Current Biology

CellPress

Review

The genomics of adaptation in birds

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https://doi.org/10.1016/j.cub.2022.07.076

SUMMARY

Organismal adaptations are the hallmark of natural selection. Studies of adaptations in avian systems have been central to key conceptual and empirical advances in the field of evolutionary biology and, over the past decade, leveraged the proliferation of a diversity of genomic tools. In this synthesis, we first discuss how the different genomic architectures of avian traits are relevant to adaptive phenotypes. A mutation's chromosomal location (e.g., microchromosomes or sex chromosomes) or its specific nature (e.g., nucleotide substitution or structural variant) will determine how it may evolve and shape adaptive phenotypes, and we review different examples from the avian literature. We next describe how the source of adaptive variation, whether from *de novo* mutations, existing genetic variation, or introgression from another species, can affect the evolutionary dynamics of a trait. Our third section reviews case studies where the genetic basis of key avian adaptive phenotypes (e.g., bill morphology or plumage coloration) have been revealed. We end by providing an outlook and identifying important challenges to this field, both by focusing on technical aspects, such as the completeness of genomic assemblies and the ability to validate genetic associations with new sources of data, as well as by discussing the existential threat posed to birds from habitat alteration and climate change.

Introduction

In a little over a decade, the study of avian evolutionary genetics transitioned from the predominant use of Sanger-sequenced mitochondrial genes and a handful of nuclear markers to whole genome datasets with high-quality species-specific annotated reference genomes[1,2]. The field had been limited by the ability to use polymerase chain reaction to amplify and sequence homologous markers across species that diverged from those for which the genetic resources had been developed[1]. Now, the ability to obtain large genomic datasets from species without existing genomic resources, together with certain properties of avian genomes (e.g., relatively small and conserved genome sizes or the low density of transposable elements) has allowed researchers to leverage the main advantages of studying evolution through avian systems[2]. These advantages derive from a long tradition of ornithological research, leading to a deep knowledge of bird taxonomy and phylogenetic relationships, diverse within-species phenotypic variation, a precise understanding of range limits, and extensive existing sampling efforts with genetic materials preserved in natural history collections (though see Rohwer *et al.*[3]). As a result, avian genomic resources, like the availability of increasingly high quality annotated reference genomes and re-sequencing datasets, have accumulated at a fast pace[2,4], and with these our knowledge of the genomics of avian adaptations. Here we review the molecular underpinnings of those adaptations, covering studies drawing upon different types of genomic data (e.g., transcriptomics, reduced-representation genomic techniques, or whole-genome re-sequencing).

There are some broad trends in the papers that we review. For example, passerines (Passeriformes) have dominated the literature to date, perhaps because they can be more easily sampled using field methods (e.g., mist-netting), their generally higher abundance compared to larger-bodied birds, or because they are the most diverse order (i.e., representing more than 60% of all avian species). Another trend is that most studies uncover statistical linkages between genotypes and phenotypes via association mapping or genome scans. However, the independent validation of these candidates through transcriptomics or functional genomics is much less common, possibly because of the logistical difficulty in bringing wild birds into a laboratory setting. Moreover, the function of many candidate genes that arise from association studies is limited to our understanding of gene functions in sometimes distantly related model species or domestic lineages. Therefore, there may be a bias towards discovering or reporting genes with already well-known functions, at the expense of uncovering novel targets of selection, for which a connection cannot be easily made with the phenotype of interest. As sequencing power continues to increase, so will the sample size that is feasible within a given budget, and therefore the statistical power to detect genetic associations. Smaller sample sizes may be underpowered to detect associations beyond genes of large effect, and thus may have biased our comprehension of the architectures of some of the studied traits. Finally, although genome scans are commonly based on summary statistics, studies are beginning to incorporate powerful model-based methods like machine learning to infer the processes behind the patterns (e.g., uncover signatures of selection)[5].

Our review is structured into four different sections. First, we discuss the different genomic architectures of avian traits and their relevance to the evolution of adaptive phenotypes. Second, we analyze the evolutionary sources of variation which ultimately lead to adaptation, and then review the genetic bases of key avian traits. We conclude by providing an outlook and discussing future challenges.

The genomic architectures of adaptive avian traits

There are general characteristics of genomes — some specific to avian genomes — that predictably facilitate adaptation. Therefore, the underlying genetic basis of a trait, known as the genomic architecture, can have implications for its evolution. For example, the specific chromosome where a gene is situated will dictate its inheritance pattern and its location within the chromosome may influence its neighbors, by determining the degree of linkage to nearby genes (through variation in recombination rate)[6,7]. Non-synonymous mutations, by definition, lead to phenotypic variation. However, in genes with multiple effects (i.e., pleiotropic), the overall selective advantage of such changes will likely depend on how the mutation influences the various functions of that gene. On the contrary, regulatory mutations may not face this constraint if changes in the regulatory network within which a gene is expressed are more specific to both tissue and developmental time[8]. Although the genetic basis of phenotypic traits are generally studied via associations with single-nucleotide polymorphisms (SNPs), the underlying causal variants may not be the SNPs themselves. For example, the architecture of the trait may be more complex, and exist within a chromosomal inversion, or involve insertions/deletions or copy number variants which are not present in SNP datasets[9]. Importantly, little is known about some genetic architectures (e.g., alternative splicing or copy number variants) that nevertheless may be important for the generation of traits that are relevant to avian evolution[10].

Microchromosomes, sex chromosomes, neo-sex chromosomes and germline-restricted chromosomes

Approximately 22% of birds have 2n=80 chromosomes, with most species showing little variation around this chromosome number, and only a few taxa departing substantially from this chromosomal complement (range 40–142)[6,11]. Notably, the largest chromosome in the chicken (*Gallus gallus*) genome (chromosome 1) subsequently underwent a fission event (i.e., split in two) in songbirds, producing two intermediately sized chromosomes[6,12]. There is considerable variation in chromosome size within any given bird species, with an approximately even split between larger macrochromosomes and smaller — below an average of 12 Mb — microchromosomes (although the size distribution is roughly continuous and therefore the distinction in the literature between 'micro' and 'macro' is somewhat arbitrary)[6,13]. Microchromosomes comprise about a quarter of the genome and show unique properties that distinguish them from macrochromosomes[13]. Microchromosomes have higher GC-content, mutation rate, recombination rates, and overall gene density[13]. They also have a lower density of transposable elements (except for woodpeckers)[14]. At least one crossing-over event is required for normal meiosis, which by definition leads to a higher per megabase recombination rate in small chromosomes[6]. This implies that linkage disequilibrium between selected alleles can be more effectively broken down in microchromosomes, making them good candidates for housing genes involved in local adaptation[15]. The *asip* gene, for example, a regulator of melanic coloration, is found in very narrow divergence peaks among closely related species in multiple taxa[16–18] and located on a microchromosome (chromosome 20).

