The Contribution of Genomics to Bird Conservation

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Abstract

The world's birds are in trouble, and scientific research, including genetic and genomic methods, can play an important role in understanding and mitigating these problems. In this review, we summarize several ways that the concepts and methods of genomics can help with bird conservation and how the dramatically increasing power and decreasing costs of these methods may allow an even greater role in the future. We assess six primary, not exhaustive, and not mutually exclusive research areas, including avian forensics, captive management, infectious disease and vector interactions, metagenomic and microbiome applications, systematics and the definition of conservation units, and the genomics of adaptation. We conclude that the uses of genomics to identify, understand, and in some cases reduce anthropogenic impacts on bird populations are well underway. And the future holds great promise that developments in our understanding of avian genomes and tools to modify them will play an increasingly important role in future attempts to alleviate these impacts.

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1 Introduction

Like the proverbial canary in the coal mine, birds serve as important model systems and umbrella taxa for conservation of ecosystems. Since 1500 as many as 183 species of birds have gone extinct, and currently about 23% of the nearly 11,000 species of birds are considered endangered, vulnerable, or threatened, and their decline continues at an alarming pace (Birdlife International 2018). While there are many scientific fields involved in understanding and mitigating these threats to birds, genomics and advanced genetic methods have much to contribute to the conservation of birds. Here we summarize several of the recent advances in genomic applications in conservation and provide examples of these approaches from the literature. We also provide a prospective view of novel future applications and approaches, given the rapidly increasing power arising from high-throughput sequencing, synthetic biology technologies, and increased bioinformatics power.

Genomics in the Broad Sense Here we consider genomics in the broad sense, that is, not just sequencing and characterizing full genomes but using genomic and advanced (e.g., next-generation) sequencing methods that can provide useful data for a wide range of applications in conservation biology. For example, transcriptomics methods can be used to identify avian genes expressed in response to environmental stressors such as disease, climate change, or pollution (Jax et al. 2018). In addition, use of metagenomic and microbiome methods can elucidate how avian symbionts influence survival, life history, and population dynamics and facilitate characterization of diets and dietary shifts (Trevelline et al. 2018). Genomic methods can also be used in such classic applications of conservation genetics as assays of genetic variation in small and captive populations (Harrisson et al. 2014), determination of species and evolutionary significant units (Robertson et al. 2014; Oyler-McCance et al. 2015; Peters et al. 2016; Ottenburghs 2019), inbreeding levels (Li et al. 2014), movements (Kawakami et al. 2014), population sizes (Nadachowska-Brzyska et al. 2015), and the presence of disease (Sun et al. 2015). And layered upon these types of issues in conservation genetics, environmental and noninvasive DNA approaches have proven useful for research on endangered and difficult-to-study birds. Below we survey several of the areas of avian conservation biology for which we believe genomic methods can and will play an important role.

Genomics Methods These can involve assessment of whole genomes or just sizable numbers of nuclear and plastid loci sampled from across the genome (Lerner and Fleischer 2010; Toews et al. 2015). Early applications of genomics have primarily involved reduced representation sequencing, in which short sequences (e.g., SNPs) are sampled from throughout the genome to represent genomic or evolutionary processes occurring across the genome (Huang et al. 2013; Kraus et al. 2011, 2012). Genomic methods can include whole-genome "shotgun" sequencing, transcriptome characterization (usually via RNA-seq methods; Wang et al. 2009), and reduced representation methods such as restriction site-associated DNA (RADs, Baird et al. 2008), ultra-conserved elements (UCEs, McCormack et al. 2012), exomes, and other sorts of capture approaches. They also often include metagenomic (Riesenfeld et al. 2004) and metabarcoding (amplicon) methods (Taberlet et al. 2012) such as those used in some eDNA (environmental DNA or DNA sampled from an environmental substrate rather than a single organism) and microbiome assessments.

2 Applications of Genomics to Avian Conservation

2.1 Forensics

One of the seemingly simplest uses of genetic and genomic data is in avian forensic analysis. Forensics involves using tools to make identifications that are relevant to the solution of a public issue (such as identification of a bird involved in an airplane bird strike) or a crime (such as poaching or illegal trade). The classical approaches involve species identifications using comparisons of feather morphology or anatomy, but more recently simple DNA barcoding has become standard (Dove et al. 2008, 2009; Johnson 2011). In addition, microsatellite or simple tandem repeat (STR) markers, which usually require higher-quality DNA samples, have been the standard for identifying individuals within a species (e.g., Dawnay et al. 2009; Bielikova et al. 2010; Jan and Fumagalli 2016; Coetzer et al. 2016) and population of origin (Weissensteiner and Suh 2019). Usually these methods are used in identification cases where individual birds have been the focus of poaching or theft. More sophisticated DNA analyses have now increased resolution of population- and individual-level identifications (Iyenegar 2014; Arenas et al. 2017), and next-generation sequencing and genomic methods hold great promise for making more accurate and inexpensive identifications.

In addition, the use of genomic methods in forensics includes identification of species and of individuals from remnant and often degraded samples, which are not usually considered "genome quality" and thus are amenable to only some next-generation sequencing methods (Yang et al. 2014). In the past, simple PCR of mtDNA and microsatellites have been the primary approaches in avian forensics (e.g., Dove et al. 2008; Coetzer et al. 2017), but next-generation methods may have a greater ability to contribute to forensic analyses. This is because very small fragments of DNA are all that are needed via shotgun or hybridization capture

methods to characterize a SNP and many dozens to thousands of SNPs can be assayed in a single analysis, assuming adequate coverage to call each SNP.

Recently, next-generation sequencing methods have been used to identify larger numbers of avian microsatellites than can be obtained from standard methods, for example, for use in parentage analyses of Bornean birds (Kaiser et al. 2015) and forensics of potentially criminally obtained endangered New World parrots (Jan and Fumagalli 2016). In this process, many thousands or even millions of random sequences are obtained from shotgun sequencing of one or more individuals on a high-throughput sequencer. Sequence platforms must be capable of producing sequences of sufficient length to include sequence repeats capable of generating variation (>10 dinucleotide to pentanucleotide repeats), plus adequate priming sites (usually >150 bp in total length). From this approach, many hundreds or thousands of loci can be identified that can then be developed and optimized for further use.

Another interesting application has been to use PCR with mammalian markers on avian specimens to assess the identity of predators of birds such as sage grouse from mammalian saliva or other remnants left behind (Hopken et al. 2016). And microbiomes may also be useful for identifying population of origin or perhaps may even have signatures useful to identify individuals (Arenas et al. 2017).

Perhaps the biggest problem with forensics as applied to birds is more in terms of policy, in that for criminal investigations specific protocols and constrained chains of custody are required, and development, troubleshooting, and application of novel methods tend to evolve very slowly in these communities because of legal constraints.

2.2 Captive Population Management

Genomic techniques are ideally suited to advance the goals of captive population management. Captive population managers seek to preserve the range of genetic diversity of a species in order to preserve adaptive variations and adaptive potential. Managers also try to avoid adaptation to captivity or other forms of selection which might influence the allele frequencies of the population. Genomic data can be applied to increase the accuracy and precision of current methods for maintaining overall genetic diversity and has the potential to enable new methods/practices to avoid adaptation to captivity and preserve adaptive variants.

2.2.1 Maintaining Genetic Diversity

One of the main goals of captive population management in zoos and other institutions is to enable conservation in situ, by providing animals for reintroduction or for release into declining wild populations (Ralls and Ballou 2013). Managers may also maintain viable captive populations for display/public education or research (Ralls and Ballou 2013). Typically, captive management includes managing both the demographic and genetic profile of the population as a whole to preserve diversity and ensure that the population will grow or retain its size. The goal of maintaining a stable or growing captive population is generally met through the use of pedigree information and planned pairings (Ballou and Lacy 1995). Each wild-

caught founding member of the captive population is assumed to be unrelated. The relationships (or kinship) between each animal and all the others in the population are determined, and a breeding plan is devised to minimize the average mean kinship within the population. This has been shown to be the optimal strategy to preserve the original genetic diversity of the founders (Ballou and Lacy 1995). Care is also taken to manage the demographic profile of the population to ensure that there will be enough breeders of both sexes in the future to maintain the population.

