SPECIAL FEATURE: Species Limits and Taxonomy in Birds

An overview of speciation and species limits in birds

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ABSTRACT

Accurately determining avian species limits has been a challenge and a work in progress for most of a century. It is a fascinating but difficult problem. Under the biological species concept, only lineages that remain essentially independent when they are in sympatry are clearly species. Otherwise, there is no clear line yet found that marks when a pair of diverging lineages (e.g., in allopatry) become different enough to warrant full biological species status. Also, with more data, species limits often require reevaluation. The process of divergence and speciation is itself very complex and is the focus of intense research. Translating what we understand of that process into taxonomic names can be challenging. A series of issues are important. Single-locus criteria are unlikely to be convincing. Genetic independence is not a species limits requirement, but the degree of independence (gene flow) needs to be considered when there is opportunity for gene flow and independence is not complete. Time-based species (limits determined by time of separation) are unsatisfactory, though integrating time more effectively into our datasets is warranted. We need to disentangle data signal due to neutral processes vs. selection and prioritize the latter as the main driver of speciation. Assortative mating is also not likely to be an adequate criterion for determining species limits. Hybridization and gene flow are more important than ever, and there is a condition not being treated evenly in taxonomy: evolutionary trysts of 2 or more lineages stuck together through gene flow just short of speciation over long periods. Comparative methods that use what occurs between good species in contact to infer species limits among allopatric forms remain the gold standard, but they can be inaccurate and controversial. Species-level taxonomy in birds is likely to remain unsettled for some time. While the study of avian speciation has never been more exciting and dynamic, there is no silver bullet for species delimitation, nor is it likely that there will ever be one. Careful work using integrative taxonomy in a comparative framework is the most promising way forward.

Keywords: divergence, gene flow, genomics, integrative taxonomy, phenotype, speciation, subspecies

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LAY SUMMARY

- The process of population divergence and speciation produces avian diversity but is very complex.
- I review this process and some of the challenges we have in translating this knowledge into taxonomic names.
- Natural and sexual selection, gene flow, time, and neutral changes affect lineages differently, creating difficult puzzles for us to solve when asking "Is it a species?"
- Avian taxonomy will continue to change as we improve our data and analyses and test historic hypotheses about species limits.
- An integrative approach using diverse datasets in a comparative framework is the most promising way forward.

Una visión general sobre especiación y límites de especies en las aves

RESUMEN

Determinar con precisión los límites de las especies de aves ha sido un desafío y un trabajo en desarrollo por más de un siglo. Es un problema fascinante pero difícil. Bajo el concepto biológico de especie, solo los linajes que permanecen esencialmente independientes cuando están en simpatría son claramente especies. De otro modo, aún no se ha encontrado una línea clara que marque cuando un par de linajes divergentes, e.g., en alopatría, se vuelven lo suficientemente diferentes como para garantizar el estatus completo de especie biológica. Además, con más datos, los límites de especies usualmente requieren ser re-evaluados. El proceso de divergencia y especiación es en sí mismo muy complejo y es el foco de intensas investigaciones. Trasladar lo que entendemos de este proceso en nombres taxonómicos puede ser desafiante. Una serie de cuestiones son importantes. El criterio de un único locus es poco probable que sea convincente. La independencia genética no es un requisito para el límite de las especies, pero el grado de independencia (flujo génico) necesita ser considerado cuando hay oportunidad de flujo

génico y la independencia no es completa. Las especies basadas en tiempo (límites determinados por tiempo de separación) son insatisfactorias, aunque se justifica integrar el tiempo de manera más eficaz en nuestras bases de datos. Necesitamos desenmarañar la señal de los datos debido a procesos neutrales versus selección y priorizar esto último como la principal fuente de especiación. Tampoco es probable que el apareamiento selectivo sea un criterio adecuado para determinar los límites de las especies. La hibridación y el flujo génico son más importantes que nunca, y hay una condición que no se trata de manera uniforme en taxonomía: las citas evolutivas de dos o más linajes unidos a través del flujo génico poco antes de la especiación durante largos períodos. Los métodos comparativos que usan lo que ocurre entre buenas especies en contacto para inferir los límites de las especies entre formas alopátricas sigue siendo el estándar de oro, pero pueden ser inexactos y controversiales. La taxonomía a nivel de especie en las aves es probable que permanezca sin resolver durante algún tiempo. Aunque el estudio de la especiación de las aves nunca ha sido más emocionante y dinámico, no existe una fórmula mágica para la delimitación de las especies, ni es probable que alguna vez haya una. El trabajo cuidadoso utilizando taxonomía integradora en un marco comparativo es el camino más prometedor a seguir.

Palabras clave: divergencia, especiación, fenotipo, flujo génico, genómica, taxonomía integrativa, subespecie

INTRODUCTION

Species delimitation is often difficult. This difficulty stems not from there being multiple species concepts, but because even under a single concept it is often a very complex problem. Probably the biggest reason that accurate species delimitation is difficult is that speciation is a process, and one that, in birds, can take tens of thousands of years to more than a million—2 orders of magnitude (Price 2008). Drawing a bright, clean line on an ongoing process to denote the completion of speciation has thus far been impossible to do across all birds. Under the biological species concept (BSC), "[S]pecies are groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1970, 1996).

The BSC is the predominant one under which avian diversity is considered globally (e.g., taxonomies, field guides). It is also conveniently, but not coincidentally, the dominant framework in which research on the speciation process occurs (Coyne and Orr 2004). There is no better test for the completion of speciation than when 2 divergent lineages occur together and reproductive isolation between them prevents these 2 populations from merging together through gene flow (reticulating). Morphological and genetic species concepts do not have such a widely accepted conceptual and evolutionarily demonstrable landmark to reference "species," and with increasingly large datasets they lack a real bottom to how finely units can be distinguished. Calling the smallest definable lineage a species does not have wide acceptance. In this review, I will not include other species concepts; there are many, and they can produce strong disagreements (Mayden 1997, Coyne and Orr 2004, Collar 2013). Different species concepts produce different criteria for species delimitation and thus different numbers of species (Barrowclough et al. 2016). The BSC is, as they say, the worst species concept, except for all the others. It works well enough.

Where the BSC is at its weakest is in determining the status of isolated (allopatric) populations in which there is no test possible to determine whether they are reproductively isolated enough to be maintained if they were to occur together (crosses in captivity are generally not considered definitive). In these cases, to apply the BSC we have to infer species limits based on what occurs between closely related taxa that *do* occur together and exhibit sufficient reproductive isolation to be full species. This comparative framework is sometimes called the yardstick method (Mayr et al. 1953, Mayr 1969, Mayr and Ashlock 1991) and is discussed in detail below.

Under the BSC, there are frequently important evolutionary units below the taxonomic level of species. Important within-species variation in phenotype (usually plumage or morphology) is usually considered as subspecies, but variation can also represent subspecific groups, evolutionarily significant units, and some phylogenetic species (and these categories often overlap). Our ability to diagnose geographically partitioned variation in a host of new dimensions below the species level has become ever more powerful. Just because a population or lineage is not recognized as a full biological species (or even a subspecies) does not mean it is unimportant. Intraspecific variation is usually included in checklists of the world's birds, and the subject was reviewed by Winker and Haig (2010).

In this review, I consider several broad issues that are currently important in avian species delimitation. Each of these topics is large and can only be summarized here. Also, each generates strong opinions and fervent discussions, a hallmark of research areas that are still being developed. Understanding the process of speciation is helpful for assessing species limits and for applying taxonomy appropriately. Following a brief background on this process, I treat each of these broad issues in its own section, discussing more details of the relevant divergence processes in the context of the types of data and interpretations used for species delimitation. Finally, I summarize the whole.

THE PROCESS OF SPECIATION

In birds and other animals, selection is considered to be the predominant driver of speciation, but the full role of stochastic processes, such as mutation and genetic drift (especially how each might contribute to speciation driven by selection), remains to be determined (Coyne and Orr 2004, Price 2008, Sobel et al. 2010, Langerhans and Riesch 2013, Payseur and Rieseberg 2016, Shapiro et al. 2016). Coyne and Orr (2004) and Price (2008) provided superb reviews on speciation. The field as a whole remains vibrant, however. In aid of species delimitation, I provide an abbreviated overview of some key aspects.

Reproductive Isolating Mechanisms

Under the BSC (defined above), reproductive isolation represents de facto species limits. Reproductive isolation does not need to be absolutely complete, however—some hybridization between species occurs; biological species must be "essentially" reproductively isolated (American Ornithologists' Union [AOU] 1983, 1998, Mayr 1996). Reproductive isolation arises indirectly through an accumulation of mechanisms (also called barriers) that diminish gene flow between 2 diverging populations. Geographic isolation alone is not considered a reproductive isolating mechanism (Mayr 1942, 1996).

In birds, reproductive isolating mechanisms are traditionally thought to arise in allopatry as a byproduct of populations adapting to different environments, sexual selection regimes, etc., and these mechanisms are often then evolutionarily tested when populations come into secondary contact (Mayr 1963, Price 2008). Under this classic view, when isolating mechanisms have built up to a sufficient degree, populations in secondary contact are effectively reproductively isolated and are classified as species. When these isolating mechanisms have not developed to a sufficient degree for this evolutionary independence (essentially independent), the 2 populations might fully reticulate through rampant gene flow and become one taxon again or, in an intermediate situation, hybridize at a substantial rate but retain some differences (e.g., some subspecies). As we come to understand ecological speciation better (speciation from divergent selection between different environments), our traditional view of speciation is expanding to include populations that diverge despite opportunities for intermittent or ongoing gene flow (i.e. not in strict allopatry; see below). In each case, it is the buildup of isolating mechanisms that enables species-level divergence to accumulate and persist.

As Mayr (1963, p. 102) observed, "[a]n isolating mechanism is rarely an all or none affair .... The differences are quantitative rather than qualitative." Importantly, the simple presence of one or more isolating mechanisms is not indicative of the completion of speciation; the accumulation of multiple reproductive isolating mechanisms is common, and isolation strong enough to prevent reticulation and thus result in speciation usually requires multiple mechanisms (Mayr 1947, Sobel et al. 2010, Abbott et al. 2013, Butlin and Smadja 2018). This becomes even stronger when mechanisms are genomically coupled, which requires that linkage disequilibrium build up between them (Smadja and Butlin 2011, Butlin and Smadja 2018). The degree of reproductive isolation achieved between 2 diverging populations is therefore the product of a potentially rich mix of mechanisms acquired as a byproduct of these 2 populations' divergence. Importantly, acquisition of isolating mechanisms between populations is not irreversible; they can be fragile and lost, for example, due to gene flow and changing environmental conditions (Barton 2020, Kulmuni et al. 2020).