Birds possess a ZW sex chromosome system with heterogametic (ZW) females, and a W sex chromosome that is mostly non-recombining, with the exception of a small pseudo-autosomal region[7]. The Z chromosome evolves faster than the autosomes in birds (i.e., the 'fast-Z effect') for multiple reasons, including a wider range of conditions that allow a mutation to increase in frequency (e.g., recessive mutations are exposed to selection in females), a slightly higher mutation rate, and increased genetic drift (as a consequence of having one-third the effective population size of an autosome)[7]. Consequently, the Z chromosome shows higher differentiation than autosomes in multiple taxa, and may be playing a disproportionate role in speciation and adaptation in birds[7]. By contrast, the W sex chromosome is significantly smaller, has the highest density of transposable elements and potentially active endogenous retroviruses of any chromosome[14,19], and is 'degrading' (over evolutionary time) due its lack of recombination, retaining few functional genes[7].

The maternal inheritance of the W chromosome — directly co-inherited with the mitochondrial genome — has also allowed it to play a role in controlling a key avian trait, egg coloration, in African cuckoo finches (*Anomalospiza imberbis*)[20]. These parasitic birds exploit a variety of host species (and populations within those species) by laying their eggs in the host's nest and, therefore, foregoing the costs associated with parental care. However, a successful *A. imberbis* female must mimic the appearance of her hosts' eggs to prevent rejection. Matrilines therefore specialize in parasitizing certain species, closely matching their egg coloration and markings. Moreover, autosomal data show ongoing gene flow between the males and females raised by different hosts, implying that the genes for matriline-specific egg coloration patterns cannot be on these chromosomes. Thus, African cuckoo finch egg coloration is thought to be mediated by W-linked genes. This chromosomal architecture likely imposes evolutionary constraints to the parasites through the lack of recombination on the W chromosome. For example, it may prevent the generation of certain coloration patterns that hosts, with the recombination afforded by autosomal control of egg coloration, can achieve.

Neo-sex chromosomes are another genomic architecture which has repeatedly influenced avian adaptation. These chromosomes are generated by reciprocal translocations or fusions of autosomes onto existing sex chromosomes, and therefore affect how these originally autosomal genes are inherited once they become linked to sex chromosomes. When genes become sex-linked, such as to the W chromosome, neo-sex chromosomes could provide an evolutionary 'escape' from sexual antagonism (e.g., beneficial genes for females which are detrimental for males), as this chromosome is only present in females[21]. Several instances of the evolution of neo-sex chromosomes have been documented in birds, but the details of how they were formed are not fully understood. For example, the

Raso lark (*Alauda razae*) and the Reunion white-eye (*Zosterops borbonicus*) both possess neo-sex chromosomes, which may involve several autosomes[22,23]. Both species belong to the passerine superfamily *Sylvioidea*, and a neo-sex chromosome involving the first 10 Mb of chromosome 4A seems to have evolved in this group's common ancestor[24]. This region contains the androgen receptor gene (*ar*), a gene involved in male sexual development, and the neo-sex chromosome may therefore have provided an opportunity to link this gene to other male-benefitting Z-linked loci[24].

A portion of chromosome 1A is sex-linked in the Australian eastern yellow robin (*Eopsaltria australis*) and is predicted to have co-evolved with the mitochondrial genome. Together the chromosome 1A region and mitochondrial genome are thought to mediate adaptation to local climatic conditions in this species[25,26]. Populations are divergent in their nuclear genomes in a north-to-south direction, while due to the history of isolation and gene flow, mitochondrial divergence is arranged perpendicularly, in line with an inland to coastal climatic gradient, and has narrow contact zones. Therefore, each mitochondrial lineage exists on both nuclear genomic backgrounds: the ancestral background with which it co-evolved and the derived type into which it introgressed. However, mitochondrial genes are located on both the mitochondrial and nuclear genomes, and these cannot diverge freely: gene products from both genomes are required to work together to maintain the cell's energetics. Consequently, it is thought that to preserve mitonuclear coadaptation, a portion of chromosome 1A, which is enriched for nuclear genes of mitochondrial function, has co-introgressed with the mitochondria, therefore preserving the original nuclear genomic background (at least at these key loci) after the introgression took place. Most of chromosome 1A is sex-linked and thus involving the nuclear-encoded mitochondrial genes in a neo-sex chromosome is thought to facilitate mitonuclear co-adaptation: these genes are linked with the W chromosome and in turn, through the shared matrilineal inheritance, to the co-inherited mitochondria. A similar pattern of co-introgression of the mitochondrial genome with nuclear-encoded mitochondrial genes has likely occurred in Audubon's warblers (*Setophaga coronata audubon*i)[27], suggesting a possible broader evolutionary solution to mitonuclear discordance.

Birds can also show chromosomal differences between the germline and the soma. All songbirds studied to date have a germline-restricted chromosome (GRC) which is entirely absent in somatic cells and is also absent in non-songbirds[28–30] (Figure 1). The GRC is usually heterochomatic, ejected after meiosis, and mostly found in a single copy in males. In females, however, it is present in two copies, recombines, and is transmitted to the progeny[31]. Depending on the species it can be a microchromosome or a macrochromosome — it is in fact the largest chromosome in the zebra finch (*Taeniopygia guttata*) genome — and has low homology across divergent species[29,31]. Although the songbird GRC has many repetitive sequences and could be a selfish (i.e., parasitic) chromosome, it is also transcriptionally active and contains paralogs for ~115 genes that are present on regular chromosomes[30,32]. It is enriched in genes involved in female gonad development and it is thought that its elimination could be an evolutionary mechanism to avoid antagonistic pleiotropy and to minimize conflicts between the germline and the soma[32]. Many genes are apparently species-specific and could have contributed to reproductive isolation among closely related species and may play an important role in avian adaptation.

