The mitonuclear compatibility species concept

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COMMENTARY

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ABSTRACT

The avian world is packaged into genetic assemblages that we call species. Although ornithologists can, with a few important exceptions, agree on the boundaries among avian gene pools that delimit species, the evolutionary process that created this structured subdivision of Aves remains uncertain and contentious. Moreover, although avian species are recognizable and diagnosable, many bear signatures of recent, often substantial, exchange of nuclear (N) genetic material. As a result, there is debate regarding the process that gives rise to and maintains the genetic structure of avian populations. I propose that a key missing consideration in discussions of speciation is the necessity of coadaptation between N and mitochondrial (mt) genes to enable core energy production via oxidative phosphorylation. Because mt genomes are non-recombining and subject to high mutation rates, they evolve rapidly. Consequently, N and mt coadaptation persists only through perpetual coevolution between mt and N genes. Mitonuclear coevolution leads to rapid divergences in coadapted mitonuclear gene sets whenever there is a disruption in gene flow among populations. As a result, once populations diverge in coadapted mitonuclear genotypes, the reduced fitness of offspring due to mitonuclear incompatibilities prohibits exchange of mt and N-mt genes and effectively isolates individuals with shared coadapted N and mt genotypes. Given these considerations, I propose that avian species can be objectively diagnosed by uniquely coadapted mt and N genotypes that are incompatible with the coadapted mt and N genotype of any other population. According to this mitonuclear compatibility species concept, mitochondrial genotype is the best current method for diagnosing species.

Keywords: DNA barcode, gene flow, Haldane's rule, hybridization, OXPHOS, speciation

El concepto de especie de compatibilidad mitonuclear

RESUMEN

El mundo de las aves esta empaquetado dentro de ensambles genéticos al que llamamos especies. Aunque los ornitólogos pueden, con algunas excepciones importantes, estar de acuerdo sobre las fronteras entre los grupos genéticos de las aves que delimitan las especies, el proceso evolutivo que creó la estructura de esta subdivisión de Aves permanece aún incierto y controversial. Más aún, aunque las especies de aves son reconocibles y diagnosticables, muchas presentan signos recientes y muchas veces sustanciales de intercambio de material genético nuclear (N). Como resultado, existe debate sobre el proceso que origina y mantiene la estructura genética de las poblaciones de aves. En este trabajo propongo que una consideración clave que no se menciona en la discusión sobre especiación es la necesidad de coadaptación entre N y los genes mitocondriales (mt) para permitir la producción de energía central a través de la fosforilación oxidativa. Debido a que el genoma mt es no recombinante y está sujeto a altas tasas de mutación, evoluciona rápidamente. Consecuentemente, la coadaptación N/mt persiste solo a través de la coevolución perpetua entre los genes mt y N. La coevolución mitonuclear lleva a divergencias rápidas en los conjuntos de genes mitonucleares coadaptados cada vez que hay una disrupción en el flujo génico entre poblaciones. Como resultado, una vez que las poblaciones divergen hacia genotipos mitonucleares coadaptados, la reducción de la adecuación biológica de la descendencia debido a incompatibilidades mitonucleares impide el intercambio de genes mt y N-mt y aísla efectivamente a los individuos con genotipos N y mt coadaptados compartidos. Dadas estas consideraciones, propongo que las especies de aves pueden ser diagnosticadas objetivamente por medio de genotipos mt y N coadaptados únicos, que son incompatibles con el genotipo mt y N coadaptado de cualquier otra población. Por medio de este concepto de especie de compatibilidad mitonuclear, el genotipo mitocondrial es la mejor manera actual de diagnosticar a las especies.

Palabras clave: código de barras de ADN, especiación, flujo génico, hibridación, OXPHOS, regla de Haldane

In the early 20th century, Ernst Mayr, the great avian biogeographer and evolutionary theorist, grappled with the question of whether avian species are real biological entities or simply a fabrication of western taxonomists (Mayr 1940). He made a compelling case that avian species are real and can be objectively and repeatably delineated. The species concept articulated by Mayr and endorsed by the American Ornithologists' Union (AOU)

Committee on Classification and Nomenclature is that "species are genetically cohesive groups of populations that are reproductively isolated from other such groups" (AOU 1998). Thus, there is at least some consensus that species are more-or-less discrete gene pools (Gill 2014, Toews 2015). A rapidly expanding literature on the genetic structure of populations, however, indicates that gene flow is common among populations recognized as species—and sometimes substantial between populations unanimously recognized as species (e.g., Toews et al. 2016b). In most birds, this flow of genes appears to be much greater for autosomal genes than for sex-linked or mitochondrial (mt) genes (Carling and Brumfield 2008, Qvarnström and Bailey 2009, Rheindt and Edwards 2011). The paradox of avian taxonomy in the early twenty-first century is that the more we learn about the genetic structure of populations, the more current theories of speciation become inadequate to explain the observed patterns (Harrison and Larson 2014).

In this essay, I present an argument that species are best defined by coadapted sets of mt and nuclear (N) genes. In presenting this new species concept, I make no attempt to comprehensively review the hundreds of papers and several books that have been written on animal and avian speciation. The state of thought regarding avian speciation and the concept of species as applied to birds was thoroughly summarized by Gill (2014) and Toews (2014), and empirical studies of avian speciation were comprehensively reviewed by Price (2007). I present only a brief overview of dominant current models of speciation in order to provide necessary context for the proposed model. Mitonuclear coadaptation was not mentioned in any recent treatments of avian species concepts, so my primary goal here is to introduce the ornithological community to the concepts of mitonuclear coadaptation and coevolution as central to understanding the process of speciation and the nature of avian species.

I present my argument for mitonuclear genomic interactions driving speciation specifically in birds—rather than all vertebrates, metazoans, or eukaryotes—because, as an ornithologist, I can articulate and assess a mitonuclear compatibility hypothesis of speciation most effectively in birds. Ornithologists have played a dominant role in the development of species concepts because birds are by far the best-known animal taxon (Scheffers et al. 2012). Birds are typically diurnal and conspicuous, so ornithologists had recorded the phenotypes (particularly coloration and song) as well as the distributions of the great majority of the world's bird populations by the early twentieth century (Sharpe 1909, Mayr 1970). While humans cannot perceive the signals used in species recognition by most animal species (Palumbi 1994), the primary sensory modalities of birds are the same used by humans, so sounds and morphological features that are conspicuous to birds—and

potentially important in distinguishing conspecifics from heterospecifics—are also apparent to humans (Hill 2006). For many decades, ornithologists stood on the platform of their knowledge of the biogeography of Aves as they speculated on the processes that gave rise to this biodiversity and debated where species boundaries should be drawn (Mayr 1940, 1982, Cracraft 1983, Zink and McKitrick 1995). If an argument can be made for the importance of mitonuclear coadaptation in avian speciation, then the full scope of the theory can be explored through extrapolation to other taxa.

The mitonuclear compatibility model of speciation requires a basic understanding of how products of the mt genome and products of the N genome co-function to create oxidative phosphorylation (OXPHOS), so I begin with a brief review of the genomic architecture of the electron transport system (ETS) and a discussion of how this architecture necessitates tight mitonuclear coadaptation that can be maintained only through perpetual mitonuclear coevolution. I then present the mitonuclear compatibility model of speciation in detail. I conclude by applying this mitonuclear perspective to the interpretation of patterns of (1) distinctiveness of mt genotypes between avian populations; (2) greater introgression of autosomal vs. mt or sex-linked genes between putative avian species; (3) chromosomal locations of genes for ornamentation, preference, and incompatibility; (4) disproportionate effects of hybridization on the heterogametic sex (Haldane's rule); and (5) hybrid speciation in birds.

