

# The pace of mitochondrial molecular evolution varies with seasonal migration distance

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## Abstract

Animals that engage in long-distance seasonal migration experience strong selective pressures on their metabolic performance and life history, with potential consequences for molecular evolution. Species with slow life histories typically show lower rates of synonymous substitution ( $d_s$ ) than “fast” species. Previous research suggests long-distance seasonal migrants have a slower life history strategy than short-distance migrants, raising the possibility that rates of molecular evolution may covary with migration distance. Additionally, long-distance migrants may face strong selection on metabolically-important mitochondrial genes due to their long-distance flights. Using over 1,000 mitochondrial genomes, we assessed the relationship between migration distance and mitochondrial molecular evolution in 39 boreal-breeding migratory bird species. We show that migration distance correlates negatively with  $d_s$ , suggesting that the slow life history associated with long-distance migration is reflected in rates of molecular evolution. Mitochondrial genes in every study species exhibited evidence of purifying selection, but the strength of selection was greater in short-distance migrants, contrary to our predictions. This result may indicate effects of selection for cold tolerance on mitochondrial evolution among species overwintering at high latitudes. Our study demonstrates that the pervasive correlation between life history and molecular evolutionary rates exists in the context of differential adaptations to seasonality.

**Keywords:** life history, seasonal migration, molecular evolution,  $dS$ , mitochondria

## Introduction

Species' traits are the product of their genome and their environment, but in turn, traits and the environment also shape the molecular evolution of the genome. For example, metabolically demanding traits influence molecular evolution of mitochondrial genes (e.g., Chong & Mueller, 2013; Shen et al., 2009; Strohm et al., 2015). More broadly, traits associated with the slow-fast continuum of life history (Stearns, 1983) are correlated with rates of molecular evolution (Bromham, 2020) such that life history evolution is thought to alter the pace of a lineage's molecular clock (Hwang & Green, 2004; Moorjani et al., 2016). Environmental pressures associated with seasonality can influence life history (Varpe, 2017) and metabolic demands (Chen et al., 2018; Weber, 2009), suggesting that variation in adaptation to seasonality could have molecular evolutionary consequences. However, the linkages between molecular evolution and differential adaptations to seasonality are rarely explored.

In this study, we investigate how patterns of mitochondrial molecular evolution are related to variation in seasonal migration distance. Migratory animals survive harsh seasonal conditions on their breeding grounds by temporarily departing until conditions improve (Winger et al., 2019). Migration distance varies across species, ranging from short-distance movements within an ecoregion to hemisphere-crossing journeys. Long-distance seasonal migration

requires high metabolic performance (Weber, 2009), with potential implications for the dynamics of selection on the metabolically-important mitochondrial genes (Shen et al., 2009; Strohm et al., 2015). Migration distance has also been recognized as an important axis of life history variation (the balance between annual survival and reproduction) in birds (Bruderer & Salewski, 2009; Greenberg, 1980; Möller, 2007; Winger & Pegan, 2021). Migration distance may therefore also influence molecular evolutionary rates through effects on life history (Bromham, 2020) that are not directly associated with metabolic demands, but this relationship has not been assessed. Here, we assess how migration distance correlates with mitochondrial molecular evolution within the community of migratory birds breeding in the highly seasonal North American boreal region, and we test hypotheses regarding the roles of life history and metabolic adaptation in mediating a relationship between molecular evolution and seasonal migration.

## Metabolic adaptation, life history, and mitochondrial molecular evolution

Reliance on locomotion (migration) for adaptation to seasonality may influence selection on mitochondrial genes, which play an important role in metabolism. Mitochondria typically experience purifying selection (i.e., selection that reduces genetic variation) because most mutations in these genes are

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deleterious to fitness (Nabholz et al., 2013; Nei et al., 2010; Popadin et al., 2013). Prior studies have shown that purifying selection tends to be stronger in the mitochondria of mobile animal species compared to less mobile relatives. This pattern has been demonstrated in comparisons between flighted and flightless birds (Shen et al., 2009) and insects (Chang et al., 2020; Mitterboeck et al., 2017), between migratory and non-migratory fishes (Strohm et al., 2015), and between amphibians (Chong & Mueller, 2013) and mollusks (Sun et al., 2017) with different locomotory modes. Within flighted birds, species with slow flight and those that rely on soaring (versus flapping) have been shown to experience relaxed mitochondrial purifying selection compared to faster-flying species (De Panis et al., 2021; Shen et al., 2009). Additionally, Montoya et al. (2022) recently demonstrated that flight habit, as represented by wing morphology, is associated with nonsynonymous mitochondrial evolutionary rate variation in a large clade of South American birds (Furnariidae). These studies suggest that mitochondrial genotype plays an especially important role in fitness for organisms that rely on high-energy locomotion, including migratory birds. Metabolic demand may be strongest in long-distance migrating species if these demands primarily arise from locomotion. However, species that breed at high latitudes and migrate only short distances for the nonbreeding season may require alternative metabolic adaptations for dealing with harsh seasonal conditions since their shorter migrations do not allow them to fully escape cold, resource-depleted winters (Winger et al., 2019). The effect of variation in seasonal migration distance on the strength of mitochondrial purifying selection is unknown.

A second and distinct way in which seasonal migration may influence molecular evolution is through its relationship with life history and, consequently, molecular evolutionary rate. The slow-fast continuum of life history is commonly characterized by “life-history traits” that underly or correlate with differing rates of growth, survival, and reproduction (Read & Harvey, 1989; White et al., 2022). Within major lineages of plants, bacteria, vertebrates, and invertebrates, species with “slow” life history (i.e., long generation time, low annual fecundity, large size; Stearns, 1983) also exhibit slower molecular substitution rate than “fast” species (i.e., those with shorter generation time, higher annual fecundity, and smaller size; Nabholz et al., 2008a; Smith & Donoghue, 2008; Thomas et al., 2010; Weller & Wu, 2015). Within migratory birds breeding in the temperate zone, seasonal migration distance covaries with annual fecundity and survival such that long-distance migrants show “slower” life history (i.e., higher annual survival, lower annual fecundity) than short-distance migrants (Bruderer & Salewski, 2009; Greenberg, 1980; Winger & Pegan, 2021). As such, variation in migration distance across species may affect molecular evolutionary rates because of its association with life history variation. Specifically, the synonymous substitution rate “ $d_s$ ” often correlates with the slow-fast life history continuum (Nikolaev et al., 2007; Bromham et al., 2015; Hua et al., 2015; Table 1). Prior studies suggest that life history may influence  $d_s$  through effects on DNA replication rate or selection for mutation avoidance (reviewed in Bromham, 2020), because  $d_s$  is thought to primarily reflect the underlying mutation rate when synonymous mutations are selectively neutral (Kimura, 1983; Lanfear et al., 2014; Nei et al., 2010). Direct estimates of nuclear germline mutation rates support the hypothesis that species-level variation in mutation rate correlates with life-history traits (Bergeron et al., 2023).

## Predicting the relationship between seasonal migration distance and molecular evolution

Long-distance migratory birds have been shown to exhibit a slower life history than sympatric breeding short-distance migrants (Winger & Pegan, 2021; Figure 1). Thus, long-distance migrants travel farther in each migratory trip than short-distance migrants and may also require more trips per lifetime to achieve the level of lifetime fitness of short-distance migrants (Møller, 2007). Owing to the metabolic demands of migration and the importance of repeated migration success for fitness in long-distance migrants, the migratory phenotypes of these species are thought to be under strong variation-reducing natural selection (Conklin et al., 2017). As such, we hypothesize that long-distance migrants exhibit both lower  $d_s$  (which could reflect selection against mutation in the mitochondria; Hua et al., 2015) and stronger purifying selection in their mitochondrial genes than short-distance migrants.