Isolating mechanisms are typically grouped into premating, postmating–prezygotic, and postzygotic factors that affect the mating, fertilization, and offspring (and backcross) fitness between individuals of these 2 populations when they have the opportunity to interbreed (Coyne and Orr 2004, Price 2008). In each of these isolation-mechanism categories, mechanisms might exist that limit gene flow, and at a zone of contact it is the total effect of all mechanisms operating that dictates how effective reproductive isolation between the 2 populations has become. If reproductive isolation is not yet complete when populations come together, some degree of gene flow occurs (or it increases if already present), and we often see a combination of isolating mechanisms in play. The interactions of these mechanisms and their relative importance vary among taxa (Coyne and Orr 2004, Price 2008).

In birds, we focus mostly on premating and postzygotic isolating mechanisms. Although postmating–prezygotic isolating mechanisms can be important in plant and insect speciation, in birds we do not know enough yet about factors such as sperm survival and competition and female choice in fertilization to know how important they might be (Birkhead and Brillard 2007, Pizzari 2007, Eberhard 2009, Birkhead and Montgomerie 2020).

Remarkably low levels of gene flow must be achieved if speciation is to occur successfully (see the Gene Flow section). And degrees of reproductive isolation can be challenging to measure. While we now recognize that there have been more gene-flow events among lineages in evolutionary time than we might have predicted (Arnold 2016, Everson et al. 2019), the phylogenetic networks that result among many genes do not negate the importance of reproductive isolation in limiting gene flow (albeit often imperfectly) and resulting in what we call species.

In birds, speciation generally happens faster than reproductive incompatibilities arise, as evidenced by

taxonomically widespread hybridization (at low levels) producing fertile offspring well after speciation has occurred (Price 2008). This indicates that premating isolating mechanisms are important in avian speciation, in that they prevent these cross-species matings from occurring more frequently. But it does not mean that postzygotic (or postmating–prezygotic) mechanisms are unimportant; these mechanisms are much more difficult to detect. Indeed, postzygotic mechanisms are probably quite important in avian speciation, but we have much to learn still about avian isolating mechanisms and their relative importance in this process (Price 2008, Hudson and Price 2014, Pulido-Santacruz et al. 2018, Hill 2019, Irwin 2020).

What is important for determining species limits is that, in this framework, measuring or estimating just one isolating mechanism, even if done effectively, is unlikely to be a reliable indicator of species limits in single cases, much less be broadly applicable. Exceptions occur in truly diagnostic cases such as 2 taxa existing in sympatry with no evidence of crossing, or with hybrid infertility or inviability (but the latter are not reliable if determined in captivity; see Coyne and Orr 2004 and Barton 2020 for examples). Directly assessing the cumulative effects of reproductive isolating mechanisms is the best approach (see the Genomics section), but this is not possible among allopatric forms and so traditionally this has been done through inference using phenotypic characters.

Allopatry

Historically, speciation was considered to be driven by differences accruing between isolated allopatric populations that would eventually cause reproductive isolation (Mayr 1963). Research is showing that speciation also occurs without the complete absence of gene flow that allopatry often provides (Rheindt and Edwards 2011, Nosil 2012, Turelli et al. 2013, Arnold 2016). Speciation with gene flow is being found often in birds, which, given their vagility, should not be surprising (Carling et al. 2010, Zarza et al. 2016, Battey and Klicka 2017, Beckman et al. 2018, Everson et al. 2019, Linck et al. 2019, Funk et al. 2020). Allopatric isolation remains an important part of avian speciation, but its historically perceived overriding importance in the classic sense of causing zero gene flow over very long periods of time is being rapidly eroded with discoveries in population and speciation genomics.

Allopatric populations can and do speciate without ever coming into secondary contact and so do not produce direct evidence (e.g., sympatry) that they are full biological species. As noted above, BSC taxonomy deals with this problem by using a natural comparative framework (considered more below). In applying taxonomic principles, it is important to strive for roughly equivalent levels of divergence among taxa (i.e. families, genera, species, and subspecies).
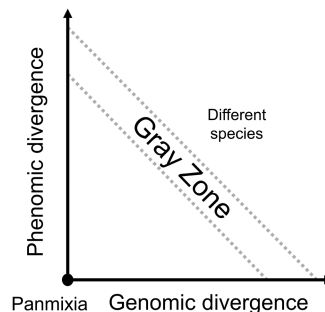

FIGURE 1. Speciation process space is shown in 2 dimensions for heuristic purposes (the process itself is highly dimensional). The 2 main axes of divergence, phenomic and genomic, represent the largely adaptive and largely neutral attributes, respectively, in the major datasets used to study and describe diversity (adapted from Moritz 2002). Differentiating pairs of populations, subspecies, and (perhaps) eventually species progress from panmixia (one interbreeding population) to and past speciation in this space. The gray zone reflects uncertainty in species delimitation.

Speciation Process Space

Visualizing the speciation process in 2 dimensions, along axes of phenomic and genomic divergence, helps us understand this differentiation in terms of the datasets commonly being used to evaluate it (Winker 2009 considered this space in more detail; Figure 1). The process of speciation will have a high dimensionality (e.g., phenotypic traits can be influenced by hundreds of loci), and differences among speciation events are likely to be rampant, but this graphic overview is a useful heuristic device when considering where diverging lineages fall relative to their own phenomic and genomic divergences and relative to other diverging taxa (Campbell et al. 2016).

GENE FLOW

Gene flow between diverging populations—its absence, or its presence, degree, and timing—plays a crucial role in the speciation process and often in our evaluations of species limits. This might be the most complex and unresolved issue that we are currently dealing with both in speciation research and in species delimitation. I suggest that this is because we are still making good progress in fully understanding it in these contexts. Four issues seem important and they are discussed as follows.

Gene Flow Cannot Be Treated as a Presence–Absence Condition

Sometimes the BSC is caricaturized as relying on the presence or absence of gene flow, but this is not how it has been used. Since the inception of this concept, some gene flow has been recognized to be both possible and, in some cases, to occur during the divergence process and after the completion of speciation (Simpson 1961, Mayr

1963, 1982). Mayr (1996) reaffirmed the importance of "essential integrity" of species gene pools, thus allowing crossing (gene flow) to a degree that does not cause a collapse of that effective independence. Introgression (gene flow) between species lineages (and almost-species such as semispecies and some subspecies; Mallet 2007) has long been considered to represent incomplete effectiveness of reproductive isolating mechanisms, and it occurs widely among plants and animals. (I use semispecies as incipient species that are subspecies on the cusp of being biological species, that is, a bit more differentiated than most subspecies. I use allospecies as allopatric entities that are probably biological species and components of a superspecies.)

Hybridization between full biological species in birds occurs surprisingly often (albeit at low rates between any given species pair), given that species are supposed to be essentially reproductively isolated (Gray 1958, McCarthy 2006). Ottenburghs et al. (2015) found that 16.4% of avian species have hybridized with at least one other species in the wild, and this number will grow with genomic research. Both phenotypic and molecular genetic evidence in birds indicates that such hybridization can occur far beyond the completion of speciation. Price (2008) calculated that fertility loss occurs at ~7 My and that hybrid inviability occurs at ~11.5 My. More importantly, in the context of species limits, hybridization "occurs in almost all proposed processes of speciation" (Abbott et al. 2013, p. 230), and, as noted above, genomic studies are revealing more gene flow during avian speciation than was historically recognized. Hybridization does not necessarily mean that gene flow is occurring between populations; postmating reproductive isolation can be strong and prevent gene flow, for example, through lowered fitness in F_2 and backcross generations (Coyne and Orr 2004, Price 2008). In determining species limits, we can thus put aside the many cases of avian hybridization after speciation—cases in which the vast majority could agree that reproductive isolation is complete enough to consider the lineages separate species. But such cases make it clear why a presence–absence view of gene flow in assessing species limits is inadequate.

Effects at Low Levels Are Highly Nonlinear

Not only are low levels of gene flow required for speciation to go to completion, but the effects of gene flow at low levels are highly nonlinear (Wright 1943, 1951, Cabe and Alstad 1994, Mills and Allendorf 1995; Figure 2). Understanding this is critical when interpreting levels of gene flow in terms of species delimitation.

Low Levels Can Be Beneficial

Small amounts of gene flow into a population can provide new genetic variation that enables new adaptations to occur or that lowers the negative effects of inbreeding

(Slatkin 1987, Morjan and Rieseberg 2004, Garant et al. 2007, Räsänen and Hendry 2008, Seehausen et al. 2014, Arnold and Kunte 2017, Stryjewski and Sorenson 2017, Edelman et al. 2019, Grant and Grant 2019, Oziolor et al. 2019).

Gene Flow Is Often Evolutionary Glue

Relatively low levels of gene flow can slow and stop adaptation and divergence, even in the presence of strong divergent selection (Wright 1943, 1951, Slatkin 1987, Rice and Hostert 1993, Hostert 1997, Lenormand 2002, Postma and van Noordwijk 2005, Polechová and Barton 2015). What "low" means is suggested in Figure 2 and considered in more detail below.

These 4 issues might be summarized as follows. When using reproductive isolation as a criterion in assessing speciation and its status, the degree to which it exists is important. Genetic independence is not a species limits requirement; it is common among allopatric populations. But the degree of independence should be part of species limits assessment when that independence is not absolute. The effectiveness of reproductive isolation is best determined through estimates of gene flow when the opportunity for that exists between 2 populations. It is our best assessment of the effects of existing reproductive isolating mechanisms (whatever they might be) in an evolutionary sense. While some gene flow can exist between avian biological species, how much is too much for full biological species? It would seem that when 2 populations are irrevocably locked together with substantial levels of gene flow they are a different entity than populations that are truly essentially reproductively isolated without the possibility of reticulation. What are these threshold levels? We cannot (yet) put a value on this, although we know the numbers are low (Figure 2), and some models and researchers are suggesting limits from around <1 individual to <1% hybridization event per generation (Drès and Mallet 2002, Mallet 2008, Nosil and Flaxman 2011, Leaché et al. 2018).