Mitonuclear Coadaptation: A Missing Fundamental Principle in Concepts of Speciation

For a eukaryote to be a functional organism, it must have coadapted mt and N genes (Rand et al. 2004, Lane 2005). The implications of this fundamental necessity of mitonuclear coadaptation are potentially huge for processes of speciation and sexual selection (Hill 2015a), yet mitonuclear interactions are virtually never considered in the formulation of species concepts related to birds. The necessity of mitonuclear coadaptation arises from the fundamental genetic architecture of complex life: The phenotype of eukaryotes is encoded by both an mt and an N genome. Basic functionality that determines individual fitness, particularly of complex animals, hinges critically on the products of N genes and the products of mt genes working in intimate functional association to enable cellular respiration and core energy production (Hill 2015a, Lane 2015; Figure 1). To understand the significance of mitonuclear interactions to the process of speciation in birds, it is critical to have basic understanding of the genomic architecture of birds, so I begin with a brief review of the sets of genes responsible for a functional ETS (Table 1).
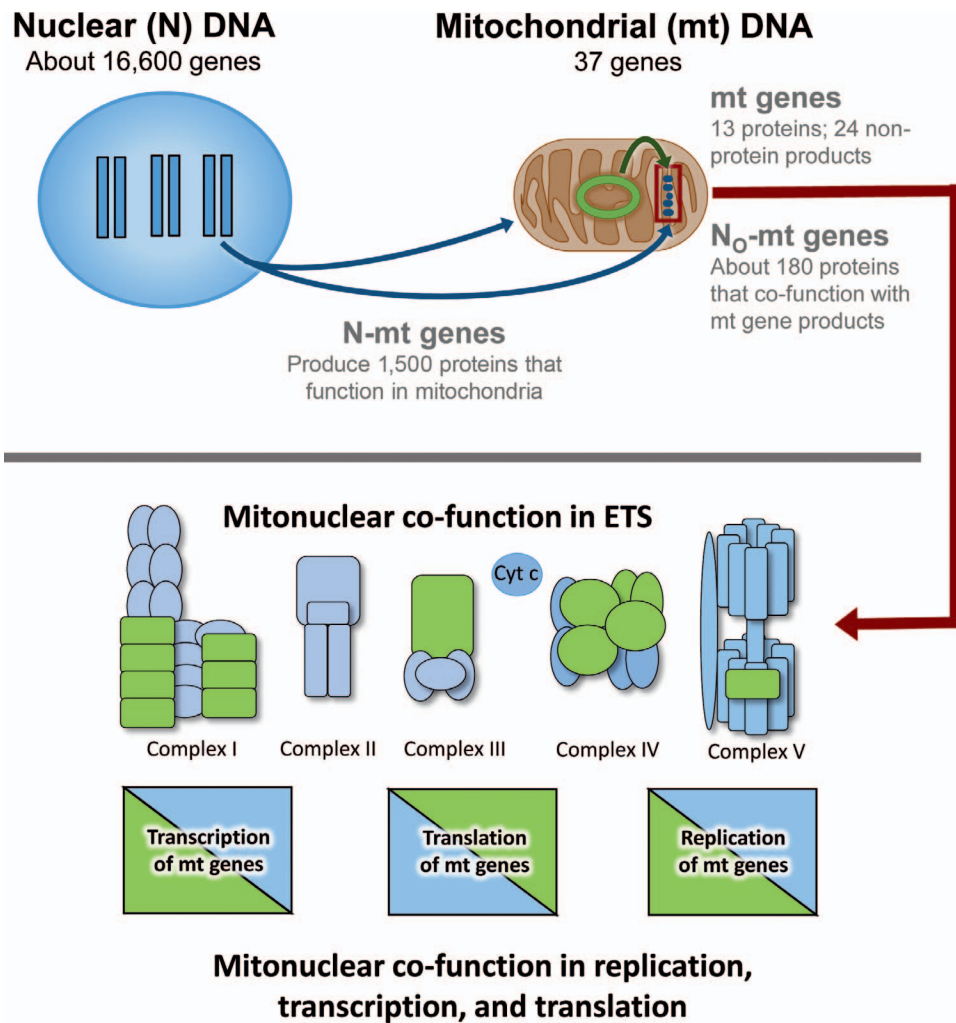
FIGURE 1. An overview of the genomic architecture of birds (top) and the functional arenas in which mitonuclear interactions are manifest (bottom). Nuclear genes and their products are shaded blue; mitochondrial genes and their products are shaded green. Sources: number of genes from Prachumwat and Li (2008); number of N-mt genes and N-mt protein-coding genes from Bar-Yaacov et al. (2012); number of N_O-mt genes from Burton and Barreto (2012).

The mt genome of vertebrates is small, with 37 genes that code for 13 proteins, 22 transfer RNAs (tRNA), and 2 ribosomal RNAs (rRNA; Rand et al. 2004, Kühlbrandt 2015; Figure 1). There is also an mt control region that interacts with N gene products. The function of all these mt-encoded components is, either directly or indirectly, energy production via OXPHOS (Lane 2005, Woodson and Chory 2008). The 13 mt proteins form core elements of Complexes I, III, IV, and V of the ETS (Pierron et al. 2012, Kühlbrandt 2015; Figure 1). The non-protein-coding products of the mt genome play essential roles in the replication, transcription, and translation of mt genes as a core part of the process to maintain OXPHOS (Burton and Barreto 2012). These mt-encoded protein and non-protein products work in intimate association with N-encoded proteins. In the ETS, the 13 mt-encoded proteins combine

with ∼75 N-encoded proteins to form ETS complexes (Burton and Barreto 2012). These 75 ETS N proteins are only a subset of the 1,500 proteins that are encoded by N genes and function in the mitochondrion (N-mt genes; Bar-Yaacov et al. 2012; Figure 1).

There are also important protein–DNA and protein–RNA mitonuclear interactions wherein the non-protein products of the mt genome and protein products of the N genome co-function in the transcriptional, translational, and DNA replication mechanisms that enable production of ETS proteins (Burton and Barreto 2012, Levin et al. 2014, Hill 2015a). These interactions involve another ∼105 N-mt proteins, including ∼80 ribosomal proteins, 17 aminoacyl tRNA synthases, mtRPOL, TFAM, TFB1, TFB2, DNA polymerase, mtRPOL, and TFAM (Burton and Barreto 2012). I will refer to all the N genes whose

TABLE 1. Abbreviations and definitions of the types of DNA discussed and the number of genes encoded by each DNA type.