To test these hypotheses, we examined the relationship between migration distance and rates of molecular evolution of the mitochondrial coding genes in a community of small-bodied migratory songbirds breeding in the boreal forests of North America. The 39 codistributed species we studied are ideal for investigating the effects of migration distance on molecular evolution because they vary greatly in migration distance (e.g., Figure 1; Supplementary Table S1), yet they otherwise share similar breeding habitat, population history, and body mass (Winger & Pegan, 2021). This system allows us to test hypotheses about migration distance while minimizing variation in other traits that could influence molecular evolution. We assessed effects of migration distance on  $d_s$  (synonymous substitution rate) and  $d_N/d_s$  (purifying selection) in a Bayesian phylogenetic framework (Lartillot & Poujol, 2011) with full mitochondrial gene sets we sequenced for 39 species. Further, we used population genetic datasets from all mitochondrial genes that we generated for 30 of the species (for a total of 1,008 samples used across all analyses) to assess effects of migration distance on purifying selection at the population level. Specifically, we assessed  $\pi_N/\pi_s$ , which is a population genetic summary statistic representing the amount of nonsynonymous versus synonymous polymorphism within a population.

## Accounting for effects of $N_e$ on substitution rates

Molecular evolution is fundamentally influenced by effective population size ( $N_e$ ), so it is often difficult to determine whether links between traits and molecular evolutionary rates are mediated by effects of traits on  $N_e$  versus other hypothesized mechanisms (e.g., Montoya et al., 2022). Therefore, we take advantage of our population-level datasets to directly test for effects of  $N_e$  on molecular evolutionary rates and purifying selection, providing valuable context for the interpretation of our results. Variation in  $N_e$  can cause variation in substitution rates because the efficiency of natural selection in purging deleterious mutations is determined by the balance between strength of selection and strength of drift, which is reflected in  $N_e$  (Ohta, 1992). Specifically, studies on empirical populations have demonstrated that populations with small  $N_e$  typically show weaker purifying selection (i.e., higher  $d_N/d_s$ , e.g., Popadin et al., 2007; Leroy et al., 2021; and higher  $\pi_N/\pi_s$ , e.g., Chen et al., 2017). Several recent studies found correlations between traits associated with life history and genetic diversity, suggesting that species with “slow”

**Table 1.** Definitions of abbreviations for molecular substitution rates and population genetic parameters and predictions for their relationships with migration distance.

Concept	Abbr.	Description and assumptions	Predictions (this study)
Synonymous substitution rate	$d_s$	Assuming synonymous sites evolve neutrally, $d_s$ primarily reflects $\mu$ (Lanfear et al., 2014; Nei et al., 2010).	Negative relationship between migration distance and $d_s$ .
Nonsynonymous substitution rate	$d_N$	Assuming nonsynonymous sites are generally deleterious, $d_N$ is influenced by both $\mu$ and $N_e$ (reviewed in Nei, 2005).	NA
$d_N/d_s$ ratio	$d_N/d_s$	Assuming nonsynonymous mutations are generally deleterious, $d_N/d_s$ reflects strength of purifying selection on $d_N$ while accounting for variation in $\mu$ . Low $d_N/d_s$ = strong purifying selection. (Kryazhimskiy & Plotkin, 2008; Nei, 2005).	Negative relationship between $\theta$ and $d_N/d_s$ , reflecting the influence of $N_e$ on $d_N/d_s$ . Negative relationship between migration distance and $d_N/d_s$ , indicating positive relationship between migration distance and purifying selection strength.
Mutation rate	$\mu$	May be influenced by life history; reviewed in (Bromham, 2020).	NA, $\mu$ not measurable in our data.
Effective population size	$N_e$	Defined as the ideal population size experiencing the same level of genetic drift as observed in the data (Waples, 2022). Estimated in mitochondrial data as $\theta/\mu$ (Nabholz et al., 2008a; Watterson, 1975).	NA, see $\theta$ .
Theta	$\theta$	Population genetic parameter representing genetic variation. Assuming low variation in $\mu$ , variation in $\theta$ primarily reflects variation in $N_e$ .	Negative relation between $\theta$ and $d_N/d_s$ and between $\theta$ and $\pi N/\pi S$ .
Synonymous nucleotide diversity	$\pi_s$	Population genetic parameter representing population-level nucleotide diversity at synonymous sites.	NA
Nonsynonymous nucleotide diversity	$\pi_N$	Population genetic parameter representing population-level nucleotide diversity at synonymous sites.	NA
$\pi_N/\pi_s$ ratio	$\pi_N/\pi_s$	Reduction of $\pi_N$ compared to $\pi_s$ is expected to reflect natural selection, but the relationship is more complex than with $d_N/d_s$ .	Negative relationship between migration distance and $\pi_N/\pi_s$ , indicating positive relationship between migration distance and selection. Negative relationship between $\theta$ and $\pi_N/\pi_s$ , indicating purifying selection on nonsynonymous polymorphisms.

life histories often have low  $N_e$  (Brüniche-Olsen et al., 2021; De Kort et al., 2021; Romiguier et al., 2014). There is also evidence that migratory behavior is predictive of population genetic diversity, a parameter associated with  $N_e$  (García-Berro et al., 2023). It is therefore important to assess whether molecular rate variation across species can alternatively be explained by confounding variation in  $N_e$ .