However, most captive populations do not meet the assumption of unrelated founders. For example, multiple individuals from the same clutch may inadvertently be collected, and multiple members of the same natural population are often selected as founders without knowing their relatedness (Bergner et al. 2014). Genetic data can be used to identify related individuals in some cases, but this is more difficult in small populations that have been subject to multiple generations of inbreeding. Historically, genetic markers could be limited in their ability to provide sufficient resolution to resolve relationships (e.g., allozymes), or large numbers of markers were required to be developed de novo for each species due to low genetic variability (microsatellites). Genome-wide data allows calculation of kinships between both larger numbers of and more related individuals and can be used to resolve or revise pedigrees (Bergner et al. 2014). Genome-wide sequencing in California condors, for example, has resulted in revised breeding strategies by revealing unknown relatedness structures between founders (Romanov et al. 2009; Ryder et al. 2016). Similarly, the ability to easily generate large number of microsatellite markers using next-generation sequencing methods (as described above) will also allow greater resolution of pedigrees and relatedness within captive populations.

For captive populations where collection of new founders is still possible, the ability to compare genomic diversity in captive and wild populations is invaluable (e.g., Rascha et al. 2016). These data would help conservation managers preserve as much wild genetic diversity as possible (Fig. 1, top right) by allowing them to target underrepresented genetic profiles for collection or translocation (Mounce et al. 2015). Even if no captive genomic data exist, studies of wild populations that identify genomic diversity or population structure can greatly benefit collection efforts (Mounce et al. 2015). Additionally, captive managers benefit from studies of wild population genomics as they learn about potential subspecies or other groups that should possibly be managed separately.

Birds that live and breed in groups, like flamingoes, also present a problem for traditional methods, as individual parents cannot be tracked to create accurate pedigrees. Parentage assignments of individuals can be done with traditional genetics techniques, but determining the relatedness between individuals in a given group or between groups has been more challenging (Smith 2010). Large numbers of genome-wide markers could be used to improve group management by allowing comparison of individuals or subgroups without needing to reconstruct a complicated pedigree using traditional genetic methods.

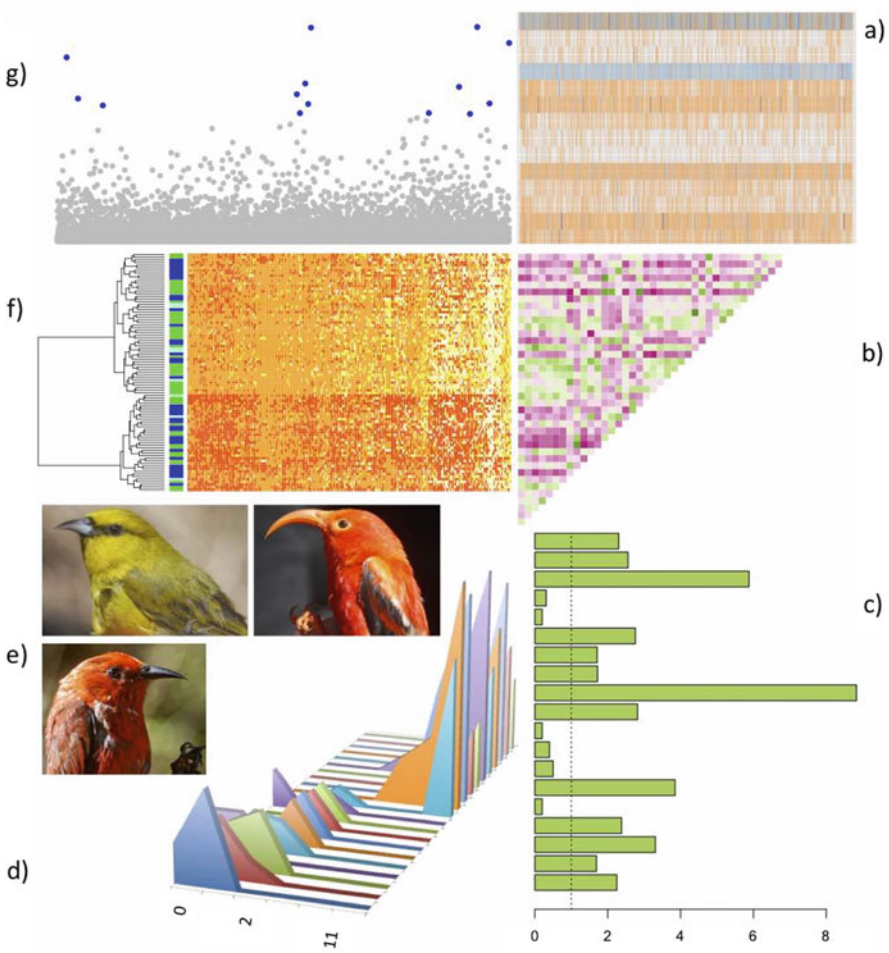

Fig. 1 Conceptual figure depicting a few ways in which genomics contributes to avian conservation. Clockwise from top right: (**a**) Individuals (rows) from a given species can be evaluated for similarity to a target sample at loci across the genome (more blue = higher similarity, more orange = low similarity, gray = no data) in forensic analysis [Section 1]. (**b**) Kinship matrix for individuals within a species, where green shows low relatedness (i.e., potential breeding pairs) and purple represents high relatedness (i.e., relatives or other individuals to avoid breeding in captive propagation) [Section 2]. (**c**) Differential expression of immune function genes can be analyzed in resistant and susceptible individuals to determine which genes control infection (greater than 1 = over-expression, less than 1 = under-expression) [Section 3]. (**d**) The distribution of bacterial OTUs in decaying chicken over time; each polygon represents an OTU, where early time points (0–2 days) indicate shortly after death and later time points are indicative of carrion [Section 4]. (**e**) Three species of variably threatened Hawai'ian honeycreepers, the Hawai'i 'amakihi (*Chlorodrepanis virens*, green), 'i'iwi (*Drepanis coccinea*, curved bill), and 'apapane (*Himatione sanguinea*, red with black bill). (**f**) Clustering of individuals (rows) across genomic loci (columns) can inform scientists of the loci involved in species divergence and reveal cryptic species [-Section 5]. (**g**) Outlier analyses can reveal loci showing significantly higher divergence (blue circles) than the rest of the genome, indicating potential adaptation to local climatic regimes or other conditions [Section 6]

2.2.2 Adaptive Variation

As more is understood about adaptive variation, genomic data could potentially be used to help preserve adaptive diversity beyond the mean-kinship method. Selection based on traits in conservation breeding has potential downfalls, such as (1) we do not know which traits will be adaptive when the captive-bred animals are released, (2) interactions between multiple loci are not well-understood for traits of interest, and (3) selection for some traits could reduce other genomic variation. Genomic studies will allow deeper investigation of some of these concerns.

With the advent of the use of genomics in evaluating adaptive variation, captive population managers will have to develop methods for integrating that information into management decisions. A first step in this direction, which is already being undertaken, is understanding adaptation to captivity. This occurs when selection pressures are unintentionally applied in the captive environment and result in genetic shifts in the population which may then be maladaptive upon release of individuals to the wild. Currently, adaptation to captivity has been identified in several species (notably fish in aquaculture; Mäkinen et al. 2015), but this work remains to be done in birds. Learning which genomic features are susceptible to selection in the captive environment will help managers reduce those effects while maintaining overall genetic diversity.

2.2.3 Future Directions

Using genomic tools for captive management is still an emerging practice, and many challenges have yet to be addressed. A few avian species, such as California condors and whooping cranes, are already being managed using data from genomic studies (Ryder et al. 2016). These methods are becoming more accessible to the conservation community, and several groups are developing consistent workflows for marker development and genotyping. However, the following questions still need to be addressed: (1) how many markers are needed to resolve pedigrees and answer kinship questions? (2) If some of the pedigree is known, how can an optimal set of individuals be chosen for genotyping? (3) In which cases is it more efficient to use genomic data to reconstruct a pedigree vs. calculate genomic kinships? As genomic tools are applied to the captive management of more species, answers to these questions should emerge.