DNA type	Abbreviation	Approximate number of genes	Definition
Nuclear	N	16,600 [a]	DNA in nucleus
Mitochondrial	mt	37 [b]	DNA in mitochondria
Nuclear mitochondrial	N-mt	1,500 [b]	DNA in nucleus that codes for products that function in mitochondria (including OXPHOS genes and 1,300 with other functions, including lipogenesis, heme synthesis, etc.)
OXPHOS nuclear mitochondrial	N_O-mt	180 [c]	DNA in nucleus that codes exclusively for products that co-function with the products of mt genes in mitochondria; includes genes for proteins in ETS and genes for replication, transcription, and translation of mt genes
OXPHOS nuclear mitochondrial—ETS proteins	–	75 [c]	DNA in nucleus that codes for proteins that are subunits of ETS complexes
OXPHOS nuclear mitochondrial—non-ETS proteins	–	105 [c]	DNA in nucleus that codes for proteins that co-function with the products of mt genes in replication, transcription, and translation of mt genes

[a] Prachumwat and Li (2008).
[b] Bar-Yaacov et al. (2012).
[c] Burton and Barreto (2012).

products have close interaction with mt genes in enabling OXPHOS—both in the ETS and in the transcription and translation of ETS proteins and replication of mitochondrial DNA (mtDNA)—as "N_O-mt genes" (Figure 1 and Table 1).

This fundamental genomic architecture of eukaryotes gives rise to a basic principle that is underappreciated in evolutionary biology and virtually never considered by ornithologists: *Mitonuclear coadaptation is essential for organism function* (Gershoni et al. 2010, Lane 2011a, Bar-Yaacov et al. 2012). Mitonuclear coadaptation may often be the aspect of an organism's genotype or phenotype that is most fundamental to fitness (Blier et al. 2001, Wallace 2009, Burton et al. 2013). If the products of mt genes and N_O-mt genes do not fit together properly or do not function well together, the result is reduced coupling of the electron transport system (Brand and Nicholls 2011), with lower ATP output and increased free-radical production (Lane 2011a, Barreto and Burton 2013a), both of which result in significant loss of fitness (Ellison and Burton 2006). Beyond the protein–protein interactions in the ETS, if mitonuclear components required for replication, transcription, or translation of mt genes do not function together properly, then production of mt ETS subunits is compromised along with redox balance in the bioenergetic membrane (Ellison and Burton 2010, Bar-Yaacov et al. 2012, Martin et al. 2015). There is perpetual strong selection on all eukaryotes to maintain mitonuclear compatibility and high ETS function (Barreto and Burton 2013b, Hill 2014). This requirement for mitonuclear compatibility is true for all metazoans but is perhaps more critical in birds than in any other vertebrate taxon, because birds (1) have the most energy-demanding life histories and hence the greatest need for highly efficient ATP production (Holmes et al. 2001, Lane 2011b) and (2) have ZW sex determination (Ellegren 2000). I will explain the relevance of ZW sex determination below.

Mitonuclear Speciation vs. Contemporary Models of Speciation

The nearly universally held model for avian speciation involves the slow accumulation of genetic changes between populations in isolation, such that the two populations eventually diverge to the point of being recognizable as species (Mayr 1942, Coyne and Orr 2004, Price 2007). Competing species concepts propose different ideas for the criteria that should be used to determine when diverging populations are distinct enough to be recognized as species (Remsen 2005, Zink 2006). The biological species concept emphasizes reproductive isolation from other populations as the key characteristic of a species, using hybridization of lineages, and presumed gene flow between populations, as evidence against such lineages being distinct at a species level (Mayr 1940). The phylogenetic species concept, by contrast, focuses on the distinctiveness of diverged populations, putting an emphasis on whether or not populations have a unique evolutionary history, as diagnosed by one or more traits (Cracraft 1983, Nixon and Wheeler 1990). The phylogenetic species concept discounts the relevance of contemporary hybridization (Zink 2006). In none of these species concepts is the need for mitonuclear coadaptation given explicit consideration.

TABLE 2. The relevance of various population interactions and characteristics to species status, according to different species concepts. "Yes" indicates that the interaction or characteristic does affect whether a population is deemed a species, and "No" indicates that the interaction or characteristic does not affect whether a population is deemed a species.

Concept	Hybridization	Contemporary diffusion of N genes	Diffusion of N genes across evolutionary history	Contemporary diffusion of mt genes	Phenotypic diagnosability of populations
Biological species concept	Yes	Yes	Yes	Yes	No
Phylogenetic species concept	No	No	Yes	No	Yes
Mitonuclear compatibility species concept	No	No	No	Yes	No

Empirical studies show that the coadapted mt and N_O-mt genotypes of animal species are distinct, even when comparisons are made between closely related sister taxa of metazoans (reviewed in Burton et al. 2013, Hill 2015a). Unfortunately, there have been no direct tests for mitonuclear compatibility between avian species, but studies with nematodes, copepods, fruit flies, parasitic wasps, rodents, and primates all show that each species of animal has a unique set of coadapted mt and N_O-mt genes (reviewed in Blier et al. 2001, Lane 2011b, Hill 2015a). The very important consequence of the rapid divergence of mt and N_O-mt genes is that "hybrid" offspring, which result from mating between populations with diverged coadapted gene sets, have reduced fitness due to reduced OXPHOS function resulting from mitonuclear incompatibilities (Gershoni et al. 2009, Chou and Leu 2010, Burton and Barreto 2012). Basic tests of species-specific mitonuclear coadaptation have yet to be conducted in birds, but because of their reliance on high performance of cellular respiration, we can expect mitonuclear coadaptation to be particularly pronounced in birds.

Building from previous essays (Gershoni et al. 2009, Lane 2009, Chou and Leu 2010, Burton and Barreto 2012, Crespi and Nosil 2013, Hill 2016), I propose that genetic divergence of avian populations leading to speciation arises not simply from accumulation of neutral genetic change, nor even from divergent selection on N genes—rather, the process of speciation is the process of divergence of sets of coadapted mt and N_O-mt genes. These species-specific sets of mt and N_O-mt genes will both define a species and maintain its identity. Fitness loss in offspring with mixed mt and N_O-mt genes will serve as a barrier to gene flow between species. This mitonuclear compatibility concept of species can be stated as follows: *A species is a population that is genetically isolated from other populations by incompatibilities in uniquely coadapted mt and N_O-mt genes.*

In the mitonuclear compatibility hypothesis of speciation, coevolution of mt and N_O-mt genes to achieve and maintain mitonuclear coadaptation drives the evolution of populations that become the discrete evolutionary entities that we recognize as species (Hill 2016). This species concept accommodates substantial levels of hybridization and exchange of N genes because such processes do not necessarily disrupt the coadapted mitonuclear gene complexes that are the essence of species identity. This model proposes that reproducing individuals in avian species suffer a severe fitness penalty for exchanging mt and N_O-mt genes with individuals from another species, assuming that a loss of fitness of hybrid offspring results from the pairing of mt and N_O-mt genes that are not coadapted (Hill 2016). If this assumption is correct (at present it is a largely untested idea in birds, based on extrapolation of observations of other animal taxa), then a species can be defined objectively and unambiguously by unique genetic coadaptation in mt and N_O-mt genes.

The mitonuclear compatibility hypothesis encompasses key elements of both the biological and the phylogenetic species concepts, while clarifying contentious issues such as the importance of hybridization among candidate species (Table 2). The central idea of the biological species concept—that species represent discrete gene pools—is also the central tenet of the mitonuclear compatibility species concept (MCSC). Rather than focusing on N genes, however, the MCSC model focuses on that small set of mt and N_O-mt genes that are uniquely coadapted to enable respiratory function. In contrast to the biological species concept, the MCSC model would not recognize hybridization and exchange of N genes as evidence against species status, so long as coadapted mt and N_O-mt genes remain distinct between populations (Table 2).