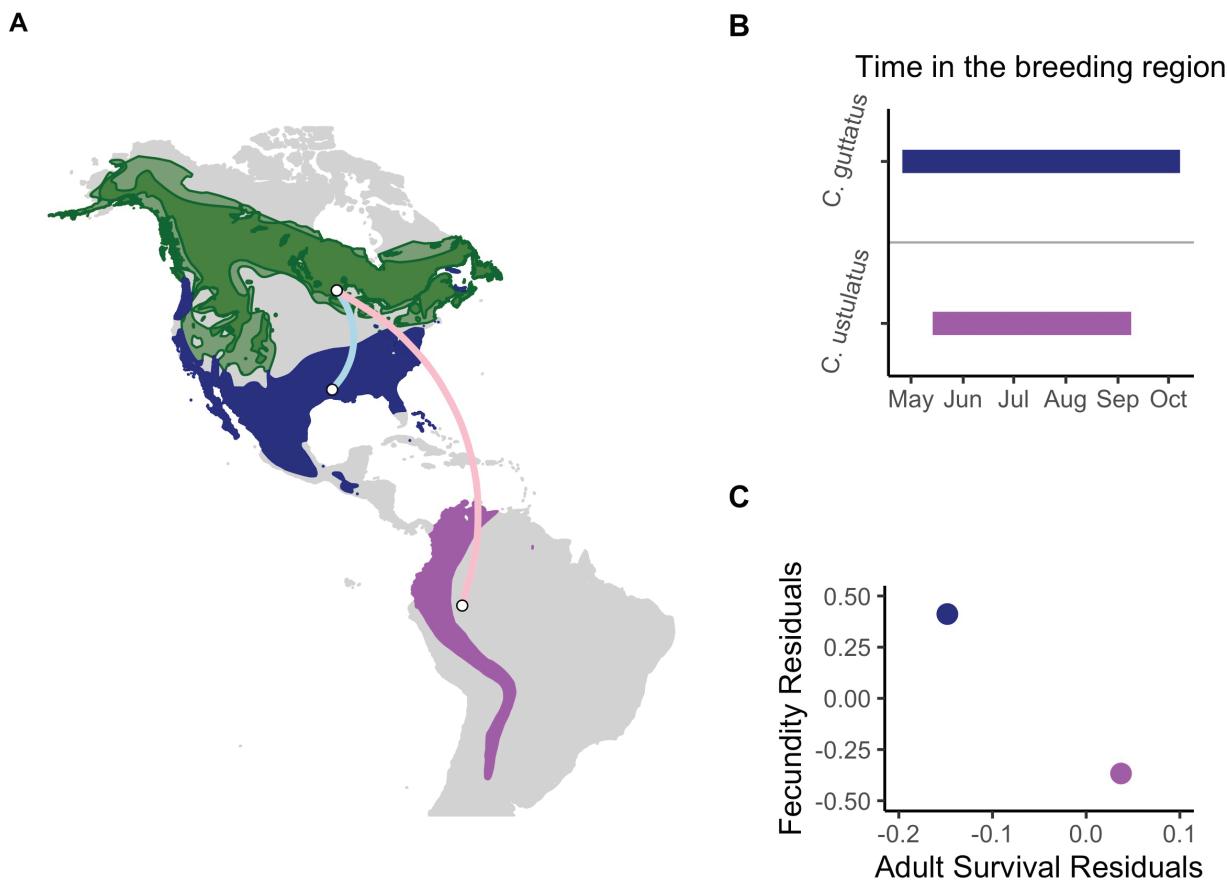
Finally, we use estimates of  $N_e$  to test the assumption of neutral evolution at synonymous sites, which is a fundamental assumption underlying the hypothesis that  $d_s$  reflects mutation rate (Kimura, 1983; Lanfear et al., 2014; Nei et al., 2010). If synonymous substitutions evolve neutrally, we expect that  $d_s$  should not show a relationship with  $N_e$  because the processes that lead to a relationship between  $N_e$  and substitution rate involve natural selection.

## Methods

### Study system

We focused on 39 species of migratory birds breeding in the North American boreal forest, representing 11 families

(Supplementary Table S1). These are the same species for which a correlation between migration distance and the slow-fast life history continuum—*independent of body size*—has been demonstrated using data on annual fecundity and survivorship (Winger & Pegan, 2021). We focus our analyses on codistributed populations of the eastern boreal belt of North America (Omernik, 1987; Omernik & Griffith, 2014; Supplementary Figure S1). Some species' breeding ranges extend into other ecoregions (e.g., the mountain west or the temperate forests south of the boreal zone), but in these cases, we only analyze samples from the boreal portion of the range to assess sympatric populations. The species in the dataset exhibit broad variation in migration distance, with their geographic range centroids shifting between 1,048 and 7,600 km between the breeding and nonbreeding periods (Figure 2; Supplementary Table S1; Winger & Pegan, 2021). These centroid shifts represent migratory strategies ranging from short-distance movements within the temperate region to the movement of an entire population across ocean and land barriers from North America to South America. All species are less than 100 g in mass (range of mean mass across



**Figure 1.** An example contrast between a shorter-distance migrant *Catharus guttatus* and a closely related longer-distance migrant *Catharus ustulatus swainsoni* illustrates the relationship between migration distance and life history in our study system. Both species have broadly overlapping breeding ranges (green ranges in northern North America), but *C. guttatus* (dark blue nonbreeding range in southern North America) migrates a shorter distance (blue migratory route) than *C. u. swainsoni* (purple nonbreeding range in South America, pink migratory route) (panel A). Accordingly, *C. guttatus* spends more time in its breeding range than *C. u. swainsoni* (panel B). With more time in the breeding range and the possibility of raising a second brood, the short-distance migrant has higher fecundity but lower adult survival—that is, faster life history—than the long-distance migrant (panel C, showing model residuals from mass-corrected analysis of fecundity and survival). The short-distance migrant spends the winter in colder, more resource-depleted regions than the long-distance migrant. Data in B and C from Winger and Pegan (2021). Species maps from BirdLife International and NatureServe (2014). Our sampling for this study occurred only within the eastern boreal belt (Supplementary Figure S1).

species is 6–87 g; Supplementary Table S1) and are broadly similar in habitat use. They are all territorial species with socially monogamous breeding systems, which suggests that they probably do not vary substantially in population sex ratio (which can affect  $N_e$ ), although empirical sex ratio data is not available for these species. Small songbirds are typically capable of breeding in their second year, and this is true of all species in our study that have been assessed (Billerman et al., 2022). Additionally, our study species share relatively similar demographic histories, with population expansions estimated to have mostly occurred during the period of glacial retreat that preceded the Last Glacial Maximum (~57,000 years before present; Kimmitt et al., 2023).

#### Life history covariates: migration distance and mass

Direct measurements of migration distance of individuals are lacking for most of the species in our system, so we used the distance between the centroid of a species' breeding range and the centroid of its nonbreeding range to represent the migration distance of the species. Although the distance between centroids does not represent individual variation in migration distance within a species, this metric captures

broad differences in migratory strategies between species. Our method for calculating the distance between range centroids is described in detail in Winger and Pegan (2021). We included mass as a covariate in our analyses because body mass and rates of molecular evolution are often associated (Figuet et al., 2014; Nabholz et al., 2016), and the relationships between survival and fecundity and migration distance demonstrated by Winger and Pegan (2021) were recovered after accounting for variation in mass. We obtained mass data from Dunning (2008) and Billerman et al. (2022).

#### Sampling and DNA sequencing

Our analysis of the relationship between migration distance and  $d_s$  requires one mitochondrial genome for each species in the study, while analyses of  $N_e$  and  $\pi_N/\pi_S$  require population-level sampling. For our analysis of  $d_s$ , we obtained whole mitochondrial genomes from one individual of each of the 39 species in our study by sequencing DNA from tissue samples associated with a museum specimen, as described below. These specimens were collected during the breeding season from near the longitudinal center of the boreal forest (Manitoba, Minnesota, or Michigan; Supplementary Tables S1 and S2). For two species (*Contopus cooperi* and *Euphagus*

*carolinus*), we used specimen-vouchered tissue samples of individuals salvaged during migration in Michigan from collision mortalities.

For our population-level analyses, we generated a large dataset of 999 additional mitochondrial genomes for 30 of the 39 species, building on a dataset of 19 species from Kimmitt et al. (2023). Our larger dataset includes complete coding sequences for 8–49 individuals per species (mean 33 individuals per species; Supplementary Table S1). These individuals were sampled during the breeding season across a longitudinal transect of the boreal forest from Alberta to the northeastern United States (Supplementary Figure S1; Supplementary Table S2). Except for 24 blood samples from New York state, all sequences we used came from frozen or ethanol-preserved tissue samples associated with museum voucher specimens provided by several museum institutions (Supplementary Table S2; Acknowledgments).