The amount of gene flow that 2 diverging populations can sustain and still achieve reproductive isolation (i.e. go to completion of speciation) varies due to a number of factors. The strength of selection in relation to gene flow is critical (Flaxman et al. 2014), as are factors such as linkage, recombination, source of selection (e.g., against immigrants or against hybrids), and epistasis among the loci causing incompatibilities (Blanckaert and Hermisson 2018). However, modeling studies consistently show that the amounts of gene flow that swamp divergence typically revolve around very low numbers (Nosil and Flaxman 2011, Bank et al. 2012, Flaxman et al. 2014, Blanckaert and Hermisson 2018, Blanckaert et al. 2020). Insofar as empirical values for the strength of selection tend to be small (Hoekstra et al. 2001), the values of gene flow used in these
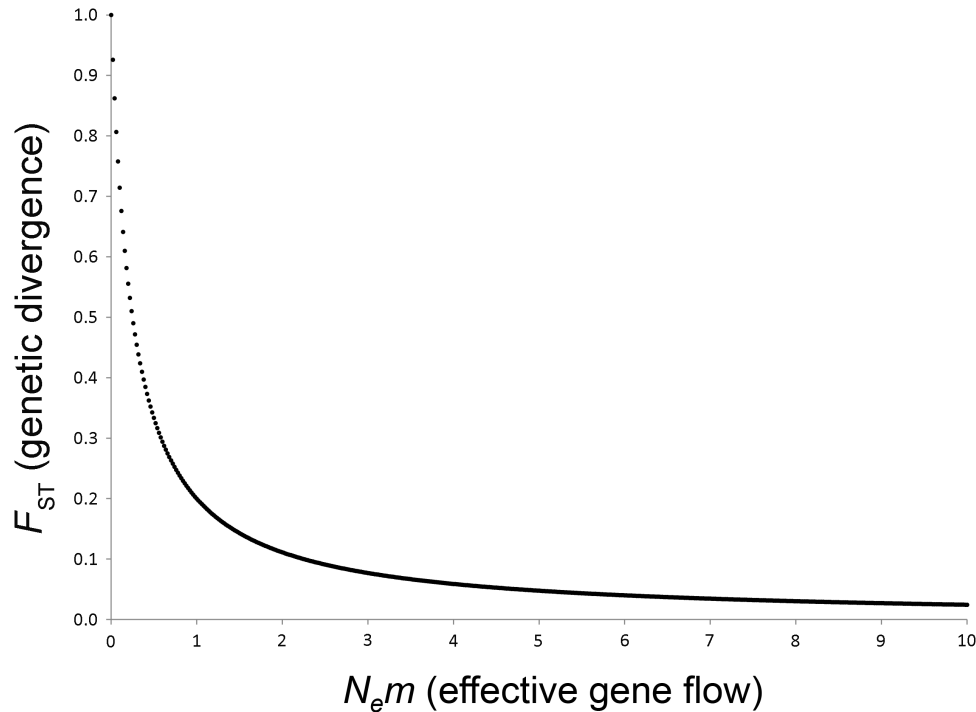
FIGURE 2. The effects of gene flow at low levels on population differentiation, shown as Wright's (1943) F_{ST} (genetic divergence) and the product of effective population size (N_e) and the rate of gene flow (m). The relationship is highly nonlinear, and under neutral conditions the inflection point at which populations switch from diverging to converging occurs at about just one migrant per generation ($N_e m = 1.0$; after Cabe and Alstad 1994).

models are probably fairly realistic. We have a lot to learn still about the mechanisms and outcomes of divergence with gene flow, but learning that even modest amounts of gene flow can exist in conjunction with essential reproductive isolation is not likely to be one of them.

SINGLE-LOCUS PERSPECTIVES AND TIME

Data from mitochondrial DNA (mtDNA) have been invaluable for illuminating evolutionary histories within and among species (Avise 2000), but the single-locus perspectives these data provide are fallible. For example, in the process of speciation, a population genetics phenomenon called lineage sorting occurs, in which alleles (variants of a genetic locus) become partitioned between populations through genetic drift, leading to population-specific alleles at that locus tracking the speciation event (i.e. giving a clear indication of relationships at the species level). (New mutations occur thereafter, too, enabling us to estimate the timing of the allelic split; more on this below.) Although this process can work well for us to reconstruct that speciation event, it often does not, because in the frequently messy genomic process of allelic divergence at these shallow evolutionary levels single loci often do not track the speciation event and

in fact provide a different history. In other words, often individual gene trees do not track the species tree (Avise and Wollenberg 1997, Funk and Omland 2003, Degnan and Rosenberg 2006, Joseph 2021). Add to this the possibility of gene flow, and the opportunity for mismatches between gene trees and species trees is even stronger. So if we make determinations based on single loci in this context, we can be assured that at times we will be wrong. The utility of mtDNA for species delimitation has been debated (Zink and Barrowclough 2008), but in this pattern-based context, our field has largely moved on to insist on more robust genomic datasets (e.g., mitochondrial and nuclear DNA data; Edwards et al. 2005, Edwards and Bensch 2009, Toews and Brelsford 2012).

Speciation takes time, and the 2 are correlated (Price 2008, Price et al. 2010, Hudson and Price 2014). In this context, genetic data can provide a clock-like measure enabling us to estimate time since divergence. MtDNA data have long been used to estimate these divergence depths. But molecular divergence levels are also not reliable indicators of species limits. Although we expect mtDNA substitutions to gradually accumulate and thus increase mtDNA distance between diverging lineages, unless speciation occurs at a constant rate (like neutral genetic divergence), we do not expect these processes to be tightly coupled. Because so much of speciation is driven

by selection (Coyne and Orr 2004, Price 2008), there is every expectation that these 2 divergence processes are not coupled, but rather only correlated over long time periods. And in birds that correlation is not tight and even varies by latitude (Price and Bouvier 2002, Weir and Schluter 2007, Price 2008, Winker 2009).

Considering the effects of mtDNA divergence alone, without the many other factors likely to be driving speciation, the effects of time are not uniform at the relatively shallow levels of divergence leading to and past speciation. MtDNA divergence does produce incompatibilities and thus reproductive isolating mechanisms, but not in a predictable, linear manner, and certainly with no obvious speciation threshold, because of the randomness of mutations and their effects (Hill et al. 2019; described in the Genomics section). Divergence thresholds for species delimitation have been suggested, but have not been widely adopted. Hudson and Price (2014), for example, suggested that allospecies limits be set at 2 My (about 4% mtDNA divergence), based on time-correlated postmating reproductive isolation and the ages of hybridizing avian taxa (Price and Bouvier 2002, Weir and Price 2011).

There is evidence that substantial levels of mtDNA haplotype divergence can accrue within bird species, either as a natural phenomenon within a large population or through total or partial reticulation of diverging populations that retains both divergent mtDNA lineages (Hogner et al. 2012, Peters et al. 2012, Pavlova et al. 2013, Benham and Cheviron 2019, Zhang et al. 2019). Within-species mitochondrial lineages with splits as deep as ~2.6 My to likely more than ~4 My (depending on data) have been documented in birds (Spottiswoode et al. 2011, Peters et al. 2012, Fossøy et al. 2016, Zhang et al. 2019, Joseph et al. 2019, Joseph 2021). Spottiswoode et al. (2011) found a divergence level of 14.8% in the mitochondrial gene ND2 within the brood parasitic Greater Honeyguide (*Indicator indicator*), and that this did not reflect cryptic species but rather maternally inherited within-species adaptations to different host species. Fossøy et al. (2016) found a 3.9% divergence in ND2 within the Common Cuckoo (*Cuculus canorus*) in a similar case of matrilineal adaptation. In a different evolutionary context, Peters et al. (2012) found that Eurasian and North American Green-winged Teal (*Anas crecca*) had mtDNA control region divergence of 6.9% but with relatively high levels of nuclear gene flow between the 2 continents.

Interestingly, it appears that substantial mtDNA splits within species can also arise spontaneously and appear as phylogeographic breaks or in a large, panmictic population (Irwin 2002, Benham and Cheviron 2019). Although these examples represent different divergence processes with respect to speciation, from the mtDNA perspective they are similar in that substantial mtDNA divergence is occurring without being accompanied by reproductive isolation and speciation.

Thus far, characteristics of mtDNA (or any other locus) have not provided reliable indicators of species limits in birds. This is likely to change only if we find molecular markers that are highly correlated with reproductive isolation and that are not error-prone in widespread application.

SELECTION, STOCHASTIC PROCESSES, AND NEUTRAL SIGNAL

As research on avian speciation and species limits has progressed, our datasets have gone from purely phenotypic—and thus rich in the signals of natural, sexual, and social selection—to include increasing amounts of genomic information, in which the signal is overwhelmingly neutral or nearly neutral through the effects of mutation and drift (Kimura 1983, Ohta 2002). For determining species limits, we need to disentangle these more than we tend to do at present and prioritize the signals of selection (Price 2008, Winker 2009). Neutral or nearly neutral genetic processes provide wonderful tools for understanding relationships, gene flow, population size, divergence timing, and more. But as long as speciation is driven largely by selection, these mostly neutral signals are unlikely to be reliable indicators of species limits. Drift alone is unlikely to cause speciation, but drift plus selection might (Templeton 2008, Fry 2009, Sobel et al. 2010).

Two diverging populations can exist in environments, biotic and abiotic, that are very similar or that are dissimilar. These different extremes create a spectrum of between-population selective forces ranging from similar to divergent. Populations diverging in similar environments can undergo mutation-order (or nonecological) speciation, eventually developing reproductive isolation through fixation of different alleles during the processes of local adaptation (Price 2008, Schluter 2009, Langerhans and Riesch 2013). They effectively find different evolutionary solutions for similar problems, causing reproductive isolation on secondary contact. At the other extreme on this spectrum, divergent selection between different environments drives ecological speciation between populations (Schluter 2009). With ecological speciation, populations experiencing different selection regimes are solving different problems in different ways, and standing genetic variation will likely be very important (Schluter and Conte 2009).

While mutation-order speciation does not require a different sequence to the fixation of advantageous mutations and can also theoretically arise only from standing genetic variation (though the latter is unlikely; Schluter and Conte 2009), it is considered on average to take longer than ecological speciation under divergent selection (Price 2008, Price et al. 2010, Langerhans and Riesch 2013). Mutation-order speciation is easily disrupted by gene flow (Price et al.

2010, Nosil and Flaxman 2011). Among island bird taxa, the relative roles of time, isolation, and mutation order and divergent selection remain uncertain, although time is particularly important (Price 2008, Price et al. 2010).

On top of these scenarios of nonecological and ecological speciation can lie sexual and social selection, which could readily be different between populations (Price 2002, Rundle and Rowe 2018), and also reinforcement on secondary contact (Langerhans and Riesch 2013, Hudson and Price 2014). Reinforcement is the evolution of prezygotic barriers to gene flow as a response to selection against hybrids. Ecological speciation and speciation involving sexual selection are widely recognized to occur in birds, but the relative frequency and roles of each of these mechanisms in driving avian speciation remain largely unknown (Price 2008, Schluter 2009, Hudson and Price 2014). If gene flow is involved or speciation is relatively fast (or both), then ecological or sexual selection are more likely routes; if long time periods and isolation occur, situations more reliant on mutations, then mutation-order speciation is likely involved.