2.3 Avian Infectious Disease

Genomic tools have enhanced our ability to understand avian disease dynamics, coevolution between avian hosts and pathogens, and the evolution of pathogen virulence and host resistance and tolerance. These technologies have enabled scientists to push the boundaries of knowledge in infectious disease research. Although many of the major questions in avian disease ecology and evolution remain the same, our ability to answer them in more nuanced ways has improved. Biologists seek to explain patterns of host susceptibility, pathogen virulence, parasite specificity, coevolution, and parasite transmission dynamics across complex

landscapes and host communities. Prior to the genomics era, studies of avian disease were focused on single "candidate" host genes thought to play a role in resisting infection (e.g., Jarvi et al. 2004), single parasite genes posited to confer virulence, reliance on parasite morphology or single genes to describe taxonomy (Križanauskienė et al. 2006), and phenotypic disease pathology in the host.

These approaches have led to significant advances in our understanding of host and pathogen ecology and evolution; however, each poses barriers to a complete understanding of host-pathogen dynamics. Prior to the advent of genomic technologies, avian disease biology experienced several primary limitations: (1) variation in virulence among parasite strains was cryptic; (2) disease pathology results from the interaction between parasite virulence and host susceptibility and thus cannot be used to infer exclusively host or pathogen processes; (3) alleles conferring both pathogen virulence and host resistance/tolerance were usually unknown; (4) a lack of variation in commonly sequenced genes hindered comparative study; and (5) individual species contributions to disease transmission were difficult to quantify. With genomic tools, avian biologists are on the cusp of making large breakthroughs in these and other areas. Here, we outline a few outstanding questions in avian disease biology and highlight some key studies that have capitalized on genomic approaches.

2.3.1 Host Susceptibility and Pathogen Virulence

Host Susceptibility

Single-gene studies, primarily focused on major histocompatibility complex (MHC, Weissensteiner and Suh 2019) genes and other genes known a priori to be part of normal immune system processes, have resulted in slow discovery of novel disease-related genes. In turn, this has biased our understanding of disease response such that non-MHC genes have largely been ignored. Negative results have likely gone unpublished, leading to an artificial inflation of the generality of importance of very few genes. Until recently, scientists had little ability to discover novel genes invoked in the infection process, as this work was economically inefficient. The single-gene approach is still of great use in systems where particular genes are known to be involved in the infection process (Chapman et al. 2016). However, there is growing evidence that a large number of host genes may be under selection from pathogens (Cheng et al. 2015; Wang et al. 2016). Of these, only some are conserved across populations (Connell et al. 2012; Videvall et al. 2015; Cassin-Sackett et al. 2018); furthermore, genetic variation at these loci can be shaped by both selection (Raven et al. 2017) and drift (Gonzalez-Quevedo et al. 2015). Thus, a priori hypotheses of relevant genes will resolve only a limited picture of the evolutionary response to infection. Moreover, with the global increase in wildlife diseases, many of which are introduced, pathogen invasion of novel hosts and host response to novel pathogens may invoke genes that co-opt cellular machinery for other purposes. A classical example of such a solution to disease is the sickling of red blood cells that prevents replication of *Plasmodium falciparum* in humans. The gene causing this abnormal hemoglobin likely would have been overlooked with a target

gene approach. Recent studies have assessed the potential for using reciprocal translocations as a means to reintroduce genetic diversity at disease resistance genes into wild populations (Grueber et al. 2017); this type of effort could be aided by a more complete understanding of which disease-related genes play a role in each system (Fig. 1, bottom right).

Pathogen Virulence

Traditionally, pathogen virulence in bacteria has been assumed to arise as a result of virulence genes contained on plasmids. This is often the case, but genomic tools have allowed us to understand that virulence is often conferred by multiple genes acting in tandem, not only on plasmids but also localized on "pathogenicity islands" (Pilo et al. 2005) or elsewhere in the genome. Often, virulence genes control some other cellular function such as metabolism and lead to virulence as a by-product (Pilo et al. 2005; Szczepanek et al. 2010; Tulman et al. 2012). Comparative genomics has been used in studies of commensal and pathogenic bacterial strains to identify unique genes as putative causes of virulence. For example, genomic tools have enabled scientists to subtract the genomes of nonpathogenic strains of *Escherichia coli* from the genomes of avian pathogenic strains, leading to the confirmation of existing virulence plasmids as well as identifying novel virulence factors (Schouler et al. 2004). The subtractive genomic approach can also be used to subtract pathogen genomes from host genomes in a single host tissue.

The identification of virulence factors in non-bacterial pathogens is even more complex. Mechanisms of host red blood cell invasion, for instance, are not conserved across *Plasmodium* species: diversification of particular host invasion mechanisms may have occurred only in mammal-infecting *Plasmodium* lineages and thus lend limited insight into invasion of avian blood cells by *P. gallinaceum* (Lauron et al. 2015) or *P. relictum*. Candidate gene studies of *Plasmodium* infection have typically focused on a small number of putative virulence genes such as the merozoite surface protein 1 (msp1), as one of the first steps in host cell invasion (Hellgren et al. 2013). However, transcriptome sequencing of *P. gallinaceum* illuminated additional parasite genes necessary for avian infection and revealed evidence of diversifying selection as a result of the host immune response (Lauron et al. 2014). As in host resistance/tolerance, parasite virulence may likewise be conferred by multiple genes, especially in systems where a coevolutionary arms race is occurring or where multiple host species exist that differ in susceptibility. For instance, attenuation of a Mexican West Nile virus lineage was conferred by both pre-membrane and envelope proteins, while either gene alone did not confer attenuation (Langevin et al. 2011).

In addition to characterization of genes involved in virulence, genomics technologies facilitate pathogen discovery when the cause of wildlife disease outbreaks is unknown. Some diseases have unknown etiologic agents: a panviral microarray led to discovery of an avian bornavirus as the previously uncharacterized causative agent of proventricular dilatation disease and mortality events in both the United States and Israel (Kistler et al. 2008). Genomics tools allow similar promise for pathogen discovery (Borner and Burmester 2017).

2.3.2 Host Specificity and Coevolution

Accurate description of host specificity relies on correct delineation of host and parasite species. Historically, parasite taxonomy was studied using parasite morphology, parasite life cycle, host species, or host pathology. More recently, mtDNA haplotypes have been used to describe taxonomic relationships. Both of these approaches have yielded great insights into pathogen specificity and coevolutionary dynamics, yet they have often led to spurious conclusions resulting from incomplete information. For instance, malaria parasites (including *Plasmodium*, *Leucocytozoon*, and *Haemoproteus* species) were traditionally classified based on their morphological characteristics and host species, leading to inferences of high host specificity. However, Beadell et al. (2006) and Martinsen et al. (2006) demonstrated that morphospecies are not always linked to particular genotypes, suggesting potentially widespread cryptic diversity or morphological convergence among haemosporidian species. The use of mitochondrial genes modified our understanding of parasite taxonomy and demonstrated wide variation in host specificity (Beadell et al. 2006, 2009) in this group, as dozens of cryptic lineages were discovered (e.g., Palinauskas et al. 2015; Nilsson et al. 2016). As more host species have been sampled and similar lineages detected across taxa, we have come to understand that many blood parasites display high host generality (Iezhova et al., 2011; Nilsson et al. 2016). Nonetheless, haemosporidians display greater levels of host specificity than other pathogens such as West Nile virus, in which lineages correspond to geography rather than host species (May et al. 2011).

In addition to their range of host specificity, pathogens also exhibit varying degrees of vector specificity that was largely undetectable prior to the genetics era. Martinsen et al. (2008) were among the first to demonstrate parasite-vector specificity: they found that major clades of malaria parasites were associated with shifts into new vectors, which then permitted weaker associations with major vertebrate host clades. While some parasites are capable of infecting multiple vector families, others may be specific to particular genotypes. For instance, the existing population of introduced *Culex quinquefasciatus* in Hawaii (a mix of North American and mostly Austral-Pacific lineages; Fonseca et al. 2006) successfully transmits the local strain of *Plasmodium relictum*, GRW4, to local avian species, whereas the North American lineage of *Cx. quinquefasciatus* was experimentally shown to be refractory to GRW4 (Fonseca, Fleischer, unpublished). The identification of these two strains was facilitated by genetic studies, and genome-wide scans of the distinct vector lineages would lend insight into the functional differences between genotypes. Wilkinson et al. (2014) used a combined 16S and targeted gene approach to reveal that pathogenic bacteria display vector specificity in seabird ticks [although this may be the case only for certain tick symbionts, as Duron et al. (2016) found minimal evidence of host specificity and a high degree of horizontal transfer]; this combined approach adds power to avian disease systems for which genomic resources have yet to be developed. Indeed, next-generation sequencing technologies can be used to develop Sanger sequencing assays (Hellgren et al. 2013).