We obtained high-depth mitochondrial genomes captured as a byproduct from low-coverage whole genome sequencing, as described in detail in Kimmitt et al. (2023). Briefly, sequencing libraries were prepared using a modified Illumina Nextera library preparation protocol (Schweizer et al., 2021) and sequenced on HiSeq or NovaSeq machines using services provided by Novogene and the University of Michigan Advanced Genomics Core. We used NOVOPlasty v4.3.1 (Dierckxsens et al., 2016) to assemble mitochondrial contigs, specifying a target genome size of 20–30 kb and using a k-mer of 21. We provided NOVOPlasty with a conspecific mitochondrial seed sequence (Supplementary Table S1) for each species. We annotated the contigs built by NOVOPlasty using Geneious Prime 2020.2.2 (<https://www.geneious.com>) with copies of mitochondrial genes from GenBank (Supplementary Table S1). Whenever applicable in the filtering and analysis steps described below, we used options specifying the vertebrate mitochondrial code.

Our initial dataset across all species contained mitochondrial sequences from 1,229 total individuals. To ensure data quality, we used BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to check species identity and we removed samples with evidence of species misidentification, chimerism, or introgression from related species (14 samples removed). We aligned and translated sequences with the R package DECIPHER v2.18.1 (Wright, 2016), and we visually inspected each alignment, ensuring that sequences contained no premature stop codons or other alignment issues. We used DECIPHER to remove partial stop codons and the untranslated C in the ND3 sequence of woodpecker (Picidae) species (Mindell et al., 1998). As our population analyses require complete data matrices, we excluded individuals with incomplete datasets (those with assemblies that were missing genes and/or with ambiguous base calls; 202 samples removed). We removed five individuals during population structure analysis, described below. This data filtering resulted in 1,008 complete mitochondrial coding sequences: 999 individuals across 30 species used in the population genomic analyses plus one sequence for each of the nine additional species we used only in the interspecific Coevol analyses. We concatenated the 13 mitochondrial coding sequences for analysis. The full list of samples, including those removed from the analyses, can be found in Supplementary Table S2.

### Estimating $\theta$ as a proxy for $N_e$

We used  $\theta$  as a proxy for effective population size ( $N_e$ ).  $N_e$  can be calculated based on  $\theta$  and mutation rate (Watterson, 1975; Nabholz et al., 2008b; Table 1), but accurate estimates

of mitochondrial mutation rate are lacking for most non-model organisms. Accordingly, many empirical studies interested in  $N_e$  focus on genetic diversity, which is thought to reflect the harmonic mean of  $N_e$  over time and which does not require mutation rate information to calculate (e.g., Ellegren & Galtier, 2016; Hague & Routman, 2016). We hereafter use the genetic diversity parameter  $\theta$  as a proxy for  $N_e$ . We used LAMARC v2.1.10 (Kuhner, 2006) to estimate  $\theta$  for each species. LAMARC estimates  $\theta$  in a maximum likelihood framework using information about the intervals between coalescence events from sampled genealogies, which the program generates from population sequence data (Felsenstein, 1992; Kuhner, 2006; Kuhner et al., 1995). We imported our population-level full mitochondrial coding sequence data into LAMARC after converting our concatenated fasta files into the phylip format for each species. We used the program's likelihood-based method in 10 initial chains (samples = 500, discard = 1,000, interval = 20) and 2 final chains (samples = 10,000, discard = 1,000, interval = 20). We used the F84 model of molecular evolution, and we provided a separate transition/transversion ratio for each species using values we calculated from population mitochondrial coding sequence datasets using the R package "spider" (Supplementary Table S1; Brown et al., 2012). All other input parameters were left at their default values. We examined the output for each species to check for chain convergence, and we ran two replicate chains for each species to make sure they produced consistent results. For five species (*Leiothlypis ruficapilla*, *Setophaga castanea*, *Setophaga coronata*, *Setophaga fusca*, and *Vireo olivaceus*), we repeated LAMARC for 25 initial chains instead of 10 to improve convergence and used the values from these longer runs.

Estimation of  $\theta$  can be biased by purifying selection, and the magnitude of this bias may vary across species due to differences in purifying selection and sample size (Subramanian, 2016). To evaluate whether these biases influence our results, we compared  $\theta$  to  $\pi_s$ , or nucleotide diversity at synonymous polymorphisms, which is not biased by purifying selection assuming that synonymous sites are evolving neutrally. We estimated  $\pi_s$  from each species using the python package egglib v3.1.0 (De Mita & Siol, 2012) and calculated Pearson's correlation coefficient between  $\theta$  and  $\pi_s$ . We also repeated Coevol models (described below) with each proxy of  $N_e$  to assess whether the choice of proxy influences our results.

### Population structure

Our population-level analyses (estimation of  $\theta$  and  $\pi_N/\pi_s$ ) assume no geographic population genetic structure within the samples used. To check this assumption, we calculated mitochondrial genetic distance between all individuals within each species using "nei.dist()" from the R package poppr v2.9.3 (Kamvar et al., 2014) and created a neighbor-joining tree with "nj()" from the R package ape v5.6-2 (Paradis & Schleip, 2019). We identified and removed four individuals from *Regulus satrapa* and one individual from *Oporornis agilis*, all from Alberta in the far western part of our sampling area, that were genetically distinct from all other samples in their respective species. Otherwise, there was little evidence of geographic genetic structure in the mitochondrial genome in these species.

### Estimating $d_s$ and $d_N/d_s$ and their correlations with traits associated with life history

We used Coevol v1.6 (Lartillot & Poujol, 2011) to evaluate associations between migration distance and molecular

evolutionary rates using a single representative of each species. Coevol uses a Bayesian phylogenetic framework to estimate  $d_s$  and  $d_N/d_s$  and to simultaneously measure the relationship between these traits and covariates of interest (migration distance, mass, and  $\theta$ ). We included mass to account for the expected relationship between mass and molecular rates (Nabholz et al., 2016). Models with mass also provide a useful point of comparison, allowing us to ask whether migration distance correlates with  $d_s$  and  $d_N/d_s$  to the same extent as (or more or less than) this well-studied life-history trait. Similarly, including  $\theta$  in the models allows us to assess whether variation in  $N_e$  accounts for differences in molecular evolutionary rates.

We provided Coevol with one complete concatenated mitochondrial coding sequence from each species and a phylogenetic tree (Figure 2) we generated with data from birdtree.org (Jetz et al., 2012) as described in Pegan and Winger (2020). In brief, we sampled 2,000 trees comprising all North American bird species from the Jetz et al. dataset, and we used the python package “DendroPy” (Sukumaran & Holder, 2010) to generate a consensus tree. We then trimmed this tree to include only the 39 species used in this study. Importantly, Coevol uses the phylogenetic tree for topological information but estimates relative branching times from the sequence data (Lartillot & Poujol, 2021). Coevol also does not require prior information about mutation rates. We investigated the potential effects of phylogenetic tree topology on our results by sampling 10 random marginal trees from the original tree dataset (trimmed to include only relevant species) and rerunning Coevol on each tree, which we found to produce consistent results (Supplementary Table S3).

We created two data subsets for Coevol models: one subset contained all species in the study and included mass and migration distance as covariates. The other subset included the 30 species for which we had population-level data available; for these, we included  $\theta$  as a covariate in addition to mass and migration distance. We also repeated these analyses using  $\pi_s$  as a proxy for  $N_e$  instead of  $\theta$ . For each data subset, we ran Coevol four times: two repeated analyses with the option “dnds” (estimating  $d_s$ ; models 1 and 2, Table 2) and two with “dsom” (estimating  $d_N/d_s$ ; models 3 and 4, Table 2). We let each analysis run for approximately 20,000 steps and examined the resulting trace files to ensure convergence and evaluate estimated sample sizes (ESS). All models converged, and all parameters had ESS > 300. We removed the first 500 steps of each analysis and thinned the posterior sample to retain every 10th step to reduce autocorrelation. Replicate analyses produced highly similar estimates, and the values we report here represent the mean value of estimates made by each replicate. We present full Coevol model output in Supplementary Tables S4–S6.