Under the MCSC, diagnosable populations with a unique evolutionary history may fail to meet the definition of species if they fail to have uniquely coadapted mitonuclear genotypes (Table 2). Conversely, populations that are not diagnosable except by genotype can be full species, provided they have uniquely coadapted mt and N_O-mt genotypes that are incompatible with the coadapted mt and N_O-mt genotypes of any other population. In practice, in the great majority of cases, the criteria of the phylogenetic species concept (Zink 2006) identify the same species boundaries as do the criteria of the MCSC (Table 2).

The utility of adopting the MCSC is supported by the emerging literature on the distinctiveness of mt genotypes (i.e. DNA barcodes) between populations regarded as

species, the patterns of greater introgression between putative species of autosomal N genes compared to mt genes or sex-linked N genes, and the chromosomal locations of genes for ornamentation, mate preferences, and hybrid incompatibility. I will consider each of these lines of evidence in turn.

Mitonuclear Coadaptation and Coevolution

The mitonuclear compatibility hypothesis proposes that the process of speciation is driven by mitonuclear coevolution to maintain mitonuclear coadaptation. Mitochondrial genes are subject to a high mutation rate (Ballard and Whitlock 2004, Lynch 2010). In the germ line of birds, the mutation rate of mt genes is ~10 times that of N genes, although mutation rates vary considerably among orders of birds (Nabholz et al. 2009). In addition, mt genes are transmitted without recombination, so slightly deleterious mutations are predicted to perpetually accumulate in mt genes (Lynch and Blanchard 1998, Neiman and Taylor 2009). The product of every gene in the mt genome plays a critical role in enabling OXPHOS, and every mt gene functions in intimate association with N_O-mt genes (Figure 1). Thus, accumulation of deleterious mutations in mt genes will lead to loss of mitonuclear coadaptation and erosion of mt function and core energy production (Wallace 2009). To maintain coadaptation, mt and N_O-mt genes must perpetually coevolve (Havird et al. 2015a, Havird and Sloan 2016). There is growing evidence that variant N_O-mt genes evolve so as to compensate for mt mutations and restore OXPHOS function (Mishmar et al. 2006, Osada and Akashi 2012, Barreto and Burton 2013b, Havird et al. 2015b, van der Sluis et al. 2015, Havird and Sloan 2016). Because the emergence of mt gene mutations is random, coevolution of mt and N_O-mt genes to maintain OXHPOS function will be unique to a population (Burton and Barreto 2012, Bar-Yaacov et al. 2015, Hill 2016). There is a tendency among molecular ecologists to focus on protein–protein interactions in the complexes of the ETS when considering mitonuclear coadaptation, but these protein–protein interactions are only a subset of the mitonuclear interactions that can affect compatibility; protein–DNA and protein–RNA interactions are also critical to OXPHOS function and are often the source of mitonuclear incompatibilities (Burton and Barreto 2012).

Mitonuclear coadaptation becomes a critical consideration in the process of speciation when coadapted sets of mt and N_O-mt genes diverge rapidly between isolated populations (Burton and Barreto 2012, Hill 2016) and when mitonuclear incompatibilities between diverged populations result in mitochondrial dysfunction and reduced fitness in hybrid offspring (McKenzie et al. 2003, Ellison and Burton 2006, Ellison et al. 2008). Consequently, mitonuclear incompatibilities are potentially the primary isolating mechanism for recently diverged taxa (Gershoni et al. 2009, Burton and Barreto 2012, Hill 2016). To date, these insights come from animal taxa other than birds, but the fundamental genomic architecture of birds is the same as that of other metazoans. I argue that a basic understanding of these key mitonuclear interactions is essential for an understanding of speciation in birds. Indeed, consideration of the process of speciation from a mitonuclear perspective clarifies several contentious issues inherent in the species concepts currently applied to birds and results in a more robust, objective, and testable means to delimit species.

High Respiration Rates and F_1 Incompatibilities

The imperative for mitonuclear compatibility is likely to be particularly acute in birds, compared to other vertebrate taxa, because of their unique combination of very high basal metabolic rates and ZW sex determination. High body temperature per se may lead to higher activity of enzymes and to less tolerance of structural changes in enzymes in birds than in other vertebrates (the "avian constraint hypothesis"; Avise and Aquadro 1982, Stanley and Harrison 1999), but it is the need for efficient energy production that necessitates highly functional cellular respiration. The energy-demanding life histories of birds hardly require detailed explanation in an ornithology journal. Birds and mammals have significantly higher standard metabolic rates than other classes of vertebrates (White et al. 2006). Birds migrate across oceans (DeLuca et al. 2015), breed in the Antarctic winter (Le Maho 1977), fly over the highest mountains (Scott et al. 2015), and generally engage in lifestyles that demand a constant high production of ATP (Lane 2011b). Birds also have lower levels of oxidative damage, on average, than mammals (Barja 2007), and evidence suggests that to maintain their high-energy lifestyles, birds must achieve a high output of ATP via the ETS while permitting the production of relatively few free radicals during OXPHOS (Lane 2011b). These characteristics of birds make high efficiency of OXPHOS critical to fitness. With greater fitness benefits for highly efficient OXPHOS comes greater selective pressure on tight mitonuclear coadaptation and higher fitness penalties for mitonuclear incompatibilities (Lane 2011b). I propose that the imperative for mitonuclear coadaptation in birds is very likely to create barriers to gene flow between avian populations that have diverged in coadapted mt and N_O-mt genotypes, because any loss of respiratory efficiency in hybrid offspring will incur severe fitness penalties.

The ZW sex determination of birds potentially reinforces a disruption in gene flow of coadapted mt/N_O-mt genes between populations if N_O-mt genes are Z-linked (Hill and Johnson 2013). The strong tendency among

hybrid offspring for the heterogametic sex (ZW females in birds) to show greater infertility or inviability than the homogametic sex (Haldane's rule; Haldane 1922) indicates that incompatibility factors are sex linked. Mitonuclear incompatibility provides a mechanism for Haldane's rule (Hill and Johnson 2013). This mitonuclear explanation requires that some coadapted N_O-mt genes are located on the Z chromosome such that the paternal Z-linked genes are forced to co-function with maternal mt genes in ZW females. The result will be core system dysfunction in females, and the loss of female hybrids when N_O-mt genes and mt genes are not coadapted. This disruption of gene flow in the F_1 generation should strictly limit the movement of mt and N_O-mt genes across species boundaries.

The mitonuclear compatibility model of speciation explains Haldane's rule as the result of incompatibilities specifically between interacting mt and Z-linked N_O-mt genes that are revealed in the heterogametic sex (Hill and Johnson 2013). The mitonuclear compatibility model predicts specifically that N genes other than N_O-mt genes should diffuse across species boundaries much more readily than mt or N_O-mt genes because they are not constrained by the incompatibilities that define species. It is especially interesting, therefore, that Z-linked genes typically diffuse across avian species boundaries at much lower rates than autosomal genes (Tegelström and Gelter 1990, Carling and Brumfield 2008, Irwin et al. 2009, Carling et al. 2010, Storchova et al. 2010, Gowen et al. 2014, Lavretsky et al. 2015, Toews et al. 2016b, Walsh et al. 2016). There are no data on rates of diffusion of N_O-mt genes in birds.