Given (1) that nonecological and ecological speciation represent endpoints on a spectrum, (2) that identical environments are unlikely, and (3) that different parts of the genome will probably be responding to different degrees of similar and divergent selection, it is understandable that ascertaining the roles of these mechanisms in speciation is ongoing. In addition, these and other mechanisms of speciation through selection, including sexual and social selection and reinforcement, can interact in complementary, coevolutionary, and multidimensional ways (Price 2008, Langerhans and Riesch 2013, Rundle and Rowe 2018). Thus, speciation under selection will often be complex, and this makes the search for genomic signatures of speciation one of the biggest challenges of the genomic era. We also expect such signatures to be only a small portion of our genomic datasets, which will be dominated by neutral and near-neutral nucleotide polymorphisms. What this means for species delimitation is that there will probably be no easy answers in genomic datasets (considered more in the Genomics section).

In addition, it seems likely that avian speciation has, on average, some latitudinal differences (Weir and Schluter 2007, Weir and Price 2011). Pleistocene glacial cycles had disproportionate effects on habitat and range shifts at higher latitudes, where seasonal migration is also more prevalent. Tropical taxa, again on average, likely had more stable ranges and are more often nonmigratory. Opportunities for gene flow are thus higher among higher-latitude taxa, whereas long-term isolation is likely more prevalent in the tropics. These are generalizations, but if mutation-order speciation is found to be important in avian speciation, it seems more likely that this will be in tropical taxa (Weir and Schluter 2007, Weir and Price 2011,

Weir et al. 2015, Pulido-Santacruz et al. 2018). However, in a taxonomically broad assemblage of 48 Philippine bird lineages, a contrast of phenotypic and genetic data showed compelling evidence for divergence primarily via inferred selection affecting phenotype (Campbell et al. 2016).

Hill (2017) has hypothesized that mtDNA divergence is an important driver of avian speciation due to mitonuclear incompatibilities. This is a form of mutation-order speciation with origins in mitochondrial mutations and is discussed in more detail in the Genomics section.

ASSORTATIVE MATING

Assortative mating is an important reproductive isolating mechanism, both theoretically and empirically (Johnson and Gullberg 1998, Kirkpatrick and Ravigné 2002, Price 2008, Servedio 2016). It is a critical ingredient in sympatric speciation theory, but alone it is not particularly effective unless present at a high level (Johnson and Gullberg 1998, Gavrilets 2006). Sympatric speciation is uncommon in birds (Phillimore et al. 2008, Taylor and Friesen 2017), but this theoretical framework is an excellent one in which to study the effectiveness of assortative mating on speciation. Importantly, both assortative mating and divergent selection together are a theoretical requirement for speciation with gene flow (Servedio and Hermisson 2020). But it is also important to recognize that assortative mating commonly occurs within species without causing speciation (discussed below), and that it is just one of a suite of reproductive isolating mechanisms that can restrict gene flow between diverging populations.

Assortative mating has been given undue prominence in assessing avian species limits, and it is often mischaracterized. For example, in AOU (1998, p. xiv) a false equivalency is made between essential reproductive isolation and "lack of free interbreeding." Similarly, Freeman and Montgomery (2017, p. 858) considered that "… 'reproductive isolation' is defined as assortative mating (i.e. nonrandom mate selection …)." Given what we currently know about speciation and assortative mating, neither of these perspectives is correct. Just what assortative mating is and how we use it to assess species limits requires closer attention.

Assortative mating is a complex topic, and it can arise through different mechanisms and how they affect mating choices. Kopp et al. (2018) reviewed the theoretical and empirical literature and found that speciation is more likely to occur when assortative mating arises from matching rules (like mating with like) than when it arises from preference/trait rules (assortment arising from divergence both in female preferences and male traits). Despite these rather stark differences in outcome, it is difficult to tease these mechanisms apart in natural populations, so

we do not yet know much about the relative frequencies of matching rules (e.g., imprinting and grouping in birds) and preference/trait rules between populations in the speciation process. What is clear, however, is that simply quantifying assortative mating will not prove widely effective in determining degrees of reproductive isolation and speciation outcomes. Note that postmating and postzygotic isolating mechanisms will also likely play an important role, and these are not estimable from measures of assortative mating alone. In fact, mating could be random and yet reproductive isolation be achieved entirely through postmating mechanisms. Assortative mating between populations that are diverged to about the full-species level can also vary geographically and temporally (Short 1969, Schumer et al. 2017).

There are a number of other reasons why the presence of assortative mating alone is not helpful in species delimitation:

(1) It is common *within* species. Many examples of within-species assortative mating that is not indicative of speciation have been found in birds (Cooke et al. 1976, Bearhop et al. 2005, Pryke 2010, Kimmitt et al. 2018). In their review, Jiang et al. (2013) showed significant assortative (55 traits) or disassortative (3 traits) mating within 33 bird species, and much more in over 200 hundred other animal species. They noted (p. E134) that "there is no simple and general set of predictions that can be made about the outcome of speciation based only on the correlation between mated pairs." From these findings, it would be unwarranted to make assumptions about what the presence of assortative mating means with respect to species status. Janicke et al. (2019) examined these data further and showed (p. 865) that "there is no relationship between the strength of assortative mating and species richness." Thus, this characteristic that is ubiquitous among animals (Jiang et al. 2013, Janicke et al. 2019) and whose strength is correlated in birds with taxonomic divergence (Freeman and Montgomery 2017) is not a particularly good predictor of speciation or of species limits (except, as noted above regarding any isolating mechanism, when 2 populations exist in sympatry without evidence of successful hybridization). This is probably because assortative mating is just one factor in reproductive isolation, and that other isolating mechanisms are as or (likely) more important, varying by case.

(2) In zones of secondary contact, the presence of assortative mating denotes a condition of ~51–100% assortative or ~49–0% disassortative mating. The degree of disassortative mating matters tremendously, because even under strong divergent selection it only takes relatively modest amounts of gene flow to maintain evolutionarily strong connections between 2 populations (Figure 2). Even among experts (Mayr 1963, Short 1969, AOU 1983, 1998),

there has been a tendency to overemphasize the presence or absence of assortative mating rather than focus on its degree, which is much more important in the evolution of divergence.

(3) Assortative mating can be nongenetic, that is, a consequence of an ecological attribute such as habitat, niche preference, or bill size (which themselves might have a genetic component; Kirkpatrick and Ravigné 2002, Coyne and Orr 2004, Jiang et al. 2013).

(4) Playback experiments to infer assortative mating are problematic in the context of assessing species limits. Using male agonistic behavior alone (as most of these studies do) leaves out the equally important factor of female mate choice. Even in a system in which males discriminate 100% between vocalizations of their population vs. the other (which would be used by some to infer perfect assortative mating), just an occasional modest preference among females for foreign males as mates (even as extra-pair fertilizations, which are common in passerines; Birkhead and Montgomerie 2020) would preclude divergence of the 2 gene pools at the whole-genome level, even with considerable levels of divergent selection (Figure 2). Considering only the mate choices of one sex can be misleading (Pryke and Griffith 2007, Lipshutz 2018, Uy et al. 2018). In a meta-analysis of playback experiments in oscine birds, Parker et al. (2018) found that the effect size of differential responses between local vs. foreign songs was stronger when the foreign song was from a different subspecies than from other individuals of the same subspecies. This indicates that, as we expect, song discrimination and thus different levels of response increase with increased evolutionary distance. However, because these local-vs.-foreign responses can be significant and even strong within the same subspecies, Parker et al. (2018, p. 12) recommended that these types of playback experiments "should not be used as primary evidence when assessing subspecies status." These problems almost certainly extend from subspecies- to species-level divergences. Considering both of these issues together (sexual bias and undependable effect-size differences), how and even whether to use playback experiments in determining species limits should be reevaluated. Playback experiments are useful in studying evolutionary divergence (Freeman et al. 2017, Weir and Price 2019), and, with care, might also prove useful when integrated with other data in assessing species limits.

(5) Alone, assortative mating does not represent the total effects of all reproductive isolating mechanisms operating between 2 populations. While it can be a strong mechanism within the basket of premating isolating mechanisms, in some cases it might not be (Kirkpatrick and Ravigné 2002). Relative to postzygotic mechanisms, assortative mating can be a weak mechanism (Irwin 2020), and the relationship between populations is defined by all

of the isolating mechanisms in operation, not a single one. This is well supported in theory and empirically (Johnson and Gullberg 1998, Butlin and Smadja 2018). For example, Brelsford and Irwin (2009) found only a narrow hybrid zone in Yellow-rumped Warblers (*Setophaga coronata*) despite little assortative mating; selection against hybrids is probably important in that system.

In their review of assortative mating in speciation, Kopp et al. (2018 p. 15–16) concluded that "… except in unusual circumstances it cannot be inferred that the presence of some current assortative mating implies that speciation will ultimately proceed." Thus, neither the presence nor the degree of assortative mating appears to be a reliable indicator of species limits, either in birds or in other taxa in which it has been studied. Given this, a reassessment of how assortative mating data have been used to infer species limits in birds is warranted.

HYBRIDIZATION AND EVOLUTIONARY TRYSTS

The degree to which hybridization occurs and the nature of this gene flow between divergent populations have long been a focus in speciation research (Mayr 1963, Short 1969, Coyne and Orr 2004, Price 2008, Abbott et al. 2013, Payseur and Rieseberg 2016). Hybrid zones can provide insight into the gradual accumulation of reproductive isolating mechanisms between populations that have been apart for varying lengths of time under varying selection regimes. In his review of avian hybrid zones, Price (2008) found that loss of hybrid fitness increased with the age of the split between the 2 interacting taxa, as we expect, and that it was likely that sexual, social, and ecological selection are all involved to varying degrees to cause these fitness losses. Despite the importance and depth of hybrid zone research and the many ways in which this work has enhanced our understanding of speciation, thus far there are no simple takeaways readily applied to species delimitation, although bimodal hybrid zones probably indicate late-stage speciation (Jiggins and Mallet 2000), and population genomics might provide something new in this regard (see the Genomics section on discontinuities).

One area of hybrid zone research that has fascinated biologists since before Darwin is relatively stable zones of secondary contact that separate 2 phenotypically distinct taxa but through which they are connected by substantial levels of gene flow. Taxonomically, we have not treated these entities evenhandedly. They have characteristics of both subspecies and species, and they have often been considered one or the other under the BSC. Historically, they have been considered part of the semispecies category (Mayr 1963, 1996) although they are not allopatric (as many semispecies are; Short 1969). When these situations show strong phenotypic divergence despite a lack of essential reproductive isolation, we have tended to consider them full species, whereas with less phenotypic divergence they are usually considered subspecies. Two examples of this are found in crows and teal. Carrion and Hooded crows (*Corvus corone* and *C. cornix*) are phenotypically distinctive Palearctic taxa. They are thought to have diverged during the Pleistocene and have a long and fairly stable hybrid zone with substantial levels of gene flow between them (Poelstra et al. 2014). As noted above, Green-winged Teal subspecies have been diverging for ~2.6 My but are still evolutionarily glued together by ongoing gene flow (Peters et al. 2012).