The method implemented in the Coevol software estimates correlation coefficients between substitution rates and each covariate, as well as partial correlation coefficients (which hold constant the effects of other covariates in the model). Each correlation or partial correlation coefficient is accompanied by a posterior probability. In the case of Coevol, posterior probabilities near 0 indicate strong support for a negative relationship, while posterior probabilities near 1 indicate strong support for a positive relationship (Lartillot & Poujol, 2021).

#### $\pi_N/\pi_s$

$\pi_N/\pi_s$  is measured by comparing polymorphisms among individuals within a species rather than by comparing between

**Table 2.** A summary of analyses. Models 1 and 2 use Coevol test our hypothesis that synonymous substitution rate ( $d_s$ ) is influenced by migration distance, with mass and  $\theta$  as additional covariates. Models 3 and 4 use the same approach with Coevol to estimate correlations between traits of interest and  $d_N/d_s$ . Models including  $\theta$  use only 30 species because we did not have population-level data available to estimate  $\theta$  for all 39 species. Coevol does not analyze molecular evolutionary parameters based on population-level data, so we used linear modeling to test whether traits of interest influence  $\pi_N/\pi_s$  (model 5). Finally, we also used linear modeling to test for potential confounding relationships between  $\theta$  and life history-associated traits of interest (mass and migration distance; model 6).

		Data subset	Method
1	$d_s \sim$ migration distance + mass	full (39 species)	Coevol
2	$d_s \sim$ migration distance + mass + $\theta$	theta (30 species)	Coevol
3	$d_N/d_s \sim$ migration distance + mass	full (39 species)	Coevol
4	$d_N/d_s \sim$ migration distance + mass + $\theta$	theta (30 species)	Coevol
5	$\pi_N/\pi_s \sim$ migration distance + mass + $\theta$	theta (30 species)	linear modeling
6	$\theta \sim$ migration distance + mass	theta (30 species)	linear modeling

species in a phylogenetic framework (and thus cannot be estimated by Coevol). We estimated  $\pi_N/\pi_s$  from each species with population-level fasta alignments, using the python package egglib v3.1.0 (De Mita & Siol, 2012) to create a “CodingDiversity” class with attributes describing nucleotide diversity at codons with synonymous or nonsynonymous polymorphisms. Predictions about the effect of purifying selection on polymorphisms are more complex than predictions about substitution rates because within-population variation can be purged by strong directional selective sweeps in addition to purifying selection (Kryazhimskiy & Plotkin, 2008). We predict a negative relationship between migration distance and the  $\pi_N/\pi_s$  ratio, indicating stronger selection (directional or purifying) on mitochondrial function in long-distance migrants. We used linear modeling to test for an effect of migration distance, mass, and  $\theta$  on  $\pi_N/\pi_s$  (Supplementary Tables S7 and S8). Prior to linear modeling, we centered and scaled our predictors using the function “standardize” from the R package “robustHD” (Alfons, 2021) with the mean value of each predictor as the center. We used a similar linear modeling approach to test whether  $\theta$  exhibits a relationship with mass or migration distance to ensure that apparent relationships between these traits and molecular rates are not confounded by correlation with  $\theta$ .

For each response variable ( $\theta$  and  $\pi_N/\pi_s$ ; Supplementary Tables S7 and S8), we first created a model with all covariates of interest. We then used the function “phylosig()” from the R package phytools v0.7-70 (Revell, 2010) to test for phylogenetic signal in the model’s residuals (Revell, 2012). For both response variables, the estimate of  $\lambda$  (phylogenetic signal) was low (<0.2), and the *p*-value for evidence of phylogenetic signal was > 0.8, so we proceeded with linear modeling rather than using models with phylogenetic covariance matrices. For each response variable, we created a null (intercept-only) model with no predictors and models with all possible combinations of our predictors of interest, and we used the function “model.sel()” from the R package MuMIn v1.43.17 (Barton,

2019) to compare the models' corrected Akaike information criterion (AICc).

## Results

For each model, we report correlation coefficients between traits of interest (migration distance, mass, or  $\theta$ ) and molecular evolutionary rates ( $d_s$  or  $d_N/d_s$ ) and assess their strength based on posterior probabilities ( $pp$ ), which are close to 0 in the case of a strong negative correlation and close to 1 in the case of a strong positive correlation. We also report partial correlation coefficients and their posterior probabilities, which indicate the relationship between variables of interest after accounting for the effects of all other covariates.

The Pearson correlation coefficient between  $\theta$  and  $\pi_s$  was high (0.77;  $p < .0001$ ), suggesting that these two variables are consistent proxies of  $N_e$ . We found that results of Coevol models with  $\theta$  as a covariate were consistent with results of models using  $\pi_s$ , so we conclude that results of analyses with  $\theta$  are not driven by biases in the estimation of  $\theta$ . We hereafter focus on models using  $\theta$ , and full results of Coevol models using  $\pi_s$  instead of  $\theta$  are presented in [Supplementary Table S6](#).

### Correlations between migration distance and molecular evolutionary rates ( $d_s$ and $d_N/d_s$ )

Our analyses show that migration distance negatively correlates with  $d_s$  across the 39 species we studied, consistent with our initial predictions ([Figure 2](#); [Supplementary Figure S2](#)). For Coevol models with the full species set, the correlation coefficient relating migration distance to  $d_s$  was -0.39 with a posterior probability ( $pp$ ) of 0.018, indicating strong support for a negative relationship. The partial correlation coefficient (which accounts for mass) between migration distance and  $d_s$  was -0.47 ( $pp = 0.0090$ ).

We did not detect evidence of a relationship between migration distance and  $d_N/d_s$  (correlation coefficient = 0.096,  $pp = 0.63$ ). The partial correlation coefficient (accounting for mass) between migration distance and  $d_N/d_s$  indicated that this relationship was not well supported (partial correlation coefficient = 0.26,  $pp = 0.82$ ).

Results from the Coevol models of the subset of 30 species for which we had estimates of  $\theta$  were consistent with results produced by the full subset (39 species) models, although support for the correlation between  $d_s$  and migration distance was slightly weaker. In the model estimating  $d_s$ , migration distance had a correlation coefficient of -0.43 ( $pp = 0.02$ ) and a partial correlation coefficient of -0.31 ( $pp = 0.11$ ). In the model estimating  $d_N/d_s$ , we did not find support for a relationship with migration distance, as this variable had a correlation coefficient of -0.15 ( $pp = 0.32$ ) with  $d_N/d_s$  and a partial correlation coefficient of -0.010 ( $pp = 0.52$ ) with  $d_N/d_s$ .

### Correlations between mass and molecular evolutionary rates ( $d_s$ and $d_N/d_s$ )

Coevol models with the full species set support the expected negative relationship between mass and  $d_s$  (correlation coefficient = -0.28,  $pp = 0.065$ ; [Figure 2](#)). This relationship weakens when effects of migration distance are accounted for (i.e., with partial correlation coefficient = -0.18,  $pp = 0.20$ ). We did not find a strong correlation between mass and  $d_N/d_s$  (correlation coefficient = -0.25,  $pp = 0.19$ ; partial correlation coefficient = -0.072,  $pp = 0.41$ ). In models of  $d_s$  with the subset of 30 species that included  $\theta$  as a predictor, mass had a

correlation coefficient of -0.17 ( $pp = 0.21$ ) and a partial correlation coefficient (which controls for the effects of migration distance) of -0.23 ( $pp = 0.15$ ). In models of  $d_N/d_s$  from this subset, mass had a correlation coefficient of 0.16 ( $pp = 0.7$ ) and a partial correlation coefficient of 0.26 ( $pp = 0.84$ ).