Structural variants: Supergenes, indels, and copy number variants

Although inter-chromosomal rearrangements are relatively rare in birds (at least in those with the typical karyotype), *intra*-chromosomal rearrangements are comparatively more common[6]. Inversions are a type of chromosomal rearrangement in which a portion of DNA is flipped in its orientation. When this occurs, crossing-over events within the inverted region in heterozygote individuals can lead to unviable unbalanced gametes (i.e., with missing or extra genes), and therefore inversions have the consequence of suppressing recombination between the ancestral and inverted haplotypes[33]. This protection from recombination allows the genes involved in the inversion to co-evolve, leading to the formation of 'supergenes'. Supergenes consist of many co-adapted genes that mediate complex traits in birds. Alternative reproductive strategies in the white-throated sparrow (*Zonotrichia albicolis*)[34] and the ruff (*Calidris pugnax*)[35,36] are controlled by either large (~10% of the genome) or small (~4.5 Mb) supergenes, respectively. Additionally, variation in sperm morphology in the zebra finch has been shown to be mediated by a large Z-linked supergene[37,38].

Insertion-deletion (i.e., 'indel') mutations are a heterogeneous class of mutation that includes short insertions, deletions, duplications, transpositions and length-change in tandem repeats[39]. Indels are correlated with SNP density in the chicken genome, yet are less common, representing ~5% of the SNP density in this species and ~2% of the nucleotide substitution rate between the chicken and the turkey. In the great tit (*Parus major*), most indels are short (<5 bp long) and tend to be deleterious[40], yet in crows (*Corvus*), where these mutations were studied using long-read sequencing technologies, they can span several kilobases[41]. Although it can be challenging to identify their ancestral state, indel mutations are likely biased towards deletions, possibly due to polymerase slippage during replication. One unique way in which indels can promote phenotypic changes is by disrupting regulatory networks, specifically by altering the spacing between cis-regulatory elements[39,42]. Regulatory regions may depend on the precise spacing (and not necessarily the specific sequence) between transcription factor binding sites or enhancers in promoter regions. By changing either the number of these binding sites or the spacing between them, indels may lead to variation in the expression levels of genes that are important for adaptation. Indels can also result from transposition events, which we discuss in the following section.

Changes in the number of copies of DNA fragments, or copy number variants, are an important source of variation in humans and are also observed between many bird species[43,44]. These rearrangements appear to be more frequent (per megabase) on microchromosomes and are predominantly found in association with genes, suggesting they are likely functionally relevant[43]. In rock pigeons (*Columba livia*), a sex-linked copy number variant encompassing the melanosome maturation gene *mlana* mediates a color polymorphism[45]. In the common murre (*Uria aalge*), there are two color morphs that are differentially adapted to their

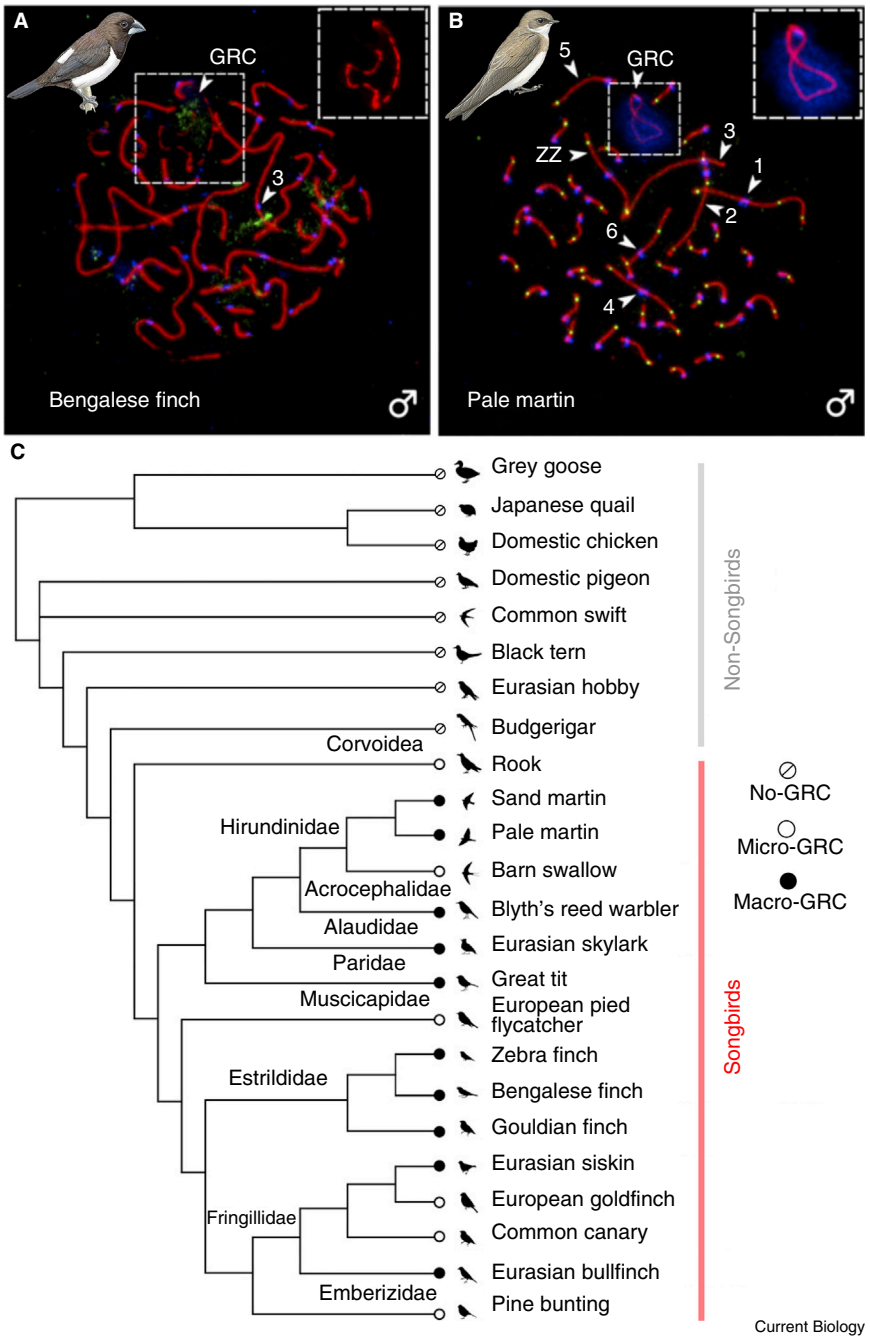

Figure 1. Germline-restricted chromosomes in songbirds.
Chromosomal spreads of (A) the Bengalese finch and (B) the pale martin immunolabeled with antibodies against SYCP3 (red), highlighting the synaptonemal complex, which is the protein structure that forms between homologous chromosomes, centromere proteins (blue) and MLH1, a mismatch repair protein marking recombination sites (green) (images from Torgasheva *et al.*[28]). (C) Macro or micro germline-restricted chromosomes (GRCs) have only been identified in songbirds, the most specious avian lineage, prompting questions about their role in the diversification process (figure from Torgasheva *et al.*[28]; this result remains true when surveying a larger number of species[30]). Bird illustrations courtesy of BOW (https://birdsoftheworld.org/bow/home).

thermal environment (cold versus warmer) and this plumage difference is associated with a single ~60 kb region containing three candidate genes[46]. Based on anomalous patterns of read depth in this area, it is likely a copy number variant, or perhaps a more complex combination of rearrangements, that underlies these phenotypic differences.