The current explanation for why mt genes do not introgress across species boundaries is that females disperse shorter distances than males and that hybrid females are less viable than hybrid males, such that introgression of mt genes will be thwarted by female hybrid inviability (Tegelström and Gelter 1990, Rheindt and Edwards 2011, Toews and Brelsford 2012). The current explanation for why Z-linked genes do not introgress across species boundaries is based on the assumption that incompatibility factors are recessive and sex-linked, such that they are revealed in the sex with the unmatched Z chromosome (i.e. females; Carling and Brumfield 2008, Rheindt and Edwards 2011). These predictions are founded on observations of inviability of the heterogametic sex (Price and Bouvier 2002, Kirby et al. 2004), and not on knowledge of specific genetic interactions. As Gowen et al. (2014) wrote, "While we may not understand why Haldane's Rule occurs … the consequences are clear."

The mitonuclear compatibility model of speciation predicts that key N_O-mt genes will be positioned on the Z chromosome to promote mitonuclear coadaptation (Hill and Johnson 2013, Hill 2014, 2016). Unfortunately, there

are no published data on the chromosomal position of N_O-mt genes in birds. In an analysis of the chromosomal position of all N-mt genes (N_O-mt plus >1,300 other N-mt genes; Figure 1) in Zebra Finches (*Taeniopygia guttata*) and Red Junglefowl (*Gallus gallus*), N-mt genes were found to be positioned on the Z chromosome at a frequency expected by chance (Drown et al. 2012, Dean et al. 2014). However, the mapping of unidentified incompatibility factors to the Z chromosome suggests that some key N_O-mt genes are Z-linked, although such factors could also be N genes that interact in a negative fashion with other N genes (Sæther et al. 2007, Pryke and Griffith 2009). Determining the specific genes involved in hybrid incompatibilities and mapping the chromosomal position of N_O-mt genes in birds should be a priority in future avian speciation research.

DNA Barcode Gaps

As predicted by the mitonuclear compatibility model of speciation, mt genotype is very good at delimiting species boundaries in birds (Zink and Barrowclough 2008, McKay and Zink 2010; but see Funk and Omland 2003). In particular, a 648 bp region of the cytochrome *c* oxidase subunit 1 (COX1) gene correctly bins North American, Eurasian, and South American birds into already recognized species with >94% accuracy (Kerr et al. 2007, 2009a, 2009b, Johnsen et al. 2010). Even more impressively, the COX1 DNA barcode accurately separates closely related sister taxa. Taveres and Baker (2008) looked at COX1 barcode sequences for 60 pairs of avian sister taxa and found that, in all 60 comparisons, there was a clear barcode gap that supported species-level divisions of populations already proposed by taxonomists. There is currently no evidence that the sometimes small divergence in mitochondrial genotype between species is linked to divergence in coadapted mitonuclear genotype as predicted by the MCSC, but testing for mitonuclear incompatibilities between lineages should be relatively straightforward.

So far, the evolutionary and ornithological communities have been reluctant to use DNA barcode similarity and DNA barcode gaps to define species, despite the success of mt gene sequences in diagnosing already recognized species (DeSalle et al. 2005, Hickerson et al. 2006, Rubinoff et al. 2006). If the essence of an avian species is a unique set of coadapted mt and N_O-mt genes that are incompatible with the coadapted mt and N_O-mt genes of any other population, then it follows that species should be diagnosed by mt and N_O-mt genotype (Hill 2016). We currently have little information on N_O-mt genotypes of birds and no direct information on mitonuclear compatibility, but we have abundant sequence data for key mt genes. Mitochondrial genotypes should be a good—but not perfect—proxy for a coadapted mt/N_O-mt genotype. Thus,

the species concept that I propose provides a logical basis for using mt genotypes to diagnose species. The success of the COX1 barcode gene in delimiting species suggests that the COX1 genotype is tightly associated with the mt genotype involved in species-specific mitonuclear coadaptation and should serve as an excellent proxy for (1) overall mt genotype and (2) mt/NO-mt genotype (Lane 2009, Hill 2016). However, the 648 bp region of the COX1 gene that is widely used as the animal DNA barcode was chosen because its conserved nature allowed a few universal primer sets to be applied to diverse taxa (Hebert et al. 2003). It was not chosen because it provides the best proxy for coadapted mt/N_O-mt genotype in birds or any taxon. Ornithologists may want to consider whether alternative or additional mt sequence data provides better information with regard to species boundaries.

Given that the COX1 DNA barcode gap is only a proxy for true species boundaries, it can fail to properly predict incompatibilities in mt/N_O-mt genotypes, even if such compatibilities define species boundaries (see discussion of Common Ravens below). When more complete knowledge of mt and N_O-mt genotypes is achieved and mitonuclear compatibility can be assessed through functional modeling of gene products, a barcode approximation can be supplanted by direct assessment of mitonuclear compatibility derived from sequencing N_O-mt and mt genotypes of putative species. In the future, if it has been proven correct that a species is best defined by its uniquely coadapted N_O-mt and mt genotype, I anticipate that avian species will be diagnosed by direct assessment of mitonuclear compatibility–incompatibility established through functional models based on nucleotide sequences. This will be particularly valuable for assessing the species status of allopatric populations. For now, I propose that the mt genotype and the COX1 DNA barcode gap should be viewed as good approximations of true species boundaries (Hill 2016).

Ornamental Traits and Species Boundaries

The mitonuclear compatibility concept of speciation does not require that individuals in a population use phenotypic markers during mate choice to correctly assort by mitonuclear type (i.e. it does not require prezygotic barriers to hybridization). Postzygotic fitness loss in hybrid offspring will provide constant selection to maintain the integrity of coadapted mitonuclear types and, hence, maintain species boundaries (Burton and Barreto 2012, Burton et al. 2013, Bar-Yaacov et al. 2015). However, it is much to the advantage of individuals engaging in sexual reproduction—both males and females—to identify prospective mates that will provide compatible N_O-mt and mt genes (Hill and Johnson 2013, Hill 2015c). Thus, under the MCSC, the evolution of signals of species identity, and of strong mating preferences for conspecific signals of species

identity, is expected. The evolution of signals of species identity is the process of reinforcement in speciation models (Servedio and Noor 2003, Hudson and Price 2014, Hill 2015b) and will lead to the evolution of species-typical coloration and song that are diagnosable by humans.

Because the MCSC defines species by coadapted mitonuclear type, signals of species identity become signals of the genotype of coadapted mt and N_O-mt genes (Hill and Johnson 2013). It follows that the mt genotype should correspond with signals of species identity. In other words, there should be an ornamentation gap between populations of birds that matches the DNA barcode gap that defines species. Consistent with this prediction, there is nearly perfect agreement between species boundaries drawn according to mtDNA types and those drawn according to species-typical song or coloration (Carling and Brumfield 2008, Tavares and Baker 2008, Aliabadian et al. 2009, Winger and Bates 2015). This association might be viewed as tautological: We define species by ornamentation and then we choose mt genotype as the primary molecular criterion for species delineation because it best supports ornament-based notions of speciation. However, I argue that any objective clustering of individuals by mt genotypes would yield the same population delineations (Baker et al. 2009, Kerr 2011), just as any objective clustering of individuals by plumage pattern or song would yield consistent groups (Mayr 1940). Population divergences in color and song are sometimes poorly associated with differentiation of N genes (e.g., Carling and Brumfield 2008, Rheindt and Edwards 2011, Toews et al. 2016b), but they seem to be invariably associated with differentiation of mt genes (Kerr et al. 2007, 2009a, Tavares and Baker 2008, McKay and Zink 2010).