Servedio and Hermisson (2020) showed that this type of situation is a stable third outcome to the divergence process. They chose the phrase "stable adaptive partial reproductive isolation" to describe these evolutionary trysts. It is wonderful to have a strong theoretical basis for this third type of outcome to the speciation process (added to reticulation or speciation), given how commonly these situations seem to occur. Some avian taxa long considered to be hybridizing semispecies include the buntings *Passerina cyanea* and *P. lazuli*, the towhees *Pipilo erythrophthalmus* and *P. ocai*, the warblers *Setophaga coronata* and *S. c. auduboni*, and others (Mayr 1963, Mayr and Short 1970). To these we can add Green-winged Teal and Eurasian and American wigeons (*Mareca penelope* and *M. americana*), both pairs of which show similar levels of gene flow across their Beringian contact zones despite in the latter case a striking degree of male plumage divergence (Peters et al. 2014, McLaughlin et al. 2020). These situations can be likened to binary stars, different but locked together, and as subspecies because they lack sufficient evolutionary independence to be full biological species. Their taxonomic status has long been debated, though, and despite historic confidence in their treatment (Mayr and Short 1970), the past 50 years have seen many changes. Some Galapagos finches might also be a more complex version of this phenomenon, lacking essential reproductive isolation and representing avian ecotypes or ecomorphs more equivalent to plant ecotypes or host races in some insects (Drès and Mallet 2002, Lowry 2012, McKay and Zink 2015, Cadena et al. 2018).

With elucidation of the theoretical underpinnings, it will be easier to study the factors causing these outcomes (Servedio and Hermisson 2020). It is likely to be a stable outcome among taxa showing heteropatric differentiation as well, a type of ecological divergence between lineages in which one or both are seasonally migratory (Winker 2010b, Winker et al. 2013). But at some point this third way, while in at least some cases evolutionarily stable for long periods, will probably break down into one of the other 2 states. I suggest this because in birds we do not seem to have very deep ones (e.g., on the order of honeyguide matrilines; Spottiswoode et al. 2011). Persistence seems limited, albeit across millions of years in some cases. Thus, my phrase "evolutionary tryst"; it is going to end, perhaps through the

extinction of one form, but probably through decreased hybrid fitness (including reinforcement). Ways in which this might occur include through increasing divergence (through pleiotropy and hitchhiking) as more genetic variation is incorporated into the process, through Haldane's rule (e.g., sterility in the heterogametic sex), or through mitonuclear incompatibilities (Rice and Hostert 1993, Innocenti et al. 2011, Peters et al. 2012, Hill et al. 2019).

Taxonomically, uneven treatment of these cases will be challenging to overcome. Simply stated, taxonomy gives us only a binary solution (subspecies or species) to a 3-state problem. Taxonomists have partially solved this by using terms like semispecies, megasubspecies, subspecies groups, etc., but there is no taxonomic designation for this tryst-like third state of divergence stalled for long periods short of speciation. It is the unbroken linkage through substantial levels of gene flow, coupled with at times very distinctive phenotypes, that causes these situations to be particularly messy taxonomically. If we are going to rigorously apply the "essentially reproductively isolated" criterion, we would seem to be forced to call them subspecies. It should not matter how phenotypically divergent they are if they are evolutionarily fairly equivalent in population genomics terms. As the population genomics of these situations is revealed, we will strive to reach some conformity in how we treat particular pairs or groups.

COMPARATIVE FRAMEWORK, YARDSTICK METHOD

The taxonomic status of isolated, allopatric populations under the BSC is determined by inference in a comparative framework. This is best done using data from congeners or other close relatives that are in contact—but essentially reproductively isolated—to judge whether related allopatric forms exhibit similar levels of divergence (and are thus species) or not (Mayr 1969, Mayr and Ashlock 1991). This comparative framework has been an important and powerful approach across zoology for making these taxonomic decisions case by case (Mayr et al. 1953, Mayr 1969, Mayr and Ashlock 1991, Isler et al. 1998, Remsen 2005, Tobias et al. 2010). But inferring species limits among allopatric taxa is arguably the most difficult area of species delimitation, and there is much room both for error and for disagreement. Sampling error alone can cause erroneous conclusions, for example through the numbers and geographic distributions of specimens available, or the amount or type of data used. It is therefore common to reevaluate species limits as datasets are improved. And while historically many workers have tried to find simple criteria that work to delimit species, we are in an era when integrative taxonomy is favored, and it is being practiced at the forefront of avian species and subspecies delimitation and description (Mayr and Ashlock 1991, Alström et al. 2008, Winker 2009, del Hoyo et al. 2013, Patten and Remsen

2017, Rheindt et al. 2020, Alström et al. 2021). Acceptable, high-quality application of one method across all of Aves, however, has not yet been accomplished.

There are 2 weaknesses of the yardstick method. One is that it requires inferential extension from a presumably accurate base (i.e. "this" degree of difference indicates reproductive isolation in this group). The second is that the accuracy of that base is subject to error, both in measurement and in the evolutionary flexibility that sympatry can cause between otherwise allopatric species. Some perfectly good allopatric species can be almost indistinguishable throughout their ranges and only come to differ where they occur in sympatry, for example, through character displacement (Brown and Wilson 1956). In such cases, the yardstick method could misclassify good allopatric species that did not have some sympatric or parapatric demonstration of reproductive isolation. Phenotypic characters can become more dissimilar or more similar on secondary contact (Price 2008), such that trait divergence in sympatry in some cases might not be an accurate indicator of reproductive isolation. More work in this area is warranted. These cases do not negate the utility of the method overall, but they do make it important to exercise caution when using it. Increasing phenotypic divergence with time (Campbell et al. 2016, Winker 2016) gives us some confidence in the basis for using such divergence in species delimitation, but developing focused yardsticks and applying them in close relatives will probably provide more accuracy.

If allopatric populations are determined not to have achieved species-level divergence, but are still diagnosable, they are usually considered subspecies. This means diagnosable under the 75% rule and not using genetics/genomics (Patten and Unitt 2002, Winker 2010c, Patten and Remsen 2017). (The latter is the case because genomics can diagnose populations at very fine scales, well below what is denoted by phenotypic descriptions of subspecies.) Not applying this comparative framework robustly can result in conclusions that are likely to prove incorrect (Sangster 2014, Freeman and Montgomery 2017). However, applying it correctly can also result in errors, with the likelihood of mistakes increasing with the breadth of application and decreasing with the applicability of the "ground-truthing" component: that is how well divergence has been measured in related taxa that are clearly full species and how effectively any yardstick used has been calibrated.

A final concern is not with the yardstick method itself, but rather with misinterpreting its use, for example, as indicative of adopting a morphological species concept. Its correct use is to infer species limits under the BSC. As Simpson (1961, p. 150) observed, "The evidence is usually morphological, but to conclude that one therefore is using or should use a morphological concept of the category (not taxon) species is either a confusion in thought or

an unjustified relapse into typology. The evidence is to be judged in the light of known consequences of the genetical situation ....." He clarified this (p. 156) thus: "The most important point here is not so much the evidence that is used as how it is interpreted."

Several developments are currently having large impacts on the application of this comparative framework: quantitative methods, genetic and genomic data, and expanded datasets. The first 2 create the most controversy.

Quantitative Methods

Since the beginnings of numerical taxonomy, how we use quantitative methods in systematics has undergone major debates (Sokal and Sneath 1963, Mayr 1965, Sneath 1995). Phenetics in particular—taxonomy based on similarities—has a terrible reputation among many systematists (Mayr 1965, Sneath 1995). Nevertheless, there are many things from the phenetics toolbox that remain useful today, and we commonly use them to understand diversity at population-to-species levels (Jensen 2009). Being aware of this largely historic controversy and its philosophical attributes can help us use the strengths and not adopt the weaknesses of methods developed originally for numerical taxonomy and phenetics. Quantitative methods have an increasingly important role to play in species delimitation, although implementation of quantitatively based guidelines has not been without controversy; see, for example, Tobias et al.'s (2010) critical examination of the Helbig et al. (2002) guidelines.

The statistics of effect size (Mayr 1969, Patten 2010) are an important positive development in the comparative framework, but how they are used is not without disagreement. Tobias et al. (2010) used effect size as an integral component of a quantitative phenotypic approach to make comparisons for all birds. Although this was an important advance of a long-held approach (Price 2008), especially in making diagnoses more transparent, it is not perfect (Winker 2010a). Its first broad implementation (del Hoyo and Collar 2014, 2016) generated strong disagreement (Remsen 2015, Cake 2016). Remsen (2015) saw this approach as a return to phenetics in taxonomy, but I would argue that this comparative framework (Tobias et al. 2010) is not part of the philosophical approach of phenetics. It is not grouping like with like; it is instead gauging degrees of difference in a rigorous quantitative framework between groups that we already know to be evolutionarily related. Where things get dicey is in choosing a single speciation divergence threshold. Such a choice gets increasingly suspect with the breadth to which it is applied (Winker 2010a). Despite these criticisms, the Tobias et al. (2010) approach has clear strengths, especially when it is used in a more taxonomically focused manner, or when applied without invoking species-limits thresholds in a broader comparative context (Campbell et al. 2016).

Genomic Data

The genomic era is producing wonderful datasets that can provide extraordinary insights. But the use of genomic data in this comparative framework for species delimitation has not yet produced any clear characteristic telling us that speciation is complete (i.e. effectively reproductively isolated if they were to occur together). This is not for lack of trying. I treat the use of genomics outside of the comparative framework in more detail below.

One area where comparative distances are particularly ripe for confusion (and illustrate uses that can deceive us) is in principal component analysis (PCA) plots of genetic or genomic distances. Although other methods might provide a better statistical model and thus be more appropriate for genomic data (Jombart et al. 2009), PCA remains popular. Using PCA plots to visualize data is useful, but accepting at face value the relative distances between full species and populations that might be species is problematic. Diagnosability itself will be discussed below under the Genomics section; here I am concerned with relative distances. Population size, divergence depths, and gene flow have pronounced effects on the relative positions of populations when visualized together in PC space. Small, isolated populations become genomically distinct relatively rapidly. Consider a thought experiment: A small, isolated population is established from a larger one (e.g., an island is colonized). We have rich single nucleotide polymorphism datasets at hundred-generation intervals for the mother and daughter populations and for a sister species (i.e. 3 entities). Now, through time (e.g., hundreds or thousands of generations), PCA plots of these 3 entities will show the isolated population rapidly moving away from the larger ones in PC space. It will fairly quickly gain distance relative to the source population across this space—due entirely to genetic drift—while the species-level populations will show less movement relative to each other. The small isolated population is undergoing evolution (primarily genetic drift), but it is not an aspect of evolution likely to be very important to speciation. There are other consequences to small population size besides accelerated drift; selection is less effective, the accumulation of deleterious alleles is faster, and the accumulation of mutations overall is slower, including favorable ones that might be important in speciation (Charlesworth 2009, Price et al. 2010). But there are multiple ways in which PCA plots require some thought when interpreting them (François et al. 2010). We need to be especially careful when considering datasets dominated by neutral signal and not well correlated with the achievement of reproductive isolation when we are trying to infer the latter. Divergence through neutral processes produces distances that may or may not be associated with speciation.