Regulation of gene expression, transposable elements, and alternative splicing

The evolution of coding sequences in pleiotropic genes may be constrained when mutations are adaptive in certain contexts but deleterious in others, depending on the tissues or the timing in which genes are expressed[8]. Variation in how or when genes are expressed may provide a solution to this constraint and be achieved with relatively small DNA sequence changes, leading to phenotypic novelty. Cis-regulatory elements (CREs) are bound by proteins which control gene expression and can be functionally modular, driving the expression of genes during specific developmental times and only in certain tissues[8]. Therefore, the evolution of CREs may allow genes to influence phenotypic changes without the potentially negative pleiotropic effects of mutations in coding regions. Coloration differences among closely related birds in the genus *Sporophila* are associated with mutations in non-coding regions close to otherwise conserved melanogenesis genes, suggesting that differences in plumage are generated by changes in the expression patterns of these pigmentation genes[17,47]. A presumable regulatory region near the gene follistatin (*fst*) mediates an intraspecific head plumage coloration polymorphism that is maintained by balancing selection in Gouldian finches (*Erythrura gouldiae*)[48,49]. Egg coloration in several duck and chicken breeds is controlled by changes in the expression of genes that modify the transport and deposition of pigments in the eggshell (Figure 2). In mallards (*Anas platyrhynchos*), for example, a SNP in a CRE increases the expression of the *abcg2* gene in the uterus[50]. This gene functions as a membrane transporter for the green pigment biliverdin, and its increased expression is thought to lead to the production of green eggs. Regulatory changes can also mediate evolution at deeper scales, as is the case with the convergent loss of flight in ratites[51].

Transposable elements (transposons, retrotransposons and the relics of old viruses known as endogenous viral elements) have played an important role in the evolution of eukaryotic

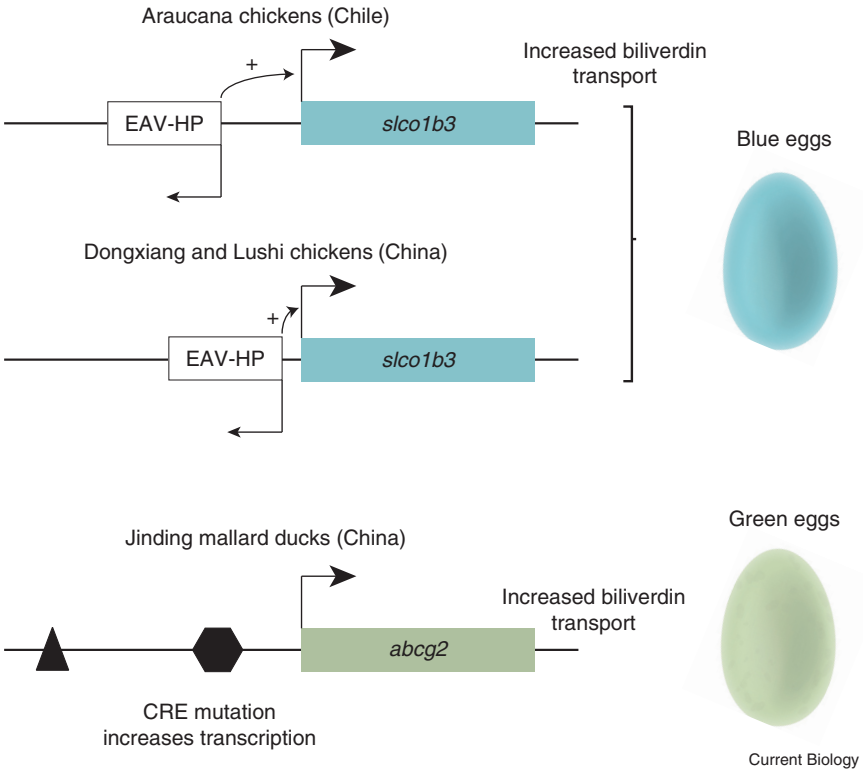

Figure 2. Gene expression differences mediate egg coloration in domestic chicken and mallard duck breeds.
Two independent insertion events of a retrovirus (EAV-HP; note the different insertion sites) with promoter activity increase the expression of the *slco1b3* gene. This gene is thought to transport biliverdin pigment to the eggshell, leading to blue eggs (obtained from Wang *et al.*[54]). In a mallard breed a mutation in a CRE increases the expression of *abcg2*, leading to higher biliverdin transport and green eggs (obtained from Liu *et al.*[50]). While these phenotypes likely arose as a byproduct of artificial selection in domestic chickens and mallards, they illustrate the role of gene regulation and transposable elements in the evolution of phenotypes that are adaptive in wild birds.

gene regulation[52]. Certain elements may become inactive after transposition and unable to mobilize, but may still contain intact promoters that affect the transcriptional regulation of the genes that are nearby[14]. In humans and mice various promoters, binding sites for regulatory proteins or polyadenylation signals are derived from transposable elements, some of which are highly conserved[52]. Transposable elements can also modify pre-existing regulatory networks by duplicating or eliminating CREs. Due to difficulties in assembling repetitive regions (especially with short-read sequencing technology), transposable elements, and perhaps their role in avian adaptation, tend to be underestimated[14,53]. In domestic chickens, the insertion of a 4.2 kb retrovirus (EAV-HP) in the 5′ flanking region of the gene for the membrane transporter *slco1b3* confers promoter activity, leading to its increased expression in the shell glands of the uterus[54]. This transporter may be responsible for increased biliverdin deposition and the production of blue eggs. The insertion sites of this retrovirus are different in different breeds with blue eggs, suggesting that the insertion occurred more than once — independently in China and Chile — where the different chicken breeds originated. Finally, a high density of DNA methylation in gene promoter regions tends to decrease gene expression by interfering with the binding of transcription factors[55]. Methylation of the *slco1b3* promoter is negatively correlated with its expression and the intensity of blue eggshell color, indicating that this phenotype can be further modulated by epigenetic modifications.