### The influence of $N_e$ on molecular evolutionary rates

In models using the subset of 30 species with population-level data, we did not find evidence for a correlation between  $\theta$  and  $d_s$  (correlation coefficient = -0.23,  $pp = 0.15$ ; partial correlation coefficient = -0.12,  $pp = 0.67$ ). This result is consistent with neutral evolution of synonymous sites among the species we studied. By contrast, we found strong support for the nearly neutral theory's predicted negative relationship ([Leroy et al., 2021](#); [Ohta, 1992](#); [Popadin et al., 2007](#)) between  $\theta$  and  $d_N/d_s$  (correlation coefficient = -0.60,  $pp = 0.025$ ; partial correlation coefficient = -0.57,  $pp = 0.031$ ; [Figure 3](#)), indicating stronger purifying selection in species with higher  $N_e$ .

### Linear modeling of $\pi_N/\pi_s$

In comparison of AICc, the highest-ranked model of  $\pi_N/\pi_s$  showed a strongly supported negative relationship between  $\theta$  and  $\pi_N/\pi_s$  ([Figure 4](#); [Supplementary Table S7](#), model weight 0.55), as predicted if purifying selection is stronger in species with higher  $N_e$ . Compared to a model with  $\theta$  alone, a model with both  $\theta$  and migration distance shows an increase in multiple  $r^2$  from 0.15 to 0.28 and a decrease in AICc by more than two units, suggesting the inclusion of migration distance improves model fit. However, contrary to our prediction, migration distance has a weak positive relationship with  $\pi_N/\pi_s$  ([Figure 4](#)). The estimated coefficient relating  $\theta$  and  $\pi_N/\pi_s$  in the best-fit model is -0.027 (std error = 0.01) and the estimated effect of migration distance from the best-fit model is 0.022 (std error = 0.01). Model comparison did not support the inclusion of mass as a predictor of  $\pi_N/\pi_s$  ([Supplementary Table S7](#)).

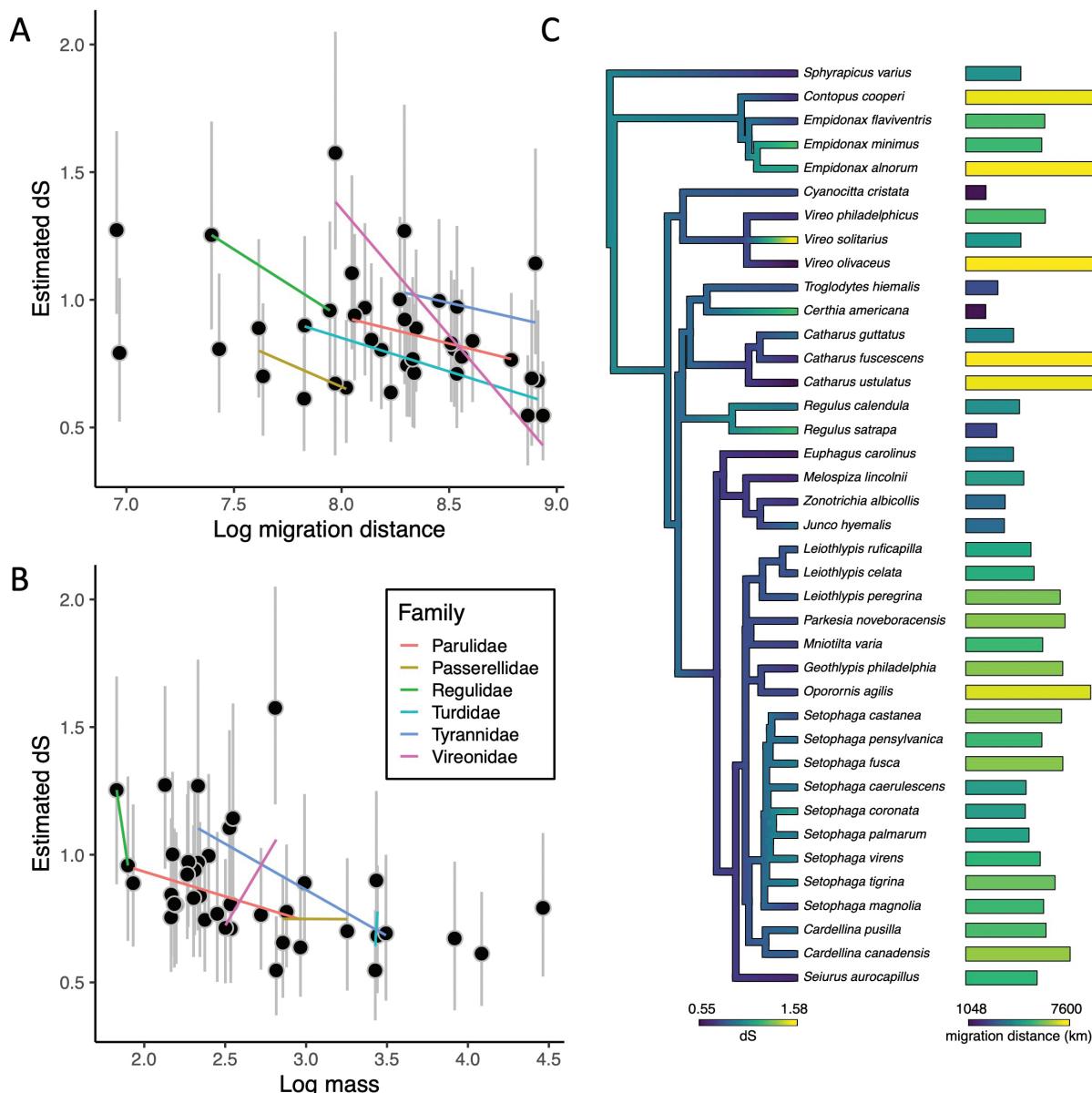
### $N_e$ does not confound inferred correlations

We used linear modeling to test whether migration distance or mass show a relationship with  $\theta$ , our proxy of  $N_e$ . We did not find strong evidence that mass or migration distance are correlated with  $\theta$  among the 30 species we studied. The null model for  $\theta$  (an intercept-only model with no predictors) showed the lowest AICc, suggesting that the addition of mass and migration distance as predictors did not improve model fit ([Supplementary Table S8](#), model weight 0.45). However, the model with migration distance as a predictor was within two AICc units of the null model and showed a model weight of 0.30, indicating considerable model uncertainty. The estimated effect of migration distance on  $\theta$  was positive but had a negligible effect size in the second-best model (estimate = 0.0017, std error = 0.0013, model multiple  $r^2 = 0.054$ ).