Expanded Datasets

This is a smaller and less controversial area of development in the comparative framework (outside of the inclusion of genomic data), but some cautions are warranted. Because museum specimens shrink in preparation and drying, measurements cannot be directly compared between specimens and living birds (Winker 1993, Totterman 2016). Inappropriately comparing or mixing specimen and living bird measurements can cause erroneous conclusions (Winker 1996). There are no simple corrections for this, because shrinkage varies among species. In a different area, it is concerning how often learned song (e.g., in the vast majority of oscine passerines) is used in taxonomic decisions without clearly addressing the variation caused by learning and cultural evolution. See the work of Tobias et al. (2010) for discussion of some of the problems involved and Weir and Price (2019) for example analyses.

Assessments of organismal characteristics that might inform taxonomy are increasingly including environmental variables (related to distribution) to examine potential differences in the niches that different taxa occupy. While niche evolution is strongly correlated with avian speciation at large scales (Cooney et al. 2016), caution is needed when interpreting these results in terms of species limits. First, few studies include the full suite of attributes that define occupied niche (i.e. both environmental and biotic factors). Biotic interactions often affect the realized niche (Alatalo et al. 1985). In their review of phylogenetic niche conservatism and speciation, Pyron et al. (2015) found that the Grinnellian niche alone (i.e. excluding attributes of biotic interactions, which are more difficult to assess) is unlikely to be an adequate evaluation of niche space, because it ignores the major phenomena of the biotic environment. Second, it is not clear what differences or lack thereof actually mean to species limits in diverging forms. Although there is a possibility that differing niche space between diverging populations might cause some immigrant and hybrid disadvantage, there are many examples of profound environmental niche differences occurring within species, both in migratory and nonmigratory taxa (e.g., *Bubo virginianus*, *Tyrannus tyrannus*, *Melospiza melodia*, and *Catharus ustulatus*).

Going Forward

In addition to the issues above, there are 2 related issues that avian taxonomists have not yet comprehensively dealt with. One is an apparent historic shift in the application of the BSC, and this segues into the other, cryptic species. When 2 sister species coexist they are not only exhibiting sufficient divergence to have achieved reproductive isolation. They have also achieved sufficient divergence (e.g., through character divergence or displacement) to not suffer from competitive exclusion—they can coexist ecologically (Brown and Wilson 1956). Ecological divergence is not necessary to achieve reproductive isolation (and thus full biological species status), but it can produce an additive effect on our phenotypic datasets such that our comparative yardstick includes more than necessary just to achieve reproductive isolation.

Mayr (1969) felt that the yardstick method should include levels of divergence that allow ecological sympatry, considering that if ecological compatibility has not evolved (i.e. competitive exclusion is not averted), then the rank of subspecies has more practical value. I view this as only an opinion; it is not part of the BSC. Indeed, Mayr apparently changed his mind on this (Mayr and Ashlock 1991), recognizing that full species could exist in parapatry (ranges abutting) because of competitive exclusion. Although I have not seen it noted before, this view (Mayr 1969) alone could have had a large effect on the overlumping of allopatric avian taxa that occurred in the 20th century as the BSC was applied globally to birds. The change of view seems to have come after the effort of the "Peters" checklists (Peters et al. 1934–1987), and Mayr and Ashlock (1991, p. 105) did consider that changing aspects within the BSC do affect our taxon rankings.

Thus, lineages that are reproductively isolated can in theory be ecologically undifferentiated geographic replacements (Mallet 2007) that are full biological species (see Freudenstein et al. 2017 for a contrary view). There are many avian allospecies (Price 2008), and some of these are undoubtedly such taxa that have achieved phenotypic differences in other ways (e.g., via social or sexual selection). However, cryptic species might arise simply through genomic incompatibilities arising through mutations and nonecological (or mutation-order) speciation without readily measurable phenotypic differences, that is, in cases with an absence of divergent selection (Price 2008, Schluter 2009, Langerhans and Riesch 2013). Avian taxonomy seems to be only beginning to adjust to this, and it will not be easy. We are likely to find many lineage pairs that are closer and closer to this theoretical possibility in coming years (especially across Animalia). How to treat them taxonomically will be problematic and not without disagreement. We ornithologists like our bird species to have some recognizable phenotypic differences. Of course, sexual and social selection and neutral processes also affect phenotype, so perhaps we will find diagnostic cues among these cryptic species, but we are likely to have to look very closely in some taxa. And it bears remembering that cryptic species can arise through divergent selection on phenotypic traits like timing and directions of migratory movements, leaving little evidence in plumage or vocal traits but causing reproductive isolation nevertheless (Winker 2010b, Oswald et al. 2016, Taylor and Friesen 2017). There are other poorly explored phenotypic traits

also, such as sperm and fertilization attributes and olfactory cues (Birkhead and Brillard 2007, Pizzari 2007, Eberhard 2009, Caro et al. 2015, Whittaker and Theis 2016).

Finally, one error we are probably making is in assuming that all members of a group are speciating in similar ways. There are numerous routes to speciation, so we expect selection to operate differently in various contexts, with various blends of ecological, nonecological, social, and sexual selection. Also, divergence under allopatry vs. divergence with gene flow is likely to produce very different looking signals and outcomes, even among closely related taxa (Winker 2010b, 2016, Everson et al. 2019). This adds to the challenges of determining species limits; it argues against the efficacy of universal approaches and for the careful calibration of comparative yardsticks.

GENOMICS

In a now data-rich environment, we are undergoing rapid changes in how we understand speciation to work at the genomic level (Campbell et al. 2018). This perspective has been termed the genic view (Wu 2001), but in the rush to strike sparks of novelty there has been some overreach, caricaturization, and inadequate coverage of historic work, leading to philosophical discussions about the framework in which speciation should be considered (Wu 2001, Wu and Ting 2004, Provine 2004, Harrison 2012, Wang et al. 2020). From research on hybridization and hybrid zones, it is clear that speciation research has fully accommodated the genic view for decades (Barton and Hewitt 1989), and that we have come a long way from the field's foundations in which genetics were absent or poorly understood (Darwin 1859, Mayr 1963). An improved understanding of the genomics of speciation has not shaken the foundations of speciation research under the BSC. This improving understanding will likely have important knock-on effects in species delimitation.

The genomic era is revealing astonishing levels of detail about speciation, relationships, gene flow, genetic diversity, divergence times, effective population sizes, and more. We have some wonderful advantages in studying avian speciation in that birds are diploid organisms with relatively small genomes having high degrees of synteny (gene arrangements conserved on chromosomes relative to other organisms; Ellegren 2013), although inversions are not uncommon (Price 2008, Hooper and Price 2017). One of the most important findings has been just how much gene flow occurs during and after speciation and how reproductive isolation accrues between lineages (Rheindt and Edwards 2011, Arnold 2016, Ottenburghs et al. 2016, Campbell et al. 2018). But many questions remain. The entities we recognize as species are remarkably variable in

how much of their genomes are involved in developing and retaining isolating mechanisms during divergence (Shapiro et al. 2016). Determining how selection and reproductive isolating mechanisms interact in behavioral, ecological, physiological, and genetic contexts, how many genes are involved, and how much divergence is required to complete speciation are important research areas (Shapiro et al. 2016).

How might developments in genomics help us in species delimitation? First, the genomic era is unlikely to provide us with a simple solution to species delimitation, despite suggestions to the contrary. Jarvis (2016), for example, suggested that whole genomes might solve the species problem by redefining species and using mostly genomic distances to determine species limits and impose a new taxonomy. This is too simplistic to function well in birds (and likely other organisms), because it prioritizes mutation and drift and does not account for the multifarious effects of selection on the speciation process. Also, in ignoring phenotype it is unlikely to be widely adopted by taxonomists. At higher taxonomic levels, however, genomic data are proving invaluable for illuminating relationships. And although we have an enormous amount yet to learn about the genomics of speciation, these tools and datasets are providing important insights into species limits that, with care, will have strongly positive impacts in species- and subspecies-level taxonomy.

Diagnosability and "Species" Delimitation
Thus far, diagnosing and defining species at the level of one or a few loci is not common (though see Barraclough 2019), and it probably should not become so or we would see things such as geographic isolation alone causing rapid "speciation." Calling these things species removes our focus on organisms and organismal lineages, the proper focus of speciation and biodiversity, and places it instead on one or a few genes. This can cause us to state, effectively, that a gene tree is equivalent to the species tree when the majority of gene trees do not conform due to recent or ongoing gene flow. In cases where selection is driving one or a few loci to high frequencies between 2 populations, it can also represent a conflation of adaptation with speciation. Currently recognized species like Carrion and Hooded crows (*Corvus corone* and *C. cornix*) and Blue- and Golden-winged warblers (*Vermivora cyanoptera* and *V. chrysoptera*) are likely relevant examples. While focusing at the level of one or a few loci can help us understand speciation, bringing that understanding back up to the organismal lineage level remains critical for a useful taxonomy producing evolutionarily comparable taxa among those lineages (e.g., bird species).

At a larger scale, with more data, it is easy for populations to acquire genomic diagnosability, especially if they are small. The stochastic processes of mutation and genetic drift drive most of this diagnosability. If it is primarily selection that drives speciation (Coyne and Orr 2004, Price 2008, Shapiro et al. 2016), then broad-scale genomic diagnosability is not likely to be especially informative about species limits. Diagnosability using speciation genes might be different, but this is undemonstrated as yet. Even in the absence of fixed genetic differences, genotyping at many loci provides a powerful diagnostic tool. Wang et al. (2003) were able to distinguish with 99% accuracy an introduced eastern population of House Finch (*Carpodacus mexicanus*) from its ancestral western founding population, despite just a century or less since their divergence. Genomic divergence through mutation and drift is an evolutionary process, but most of the signal will arise from aspects of evolution less important to speciation than the genomic changes that cause reproductive isolation. It is very difficult in genomic datasets to focus only on the latter.

Despite these inherent difficulties, there has been a huge growth in "species" delimitation efforts using genomic data (Fujita et al. 2012, Carstens et al. 2013). We are increasingly able to find evolutionarily independent lineages in genomic datasets, but evolutionary independence is not equivalent to speciation. So I consider "species" delimitation in this area (particularly coalescent-based species delimitation programs) to be a misnomer and effectively false advertising. Speciation is a process, and it is really easy to diagnose structure between groups that are unlikely to be equivalent to species in many cases (Sukumaran and Knowles 2017). Genomic analyses for this type of delimitation thus have numerous problems, from unrealistic assumptions to oversplitting (Sukumaran and Knowles 2017, Chambers and Hillis 2020).