In complement to pathogen vector specificity, host preference of vectors has been illuminated by bloodmeal analysis (Kilpatrick et al. 2006; Savage et al. 2007),

reinforcing the idea that hosts are not chosen from a community randomly (Paull et al. 2012). Genomics offers the potential for addressing specificity at various levels using high-throughput analysis of bloodmeals in both hosts and vectors.

Cryptic variation in avian host species (Stervander et al. 2016) could lead to erroneous conclusions such as overestimating host specificity (e.g., one parasite lineage in two cryptic host species) or the diversity of parasites infecting host species (e.g., two parasite lineage in two cryptic host species). In addition, host-switching events would not be detected when parasite lineages infected a new, cryptic host taxon. In the case of multiple parasite lineages infecting cryptic host species, an incomplete understanding of host taxonomy could beget the false inference of stable parasite species coexistence when in fact two parasite lineages competitively exclude each other in different host species (specialization on cryptic host taxa). Using genomic data to inform avian and parasite taxonomy solves many of these problems; in fact, whole-genome information resolves many taxonomic uncertainties (Jarvis 2014; Hug et al. 2016; Ottenburghs 2019) and should enable a better understanding of host specificity, host and vector preference, and parasite diversity.

With the ever-increasing amount of genomic data available on parasite lineages, more variation among strains will be detected, posing the problem of how to classify parasite species. Historically, species were delineated based on the existence of one or a few nucleotide differences in mitochondrial genes, but it is now trivial to discover dozens of novel variants within a single parasite species, lending the problem of delineation based on molecular information. Thus, biologists studying parasites will have to find common ground on which to define and delineate taxa (Outlaw and Ricklefs 2014) to facilitate comparative studies of host specificity and coevolution. It is now possible to identify specific parasite genes that are differentially expressed in different host species (Videvall et al. 2017), allowing for a functional understanding of host specificity that may contribute to our understanding of parasite taxonomy.

2.3.3 Phylogeography, Population Genetics, and Within-Host Evolution

After some limitations of mtDNA for resolving phylogeographic patterns became evident (e.g., the introgression of entire mitochondrial genomes (Bellemain et al. 2008) can lead to discordance between mtDNA and species trees), scientists started leveraging virulence genes or host-specific parasite attack genes to describe host-parasite relationships, host-switching events, parasite phylogeny, and parasite phylogeography (Taubenberger et al. 2005; Hellgren et al. 2013, 2014; Harkins and Stone 2015). These single-gene studies have begun to clarify some phylogenetic relationships but have obscured others (Harkins and Stone 2015).

In contrast to drawbacks with morphological approaches, single-gene studies confront the problem of poor detection of mixed infections (Valkiūnas et al. 2006). The cost efficiency of multi-gene Sanger sequencing is declining, particularly in systems where suitable genes for taxonomy, phylogeography, or functional ecology and evolution have not yet been identified. However, single-gene studies can overlook functional diversity at loci not typically used for taxonomy. These

drawbacks have the potential to be resolved with parasite genomics (Nilsson et al. 2016). Genomic tools can reveal cryptic diversity that can be leveraged for fine-scale studies of parasite phylogeography and within-host evolution.

Introduced pathogens can be traced using genomics to identify the origin of introduction. For instance, whole-genome sequencing of West Nile virus strains in the western Mediterranean revealed that the present diversity stems from a single introduction followed by local maintenance in the region (Sotelo et al. 2011). This study also revealed a meaningful insight about pathogen evolution: a single mutation linked to virulence (Brault et al. 2007) occurred multiple times in distinct geographic regions during the evolutionary history of West Nile virus (Sotelo et al. 2011). The ability to trace pathogen introduction history both to a particular host species and geographical location enables quarantine or vector control measures to be put in place to conserve vulnerable avian species. For example, Usutu virus strains from various locations in Africa were subjected to whole-genome sequencing, and the country of origin of European introduced Usutu virus was inferred due to high similarity between a native strain from Senegal and introduced strains in Europe (Nikolay et al. 2013).

2.3.4 Insights from Genomics

In summary, many broad evolutionary patterns have been revealed by the availability of genomic data, including several major insights in avian disease. Table 1 presents a selection of several major themes in host and parasite biology for which our understanding has changed as a result of genomic advances.

2.3.5 Limitations

One area in avian disease research that still lags behind, as in other fields in avian biology, is verification of gene function in non-model organisms. Experimental infection studies are often impractical or unethical in non-model species, particularly those of conservation concern. As a result, most of the data on genes underlying pathogen and host phenotypes are correlational. Comparative genomics with well-annotated genomes, in conjunction with experimental challenges of widespread related species, will assuage this shortcoming. In addition, improvements in gene prediction, genome annotation, and characterization of noncoding DNA will facilitate prediction of how genetic changes will interface with different genomic backgrounds. These improvements can be made not only via experimental gene knockout studies and germ cell editing to assess gene function (Park et al. 2014) but with a better understanding of genome evolution. Synteny is higher in avian genomes than in other vertebrate taxa (Zhang et al. 2014), enabling these types of advances more readily in birds.

A limitation that is biological and not technical emerges from recent findings in genomics studies that resistance is conferred by multiple, often unpredictable loci that vary across populations (Connell et al. 2012; Videvall et al. 2015; Cassin-Sackett et al. 2018). This suggests a continual need for species-specific studies of host and parasite evolution. However, this shortcoming is ameliorated by genomics,

Table 1 Summary of applications of genomics to major themes in the study of avian disease

Topic	Previous understanding	Current understanding	Relevant contributions
Parasite taxonomy	Morphology- or cytochrome b-based	A large degree of cryptic diversity exists, some of which may have functional consequences	Beadell et al. (2006), Martinsen et al. (2006), Palinauskas et al. (2015) and Nilsson et al. (2016)
Host specificity	Most parasites are host-specific	There is wide variation in the degree of host specificity; some parasites exhibit vector rather than host specificity	Fonseca et al. (2006), Martinsen et al. (2008), Iezhova et al. (2011), May et al. (2011), Wilkinson et al. (2014), Nilsson et al. (2016), Duron et al. (2016) and Videvall et al. (2017)
Mechanisms of host resistance/ tolerance	Primarily MHC-based	Many additional immune genes are involved (and some genes not in the immune pathway)	Cheng et al. (2015), Wang et al. (2016) and Cassin-Sackett et al. (2018)
Evolution of MHC	Diversifying selection	Diversifying selection, balancing selection, neutral evolution	Grueber et al. (2013), Raven et al. (2017) and Gonzalez-Quevedo et al. (2015)
Pathogen virulence	Decreases over evolutionary time	Increases, decreases, or remains stable over evolutionary time	Szczepanek et al. (2010), Tulman et al. (2012), Murray et al. (2017a, b) and Fan et al. (2017)

which enables whole-genome and transcriptome data to be gathered from increasingly smaller biological samples. As whole-genome data are acquired with increasing efficiency and decreasing cost, this limitation is expected to decline.

Although genomic technology is changing rapidly, it remains difficult to isolate parasite DNA from host tissue due to the lower cellular representation of parasite DNA. Nonetheless, some promising approaches have proven successful in recent parasite studies: *Plasmodium relictum* was isolated from blood smears using lasers and subsequently subjected to whole-genome sequencing (Lutz et al. 2016). This technology offers the potential to acquire genomic information from archived blood smears and other valuable sources.

2.3.6 Future Directions

Future genomics work on parasites and vectors of avian disease are needed to characterize the ecological and evolutionary linkages in vector-borne diseases. These studies will illuminate the mechanisms underlying vector adaptation to parasite lineages as well as parasite influence on vector behavior (e.g., how *Plasmodium relictum* invokes a feeding preference of infected birds on uninfected mosquitoes; Cornet et al. 2013) and therefore disease dynamics.