I contend that the match between the ornamentation gap and the mt genotype (DNA barcode) gap between species is the result of selection for reinforcement of species-typical coadapted genotypes (Hudson and Price 2014, Hill 2015b). When populations that have diverged in coadapted mt and N_O-mt genes come back into contact, a reproducing individual is at risk of choosing a mate that has mt and N_O-mt genes incompatible with its own (Morales et al. 2015, Hill 2016), but there should be very strong selection against those incompatible mate choices. Such selection should lead to the evolution of unambiguous signals of the identity of mt/N_O-mt genotypes, here defined as species identity (Hill and Johnson 2013, Hill 2015c). By this argument, the need for proper sorting of mt/N_O-mt genotypes leads to the evolution of species-typical patterns of coloration and song in birds (Hill and Johnson 2013). The implication of the MCSC is that sexual selection will follow rather than drive speciation. I propose that the species-typical coloration, patterns, and songs evolve as signals of coadapted mitonuclear genotype (Hill 2015c).

Linkages

To serve as reliable signals of species identity, genes for female mating preferences and genes for species-specific plumage pattern or song should be linked to key compatibility genes (Sæther et al. 2007, Qvarnström and Bailey 2009), which I propose to be N_O-mt genes. Without such linkage, these genetic elements could be inherited independently, and there would be mismatches between preferences, ornamentation, and N_O-mt genes. With such mismatches, signal reliability would falter. Because N_O-mt genes are predicted to be Z-linked in birds (as discussed above; Hill and Johnson 2013, Hill 2014), both genes for traits used in species recognition (species-typical plumage pattern and song) and genes for mating preferences are predicted to be Z-linked. Currently, there are few data on the genetic architecture of ornaments or mating preferences—and, hence, few data for assessing the prediction of linkages of such traits (Qvarnström and Bailey 2009). The limited data that do exist, however, are intriguing.

In the genus *Ficedula*, including the closely related Pied Flycatcher (*F. hypoleuca*), Collared Flycatcher (*F. albicollis*), and Semi-collared Flycatcher (*F. semitorquata*), species have distinct plumage patterns that enable assortative mating by species (Sætre and Sæther 2010). Genes for species-typical plumage pattern and genes for mate preference for conspecific plumage pattern are both Z-linked (Sætre et al. 2003, Sæther et al. 2007). Moreover, genes that determine incompatibility in hybrid offspring in *Ficedula* are also Z-linked (Sætre et al. 2003). Taken together, these data indicate that a sex-linked incompatibility factor reinforces female choice for species-typical plumage pattern and that such choice maintains species identity in *Ficedula* even when there is little divergence in the ecology of the four western Eurasian species in the genus (Sætre and Sæther 2010). No direct link to mitonuclear incompatibility has yet been made—although hybrid *Ficedula* have dysfunctional respiration (McFarlane et al. 2016)—but the observations are consistent with the mitonuclear compatibility model of speciation.

The Gouldian Finch (*Erythrura gouldiae*) presents a similar example of sex linkage of ornaments, preferences, and incompatibility factors—but within a single species. Ignoring a rare yellow morph that occurs in <0.1% of wild birds, wild male Gouldian Finches come in two very distinct head-color morphs: red and black (Franklin and Dostine 2000). The genes for morph color are Z-linked (Pryke 2010). In captivity, Gouldian Finches have a strong tendency to mate assortatively according to head color, and the genes that determine female head-color preference are also Z-linked (Pryke 2010). Most amazingly, between-morph pairings result in loss of viability of both male and female offspring, but the effects are twice as severe in females as in males (Pryke and Griffith 2009, Pryke 2010);

in other words, between-morph crosses follow Haldane's rule. Thus, the incompatibility factor that causes loss of fitness in hybrid offspring is also Z-linked (Pryke and Griffith 2009). The prediction from the MCSC is that the Z-linked incompatibility factor will be N_O-mt genes. Interestingly, there is little variation in mt genotype among wild populations of Gouldian Finches (Bolton et al. 2016), which all support both red- and black-morph individuals, and the incompatibilities between black and red morphs that are seen in captivity are not observed in the wild (Bolton et al. 2017). One possibility is that black and red morphs were formerly allopatric species with mitonuclear incompatibilities, but when the populations coalesced, the color morphs persisted while incompatibilities were selected against. Domestication seems to have reactivated incompatibilities linked to color morph (Bolton et al. 2017). The Gouldian Finch may prove to be an outstanding model system for studying the role of mitonuclear incompatibilities in speciation.

Blue-winged and Golden-winged Warblers: A Test Case for the Mitonuclear Compatibility Species Concept

In at least one species pair—Blue-winged Warbler (*Vermivora cyanoptera*) and Golden-winged Warbler (*V. chrysoptera*)—a gene for a species-typical plumage pattern is not Z-linked. These two sister taxa have been recognized as distinct species since they were first described in the 19th century (Gill 1997). No taxonomic revision has ever lumped them into a single species. And yet, in a recent comparative genomic study, it was found that these two warbler populations share essentially the same N genotype (Toews et al. 2016b). Golden-winged and Blue-winged warblers are much less divergent in overall N genotype than many avian populations within species that have never been considered distinct species. Importantly, within the few N genomic regions that are divergent between the warbler populations are genes for species-typical plumage pattern (Toews et al. 2016b). In contrast to the high level of introgression of N genes, the mt genomes of the two taxa are highly differentiated. The mt genomes of the Blue-winged and Golden-winged warblers differ in nucleotide sequence by >3%, a degree of divergence typical of sister species (Gill 1997).

Given the extent of introgression of N genes between Blue-winged and Golden-winged warblers, it is interesting that the gene that determines species-typical black vs. yellow throat coloration is not Z-linked in these warblers; it is located on an autosome (Toews et al. 2016b). The prediction from the MCSC model is that without linkage between species-typical plumage genes and N_O-mt genes, there will be mismatches between plumage traits and mitonuclear genotype. Indeed, male warblers with mis-

matched species-typical plumage type and mt haplotype have been reported (Confer et al. 2010). Furthermore, high levels of hybrid pairings shift selection for the maintenance of coadapted mt and N_O-mt genes from prezygotic processes to postzygotic processes. Postzygotic selection should prohibit introgression of mt and N_O-mt genes but not N genes, so the high level of introgression of N genes in these warblers is perhaps a consequence of the genetic architecture of the species.

I argue that the taxonomic situation of Golden-winged and Blue-winged warblers underscores the value of adopting the MCSC for birds. The instinct of generations of ornithologists is that Blue-winged and Golden-winged warblers are good species. They are highly divergent in plumage and song, and they occupy distinct climatic regions. Yet they do not meet the requirement for a species according to either the biological species concept (Table 2) or the stated criteria of the AOU Checklist Committee. They also do not meet the criteria of the phylogenetic species concept, because there is evidence that they have exchanged autosomal genes for millennia (Toews et al. 2016b)—hence they lack a "unique evolutionary history" (Table 2). Under the MCSC, however, they are distinct species so long as they have uniquely coadapted mt and N_O-mt genotypes. Their highly divergent mt haplotypes suggest that such divergence in mitonuclear genotype exists. Intriguingly, a majority of the N gene differences between the two taxa are clustered in two regions on the Z chromosome. The clear prediction from the mitonuclear compatibility speciation hypothesis is that N_O-mt that are uniquely coadapted with respective mt genotypes will be among the divergent genes on the Z chromosome. The scarcity of F_2 "Lawrence's Warblers," despite locally high rates of interbreeding, makes a strong case for low fitness of hybrids and postzygotic sorting by mt/N_O-mt genotype.