Alternative splicing may evolve faster than the regulation of gene expression and can therefore lead to structurally variable transcripts from a single gene by various processes, like including mutually exclusive exons, skipping exons, retaining introns, or having alternative 3′ or 5′ splice sites[10]. Transcription level and alternative splicing appear to be regulated independently, providing different evolutionary avenues for adaptation. As is the case for gene expression, *cis*- and *trans*-acting factors — as well as epigenetic modifications — can regulate splicing. Most genes predominantly express a single dominant isoform and multiple alternative isoforms at much lower levels which, in an analogous way to gene duplicates, are free to evolve new functions. Mechanisms like alternative transcription start or polyadenylation sites can also contribute to the formation of alternative isoforms[10]. Alternative splicing may be an evolutionary avenue to resolving sexual conflict. In the mallard, turkey (*Meleagris gallopavo*), and helmeted-guineafowl (*Numida meleagris*) there are sex-specific splicing differences in gonads that correlate with phenotypic differences between the sexes, and have evolved rapidly as a product of sex-specific selection[56]. However, the proportion of sex-specific spliced genes is an order of magnitude less than that of those that are differentially expressed, suggesting the latter process could be more relevant in resolving sexual conflict.

The sources of adaptive variants

The ultimate source of genetic variation has implications for the evolutionary dynamics of a given adaptive trait, determining aspects like the waiting time until an adaptive mutation occurs, or the number of mutations involved in generating the phenotype, which may determine its complexity. While *beneficial* mutations are rare, deleterious or neutral mutations occur more commonly[57]. Therefore, adaptation from *de novo* mutations may take many generations. Existing genetic variation (i.e., 'standing' genetic variation) or introgression of adaptive traits from other species or divergent populations (i.e., adaptive introgression or gene flow) are two alternative sources of variation on which selection can act[58–60], allowing adaptation to proceed at a potentially much faster pace than from *de novo* mutations. Moreover, adaptive introgression can provide mutations which have already been 'tested' by natural selection in a different species or population, potentially leading to novel complex traits involving several mutations. Finally, both gene flow among

incipient species and the mixing of variants from standing genetic variation may allow old genetic variants to reassemble in novel combinations, and therefore this 'combinatorial mechanism' can be an additional source of adaptive traits[59].

Identifying the sources of adaptive variation poses additional challenges beyond simply associating genotypes with phenotypes. While the latter can be done through different types of outlier analyses, understanding the evolutionary *origin* of a variant requires a broader knowledge of the phylogenetic context or the molecular signatures around the variants of interest. To detect adaptive introgression among multiple putatively hybridizing species, phylogenies from a locus of interest can be compared to the genome-wide topology. This was shown for the complex differences in morphology and reproductive strategies in the white-throated sparrow, which are determined by a large supergene. Phylogenetic analyses showed that this supergene is older than the species itself, and this genomic region is thought to have introgressed from a now extinct species[34]. Additionally, in *Setophaga* warblers, the topology at the carotenoid processing gene *bco2* is highly discordant with the species tree inferred from the rest of the genome, consistent with one or more bouts of historical introgression of this gene among different species[61].

Adaptations from new mutations, and possibly introgressed variants, are initially found at low frequency, and therefore should show signatures of hard selective sweeps. We are not aware of conclusive examples of avian adaptations from *de novo* mutations, such as in *Peromyscus* mice[62]. By contrast, standing variants may be at higher frequencies and found in different haplotype backgrounds before the onset of selection, leaving behind a signal of soft selective sweeps[63]. In *Sporophila* seedeaters, variants near melanogenesis genes associated with coloration differences among recently diverged taxa show signatures of soft selective sweeps from standing genetic variation[64]. Moreover, phylogenetic trees derived from these loci suggest that novel plumage phenotype likely originated through the reassembly of standing genetic variation in novel combinations[65]. Finally, there is evidence that some of the genomic regions associated with changes in beak morphology in Darwin's finches represent haplotype blocks which are older than many of the species[66]. Different combinations of variants at these loci are suggested to shape beak morphology across the radiation.

The genomic basis of key avian traits

Our goal here is not to provide an exhaustive accounting of all the studies that have linked genes to adaptive phenotypes in birds. Instead, we focus on several key avian traits and highlight the power of genomic tools to examine their genetic basis (Figure 3).

Bill morphology

One of the most iconic phenotypic adaptation in birds involves variation in bill shape and size[67–69]. As the direct anatomical link to resource acquisition — that also has implications for song production and mate signaling[70] — ecomorphological variation in bills is exceptionally high in some avian clades, particularly in seed eating species. In many cases, including in Darwin's finches, *Pyrenestes* seedcrackers[71], and *Loxia* crossbills[72], studies have explicitly shown this variation to be the result of divergent natural selection. Bird beaks can also change through anthropogenic causes, like food supplementation using bird feeders, which could have contributed to shaping longer bills in the great tit (*Parus major*)[73].

Several developmental genes have been associated with different aspects of bill morphology (e.g., length, width, or overall size). Early studies of Darwin's finches, for example, identified expression differences among species in *calm1*[74] and *bmp4*[75] during early development of the bill, presumably playing a key role in craniofacial development in these birds. Using a combination of whole-genome sequences and divergence analyses, variation in *alx1* and *hmga2*, among other genes, was subsequently implicated in driving size and shape variation[68,76], with the *hmga2* 'large variant' explicitly associated with survival during a drought period in the Galápagos Islands[77]. The variation in beak morphology in Darwin's finches is remarkable, with many species showing differences across a comparably large number of islands. Many of the studies on beak morphology focus on subsets of species and specific islands; however, one study sampled four species on over a dozen islands and found hundreds of associated developmental genes, suggesting that this trait is polygenic, despite the focus on a few genes of large effect[78].