The unprecedented ability to obtain whole-genome sequence data rapidly and with increasing cost efficiency paves the way for effective conservation actions to take place in near real time. In the coming years, whole-genome sequencing or

reduced representation screening at disease resistance loci can be carried out prior to management actions such as captive breeding or relocation, effectively increasing the disease resistance or tolerance of vulnerable host populations.

Exciting opportunities for conservation have emerged with the discovery of CRISPR (clustered regularly interspaced short palindromic repeats) defense systems in bacteria and archaea and the associated advent of genome-editing technologies (Jinek et al. 2012). With CRISPR-associated (Cas) systems, genetic information can be permanently edited, which can be leveraged in disease systems by modifying pathogen virulence, vector competence, or host tolerance. Indeed, disease systems have been some of the first real-world applications of this tool (Kistler et al. 2015; Hammond et al. 2016). Moreover, the technology allows for multiple loci to be edited simultaneously (Jao et al. 2013), which would simplify genome editing in the case of multilocus tolerance or virulence. Recent developments facilitate CRISPR-Cas gene editing in non-model avian species (Cooper et al. 2017, 2018). As pathogens are increasingly moved around the globe and naïve host populations are exposed to novel pathogens, CRISPR could allow for emergency management of critically endangered populations when there is not sufficient time for captive breeding or other conservation strategies.

2.4 Avian Conservation Genomics and Heterogeneous Samples: Metagenomics and Metabarcoding

In the traditional sense, genomics has involved the discrete analysis of one or a few samples, usually collected from individuals with known taxonomy or other characteristics of particular interest. Recently, however, there has been increasing interest in the *simultaneous* analysis of mixed samples that represent the "community" (Xu 2006). These heterogeneous community samples can include, for example, feces, microbiota, or mixtures of the remains of several individuals/species. High-throughput sequencing of these samples provides the power and sequencing depth required to obtain genomic data from those individuals, without the necessity of time-consuming approaches such as molecular cloning to first separate them by species or genotype. These data can be used for a wide variety of purposes in avian conservation, which can include (but are not limited to) biodiversity detection, investigating bird diets (which may limit aspects of the annual cycle such as breeding or survival during migration), detecting endangered birds as dietary items of predators, and understanding how avian microbiota contribute toward adaptation and health (see below).

In the strictest definition, metagenomics involves shotgun (i.e., theoretically random) sequencing of DNA—potentially to the scale of whole genomes—directly from a heterogeneous sample (Taberlet et al. 2012). This approach can be used to characterize the (often microbial) community in terms of taxa present and investigate the content and function of the genes sequenced. Similar approaches analyzing RNA instead of DNA can investigate further questions such as how gene expression changes in interacting communities under different conditions or states (Fierer

et al. 2012; Poretsky et al. 2009). In a related technique, often termed metabarcoding, DNA from an environmental sample is first PCR amplified for a particular region (e.g., 16S ribosomal RNA or COI, the cytochrome oxidase I gene), and then these amplicons are sequenced on a high-throughput platform, usually with the aim of deeply characterizing the community composition (Taberlet et al. 2012).

Below we describe some recent and potential applications of these two general approaches with birds, some strengths and limitations of each, and discuss possible future directions for the field.

2.4.1 Metabarcoding

Perhaps one of the greatest potential areas of impact for metabarcoding in avian conservation is through biodiversity monitoring and discovery. Theoretically, any sample mixture could be screened to identify the presence of avian species. This type of work is routinely taking place for mammals and aquatic animals (e.g., Bohmann et al. 2014), but so far has been less often applied to birds. One current application is "bulk-bone" metabarcoding of partial, undiagnosable bones from archeological and paleontological sites (Murray et al. 2013; Honka et al. 2018). This work allows identification of the presence of previously unreported bird species through time and may be particularly beneficial in documenting changes in diversity of small-bodied birds whose bones are easily fragmented and therefore often unrecognized. Thus, metabarcoding may help in determining a baseline for conservation actions, establish the former presence of currently extinct or extirpated species, and identify the former range of species for possible reintroduction. Another potential application could arise from metabarcoding remains after bird collisions. In cases where collisions with aircraft result in samples lacking sufficient feather material to allow morphological analyses, or in cases where mixed species flocks were involved in the strike, metabarcoding may provide important information on the avian taxa involved in collisions, thus allowing implementation of more effective management strategies (Dove et al. 2008). Metabarcoding could also be implemented to study collisions with turbines and wind energy technologies, where multiple birds may be struck through time and where the individuals struck did not die within the immediate vicinity and therefore cannot be identified through visual monitoring. The approach could also be applicable to medicines and foods derived from illegal harvests of birds, where DNA is present from multiple species or too degraded for traditional barcoding (Staats et al. 2016).

Another area for which metabarcoding demonstrates great potential to contribute to avian ecology and conservation is through analysis of diets. A better understanding of diets could provide key insights, for example, about interspecies competitive interactions and predator-prey dynamics, which would in turn inform our understanding of factors limiting reproductive success or survival. Ornithologists have long struggled to characterize diets by making foraging observations and analyzing gut contents or regurgitation. Metabarcoding can potentially yield vast quantities of data on these questions in a relatively short amount of time. Such analyses, however, have only recently been successfully applied to birds (Vo and Jedlicka 2014), likely due to the high acidity of bird feces, which can make obtaining DNA a challenge.

Jedlicka et al. (2017) used a metabarcoding approach to characterize the diets of western bluebirds (*Sialia mexicana*) in vineyards and found that they consumed primarily herbivorous insect taxa, rather than the predator or parasitoid taxa that may also be contributing to pest control. Also, McClenaghan et al. (2019) investigated the diet of a declining avian insectivore, the barn swallow (*Hirundo rustica*). They found that this species is a broad-scale generalist and is able to feed nestlings a varied diet and take advantage of opportunistic food resources. Thus, this species should theoretically be resilient to changes in food availability. Other studies have investigated the use of aquatic insect prey sensitive to pollution by Louisiana waterthrush (*Parkesia motacilla*) (Trevelline et al. 2016), as well as prey usage by semipalmated sandpipers (*Calidris pusilla*) during migratory stopover (Gerwing et al. 2016). Alternatively, diet metabarcoding could help identify when birds are the prey items, for example, in the diets of felines, mustelids, rodents, and other invasive predators (Zarzoso-Lacoste et al. 2016).

Metabarcoding has also been used to understand the bacterial communities of birds and impacts on their physiology and health (Waite and Taylor 2015). For example, hoatzins (*Opisthocomus hoazin*) consume leaves, and fermentation is thought to take place in their enlarged crops, reminiscent of cattle and horses rather than other birds. A comparison of hoatzin and cow foregut and hindgut bacterial taxa suggested that indeed, the foregut taxa of hoatzin and cow were more similar to each other than to their own hindgut community (Godoz-Vitorino et al. 2012). This suggests that the bacterial community of the hoatzin crop may represent a convergent, evolutionary adaptation for dealing with a herbivorous diet. In contrast to this, the critically endangered kakapo (*Strigops habroptilus*), which also consumes plant material, but not entire leaves, has a very different foregut bacterial community and therefore is unlikely to perform fermentation and has adapted to its diet in different ways (Waite et al. 2013, 2014). Similarly, black (*Coragyps atratus*) and turkey vultures (*Cathartes aura*), which scavenge decaying carcasses rife with toxic bacterial compounds, host a unique gut microbiome, which likely originates from their diet (Roggenbuck et al. 2014). Additional analyses of the avian microbiome could also play a role in understanding, for example, the bacterial communities which may be critical for coloration and degradation of feathers, mate attraction, nestling development, reproductive investment, as well as migratory ability (Jacob et al. 2014, 2015). All of these aspects influence survival and reproduction and therefore would be particularly important to understand for endangered avian taxa.