## Discussion

### Seasonal migration distance correlates with mitochondrial $d_s$

We examined the relationship between life history and patterns of mitochondrial sequence evolution within North American boreal birds. These species occupy a region where strong seasonality demands specialized adaptations that carry life history tradeoffs ([Varpe, 2017](#); [Winger & Pegan, 2021](#)). Our results implicate the life-history axis of seasonal



**Figure 2.**  $d_S$  versus traits associated with life history (A, B) and a phylogenetic tree showing  $d_S$  and migration distance for each species (C). In panels A and B, posterior mean tip estimates of  $d_S$  (black dots) from Coevol are shown compared to migration distance (A), and mass (B) from models using our full species set. Gray vertical bars indicate 95% credible intervals for each estimate. These analyses reveal that both migration distance and mass have a negative relationship with  $d_S$ . Plotted lines use linear models to visualize the relationship between estimated tip  $d_S$  and a given covariate within each family of birds (when represented in our dataset by two or more species), demonstrating a consistently negative relationship between  $d_S$  and migration distance within and among major clades in our system. In panel C, the phylogenetic tree was created in phytools (Revell, 2012) and is colored based on posterior mean tip and node estimates of  $d_S$  from Coevol.

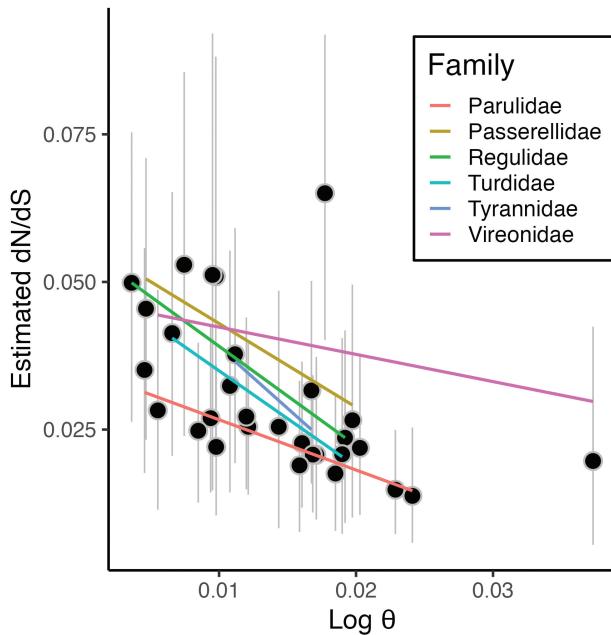
migration distance as a novel correlate of mitochondrial synonymous substitution rate ( $d_S$ ). Previous work demonstrates that, even after accounting for body size, long-distance migrants in this system have slower life history strategies than short-distance migrants, showing higher annual adult survival and lower fecundity (Winger & Pegan, 2021). Here, we find that the slow life history of long-distance migrants is accompanied by a slower rate of neutral molecular evolution in the mitochondria of these species compared with that of shorter-migrating species in the region. Indeed, among the 39 species we studied, the correlation between migration distance and  $d_S$  is stronger than the correlation between mass and  $d_S$ , which is notable given that the relationship between

mass and substitution rate has been documented in previous work (Nabholz et al., 2016). As such, we suggest that the association between migration distance and the slow-fast life history continuum extends to effects on  $d_S$ .

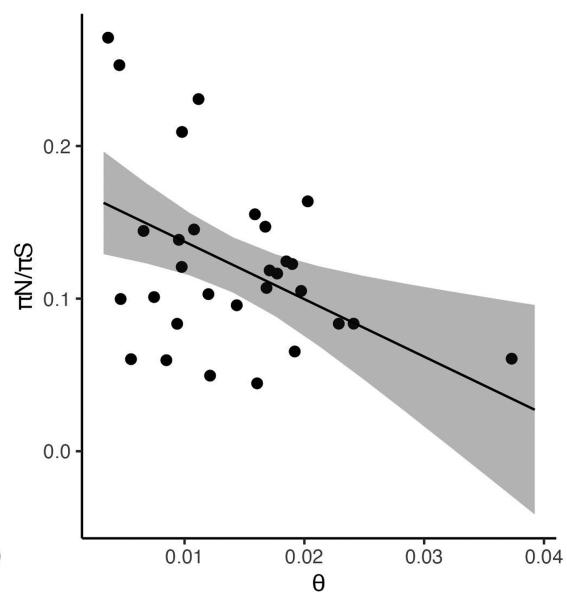
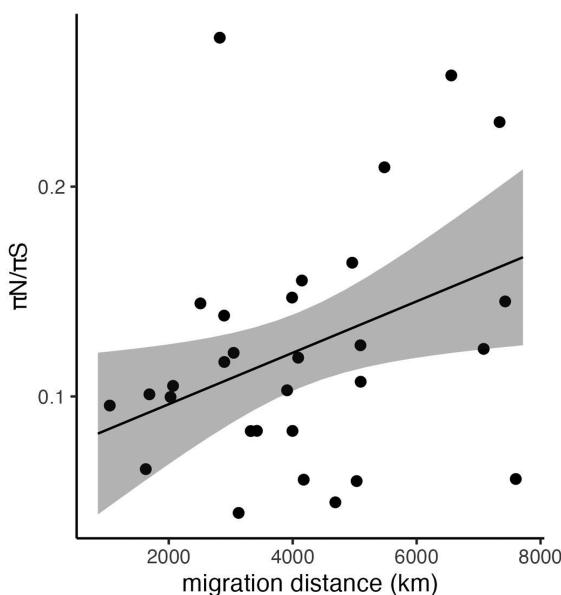
#### What evolutionary processes link migration distance with mitochondrial $d_S$ ?

Substitution rates are fundamentally influenced by mutation rate, which provides new molecular variants with potential to become substitutions, and by natural selection, which influences whether variants are fixed as substitutions or lost. The correlation between migration distance and  $d_S$  therefore reflects one or both processes.  $d_S$  is often treated as a proxy

for mutation rate alone based on the assumption that natural selection does not operate on synonymous sites (Nei et al., 2010), but in some cases, synonymous sites are known to evolve nonneutrally (Chamary et al., 2006; Künstner et al., 2011; Wei et al., 2014; Wynn & Christensen, 2015). If



**Figure 3.**  $d_N/d_S$  versus  $\theta$ . Posterior mean tip estimates (black dots) of  $d_N/d_S$  are shown compared to  $\theta$  from a Coevol model including species for which we could estimate  $\theta$ . Gray vertical bars indicate 95% credible intervals for each estimate. As in Figure 2, plotted lines use linear models to visualize the relationship between mean tip  $d_N/d_S$  and  $\theta$  within each family of birds (when represented in our dataset by two or more species), demonstrating a consistently negative relationship between  $\theta$  and  $d_N/d_S$  within and among major clades in our system.



**Figure 4.** The relationship between  $\pi_U/\pi_S$  and migration distance (left) and  $\theta$  (right).  $\pi_U/\pi_S$  is strongly influenced by  $\theta$ , as expected if purifying selection removes more nonsynonymous variation in species with larger  $N_e$ .  $\pi_U/\pi_S$  increases with migration distance, after accounting for effects of  $\theta$ . Regression lines and 95% confidence intervals show the marginal effect of each variable as calculated by “ggpredict()” from the R package ggeffects v0.16.0 (Lüdecke, 2018) using the best-fit model, which included both predictors.

synonymous sites are not evolving neutrally, nearly neutral theory suggests that the relationship between  $d_s$  and migration distance could be explained by larger  $N_e$  in long-distance migrants (Ohta, 1992). We tested the key assumption that synonymous sites evolve neutrally by assessing the relationship between  $d_s$  and our proxy for  $N_e (\theta)$  (Supplementary Table S4). We found no correlation, suggesting that synonymous sites are indeed evolving neutrally in our system. We also found no correlation between  $\theta$  and migration distance (Supplementary Table S8). Together, these results suggest that variation in  $d_s$  among species with different migration distances is not well explained by variation in natural selection or effective population size. Rather, we suggest that the negative relationship between migration distance and  $d_s$  may reflect a negative relationship between migration distance and mutation rate.