Beyond *Geospiza* finches, variation in *igf1* has been associated with large or small-billed *Pyrenestes* seedcrackers[71]. In this case, unlike *Geospiza* finches where there is moderate reproductive isolation among taxa, the *Pyrenestes* bill size polymorphism is seemingly maintained within randomly-mating populations. High linkage disequilibrium within the chromosomal region that houses *igf1* is suggestive of a chromosomal inversion, which may help maintain the polymorphism without assortative mating. Finally, a third *Pyrenestes* morph, dubbed the 'mega-billed' form, appears to have evolved using a more complex genetic architecture that is semi-independent of the variants associated with the smaller-billed forms.

Wing growth and flightlessness

Among vertebrates, powered flight has evolved only three times and deep in the past (in modern birds, bats, and pterosaurs), and thus identifying the genes associated with the initial adaptive steps in the evolution of flight in birds is challenging (if not impossible). However, the subsequent *loss* of flight has been observed in several avian lineages, both deep in the avian tree and at its tips; genomic studies have started to reveal the genetic changes associated with flightlessness in the latter cases[79,80]. For example, the flightless cormorant of the Galápagos islands (*Phalacrocorax harrisi*) has extremely short wings that are not capable of flight, although it is an agile diver. *P. harrisi* diverged from its flighted relatives within the past 2 million years, recent enough for it to be possible to use whole genomes to identify several candidate changes associated with flightlessness[79]. Most notable were amino acid changes in CUX1 and IFT122, which are both involved with ciliary function and bone growth. In an impressive application of integrative methods, the cormorant *ift122* variant was experimentally shown to affect the ciliary function of *Caenorhabditis elegans in vitro.*

South American *Tachyeres* steamer ducks are known for their conspicuous swimming behavior of vigorously flapping their wings in the water while propelling themselves forward with their feet. They are in fact a group of closely related species and are unusual among birds in that the ability to fly varies both inter- and intra-specifically. One *Tachyeres* species is

Feather coloration

Coding and regulatory changes in genes of large effect are repeatedly and independently involved in pigmentation changes across many species

Egg coloration

Increased expression of biliverdin transporters leads to green and blue eggs in domestic birds

W-linked loci generate host-specific parasitic matrilines in cuckoo finches

Alternative mating strategies

Clusters of co-adapted genes protected from recombination by inversions (supergenes) mediate reproductive strategies in ruffs and white-throated sparrows

Sweet taste reception

Independent co-opting of the ancestral *umami* receptor in hummingbirds and songbirds enable sweet perception

Flightlessness

Developmental genes affecting bone morphogenesis in the Galápagos cormorant and steamer ducks lead to shorter wings

Osmoregulation

Shared and lineage-specific targets of selection across multiple sparrow species contribute to saltmarsh adaptation

Migration

Large genomic regions and multiple genes implicated across many migratory birds

Sexual dimorphism

Sex, neo-sex, and germline-restricted chromosomes help resolve sexual conflict together with differential gene expression and splicing

Altitude and elevation

Shared pathways among bird and other animals facilitate adaptation to low oxygen and temperatures

Beak size and shape

Multiple developmental genes implicated in shaping bill morphology known predominantly from seed eating birds

Climate change and urbanization

Shifts in climate, anthropogenic habitat alterations, and novel parasites generate strong selection pressures and conservation concerns

Current Biology

Figure 3. Examples of avian adaptations and their genetic basis.
We do not cover bird migration here as a primer on the subject by Justen and Delmore[126] is also published in this issue of *Current Biology*. Illustrations by Charlotte Holden.

predominantly flighted whereas three are mainly flightless. Using a cross-species genome-wide association study (GWAS), two narrow candidate genomic regions were shown to be associated with the morphological changes leading to flightlessness[80]. One of the genes in these regions with the highest association, *dyrk1a*, is implicated in human genetic disorders that include bone length abnormalities and knockouts in mice show altered growth and bone morphogenesis.

Plumage coloration

Birds use a variety of pigment molecules to color their feathers, primarily melanins (eumelanin and pheomelanin, which give rise to different black, gray, brown or yellowish tones) and carotenoids (which produce a range of yellow, orange, and red colors)[81]. Unlike in *Peromyscus* mice or peppered moths, where color variation has been explicitly linked to survival and fitness, coloration research in birds has also been viewed through the lens of sexual selection[82]. In particular, studies of melanic variation in *Sporophila* seedeaters[17,47], *Monarcha* flycatchers[83], *Lonchura* munias[84], *Motacilla* wagtails[18], and parulid warblers[16] have all implicated common targets of selection, most notably *asip* and, to a lesser extent, *mc1r*. Both coding and presumably regulatory mutations are thought to mediate coloration differences and, in some cases, specific variants have been linked to changes in the color or pigment concentration of particular body patches[47,85,86]. Moreover, the combined variation of these genes and a few others from the melanogenesis pathway are responsible for the concerted variation across multiple body parts, leading to emergent patterning[47,87]. These genes are also involved in pelage variation in *Peromyscus* as well as other vertebrates[82], suggesting the shared melanogenesis pathway is commonly targeted by natural and sexual selection.

Beyond the presence and absence of melanin molecules, birds also vary how pigment molecules are arranged and packed into the developing feather — producing structural coloration differences — as well as incorporating a wider range of other pigment molecules into their feathers, which together produce a broad diversity of colors[81]. Recent discoveries on different pigment molecules have focused on, for example, the sequence differences which mediate psittacofulvin variation, a pigment which is specific to parrots[88] (responsible for green and blue tones), as well as the gene expression patterns associated with iridescence in African starlings[89]. However, carotenoid molecules have received the most attention, as they are thought to act as 'honest signals' in avian systems. An honest signal refers to when an individual (i.e., the signaler) deposits a metabolically costly compound in its integument such that a potential mate (i.e., a receiver) can easily assess the quality and potential to produce high-fitness offspring of the signaler[90]. Carotenoids must be acquired through the diet and later modified, which is metabolically costly, thus having the potential to become an honest signal of resource acquisition.

Evolution in *bco2* — a gene involved in the breakdown of full-length carotenoids into shorter apocarotenoids — has been linked to carotenoid-based coloration in canaries[91], the nestlings of Darwin's finches[92], and *Vermivora* warblers[61]. Whereas *cyp2j19* — involved in ketolation of yellow dietary carotenoids to red ketocarotenoids — has been linked to red coloration in *Pogoniulus* tinkerbirds[93], zebra finches[94], *Colaptes* flickers[86], and red-backed fairywrens[95]. The fact that both genes (*bco2* and *cyp2j19*) have also been directly[96] or indirectly[97] implicated in adaptive color differences among reptiles is consistent with a single evolutionary origin among non-mammalian tetrapods for the role of these genes in the deposition of carotenoids in the integument. The Honduran white-bat, *Ectophylla alba*, is the only mammal documented to deposit carotenoids in its skin, but the mechanism is unknown[98].