2.4.2 Metagenomics

While implementation of metabarcoding approaches is becoming more common, few metagenomics studies have involved birds, particularly wild birds. One such recent paper examined the cecal metagenome of the greater sage grouse (*Centrocercus urophasianus*, Kohl et al. 2016). The greater sage grouse regularly (and at some points in the annual cycle exclusively) consumes sagebrush (*Artemisia* sp.), a plant that contains toxic secondary compounds. Kohl et al. (2016) sequenced total DNA from the cecal microbiota and assembled gene sequences (rather than full genomes). They found that the sage grouse cecal metagenome was enriched for

genes related to breakdown of these compounds, as compared to the microbiota of chicken and mammalian herbivores. This suggests that greater sage grouse and their microbiota may be specially adapted for a diet of sagebrush and other plants with similar toxic secondary compounds.

Metagenomics could be particularly useful for understanding pathogens and disease in wild birds. Disease is an important factor limiting population growth rates and carrying capacity; therefore, gaining a better understanding of the infections that birds carry and how they spread could have important management and conservation implications. Beyond this, wild birds may serve as the reservoir for diseases that impact humans (e.g., avian influenza and West Nile virus), so a better understanding of bird diseases would benefit people as well (Kapgate et al. 2015). In these cases, having a more complete, less-biased picture of the bacteria, viruses, and fungi present (and potentially accurate representations of their abundance) would be especially important. A metagenomics approach was recently taken to investigate the virus community in domestic turkeys (Day et al. 2010), and a similar approach could be taken for monitoring and surveillance of wild and endangered birds.

2.4.3 Strengths and Challenges

While the above examples provide insights into the current and potential future applications of metagenomics and metabarcoding studies, it is useful to have an understanding of the challenges these studies face.

In any metabarcoding study, one of the early steps involves PCR amplification with theoretically universal primers. Unfortunately no primer is truly universal, and this step may introduce taxonomic biases during amplification (Deagle et al. 2014), which can be difficult to predict ahead of time. These biases include not only amplification success of some species and failure for others but also biases in the strength of amplification with some species amplifying strongly and others amplifying weakly. This makes interpretation of metabarcoding sequence abundance challenging (Elbrecht and Leese 2015). Beyond this, sequences obtained from metabarcoding are often compared to available DNA barcode sequence databases, such as the Barcode of Life (Ratnasingham and Hebert 2007). Taxonomic biases in the database can lead to biased sequence identification or identification to higher taxonomic levels only (such as family or order; Kvist 2013). A related problem is that DNA barcoding loci may in some cases lack sufficient resolution (particularly for degraded samples which make use of shorter barcode loci), and thus two or more taxa may share the same barcode sequence and thus could not be identified uniquely to the species level. This is a common issue for bird diet items such as plants and may exist for arthropods and some higher vertebrates as well (Starr et al. 2009; Janzen et al. 2005). In general, data on presence of taxa may be more reliable than data on absence or abundance of taxa.

The benefits of the metabarcoding approach, however, offset the challenges. If characterizing biodiversity is the goal, then this approach targets sequencing to limited areas of the genome that are comparatively well covered in online databases (e.g., the COI gene, Ratnasingham and Hebert 2007). Also, by targeting the sequencing effort, many samples can be combined within a single sequencing run,

and smaller instruments with a cheaper total run cost may be sufficient (e.g., the Illumina MiSeq), making this work feasible for projects with limited budgets or very large sample sizes. Beyond this, because the protocols generally start with a PCR amplification step, it becomes possible to append overhanging sequences to the primers that match sequencing adapters and thus more quickly and efficiently conduct library preparation for sequencing, though these overhangs may also cause taxonomic biases during amplification (Berry et al. 2011).

One of the benefits of the metagenomics approach is that it removes the initial PCR step that generates much amplification bias. Because sequencing may yield multiple and/or longer regions, taxonomic resolution may be increased with this approach, and therefore result in more comprehensive characterization of the community of interest (Srivathsan et al. 2015). However, it should be noted that few extensive tests of metagenomics sequencing for community characterization have been conducted and these approaches may still suffer from technical problems, such as the presence of inhibitors or biased ligation of sequencing adapters. Another strength of the metagenomics approach, as mentioned above, is that when sequencing is no longer targeted to one or a few genes, a wider range of questions may be addressed, leading to deeper insights about microbial community interactions, gene expression, and gene function (Fierer et al. 2012; Poretsky et al. 2009).

Metagenomics studies also face several challenges. Because sequencing is not targeted, more sequencing is necessary, and therefore run costs are likely to be higher. In addition to this, datasets are likely to be less complete, as compared to a traditional genomic study, because rather than sequencing a single individual, hundreds or thousands of individuals/species may be sequenced at the same time. This also leads to bioinformatic and computational challenges of assembling reads from many individuals into useful datasets (Wooley et al. 2010).

2.4.4 Future Directions

There are several areas where advances in metagenomics and metabarcoding could make a significant impact on our knowledge and understanding of avian conservation. Gaining a better understanding of the factors that influence the accuracy of the relationship between abundance in the sample and the proportion of sequencing reads would yield many new insights. For example, the inclusion of accurate abundance information into analyses of food web dynamics would indicate not only that a prey taxon was consumed but its relative importance in the diet. Similarly, for biodiversity monitoring, abundance of a species' sequences could indicate the relative numbers of individuals living in different areas or the relative abundance of pathogenic microorganisms compared to commensal or beneficial taxa. For metabarcoding, many factors may influence abundance, including but not limited to storage and DNA extraction methods, primer bias, polymerase bias, and even bioinformatics approaches for quality control and taxonomic assignment (Vo and Jedlicka 2014; Deagle et al. 2013, 2014; Kopylova et al. 2016; Krehenwinkel et al. 2017; Nichols et al. 2018). Metagenomic studies may suffer from less-biased sequence abundance, but little is known. Potential confounding factors could include copy number issues, biased digestion, and biased databases for

taxonomic comparison and functional annotation. Despite these potential confounding factors, several studies are beginning to suggest that a relationship may exist between abundance and sequence proportions (e.g., Srivathsan et al. 2015; Evans et al. 2016, and others). Further, for metagenomic studies, improvements in sequencing (number, quality, and length of reads) and reduction in cost would be valuable, but perhaps the most beneficial would be continued improvements in our computational abilities, both with regard to the raw computing power and software to handle complex datasets.

2.5 Systematics and Species Limits

To conserve species, scientists and managers need to know what are distinct species—that is, how do data inform us of species status and limits based on accepted species definitions. In addition, an understanding of hierarchical levels of genetic structure below the species level is necessary to also optimally conserve genetic variation. Thus we also need to have clear-cut concepts and definitions of such entities as subspecies, evolutionarily significant units (ESU), and the Endangered Species Act (ESA) legally defined "distinct population segments" (DPS). While there are problems with each of these categories because of differences in the definitions that have been proposed (Frankham et al. 2012; Garnet and Christidis 2017; Haig et al. 2006; Funk et al. 2012) and difficulties obtaining relevant data (such as hybrid fitness and local adaptation), the roles of genetic, and now genomic, data have proven useful to elucidate these categories for policy and management decisions. Note, the chapter "Avian Species Concepts in the Light of Genomics" in this volume is devoted to species concepts and how genomic data can be incorporated into criteria to distinguish species, so our discussion here will largely be concerned with their applications to conservation.

2.5.1 Species Definitions and Limits

These can be tested with genomic data to determine species boundaries and relationships and amounts and directions of introgression at contact zones (Ottenburghs et al. 2017). The expansion of data to the genomic level has greatly increased our ability to determine these variables (Toews et al. 2016) to better test species limits. The ability to discover chromosomally local "islands of divergence" between taxa that are otherwise very closely related genetically can reveal the presence of barriers to unrestricted gene flow, the directionality and timing of introgression, and the roles of sexual selection and local adaptation in speciation. In addition, these data are also very useful for assessment of different subspecies, ESU and DPS definitions, and could also provide information on the role of adaptive divergence that might limit a taxon or populations to a particular range or habitat.

Although biologists have defined a plethora of different species concepts (Frankham et al. 2012; Garnett and Christidis 2017), one in particular, the Biological Species Concept (BSC, Mayr 1942), has largely dominated the systematics of birds. More recently, a set of determined ornithologists have advocated for the application

of the Phylogenetic Species Concept (PSC, Eldridge and Cracraft 1980), which would potentially double the number of described avian species to over 20,000 (Barrowclough et al. 2016). Conservation biologists argue that species definitions and designations need standardization and better control (Mace 2004; Frankham et al. 2012), even proposing an official body of scientists be established to carry out this role (e.g., Garnett and Christidis 2017).

