordance, as well as multiple different tests that allow us to distinguish discordance from dissonance. Importantly, comparisons involving tree topologies do not necessarily tell us much about the particular events or processes that have occurred, and there are often multiple possible combinations of ILS and introgression that could lead to similar sets of empirical evidence for or against dissonance. Here, we discuss what the evidence can tell us about the histories of the nuclear and cytoplasmic genomes, focusing our discussion on five scenarios implied by the tests described in the previous section (Figure 2).

Scenario 1: No cytonuclear discordance

If one lacks support for cytonuclear discordance, the most straightforward interpretation is that the cytDNA has the same history as much of the nuclear genome (Figure 2). However, even introgressed loci do not necessarily have a discordant tree topology (Hibbins & Hahn, 2019). It is still therefore possible that introgression (and ILS) has occurred in one or more genomic compartments in this, or any other scenario.

Scenario 2: Cytonuclear discordance, but no cytonuclear dissonance in the absence of nuclear introgression

If there is support for cytonuclear discordance, but nuclear introgression is not suspected, one can use the tests outlined above to establish whether the cytDNA tree could be produced by the inferred species tree. If the cytDNA tree is sufficiently similar to the nuclear gene trees observed, only ILS is needed to explain the discordance (Figure 2). Even if the inferred cytoplasmic tree is not shown to match any particular nuclear gene tree, pervasive ILS can lead to a large number of possible trees, not all of which will be observed in the nuclear genome. Such cytonuclear discordance can therefore simply be due to ILS (e.g. DeRaad et al., 2023).

Scenario 3: Cytonuclear dissonance in the absence of nuclear introgression

If one has established that the cytDNA tree is dissonant with the species tree in the absence of nuclear introgression (Figure 2), can one conclude that the cytoplasmic element is the one that introgressed? The analyses to assess dissonance described in the previous section simply show that introgression is necessary to explain the data, not which genome introgressed. There may be some cases where one can argue for one scenario or the other, but the biology of cytoplasmic genes alone does provide a strong argument for or against introgression of the cytoplasmic compartment (Sloan et al., 2017 and see next section). However, well-designed geographic sampling can provide compelling evidence for cytoplasmic gene flow in the complete absence of nuclear introgression (see next section).

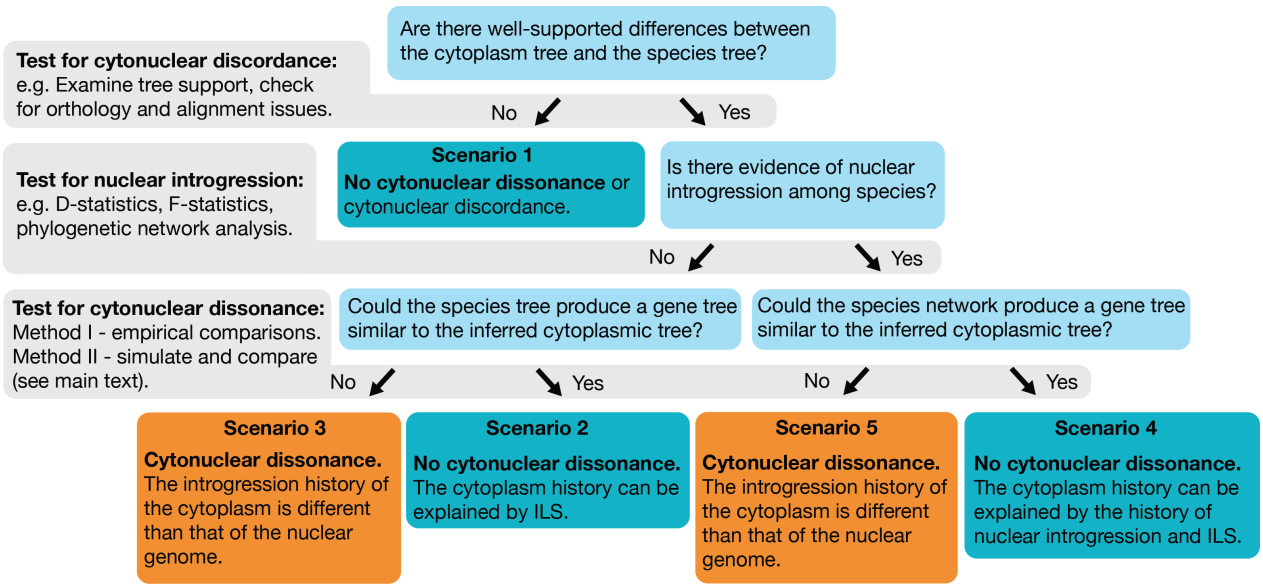

Figure 2. Decision tree for assessing cytonuclear dissonance.