Brood Parasites

One of the most interesting challenges with regard to any species concept is the pattern of mitochondrial divergence but nuclear panmixia between populations of brood parasites specialized on different hosts. Perhaps the most intriguing and perplexing of these brood parasites is the Greater Honeyguide (*Indicator indicator*), which was discovered to have a clade specialized on cavity-nesting hosts that is 14.8% divergent in mt genotype compared to a sympatric clade specialized on cup-nesting hosts. There is virtually no mitochondrial introgression between these host races, and these clades also have fixed differences in egg morphologies. Paradoxically, the two clades show almost complete introgression of N genes (Spottiswoode et al. 2011). On the basis of current species concepts, Spottiswoode et al. (2011:17738) concluded that "a complete lack of differentiation in nuclear genes shows

that mating between individuals reared by different hosts is sufficiently frequent to prevent speciation." Under the MCSC, however, these two divergent host races of Greater Honeyguide are potentially different species, because species are defined not by overall nuclear genotype but by coadapted mt and N_O-mt genotypes. Thus, species status would depend on whether or not there were unique sets of N_O-mt that are coadapted to the distinct mt genotypes and that would create incompatibilities if the genotypes were mixed.

Two explanations for the honeyguide genetic data seem possible. First, each mt genotype may be coadapted with a unique set of N_O-mt genes. For this scenario to work, N_O-mt genes would have to be linked to the genes that create egg morphologies and to host-preference genes, if such genes exist. If there is no assortative mating among individuals from the two populations, then recurring incompatible combinations of mt and N_O-mt genes that emerged from random mixing of gentoypes would be culled each generation via postzygotic selection, which would maintain the links between egg morphology and mt genotype.

Alternatively, the two divergent mt genotypes may both be compatible with a single pool of N_O-mt genotypes common to both clades. This explanation requires (1) that genes coding for egg morphology are W-linked and (2) either that genes for host preferences are W-linked or that there are no such genes and host preference is the result of imprinting (Fossøy et al. 2016). W-linkage of egg traits is necessary to maintain a perfect association between mitochondrial type and egg type because mt genes and W-linked genes co-transmit across generations. By this explanation, the clades would lack uniquely coadapted genotypes, and they would not be species according to the MCSC.

Compatibility between a single pool of N_O-mt genotypes and two mt genotypes that are 14.8% divergent may seem untenable, given the strong case that I have laid out for unique mitonuclear coadaptations among populations. However, so long as there is mitonuclear coevolution, mitonuclear coadaptation should be maintained. Through-out the evolution of the divergent mt genotypes in the two clades of honeyguides, each would have continued to coevolve with the N_O-mt genotypes and coadaptation would have been maintained in both gene sets. Having two divergent mt genomes coadapted with a common pool of N_O-mt would create the potential for interesting conflicts among the three genomes if compensatory changes in the nuclear genome that benefit one mitonchodrial type were detrimental to the other mitochondrial type. Also, I predict that the rate and extent of sequence evolution of N_O-mt genes will be higher in Greater Honeyguides and other brood parasites, compared to the rate of change in species with a single mt genotype. This story can be resolved by

mapping the chromosomal position of the genes responsible for eggshell morphology and host preference, or eventually by demonstrating whether there is assortative mating by N_O-mt genotype. Mitochondrial divergence among host-specific subpopulations seems to be a common pattern in brood parasites (Fossøy et al. 2016), and an appreciation of the need for coadaptation of mt and N_O-mt genes will help guide interpretation of the evolution and maintenance of these fascinating systems.

Contradictory Examples: Ravens and Redstarts

The greatest challenges to the MCSC are cases where highly divergent mt haplotypes exist within a single avian population. Given the arguments outlined in this essay, there should be strong selection against introgression of significantly divergent mt genotypes between populations. Paradoxically, there are reports of mitochondrial introgression between numerous bird species (Funk and Omland 2003), but the frequency of perceived introgression of mitochondria depends on assumptions made about the accuracy of current species boundaries (McKay and Zink 2010). Beyond birds, introgession of mt genotypes between species is well documented (for a discussion of the causes and consequences of mitochondrial introgression, see Sloan et al. 2017). Introgression of mt genotypes between species poses a major challenge to the MCSC, and it is worth considering well-documented cases involving birds.

In this regard, the Common Raven (*Corvus corax*) is the taxon that presents the most significant contradictions to the concepts treated in this overview. Large black corvids that have long been classified as *C. corax* have a Holarctic distribution. In size, shape, ecology, and voice, these northern populations of large black ravens appear to be an undifferentiated population circling the top of the Earth (Vaurie 1959). Analysis of mitochondria from animals collected across the range of *C. corax*, however, revealed two highly divergent (>4% divergent in nucleotide sequence) mt haplotypes within this population: a haplotype restricted to western North America (the California clade) and a haplotype found in Eurasia and the rest of North America (the Holarctic clade; Omland et al. 2000). These mitochondrial clades appear to represent two formerly allopatric populations of ravens that diverged in isolation over a million years or more (Omland et al. 2000). Contrary to predictions of the mitonuclear compatibility model of speciation (Hill 2016), there now appears to be unrestricted flow of both N and mt genes between these now broadly sympatric raven populations (Feldman and Omland 2005, Webb et al. 2011). This flow of genes between the California and Holarctic populations of ravens appears to be possible because there are neither prezygotic nor postzygotic barriers to gene flow: There is no evidence of assortative mating by mt haplotype and no apparent loss of fitness from "hybrid" matings (Webb et al. 2011). These observations indicate that there are two divergent mt genotypes—and presumably two divergent N_O-mt genotypes—being mixed and matched with the western North American population of ravens, with no apparent fitness consequences (Webb et al. 2011).

To further complicate the taxonomic status of ravens, two populations that are nested within the *C. corax* clade—the Chihuahuan Raven (*C. cryptoleucus*) and the Pied Crow (*C. albus*)—appear to be reproductively isolated from both the California and Holarctic clades of *C. corax* (Omland et al. 2000, Feldman and Omland 2005). Webb at al. (2011) propose that the most plausible history of these taxa is "species in reverse." An ancestral raven population diverged into a New World ancestor of the California clade–Chihuahuan Raven and an Old World ancestor of Holarctic clade–Pied Crow. The former then differentiated into the Chihuahuan Raven and the California population of the Common Raven. The latter differentiated into the Pied Crow and the Holarctic population of Common Raven. Subsequently, the California and Holarctic clades of ravens came back into contact and there were insufficient prezygotic or postzygotic barriers to inhibit gene flow of N, mt, or N_O-mt genes. In this case, mt genotype appears to be a poor proxy for uniquely coadapted mt and N_O-mt genotypes and a poor predictor of species boundaries. The question is this: Why are there no apparent mitonuclear incompatibilities between raven populations diverged by 4% in mitochondrial hypolotype? For virtually all other bird species that have been assessed, that level of divergence in mt genotype corresponds to species-level differentiation with loss of fitness in hybrid offspring. The resolution of this question will require study of the functional evolution of mt and N_O-mt genes through the range of the Common Raven.