For both of ornithology's primary species concepts and others that could be applied (Hill 2017; Kraus et al. 2012), genomic approaches would provide greater resolution of species limits. Assessment of fine-grained genome-wide sequence variation across avian contact zones would provide information on divergence (Fig. 1, middle left) but also on the level and pattern of introgression across the zone and into the parental taxa (e.g., Poelstra et al. 2014; Baldassare et al. 2014; Toews et al. 2016; Kearns et al. 2017). For example, only six small genomic regions were differentiated between the morphologically and mitochondrially (Gill 1997; Shapiro et al. 2004) distinct blue-winged (*Vermivora cyanoptera*) and golden-winged (*Vermivora chrysoptera*) warblers (Toews et al. 2016), and in proximity to four of those regions were genes potentially involved in the plumage differences between the two taxa. Based on the genomic sequence analyses, these two "species" have had a long and complicated history of interaction and, despite this interaction and their high genomic similarity, are likely best viewed as distinct species. There are a number of other cases emerging for birds in which evidence of apparently reproductively isolated taxa show minor, but detectable, overall genomic-level differentiation (e.g., differentiated subspecies in the barn swallow *Hirundo rustica*, Safran et al. 2016) or only a few islands of differentiation (as the above warbler case), and these will likely necessitate modification in the criteria used for species and subspecies designations.

2.5.2 ESU and DPS Definitions

Other definitions of importance in conservation management are what have been defined as evolutionarily significant units (ESU) and distinct population segments (DPS). The latter is actually incorporated as a defined unit of conservation for only vertebrates under the US Endangered Species Act (see Pennock and Dimmick 1997 and follow-ups for a discussion of DPS and how employing ESU alternatives could muddy the waters).

For a DPS to be defined and listed, the population under consideration must be discrete and significant and have an appropriate level of threat or endangerment. Although little guidance on these three criteria were provided in the act itself, additional legislation and discussion have indicated that the discrete population segment should "differ markedly from other populations of the species in its genetic characteristics" and genetics has often played a role in these designations.

There have been many definitions of ESU proposed since it was first discussed in a meeting review by Ryder (1986), including ones that involved mitochondrial DNA reciprocal monophyly and significant allele frequency differences (Moritz 1994) and identification of loci (and/or morphological or ecological traits) that reflect adaptation to local environments (Waples 1995; Crandall et al. 2000).

While genetic markers have been used in ESU and DPS approaches to a great extent (see examples in Fleischer 1998; Phillimore and Owens 2006), only recently have genomic methods been applied to confirm subspecies or ESU designations. A nice example of genomic differentiation and even potential local adaptation among subspecies and populations is that of barn swallows in Eurasia and North America (Safran et al. 2016; Scordato et al. 2017). These studies show clear genomic-level divergences based on PCA and structure analyses, with varying levels of hybridization or gene flow among the taxa based on hybrid analyses. Isolation by distance versus isolation by adaptation analyses suggest a strong component of the divergence is due to the latter, but only on a macrogeographic level. Another recent study showed only minor genomic-level differentiation using RAD-seq markers (and morphology) between Japanese and Hawaiian populations of the black-footed albatross (Dierickx et al. 2015), although the authors could not exclude the possibility that the slight differentiation detected might be meaningful for adaptation and thus conservation management. In most cases, genomic markers enable a much greater degree of resolution of taxon- or population-level differentiation than analyses based on mtDNA or nuclear sequences or microsatellites, but we must caution that the greater resolution of genomic differences attained by large numbers of markers may not necessarily imply biological significance.

2.5.3 Future Directions

Fitting structured and legal definitions like species, ESU, and DPS to biological data can be difficult, and genomic data may not really add to our ability to elucidate these concepts. Perhaps the key point of utility for avian conservation is really to understand how populations or species are related to each other, how they may be locally adapted, and what are the current and historical levels of gene flow among them. Population genomics is enhancing our ability to quantify these aspects and would allow us to determine how these entities would need to be managed. It is now possible to genotype hundreds of individuals at thousands of markers without needing a reference genome (although many more reference genomes are becoming available; Callicrate et al. 2014; Jarvis et al. 2014; Zhang 2015). Such a plethora of information enables us to explore how genome structure and evolution contributes to differentiation and speciation. We are now able to assess functional and adaptive differences between populations using genomic and transcriptomic data (e.g., Safran et al. 2016; Taylor and Mason 2015). Genome-wide markers such as single nucleotide polymorphisms (SNPs) can be used to genotype the same loci in ancient and modern samples, and although working with ancient DNA presents many challenges, genomics helps us overcome some of the problems with fragmented DNA. Thus, ultimately we will be able to document the historical contexts (past population size, structure, migration levels) using approaches such as Pairwise Sequentially Markovian Coalescent (PMSC; Li and Durbin 2011) and similar analyses for present-day situations (e.g., Nadachowska-Brzyska et al. 2015, Murray et al. 2017a, b). These can provide information useful for the management and recovery of endangered avian species.

2.6 Adaptation to Climate Change and Other Stressors

Adaptation occurs in response to various biotic and abiotic factors. As anthropogenic stressors increase, studying species response has become essential. Understanding how populations react to climate, habitat loss, and other compounding stressors can help us conserve species as a whole. In the past, studying adaptation required previous knowledge of genes involved in specific pathways. Early work focused on identifying candidate genes responsible for the change in phenotype through methods such as quantitative trait locus (QTL) analyses. With the advent of next-generation sequencing, our capacity to study adaptation in response to anthropogenic stressors has drastically improved. The field has expanded to include multiple pathways of adaptation, such as transcriptional regulation, selection in exonic regions, and posttranscriptional regulation. Next-generation sequencing allows us, without any a priori knowledge, to identify loci under selection that may be important in adaptation (Fig. 1, top left; Stillman and Armstrong 2015). Additionally, it enables us to study responses within a single generation (e.g., phenotypic plasticity) using methods such as RNA-seq (Wang et al. 2009; avian transcriptomics review: Jax et al. 2018).

2.6.1 Species Response to Climate Change

When faced with a stressor, species respond in one of four ways: (1) evade, (2) die (local extinction), (3) acclimate, or (4) adapt. While it is more straightforward to test for evasion or local extinction, the latter two have been historically more difficult to tease apart (Gienapp et al. 2008). Climate change-related range shifts entail an overall shift toward higher latitudes and higher elevations (Chen et al. 2011). Range shifts are considered to have two components—the cool-edge expansion (evasion) and the warm-edge contraction (local extinction; Wiens 2016). Range expansion and local extinction have been a primary focus in the response to climate change, whereas the importance of evolution occurring at the warm edge is often overlooked (Hoffmann and Sgrò 2011; Vedder et al. 2013).

Population response in the warm edges of a species' range can occur through adaptation and acclimation or genotypic specialization and phenotypic plasticity (Box 1). A well-documented example of population response to climate change is the occurrence of earlier mean breeding dates in many bird taxa (Charmantier and Gienapp 2013). Breeding timing is often based on prey abundance, which is in turn dependent on climatic factors. In great tits (*Parus major*), breeding season depends on a brief annual peak in caterpillar abundance, their offspring's food source, which is determined by spring temperatures (Visser et al. 2004). Vedder et al. (2013) found evidence of plastic response in breeding time in Wytham Woods great tits. Based on these data, the authors modelled extinction risk and found that the absence of this phenotypic plasticity would increase the likelihood of population extinction by 500-fold. They, however, note that little is known about the underlying causes and limits of this plasticity. The usage of next-generation sequencing methods could help identify the genomic basis of this response.