Scenario 4: Cytonuclear discordance, but no cytonuclear dissonance in the presence of nuclear introgression

If there is evidence of both cytonuclear discordance and nuclear introgression, dissonance requires showing that the cytDNA tree could not result from the proposed species network. If the species network could generate a similar tree through a combination of ILS and introgression, then there is no evidence that the history of the cytoplasm differs from that of the nuclear genome, and therefore no evidence of cytonuclear dissonance (Figure 2). Several studies have found cytonuclear discordance that is well-explained by a history of introgression also observed among nuclear loci, including in bats (Foley et al., 2024), seabirds (Mikkelsen & Weir, 2023), and wild pigs (Frantz et al., 2013).

Scenario 5: Cytonuclear dissonance in the presence of nuclear introgression

If one has established cytonuclear discordance and shown that the nuclear history of introgression cannot reasonably explain the cytDNA history, then there is evidence of cytonuclear dissonance involving a complex history of introgression (Figure 2). There are several studies that have inferred cytonuclear dissonance in the presence of nuclear introgression. Folk et al. (2017) used empirical tree comparisons and simulations (i.e. Methods I and II here) to show that both of the cytDNA trees in the plant genus *Heucera* were dissonant with the species tree. The chloroplast and mitochondrial trees were similar to one another, but shared few clades in common with the nuclear-derived species tree. Similarly to scenario 3, however, one cannot simply conclude that the cytoplasm has introgressed to generate this dissonance.

Which cellular compartment introgressed?

In most cases of cytonuclear dissonance, the topology of a phylogenetic tree alone is not sufficient to distinguish which genome introgressed. However, some particularly clear examples of cytonuclear dissonance come from population-level analyses, where cytoplasmic genome variation within a species allows inference of the directionality of introgression (Denton et al., 2014; Soltis et al., 1991; Toews & Brelsford, 2012). For example, Good et al. (2015) used targeted sequence capture to show that there was no evidence of nuclear introgression, despite clear, unidirectional introgression of the mitochondrial genome in populations of *Tamias* squirrels. It is important to note that monophyly of a gene tree within a species does not necessarily rule out introgression, since an introgressed genome can also fix within a species (e.g. Bossu & Near 2009).

The tests we describe above rely mainly on identifying differences in tree topologies; however, branch length information can also be useful for determining evidence of introgression (e.g. Hahn & Hibbins 2019). Under ILS alone, the divergence time of cytDNA should be older than a given species divergence, whereas introgression can result in cytDNA divergences that are more recent than the species divergence (Joly et al., 2009; Rosenzweig et al., 2016). However, it is important to ensure that genetic distances or branch lengths are comparable between compartments, as both mutation rates and effective population sizes differ. Fair comparisons require making clear distinctions between allelic divergence times and species divergence times (cf. Edwards & Beerli 2000) and making use of scaling factors that account for differences in mutation rates between compartments (e.g. Mikkelsen & Weir 2023; Lee-Yaw et al., 2019).

Because it is so hard to establish which compartment has introgressed, many researchers have made arguments for one interpretation or the other. There are many explanations in the literature for why genes in one or the other compartment might have introgressed, given that cytonuclear dissonance can have important functional consequences. For example, mismatches between cytoplasmic genes that cause male sterility and nuclear restorer elements underlie hybrid male sterility in several groups of plants (Fishman & Willis, 2006). Furthermore, several organellar protein complexes are derived from subunits that originate from both the cytoplasmic and nuclear genomes, which coevolve to maintain the structure and function of the protein complex (Rand et al., 2004; Sloan et al., 2018; Weaver et al., 2022; Yan et al., 2019). Mismatches between these co-adapted subunits can result in genetic incompatibilities through poor physiological performance or lethality in hybrids (Barnard-Kubow et al., 2017; Lamelza & Ailion, 2017; Moran et al., 2024; Willett & Burton, 2001). Therefore, some arguments point to the central role of mitochondria and chloroplasts in metabolism and emphasize that their genomes (and any nuclear genes to which they are co-adapted) should generally be resistant to gene flow due to reduced fitness of early hybrids. Thus, from this point of view, introgression of the cytoplasmic genomes should be rare relative to the nuclear genome.

Other arguments emphasize that the lack of appreciable recombination in cytoplasmic genomes could lead to the accumulation of deleterious mutations, with no mechanism to remove them (except perhaps very rare back-mutations). In such cases, a species could benefit from acquiring an overall less-impaired cytoplasmic genome through introgressive hybridization with another species (reviewed in Sloan et al., 2017). However, as mentioned above, discordance by itself is also usually uninformative about the direction of introgression, so determining who "captured" whom requires additional data. Other arguments highlight the possibility that during a species' range expansion, introgression allows the acquisition of more locally adapted cytoplasmic genomes (Hill, 2019). In these cases, one might argue that the cytoplasmic genome is more likely than most nuclear genes to introgress.