There are additional problems, such as not accounting for gene flow, changes in population size, and excluding phenotype. Also, as with other methods, these delimitation analyses are subject to sampling issues. For example, increasing sample sizes of individuals and especially loci can make even subtle genotypic differences between populations more diagnosable (O'Dushlaine et al. 2010). Another example is that isolation by distance can create "species" when clines and contact zones are not sampled. Even in well-known groups, accuracy seems poor (Campillo et al. 2020), and advocates are focusing on its utility when the discovered lineages are in sympatry (Leaché et al. 2018); such phenotypically cryptic lineages have a much higher likelihood of having achieved reproductive isolation and thus represent genuine species. When these methods are used alone for lineage diagnosis it is important to think of them not as species delimitation, but as *lineage delimitation* only, and lineages can span the entire divergence continuum.

A different genomics approach to determining species limits was proposed by Hey and Pinho (2012), using what they considered to be objective population genetics criteria. But by excluding selection, phenotype, and a way to treat allopatric populations, their method will not have broad applicability. Like Leaché et al. (2018) in applying species delimitation methods for populations in sympatry (i.e. with definite opportunities for gene flow), such approaches do have a narrower utility and might serve as a basis for deeper investigation that includes phenotype and the signatures of selection.

At this time, genomics alone cannot be used to reliably define species or subspecies. These data are excellent for diagnosing groups, but where exactly those groups fall out in the divergence process (e.g., populations, subspecies, species) requires other information. This argues for continuing to integrate genomic and phenotypic datasets (Winker 2009, Carstens et al. 2013, Patten and Remsen 2017) and understanding the results in a comparative framework using a biologically relevant touchstone for relative degrees of difference to know what groups or lineages belong in each of our categorical bins on the divergence continuum.

The Permeable and Heterogeneous Genome

Locally adaptive portions of the genome are more resistant to gene flow at a contact zone, whereas neutral and beneficial alleles will move much more freely between interbreeding populations (Rheindt and Edwards 2011, Abbott et al. 2013). This is visible at the population level through examining the genomic patterns that result when hybrids cross and backcross. This often creates clines of different shapes (e.g., width, steepness; Price 2008) at different portions of the genome at contact zones (Carneiro et al. 2013). This heterogeneous genomic landscape contributes to the complexity of signals from the speciation process and to the difficulty of applying these data to taxonomic questions. We are still left asking how much selection is needed on how many genes to cause speciation, and how much divergence across the whole genome will this represent (Shapiro et al. 2016)?

Another aspect of genomic heterogeneity is also important when using genomic data to understand speciation and species limits. In any genomic sample of 2 populations, a lot of the variation will predate the divergence event and thus reflect older variation spread throughout the ancestral population (Charlesworth et al. 2003, Linck and Battey 2019). Coalescent theory and analyses have improved our abilities to distinguish more recent, post-divergence events from this older signal (Gutenkunst et al. 2009, Sethuraman and Hey 2015).

Genetic Incompatibilities

Diverging populations gradually accumulate genomic differences that can cause decreased fitness in hybrids and, thus, postzygotic reproductive isolation to varying degrees. The genomic basis for reproductive isolation is an intense area of speciation research and much remains unknown. The traditional focus has been on searches for genes that cause sterility or inviability, but these extremes are less important in bird speciation than in, for example, insects (Coyne and Orr 2004, Price 2008). Haldane's rule (sterility, rarity, or absence of the heterogametic sex, which in birds is the female), occurs in birds among hybrids of more divergent lineages, beyond full biological species level (Price 2008). Incompatibilities in hybrids can also be caused by differences in sex chromosomes (the "large-Z effect" in birds), but evidence for this is mostly indirect and is also concordant with the effects of enhanced drift due to smaller effective population size (0.75 for sex chromosome loci vs. 1.0 for autosomal loci) and other phenomena (Presgraves 2018). Initial work on postzygotic genetic incompatibilities in fish and *Drosophila* flies showed that ordinary genes with normal functions are involved, that rapidly evolving genes predominate, and that these genes are undergoing positive Darwinian selection (i.e. selective pressures favor change; Coyne and Orr 2004, Orr et al. 2007).

But because birds tend to speciate well before hybrids exhibit sterility or inviability (Price 2008), it is not yet clear how much relevance that research has for ornithology. Avian speciation tends to involve less extreme losses of fitness in hybrids, and genetic incompatibilities between diverging populations can arise from something as simple as different alleles at the same locus to genomic rearrangements such as chromosomal inversions and transposable elements (TEs; Price 2008, Manthey et al. 2018, Serrato-Capuchina and Matute 2018). The mechanisms of Haldane's rule bear consideration, especially in cases with long divergence times and low divergent selection between populations. But given present knowledge, our focus for postzygotic reproductive isolating mechanisms in birds should instead be on losses of fitness in hybrids caused by Dobzhansky–Muller (DM) incompatibilities. Simply put, these are gene combinations between 2 populations with different evolutionary histories that cause lowered fitness in hybrids (Orr 1996). Such fitness decreases can be caused by extrinsic factors (e.g., environmental) and by intrinsic ones (e.g., physiological performance regardless of the environment), and these 2 can be linked (Langerhans and Riesch 2013, Kulmuni and Westram 2017). DM incompatibilities tend to arise as byproducts of selection operating in 2 allopatric populations and their respective environments, but these environments do not have to be different and the changes do not have to be adaptive (Schluter 2009, Unckless and Orr 2009, Presgraves 2010a, Langerhans and Riesch 2013).

A potentially important source of intrinsic postzygotic isolating mechanisms comes from mitonuclear incompatibilities, which are a subset of DM incompatibilities. Mitonuclear incompatibilities arise when coadapted mitochondrial and nuclear genomes (which are critical for cell energetics) diverge between populations and cause fitness losses when combined in hybrids. These incompatibilities are important in arthropod speciation (Barreto et al. 2018) and are more likely to develop in isolated populations (Burton and Barreto 2012). The effects are more pronounced in taxa with female-biased dispersal (Telschow et al. 2019), which is the case in most birds. Pavlova et al. (2013) found a 6.6% divergence in ND2 between interior and coastal Eastern Yellow Robin (*Eopsaltria australis*) populations and estimated ~1.5 My divergence. Morales et al. (2018) found corresponding selection in the nuclear genome, evidence that mitonuclear incompatibilities can be an important mechanism of divergence. Hill (2017, 2019) has advocated that mitonuclear incompatibilities are of core importance in avian speciation, but this seems to require changes in our concepts. Thus far we lack data to know how important these incompatibilities are in birds, and there is substantial evidence to cause doubt about their importance in avian speciation (e.g., highly divergent mtDNA lineages within species). The fragility of mutation-order speciation to gene flow might explain how relatively deep mtDNA divergences can accumulate within biological species without apparent effects on reproductive isolation (as discussed above). Nevertheless, as we seek genomic bases for postzygotic reproductive isolation, this is an area to search, and this subset of DM incompatibilities might be prevalent in tropical and especially island taxa where the speciation process is long, divergent selection relatively weak, and gene flow is very low (Weir et al. 2015, Pulido-Santacruz et al. 2018). As this area of research develops, it will be important to remember that the presence of one type of isolating mechanism does not mean that others are not present and also important; in fact, we should expect the latter (Mayr 1947, Price 2008, Sobel et al. 2010, Abbott et al. 2013, Butlin and Smadja 2018).

Chromosomal rearrangements such as inversions can also cause genetic incompatibilities by preventing recombination in the inversion and inhibiting the transfer of those loci between divergent populations (Kirkpatrick and Barton 2006, Price 2008). They become particularly effective as a postzygotic isolating mechanism as more adaptive alleles accrue within them. Hooper and Price (2017) found that inversions are common in passerine birds and that they often separate closely related species, being especially well correlated with range overlap. This suggests that inversions can be important isolating mechanisms on secondary contact. Their study focused on large pericentric inversions and is therefore probably conservative. The full

role of chromosomal inversions in avian speciation is not yet known, but should become clearer as more complete avian genomes are developed.

Two other genomic attributes bear watching as our understanding develops. First, Piciformes have a substantially larger proportion of the genome containing TEs than other birds, and this is likely to increase the importance and nature of postzygotic reproductive isolation in this group (Manthey et al. 2018). Second, global recombination rates can vary between populations, and the effects of this on the genomics of speciation are similar to those of gene flow (White et al. 2019). This variation can be strikingly high, within and among species. Dumont and Payseur (2011) found a 30% difference between males of different house mouse (*Mus musculus*) subspecies. Kawakami et al. (2014) found that the recombination rate of the Collared Flycatcher (*Ficedula albicollis*) was 200% that of the Zebra Finch (*Taeniopygia guttata*). How often these differences will be important in avian speciation is unknown.

Might we find some key genomic attributes among these many incompatibilities that widely reflect the completion of speciation in birds? This seems unlikely, for several reasons. If speciation in birds is largely driven by selection (Price 2008), consider that this selection is operating largely on standing genetic variation and new mutations to cause increased adaptation in a population, and that reproductive isolating mechanisms arise as a byproduct of this process occurring between 2 diverging populations. Adaptation in these lineages is independent—with different genetic variation and different responses to selection. This effectively doubles the number of loci that might be involved in later incompatibilities that cause partial or complete reproductive isolation. These mechanistic byproducts to independent adaptation can be premating, postmating–prezygotic, and postzygotic; it usually takes more than one to cause effective reproductive isolation (Mayr 1947, Price 2008, Sobel et al. 2010, Abbott et al. 2013, Butlin and Smadja 2018); and we know of many different kinds of these mechanisms across all of Aves. We should therefore expect discordance among avian speciation events in where and how (genomically) these isolating mechanisms accrue, and thus far evidence suggests this is the case (Seehausen et al. 2014). As Sobel et al. (2010) considered, with multiple barriers causing reproductive isolation, each with a different effect and genetic basis, commonality among speciation events will likely be rare. Even in closely related taxa, the lack of concordance is noteworthy, particularly early in the process (Feulner et al. 2015, Delmore et al. 2018). Concordance should be rare, but it will be interesting where it appears (Kanippayoor et al. 2020).

Given what we know about speciation, then, it seems an "all of the above" outcome is likely—among all avian divergences and speciation events, we are likely to find some representation of each of these mechanisms present in the genome, with varying presences, absences, and signatures, with a lot of variation among lineages. So the genomics of avian speciation will likely reveal a highly complex assemblage of characteristics with no widely applicable condition useful for species delimitation.