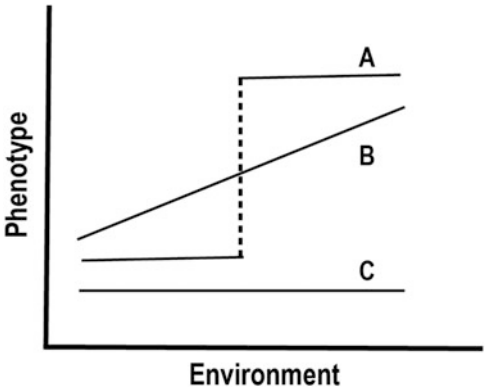

Fig. 2 Representation of reaction norms of three genotypes for a single trait. Genotypes A and B show discontinuous and continuous phenotypic plasticity, respectively. Genotype C shows no plasticity for this trait since its phenotype remains consistent across environments

Box 1: Genotypic Specialization Vs. Phenotypic Plasticity

Genotypic specialization is the process by which canalized, locally adapted phenotypes are present in an environment, and phenotypic specialization (or plasticity) is differentiation by regulated gene expression in response to the given environment (Wahl 2002). Genotypic specialization involves the genetic makeup of a population changing over generations due to the differential survival or reproductive success of certain genotypes in an environment or natural selection (DeBiasse and Kelly 2016). Conversely, phenotypic plasticity involves a change in phenotype in response to the environment within an organism and within a single generation (DeBiasse and Kelly 2016). Historically, the influence of the environment on the genome was assigned primarily to natural selection or gene-by-environment (GxE) interactions. A GxE interaction is when two genotypes respond to the same environment differently. Recently, however, the response of a single genotype in different environments has been attributed a more dynamic role (Fig. 2). For example, given the same genotype, an individual's phenotype can be a result of the environment it is exposed to from development through adulthood (Fusco and Minelli 2010); this is phenotypic plasticity.

Phenotypic plasticity comes with a cost, as species have to maintain the genetic and cellular mechanisms required to produce plastic responses, e.g., regulatory genes and/or enzymes (Scheiner 1993). Additionally, there could be costs associated with information gathering about environmental conditions and genetic costs involving linked genes, pleiotropy, and epistatic interactions (DeWitt et al. 1998). Therefore, plasticity is not likely to evolve in a population that does not experience a variety of environments or does not possess the genetic variance required for a plastic response in that specific trait. When environmental heterogeneity is fine-grained, individuals will experience various environmental conditions during their lifespan, requiring an increased

(continued)

acclimation capacity, and therefore phenotypic plasticity is more likely to evolve (Storz et al. 2010). However, when environmental heterogeneity is coarse-grained, individuals experience a limited range of environmental conditions, and therefore phenotypic plasticity is less likely to evolve (Storz et al. 2010).

When an individual's phenotype is altered in the direction of the local optima, plasticity is said to be adaptive. An adaptive plastic response can later become genetically encoded via natural selection through a process known as genetic assimilation (Ghalambor et al. 2015). This process allows an organism exposed to a constant stressor to eventually develop a biologically robust response in the direction of its previously plastic response (DeBiasse and Kelly 2016).

2.6.2 Approaches for Assessing Response to Climate Change

The genomic basis of response to climate change can be studied via multiple approaches (Table 2). It can also be studied on multiple levels: genomic, transcriptomic, and epigenomic. Reduced representation methods, such as RAD-seq (Baird et al. 2008), and sequence capture provide genomic information, whereas RNA-seq and reduced representation bisulfite sequencing (RRBS; Meissner et al. 2005) are used for transcriptomics and epigenomics, respectively. Multilevel approaches are important in parsing the relative effects of adaptation and acclimation or genotypic specialization and phenotypic plasticity.

In populations where candidate genes are responsible for most of the variation in potentially evolving traits, sequence capture can be employed to isolate and enrich those genes for analysis. This approach can be used to identify genotypic specialization if there is a priori knowledge of the species' genome. A classic example of this method is the usage of exome capture to identify the genetic basis of high-elevation adaptation in Tibetans (Yi et al. 2010). Long-term study of a single population allows researchers to differentiate between genetic and environmental contributions to trait change. Using whole-genome resequencing or reduced representation genomics, researchers can look for signatures of selection that correspond to climate-related phenotypic changes. This method, while effective, requires studying a population over multiple years, which is not always possible.

Common garden experiments and transplant experiments offer a method by which one can tease apart the roles of local adaptation and plasticity in adaptive success. In a single generation, RNA-seq and RRBS can be used to identify plastic responses to environmental change. In a multi-generation experiment, researchers can identify signatures of selection and heritability of epigenetic modifications. For example, to test for plasticity in high and low elevation populations of the rufous-collared sparrow (*Zonotrichia capensis*), Cheviron et al. (2008) transplanted individuals to a low elevation common garden. None of the transcripts that were

Table 2 Next-generation approaches for assessing the genetic basis of response to climate change in wild avian populations (Adapted from Hoffman and Sgro 2011)

Approach	Advantages	Limitations	Next-gen sequencing method
Assessing variation in candidate genes for relevant traits	Useful when candidate genes are responsible for most of the variation in relevant trait	Requires a priori knowledge of both the loci and the mechanism of adaptation	Sequence capture
		Useful only if few genes are responsible for most of the variation in the relevant trait	
Long-term study of a single population	Useful in long-term study populations	Time-consuming because it requires repeated sampling over multiple years/seasons	Sequence capture
	Can parse out genetic and environmental contributions		RAD-seq
			Genome resequencing
Common garden and transplant experiments across a climatic gradient (single generation)	Can differentiate between genotypic specialization and phenotypic plasticity	Not all species can be transplanted and/or live in captivity	RNA-seq
			RRBS
Common garden and transplant experiments across a climatic gradient (multi-generation)	Can identify signatures of selection	Not all species can be transplanted and/or live in captivity	Genome resequencing
			RAD-seq
			RNA-seq
			RRBS
Experimental evolution in artificial environments designed to simulate climate change	Can differentiate between genotypic specialization and phenotypic plasticity	Not all species can be transplanted and/or live in captivity	Genome resequencing
	Can identify signatures of selection	Difficult to extrapolate to wild populations experiencing compounding stressors	RAD-seq
			RNA-seq
			RRBS

differentially expressed at native elevations remained different in the common garden, demonstrating a considerable level of phenotypic plasticity in cold and hypoxia response (Cheviron et al. 2008). While common garden and transplant experiments yield valuable insights into population response to stressors, they are limited to species that can be transplanted and survive in a captive environment.

Experimental evolution in artificial environments designed to simulate climate change provides a unique opportunity to study adaptation and acclimation to a controlled stressor. For example, wild guinea pigs that were exposed to heat treatments showed epigenetic modifications that were passed on to their offspring, providing them with improved resilience to environmental temperature increase (Weyrich et al. 2016). Simulated environment experiments allow researchers to

study response along a single axis; however, whether these data can be extrapolated to wild populations experiencing compounding stressors remains to be seen.

2.6.3 Future Directions

Studying the genomic basis of population response to climate change is a relatively new field in ornithology. Future studies that use transplant and/or simulated experiments and next-generation sequencing are needed to understand the relative roles of adaptation and acclimation in response to climate change. Additionally, research on rapid evolution in invasive species could provide some insight into how species will respond to climate change in the future (Moran and Alexander 2014; Chown et al. 2015). Nonetheless, the function of many avian genes is still unknown, particularly for genes with multiple functions and small effect sizes. Studies that work to characterize gene function across avian taxa are greatly needed. Still, as sequencing becomes more cost-efficient, multilevel studies that compare genomics, transcriptomics, and epigenomics of a population will become possible, thereby creating a more complete picture of the avian evolutionary response to climate change.

3 General Conclusions

The advent of genomics and next-generation sequencing methods has enabled significant advances in our understanding of avian conservation. It has the potential to facilitate the preservation of adaptive diversity—particularly as technology progresses and our understanding of gene function improves. In combination with new computational approaches, we have gained incredible power to reconstruct population and evolutionary histories of endangered species and to define units for conservation. In addition, new high-throughput methods increase our ability to learn about the biology of endangered species often without handling or disturbing them (such as noninvasive analyses of population size, inbreeding, movements, diet, and disease). Some challenges remain, most notably that we lack detailed information about gene function across taxa. Nonetheless, the increasing availability and decreasing cost of obtaining large-scale genomic data are resulting in a burgeoning number of studies that address this shortcoming, and our understanding of gene function stands to improve dramatically in the next few years. Our ability to harness the power of genomics will be amplified by using multiple genomic approaches to creatively answer pressing questions in avian conservation.

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