Taste reception

Unlike mammals, which have evolved taste for both savory and sweet diet items, avian taste receptors were long thought to be primarily restricted to detecting savory foods, as their genomes lacked a key sweet taste receptor (the *t1r2* gene, which encodes one of two proteins that combine to produce the sweet taste receptor heterodimer)[99]. Yet, nectivores, and especially hummingbirds, belied this pattern (Figure 4). Hummingbirds have evolved sweet taste reception, but by co-opting an ancestral savory (umami) receptor (a heterodimer of T1R1–T1R3)[99].

Nectivory and sweet taste have also evolved in songbirds (Passeriformes) independently of hummingbirds (Apodiformes). Using synthesized proteins of ancestral reconstructions and functional experiments, carbohydrate sensitivity in songbirds was also found to have evolved through co-option of the T1R1–T1R3 umami receptor[100]. However, this occurred independently of hummingbirds, as most of the functionally important amino acid sites for the songbird sweet taste involve T1R1, instead of T1R3, as in hummingbirds. That said, the multiple presumably functional and adaptive changes in both lineages involved the ligand-binding region of the heterodimers, implying parallel adaptation at the level of tertiary protein structures.

Elevational and altitudinal adaptation

How birds have adapted to living at high elevations and flying at high altitudes has been of interest for decades, yet logistically challenging to study in the wild. Research on the molecular and physiological adaptations in this realm has been exemplified by work on bar-headed geese (*Anser indicus*), which migrate over the Himalayas, recorded at altitudes of 6,000 meters, where the partial pressure of oxygen is significantly reduced[101]. Early research showed that these geese have an inherently higher haemoglobin O_2 affinity[102], but also their mitochondria are distributed towards the cell membrane[103], presumably both adaptations to improve oxygen transport efficiency. More recently, genomic analyses of this species — as compared to other low-altitude species — showed that a number of genes in the hypoxia inducible factor (HIF) pathway are under strong positive selection[104]. Notably, genes in this pathway are also involved in the transcriptional response of high-altitude adapted Tibetan human populations and in different duck species[105].

Adaptations to high elevation in the Qinghai-Tibet Plateau were assessed by comparing transcriptomic data for three high elevation passerine species paired with related low elevation species[106]. The study showed a large difference in the expression profiles of putative elevation-adapted genes, with similar genes showing evidence of positive selection among the pairs, while only sharing a small number of common amino acid changes, suggesting convergent evolution.

While many studies have looked explicitly at avian adaptations to the most conspicuous abiotic changes to elevation (i.e., temperature and oxygen pressure), work in mountain chickadees

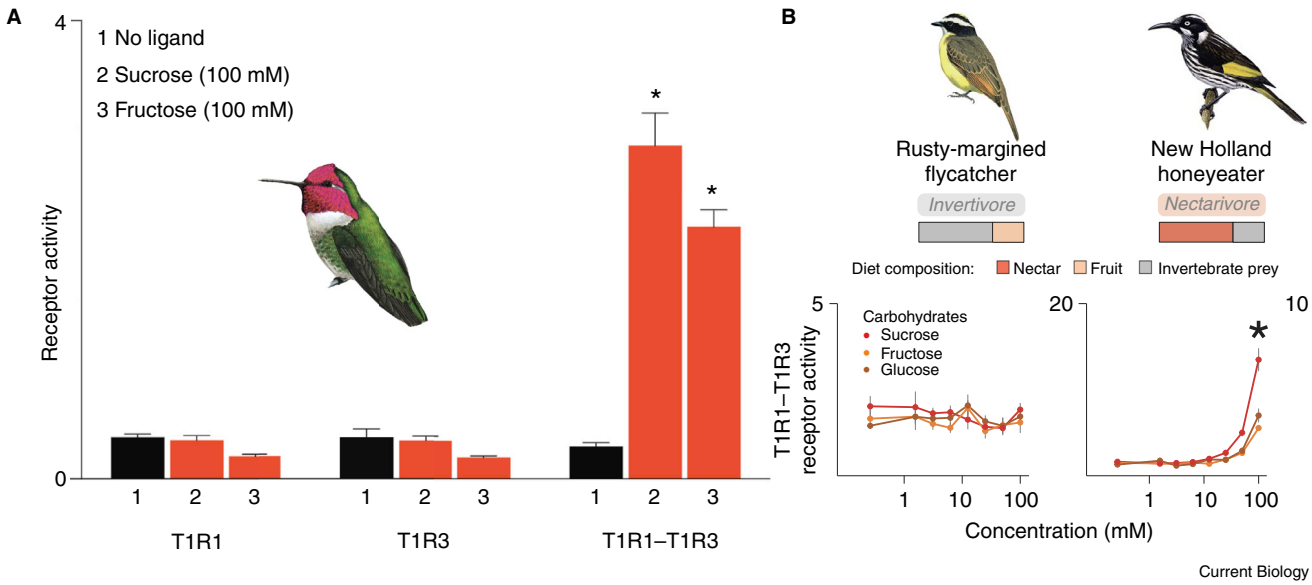

Figure 4. Sweet taste receptor evolution in birds.
(A) Responses of hummingbird taste receptors to sucrose and fructose, showing the very strong sensitivity of the T1R1–T1R3 heterodimer (figure from Baldwin *et al.*[99], © The American Association for the Advancement of Science). (B) Other nectarivores, like the New Holland honeyeater, have independently evolved sweet taste reception using the T1R1/T1R3 heterodimer, while in insectivores, illustrated by the Rusty-margined flycatcher, this receptor is not sensitive to sugars and is only activated by amino acids (figure from Toda *et al.*[100], © The American Association for the Advancement of Science).

(*Poecile gambeli*) has studied variation in cognitive phenotypes. These species are food-caching, and therefore require impressive spatial cognitive adaptations to recover their food stores, particularly at higher elevation where snow precipitation can be very high. Significant associations with several genes, including those involved in neuron growth and development, were found by comparing the genomes of wild chickadees that differed in their ability to solve a spatial cognitive task at both low and high elevations[107]. These genes may have been involved in the polygenic cognitive adaptation needed to survive in these novel environments.