Another example of deeply diverged mt haplotypes within a single population is in the Common Redstart (*Phoenicurus phoenicurus*). As with Common Ravens, within the population of Common Redstarts there are two mt haplotypes diverged by ~5% (Hogner et al. 2012). These two mt haplotypes are dispersed throughout Common Redstart populations, with no evidence of assortative mating by mitotype nor any fitness effects associated with either haplotype (Hogner et al. 2012). Moreover, the two divergent Common Redstart mt haplotypes are distinct from the mt haplotypes of other birds in their genus, so it appears not to be a case of introgression of mt from a sister taxon.

The existence of deeply diverged mitochondrial types within Common Raven and Common Redstart populations underscores that a divergent mtDNA genotype is only a proxy for a diverged coadapted mt/N_O-mt genotype. How divergent mitochondrial types can coexist within a popu-

lation and achieve OXPHOS function with a common set N_O-mt genes is a key unresolved question in avian functional genomics.

Hybrid Mitonuclear Speciation in Birds

The core premise of the MCSC is that species are defined by uniquely coadapted sets of mt/N_O-mt genes, which prevents exchange of mt or N_O-mt genes with any other species. Therefore, "hybrid speciation," whereby new species evolve as a product of novel combinations of genes from two parent species (Abbott et al. 2013), seems a direct contradiction of the MCSC. In rare instances, however, hybridization events between species may create novel combinations of mt/N_O-mt genes that are both highly functional—as documented, in detail, in copepods in the genus *Tigriopus* (Pereira et al. 2014)—and less compatible with either parental type.

A study of species in the genus *Passer* in southern Europe revealed a case of hybrid speciation that appears to be driven by mitonuclear incompatibilities (Hermansen et al. 2014, Trier et al. 2014). The two parent species, Spanish Sparrow (*Passer hispaniolensis*) and House Sparrow (*P. domesticus*), are ~4% divergent in nucleotide sequence, but there is no unique mt haplotype associated with the putative hybrid species Italian Sparrow (*P. italiae*), which is distinct in plumage pattern; rather, Italian Sparrows carry House Sparrow mt haplotypes (Hermansen et al. 2014). Italian Sparrows form a narrow zone of contact and hybridization with both parent species, and mtDNA and Z-linked N-mt genes show steeper clines at the species boundaries than autosomal N genes (Trier et al. 2014). Specifically, the Italian Sparrow is intermediate in overall nuclear genotype in relation to its parental species (Hermansen et al. 2014), but there is an excess of Z-linked House Sparrow alleles at the boundary with Spanish Sparrows, and an excess of Z-linked Spanish Sparrow alleles at the House Sparrow boundary (Trier et al. 2014). Z-linked and mitonuclear reproductive barriers appear to limit gene flow and maintain the integrity of the three populations (Trier et al. 2014). Thus, it appears that a novel and adaptive combination of mt/N_O-mt genes arose through hybridization, and that postzygotic selection involving mitonuclear incompatibilities now limits gene flow between the three populations (Trier et al. 2014). Prezygotic assortative mating may also contribute to the maintenance of coadapted mt/N_O-mt genes (Hermansen et al. 2014).

Hybrid speciation seems to be very rare in birds, or COX1 DNA barcoding would not be so effective for delimiting species, but there is at least one other well-publicized case of putative hybrid speciation in birds: the Yellow-rumped Warbler (*Setophaga coronata*) complex. Audubon's Warbler (*S. c. auduboni*) is hypothesized to be a hybrid taxon (currently a subspecies in the AOU checklist) that evolved from a hybridization event between Myrtle Warbler (*S. c. coronata*) and Black-fronted Warbler (*S. c. nigrifrons*), the parent species being ~2% divergent in mt nucleotide sequence (Jacobsen and Omland 2011). All three taxa have a distinct male plumage pattern. In contrast to the Italian Sparrow example, however, Audubon's Warblers carry both Myrtle and Black-fronted warbler mt haplotypes, a sharp cline in mt haplotype occurring within the range of Audubon's Warbler (Brelsford et al. 2011). Thus, a sharp cline in plumage pattern between Myrtle-type and Audubon's-type warblers is not concordant with a sharp cline in mitochondrial type within Audubon's Warblers. There is some evidence that plumage traits are Z-linked, but there is little evidence of assortative mating by species plumage type (Brelsford and Irwin 2009). An analysis based on extensive genotyping of N genes uncovered some fixed nuclear differences between the Myrtle and Audubon's populations (Toews et al. 2016a), but the populations as defined by plumage coloration do not appear to have uniquely coadapted mitonuclear genotypes.

Interestingly, the taxa in the Yellow-rumped Warbler complex show discrete differences in OXPHOS function. The mitochondrial type of the highly migratory northern Myrtle Warbler shows greater efficiency of OXPHOS in the flight muscles compared to the mitochondrial type of the southern, nonmigratory Black-fronted Warbler (Toews et al. 2014). Thus, the northern populations of Audubon's Warblers that have Myrtle Warbler mt haplotypes appear to be well adapted for long-distance migration. The southern populations of Audubon's Warblers that have Black-fronted Warbler mt haplotypes are better adapted for short-distance or no migration. More work is needed to unravel this fascinating complex, but one possibility is that we are seeing hybrid speciation in progress, with mitochondrial introgression driven by selection for novel mitonuclear genotypes related to migration (Toews et al. 2014). It would be very informative to know the role of coadapted mt/N_O-mt genes in this warbler system.

Summary

The mitonuclear species concept that I advocate for determining avian species boundaries provides a coherent explanation for a host of empirical observations related to avian speciation, including the distinctiveness of mt genotypes between populations, the common pattern of introgression of autosomal but not mt or sex-linked genes between putative species, the chromosomal locations of genes for ornamentation, mate preferences, incompatibility factors, and, finally, Haldane's rule of greater female inviability in hybrid crosses. This species concept is an extension and expansion of cytonuclear species concepts

presented in previous essays on the fundamental role of cytonuclear interactions in creating barriers to gene flow among populations (Gershoni et al. 2009, Lane 2009, Chou and Leu 2010, Burton and Barreto 2012, Bar-Yaacov et al. 2015, Hill 2016). Because species are defined by uniquely coadapted mt and N_O-mt genotypes, mitochondrial genotype becomes a good proxy for diagnosing species under the MCSC. For a few avian taxa, however, divergent mt haplotypes exist within a single population, and explaining how such diverged mt haplotypes function within different N_O-mt backgrounds should be a priority for future research.

I present the mitonuclear compatibility species concept in confident terms, with an emphasis on supporting data to clearly lay out ideas and to add this hypothesis to discussions of speciation. At present, however, key elements of this hypothesis are entirely hypothetical in regard to birds, because few avian data exist with which to assess predictions and assumptions. We know that the protein-coding regions of avian mtDNA are under strong purifying selection, such that the great majority of changes to protein-coding mt genes do not change amino acid sequences (Kerr 2011). However, most functional interactions of the products of mt and N_O-mt genes involve DNA–protein and, especially, RNA–protein interactions (Burton and Barreto 2012), and such interactions present a new frontier for studies of avian genomic evolution. The tools now available—for genome sequencing, for studying transcriptional regulation, and for modeling the functional interactions of three-dimensional gene products—throw open the door for heretofore unimaginable analyses of the genetics of speciation and make the ideas presented here entirely testable.

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