Finally, it may be that nuclear genes can co-introgress with co-adapted cytoplasmic genes (e.g. Forsythe et al., 2020). If a small number of nuclear genes appear to share an introgression history with the cytoplasm, should one consider there to be cytonuclear dissonance? The threshold for how many nuclear genes should be allowed to introgress alongside the cytoplasm is largely a matter of terminology: what is more relevant is whether this pattern is due to co-evolution and/or selection for co-introgression between nuclear and cytoplasmic genes. One approach to detecting such scenarios is to test whether introgressed nuclear loci are enriched for genes involved in plastid or mitochondrial interactions (e.g. Lee-Yaw et al., 2019; Forsythe et al., 2020). If there are more genes involved in cytonuclear interactions than expected by chance, this could be evidence that selection has caused these genes to be preferentially introgressed after or during the introgression of the cytoplasm. One should also be aware of the possibility that these genes are among the only ones that have not introgressed within a background of near-total nuclear replacement. Determining which situation is more likely requires additional information.

Conclusions and Future Directions

We have argued for a clearer distinction between cytonuclear discordance and cytonuclear dissonance. Such a distinction will allow researchers to differentiate between patterns observed in phylogenetic analyses and evolutionary processes, including introgression and interactions between cytoplasmic elements and nuclear genomes. Cytonuclear discordance is relatively easy to demonstrate; cytonuclear dissonance requires much more work. Advances in DNA sequencing have provided a broader view of the frequency with which nuclear gene trees will be discordant with the species tree. Depending on the tempo of speciation and the history of introgression, many cases of cytonuclear discordance may be well-explained by processes that affect all cellular compartments.

There are several areas of research that may yield advances in our understanding of the causes of cytonuclear discordance and dissonance. One pattern that we find

particularly interesting is that cytoplasmic gene trees often appear to be more different from the species tree, and with higher support, than any particular nuclear gene tree (e.g. Zhou et al., 2022; Gardner et al., 2023; Hendriks et al., 2023). The reasons for this pattern are unclear: it could be because cytDNA introgresses more often, or simply that the signature of introgression is more easily observed in the relatively long, nonrecombining cytoplasmic genome. In other words, it could be that the length of recombination-free loci within the nuclear genome are generally too short to yield strongly supported, highly conflicting tree topologies. Another explanation could be that the conserved functions of chloroplasts and mitochondria mean that their genomes are capable of introgressing across further evolutionary distances without experiencing the same levels of genetic incompatibilities as the average nuclear gene.

Furthermore, scientists cannot investigate the potentially powerful consequences of cytonuclear discordance until it is defined and reliably identified. A robust definition of cytonuclear dissonance will allow empirical studies to assess other related processes, such as the prevalence of co-introgression of nuclear and cytoplasmic loci, signatures of compensatory molecular evolution, or the functional costs of cytonuclear mismatch. Taxonomic systems that demonstrate strong signals for cytoplasmic dissonance can be used to test for potential negative effects on these types of traits (e.g. mitochondrial efficiency, organismal metabolic rate, fertility, etc). Several studies have demonstrated that cytonuclear mismatch can carry a negative or lethal consequence, particularly in F1 hybrids or asexual lineages of animals (Cullum, 1997; Willett and Burton, 2001; Denton et al., 2017; Klabacka et al., 2022; Moran et al., 2024). However, it remains unclear how common these cytonuclear scenarios are—especially compared to nuclear incompatibilities—or if the fitness costs are meaningful. Systems with a history of cytonuclear dissonance, particularly without co-introgression, should provide better insight to the ubiquity of this phenomenon.

Reliably differentiating between patterns of cytonuclear discordance and cytonuclear dissonance is an important step forward, especially as these concepts become applied to other biological disciplines. For example, cytonuclear dissonance is increasingly studied as a contributor to phenotypes associated with aging (e.g. Serrano et al., 2024). These studies often conduct crosses among strains to create new combinations of mitochondrial haplotypes on different nuclear backgrounds (Serrano et al., 2024) or to quantify selection between or within individuals that are heteroplasmic (Battersby & Shoubridge, 2001; Jenuth et al., 1997). In these instances, phylogenetic methods could help to quantify cytonuclear dissonance to contextualize experiments that measure putative physiological outcomes. The above guidelines could also contribute to the further development of ecological models that consider cytonuclear dissonance as a parameter that influences community composition, abundance, and distribution (e.g. Princepe et al., 2022). The dizzying array of terminology surrounding cytonuclear discordance and dissonance (Box 1) makes these connections across fields challenging, but we hope that the conceptual clarifications offered here make them more likely.

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