Water regulation and climate change

The genomics of adaptation of osmoregulatory pathways have generally been studied in non-avian vertebrates, such as anadromous salmonids[108]. However, research in the Karoo scrub-robin (*Cercotrichas coryphaeus*), distributed across an aridity gradient, as well as work among four sparrow species that independently colonized coastal habitats, have highlighted these adaptations in birds[109,110]. Whole-genome data from sparrow saltmarsh and upland groups revealed strong genetic evidence of adaptations to these challenging environments[110]. While many genomic regions were independently divergent between the pairs, several others showed evidence of parallel adaptation across all pairs. One of those regions included the gene *slc41a2*, which in teleost fishes has been shown to be a Na^+/Mg^{2+} transporter, and thus possibly involved in osmoregulation in these saltmarsh-adapted sparrows.

Recent advances integrating genomic and environmental data via machine learning (ML) approaches have opened new avenues for growth in this field. For example, work on yellow warblers[111] (*Setophaga petechia*) and willow flycatchers[112] (*Empidonax traillii*) both combined reduced-representation genome sequencing and gradient forest ML analysis of remote sensing environmental data. While the demographic history of a species, the genomic architecture of the trait, or the nature of the environmental gradient can impose limitations to interpreting results from this approach[113], both studies were able to identify — and subsequently validate in a broader sample — adaptive loci, for example, SNPs upstream of the *drd4* gene associated with high precipitation[111]. This gene had been previously associated with the 'boldness' phenotype across a range of vertebrate taxa, although it is unclear how it is directly related to climate adaptation in birds. An additional study reported significant associations with the mean temperature of the warmest annual quarter; however, the top SNP was not linked to any genes with known functions in thermal tolerance[112]. Importantly, both studies used genotype–environment relationships to measure the mismatch between the current and predicted future genomic variation (or 'genomic offset') to forecast how much populations needed to adapt to respond to a changing climate, providing an important predictive framework for future genomic studies of adaptation in birds.

Outlook and future challenges

We end by turning to what we believe are important challenges to this field, by both focusing on the technical aspects of studying avian genomics, as well as discussing the existential threat posed to biodiversity from habitat alteration and climate change.

Reference genome assemblies and structural variants

Genome assembly quality remains a limiting factor in the identification of the genetic basis of adaptation in birds. Whereas the number of assembled avian genomes using short-read sequencing has dramatically increased in the last few years, there are still regions of the genome that are not recovered, and these may contain 'hidden genes' involved in shaping different phenotypes. Long-read sequencing technologies lead

to more complete assemblies, which can include repeat or GC-rich regions such as microchromosomes, telomeres, centromeres, multicopy genes, or heterochromatin[53]. As the field progresses towards telomere-to-telomere assemblies, so will our ability to understand how different regions of the genome contribute to adaptation, as well as developing more robust and species-specific gene annotation information. Long-read sequencing technologies will also enable the characterization of different types of structural variants, and with this a better understanding of how different types of causal mutations contribute to phenotypic variation[9].

Beyond association studies

Many of the genomic studies of avian adaptation began with a clear phenotype, segregating either within or between species, that has a demonstrable connection to fitness, and the genetic basis for those traits are identified by GWAS, F_{ST} scans, or related outlier methods. However, unlike in other taxonomic groups where subsequent validation of associations would be readily feasible by bringing the organism into the laboratory, many of the tools of functional genomics remain out of reach for most avian taxa. For instance, the CRISPR/Cas9 system for gene editing is still only emerging in birds, with the chicken and quail as the two species showing significant advances[114,115]. Recently, however, CRISPR/Cas9 was used on an immortalized cell line from the zebra finch, which will allow for more comprehensive molecular studies, at least for that songbird species[116].

Transcriptomics has been, and will likely continue to be in the near future, the more fruitful avenue for validating the connection between gene associations and adaptive phenotypes in birds[117]. Moreover, many studies of adaptation which only look at segregating sequence variation have identified non-coding, putatively regulatory regions, as likely underlying causal phenotypes. This suggests that gene expression differences may underlie many of the adaptive differences within and among bird species, as has been documented in other taxonomic groups like *Gasterosteus* sticklebacks[118]. We also believe that valuable genomic insights of avian adaptation will come from highly integrative research. For instance, studies that combine natural history, comparative phylogenomics, and molecular biology, make the most compelling cases for adaptive evolution with explicit functional connections[79,99,100]. Thus, with new candidates for other molecular processes that underlie avian adaptation coming from this first generation of association studies, similar work will likely be feasible for coloration, migration, morphology, and many other adaptations. We also envision that the incorporation of machine learning methods will be able to overcome some of the challenges for combining large and diverse datasets, allowing for example the identification of mutations under selection or associations between the genome and the environment, and will likely be a central tool for future studies of avian genomics[5,64,111,112].

Conclusion and conservation challenges

The study of wild birds — like many vertebrate taxa — is framed within a broader context of population declines and conservation concerns[119]. We have already observed genomic evidence of anthropogenic influence on the adaptation of bird populations[73,120], which can obscure our understanding of how evolution takes place in natural populations. For example, the degree of hybridization may increase as populations are forced to coexist in patches of remaining habitat, and this may influence their evolutionary fate[121]. Genomic offset to climate change has also been identified as an important factor to consider across several systems[111,112,122]. Anthropogenic change, like the introduction of novel parasites[123,124], generates strong and novel selective pressures which directly threaten the survival of entire species groups, like Darwin's finches, which have been foundational in our understanding of the genetic underpinnings of avian adaptation. Finally, admixture between wild and domesticated individuals can threaten to modify the evolutionary trajectory of a species[125]. It is likely that other systems, less well known than some of the iconic examples discussed above, are already facing extinction or extirpation, and will be lost without the ability for us to gain insights regarding their evolution. While genomic data play a key role in better understanding population structure and the effects of declines, because of these clear conservation concerns, we see that the study of genomic adaptation in wild birds is at both an exciting and precarious crossroads.

ACKNOWLEDGEMENTS

We thank Alexander Suh and an anonymous reviewer for valuable suggestions on a previous version of the manuscript. We also thank Charlotte Holden and Jillian Ditner from the Bartels Science Illustration Program of the Cornell Lab of Ornithology for the illustrations in Figure 3. Support for D.P.L.T. was provided by Pennsylvania State University, startup funds from the Eberly College of Science and the Huck Institutes of the Life Sciences, and the NSF-DEB (award# 2131469).

DECLARATION OF INTERESTS

The authors declare no competing interests.

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