Discontinuities on the Divergence Continuum

Although divergence is a continuous process in which differences gradually accumulate between 2 populations, there is evidence that the cumulative effects of these differences and the incompatibilities that arise from them can cause an acceleration in reproductive isolation. The mechanisms for this are still not clear, but a relatively rapid, nonlinear approach to completion of the speciation process can occur (Presgraves 2010b, Feder et al. 2014, Nosil et al. 2017, Dagilis et al. 2019). This phenomenon might be reflected empirically in one of the most interesting aspects of Price's (2008) review of avian hybridization. He found 2 general groups of diverging taxa when graphing the age of hybridizing taxa that have not come together recently and the width of their hybrid zones (Price 2008, figure 15.3, right). This also might suggest the completion of speciation through reinforcement. The role of reinforcement in speciation generally is uncertain (Servedio and Noor 2003, Butlin and Smadja 2018), but it seems likely to be important in avian speciation (Price 2008, Hudson and Price 2014).

Another important development is that there is both theoretical and empirical support for a threshold (or fairly rapid phase change) between populations at low levels of gene flow using neutral markers. From a speciation genomics perspective, it appears that the speciation continuum is remarkably discontinuous, with species and not-species (including evolutionary trysts) falling into 2 groups (Flaxman et al. 2014, Roux et al. 2016, Riesch et al. 2017, McLaughlin et al. 2020). These discontinuities are being found in process space with widely applicable axes (e.g., gene flow, F_{ST}). We can likely anticipate clarification of these relationships in the coming years. It might be that the inflection point in Wright's F_{ST} in relation to gene flow (Figure 2) is a fleeting and difficult place to land between lineages that have diverged to that point, with increased divergence following relatively quickly thereafter due to fitness losses in hybrids. I suspect this would come as no surprise to Wright.

In taxa with the opportunity for gene flow, these discontinuities might provide a broadly applicable line denoting species limits. The population genomics approach provides an opportunity to effectively set aside all the complex details of reproductive isolation (usually unknown) but see their overall effects in an evolutionary

sense between 2 diverging populations with the opportunity for gene flow. At present, these discontinuities seem one of the most promising signals of speciation to emerge from the genomics era thus far. But that might be because we do not yet know enough about them. And we still have the problem of figuring out what genomic attributes strictly allopatric taxa achieve when they diverge enough to represent full species. That seems unlikely to be something obvious. And despite the importance of genomics, there are other factors that might be important in avian speciation that genomic data do not illuminate, such as phenotypic plasticity, epigenetics, and learning (Smadja and Butlin 2011). However, genomic data together with other data will help us get our taxonomies closer to reality.

Adaptation vs. Speciation

Evidence of adaptation is not evidence of speciation. In systematics terms, anagenesis is not equal to cladogenesis. In avian speciation, the bulk of evidence suggests that one precedes the other, but under the BSC the collective adaptations between 2 populations must include sufficient reproductive isolating mechanisms to prevent reticulation (i.e. retain essential reproductive isolation) between those 2 lineages for speciation to be complete.

Currently, taxonomy is probably experiencing oversplitting because adaptation and speciation are being confused, using both phenomic and genomic data. We have had more experience with phenomic data (e.g., recognizing subspecific differences), but as we employ genomic datasets to help us determine species limits, we need to ask when divergences are just adaptation and not really speciation. Adaptive alleles or haplotypes often undergo such strong selection that they go to very high frequencies in populations. Lactase retention in adult humans, for example, reaches high frequencies in western European populations (Gerbault et al. 2011), and some of the alleles affecting human pigmentation also show strong geographic structure (Sturm and Duffy 2012). Cases of strong selection pushing an allele to high frequency or fixation in a population do not make a species, whether or not populations are connected by gene flow. Such selective sweeps are not speciation events. Genetic drift can cause similar genomic patterns, and this is also not speciation. Adding to the complexities, distinguishing between drift and selection can be impossible (Hughes 2007, Bank et al. 2014). In birds, sufficient genomic data are accumulating to untangle species-level taxonomy by likely demoting some full species into subspecific entities that have some interesting—but not species-level—adaptations exhibited in few loci or in gene regulation. Examples include Blue- and Golden-winged warblers (*Vermivora cyanoptera* and *V. chrysoptera*; Toews et al. 2016) and Common and Hoary redpolls (*Acanthis flammea* and *A. hornemanni*; Mason et al. 2015). Neither of these examples has yet experienced

a recent taxonomic demotion to one species. But as we learn more about how phenotypic traits can mask underlying gene flow and we better understand adaptive loci in relation to population-level gene flow, taxonomic changes like these will likely occur (Toews et al. 2021).

CONCLUSIONS

Describing avian diversity and delimiting species and subspecies are important ongoing tasks. It might surprise some that after studying speciation for over 150 years we still do not know enough about it to be able to reliably make these determinations. To those of us close to the subject, it is a fascinating and complicated problem, and placing lines on a continuous divergence process that differs widely in its details among lineages is an inherently difficult exercise. Each of the major issues I have briefly covered is complex, with a vast literature, and I have not been able to fully explore their complexities here. Also, many of these areas are hotbeds of research, so it is an exciting time in speciation research and taxonomy. As our understanding of the processes improves and more data are obtained, we can anticipate ongoing changes.

Much progress on avian species limits was made during the 20th century as "Morphological, ethological, physiological, biochemical, and bioacoustic characters and their geographical variation were investigated in ever increasing detail," and this approach was widely adopted across systematics (Haffer 1992, p. 139). Mayr (1996) held that mosaic evolution, the variation among lineages in the rates of character evolution, was the biggest problem in species delimitation. This phenomenon often produces discordance in signal between characters and species limits. Genetic and genomic data have not provided a ready solution. More data are usually the best way to resolve these problems, and many have advocated what we today call integrative taxonomy (Mayr 1969, 1996, Mayr and Ashlock 1991, Alström et al. 2008, Winker 2009, Padial et al. 2010, Carstens et al. 2013, Patten and Remsen 2017). The cutting edges of this work usually involve the use of all data available in a classic comparative framework under the BSC (del Hoyo et al. 2013, Rheindt et al. 2020). Even if we disagree on species concepts, taxonomists tend to continue to agree on the importance of an integrative approach (Sangster 2018).

I have largely omitted big topics (e.g., pleiotropy, epistasis, linkage disequilibrium, gene regulation, reinforcement, sex chromosomes, variation in effective population size across the genome, learning, phenotypic plasticity, epigenetics) and big toolboxes (e.g., quantitative genetics) that are important in speciation research but which have not (yet) been important in avian species delimitation. I note this as a reminder that there is still much to learn

about speciation and, thus, about how to better categorize diverging lineages. I have also omitted important topics in avian speciation (e.g., sexual and social selection, heteropatric and allochronic speciation) where we also have much to learn about what isolating mechanisms are important in divergence and how they work relative to each other and among avian lineages.

The theory, tools, and data we have and how we use them change through time, adding our own certainties and uncertainties to the process of determining species limits. An example of this is that the principles of phylogenetic systematics have to be able to break down at the species level, because they do in the process of species-level divergence. Reticulations happen, and not necessarily between sisters, and founding populations can arise from the middle of a group of related populations and speciate while the source populations do not (Haffer 1992, figure 3). In both scenarios, genuine biological and population genetic processes are not well served by rigid adherence to our conceptual tools. As phylogenetic networks become increasingly recognized as more reflective of evolutionary processes than bifurcating trees at these levels, this should be an easier mental hurdle for us to overcome (Arnold 2016, Ottenburghs et al. 2016, Shapiro et al. 2016). But conceptual issues aside, abundant and increasing evidence of gene flow during and after avian speciation—though rich evolutionary ground—makes species delimitation even more complicated.

Another example is that using degrees of genetic or genomic divergence and diagnosability incorporates a lot of neutral signal, thus emphasizing things unlikely to affect actual speciation, such as effective population size and genetic drift. So simple genetic or genomic distance measures, while increasing generally with divergence, do not work at the individual case scale, and time alone is a poor surrogate for determining when speciation is complete. This is the case because selection is usually involved in speciation and its effects are not incorporated in genetic distance measures, and because the accumulation of effective isolating mechanisms through mutation is also a temporally uneven process (Hill et al. 2019). Cladistic patterns and genetic divergence values are therefore unreliable criteria for species delimitation. However, the associated approaches have been invaluable in revealing cryptic species (the "sibling species" of Mayr and earlier authors; Bickford et al. 2007) that, with other data, often are recognized as full biological species (Singhal et al. 2018).

The sociopolitical aspects of avian taxonomy have grown enormously in the past 50 years, even to uses of the terms "tyranny" and "democracy" (Collar 2013, 2018). Globalization, the increasing popularity of birdwatching, and social media are all correlated with this change. People feel strongly about birds. As a member of the American Ornithological Society's North American Checklist Committee, I consider that we probably make mistakes in our taxonomic and nomenclatural decisions every year (results appear annually; Chesser et al. 2019). We review many challenging cases, rarely with perfect data, and we achieve consensus through discussions and voting. But laws and regulations protecting avian diversity also increase the political ramifications of avian taxonomy, and being able to meaningfully engage professional expertise is critical (National Academies of Sciences, Engineering, and Medicine [NASEM] 2019). Much of our sociopolitical wrangling is internal, among experts. "The problem is solved if you approach it in my preferred way" is a common theme in species delimitation, usually representing strong and often reasonable views about components of a complex larger whole.

A positive aspect of our situation is that we humans, *Homo sapiens*, are one of the best-studied biological species. As a vertebrate species with geographically partitioned variation, we have similarities useful in the context of avian divergence and speciation. Insights into our own diversity and relationships can help us understand those of other species. Mayr (1963) considered humans as a biological species, providing an entire final chapter on that concept. Although cultural evolution in humans is rather pronounced, it is also common in other vertebrates, including birds (e.g., learned behaviors). Mayr (1982, p. 622) considered that "even in the evolution of culture there is not a sharp break between animal and man." This view has the advantage of orienting "species" on an entity already widely understood and accepted as such. As biodiversity experts, we depart from that substantial basis of buy-in at our own peril.

Work in bird species delimitation is littered with errors of overlumping (false negatives)—for example, from the heavy-handed implementation of the BSC (Peters et al. 1934–1987). And it is also likely becoming littered with examples of oversplitting (false positives)—for example, overeager implementation of genomic "species" delimitation analyses and guidelines that enable overly fine splitting (Helbig et al. 2002). Oversplitting seems likely in avian allospecies generally (Hudson and Price 2014). And we still have major unanswered questions, such as the relative frequencies and importance of various reproductive isolating mechanisms (e.g., behavioral, ecological, or physiological) and how much independence populations' genomes should have. There is much work remaining to be done, especially among difficult cases, where there is room for disagreement even among experts. One thing is clear, however. There is no silver bullet for species delimitation, nor is it likely there will ever be one. But it has never been a more exciting time to study, discover, and classify biodiversity. Careful work

using integrative taxonomy in a comparative framework is the most promising way forward.

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