#### Why might long-distance migrants have a lower mitochondrial mutation rate?

We predicted that migration distance would correlate with  $d_s$  because of its relationship with the slow-fast continuum of life history in these species independent of body size (Winger & Pegan, 2021). In turn, a species’ position on the slow-fast life history continuum is hypothesized to affect mutation rate (Bromham, 2020). There are several potential mechanisms to explain the link between life history and mutation rate, and the relative importance of each is not clear (Bromham, 2020). The “copy error effect” hypothesis suggests that the explanation is related to generation time, assuming that “fast” species with short generation times and young age at first reproduction experience higher rates of germline replication (and thus replication-induced mutation) than species with “slow” life histories (Lehtonen & Lanfear, 2014; Li et al., 1996; Thomas et al., 2010).

However, recent studies comparing cell division rates with directly-measured mutation rates suggest that replication-induced copy errors may not be the only driver of differences in

mutation rate between lineages (Wang et al., 2022; Wu et al., 2020). The “mutation avoidance” hypothesis offers another nonexclusive explanation for lower  $d_s$  in organisms with slow life history based on higher mutation costs in longer-lived species (Bromham, 2020). Under this hypothesis, organisms with slow life history are predicted to have adaptations that reduce the introduction of mutations from DNA damage or DNA replication and repair processes (Cagan et al., 2022; Galtier et al., 2009; Tian et al., 2019; Zhang et al., 2021). Long-distance migrants may be especially sensitive to the costs of mitochondrial mutation, which may cause mitochondrial senescence (Galtier et al., 2009; Hua et al., 2015), because of the high physical performance demanded by their migratory behavior across their entire lifespans (Conklin et al., 2017; Møller, 2007). Further research is necessary to understand what processes contribute to the apparent reduction of mutation rate in species at the slow end of the slow-fast continuum of life history.

Another possible link between migration distance and mutation rate is oxidative damage from metabolism, which is recognized as a potential source of mutation rate variation (Martin & Palumbi, 1993; Gillooly et al., 2005; Berv & Field, 2018; but see Galtier et al., 2009; Lanfear et al., 2007). Thus, a potential explanation for our results—lower mitochondrial  $d_s$  in long-distance migrants—is that long-distance migrants incur less metabolically-induced DNA damage than short-distance migrants. This explanation is initially surprising in light of studies showing that migratory birds experience oxidative damage from endurance flight (Jenni-Eiermann et al., 2014; Skrip & McWilliams, 2016). However, we suggest that three plausible and nonexclusive scenarios could lead to lower metabolically-induced DNA damage in long-distance compared to short-distance migrants. First, long-distance migrants may have better adaptations for flight efficiency (Elowe et al., 2023; Weber, 2009), reducing the oxidative damage they experience per mile traveled. Second, the mutation avoidance hypothesis predicts that long-distance migrants may have more efficient DNA repair mechanisms than short-distance migrants, which could reduce metabolically-induced mutation rates even when long-distance flight does induce high oxidative stress. Last, short-distance migrants in our boreal study system may experience greater oxidative damage arising from their increased need for winter cold tolerance than long-distance migrants that winter in the tropics. The mitochondria also play an important role in the metabolic challenge of maintaining homeostasis during cold weather and resource shortages (Bicudo et al., 2001; Chen et al., 2018). Short-distance boreal migrants likely face more of these kinds of challenges than long-distance migrants during migration and winter (Winger & Pegan, 2021). Despite the view that long-distance migration is an extreme performance challenge, its alternative—spending the winter within the temperate zone—is also an extreme metabolic challenge for small-bodied homoeothermic endotherms that do not hibernate (Dawson & Yacoe, 1983; Winger et al., 2019). Further investigation of the comparative metabolic challenges faced by short versus long-distance boreal migrants is needed to clarify whether and how migration distance influences metabolically-induced mutation in the mitochondria.

### Purifying selection is not stronger in long-distance migrants

Whereas evolutionary rate at synonymous sites ( $d_s$ ) may primarily reflect mutation rate, evolution at nonsynonymous

sites is expected to strongly reflect natural selection because nonsynonymous mutations alter the amino acid sequence of a gene’s protein product. We found that the ratio of nonsynonymous to synonymous substitutions ( $d_N/d_s$ ) among our species is universally much less than 1 (Figure 3), indicating that the mitochondrial genes we studied are under purifying selection in all species in the system. We similarly found low ratios of nonsynonymous to synonymous polymorphisms within each population ( $\pi_N/\pi_s$ ; Figure 4), which is also consistent with purifying selection. Moreover, both  $d_N/d_s$  and the  $\pi_N/\pi_s$  ratio are strongly correlated with  $\theta$ , our proxy for  $N_e$  (Figures 3 and 4), as expected under nearly neutral theory (Ohta, 1992). A nuance of our results is that  $d_N/d_s$  reflects the accumulation of substitutions across the entire history of a lineage, whereas population parameters such as  $\theta$  and  $\pi_N/\pi_s$  may be more strongly influenced by recent demographic processes. However, that we and others (e.g., Leroy et al., 2021; Popadin et al., 2007) find empirical evidence for the relationship between  $\theta$  and  $d_N/d_s$  predicted by nearly neutral theory, despite this potential mismatch in evolutionary timescales, suggests that similar demographic processes may shape empirical estimates of genetic diversity and molecular evolutionary rates.

Our results are consistent with the general finding that mitochondrial genes tend to experience strong purifying selection (Nabholz et al., 2013; Popadin et al., 2013). However, we did not find evidence supporting our prediction that long-distance migrants would show stronger purifying selection (i.e., lower  $d_N/d_s$  and  $\pi_N/\pi_s$ ) than short-distance migrants. This finding may reflect the reality that all species in our system face generally strong mitochondrial purifying selection, such that the endurance flights of long-distance migrants do not incur much stronger selection than the level that exists among all the species we studied. Our results also imply that short-distance migrants in the boreal region do not experience relaxed purifying selection on mitochondrial genes compared to long-distance migrants. As noted above, short-distance boreal migrants contend with metabolic challenges associated with cold winter temperatures which may also exert selection on the mitochondria (Chen et al., 2018), as well as the metabolic demands of flight.

### Migration distance and the costs of mitochondrial mutations

In this study, we based our predictions on several complementary hypotheses about the costs of mutation in species with slow life history and high demand for physiological performance, such as long-distance migrants. From the perspective of molecular evolution, the mutation avoidance hypothesis (Bromham, 2020) and studies on the relationship between lifespan and mutation rate (Galtier et al., 2009; Nabholz et al., 2008a; Tian et al., 2019; Zhang et al., 2021) predict that phenotype-altering genetic variation is harmful enough to induce selection for mutation avoidance in organisms with slow life history. From the perspective of population biology, the hypothesis proposed by Conklin et al. (2017) predicts that “slow” species with high performance demands experience a strong selective filter on phenotypic performance in early life, reducing phenotypic variation in these populations. While Conklin et al. (2017) frame their hypothesis around reduction of phenotypic variation, a similar prediction about reduction of genetic variation emerges from a series of studies showing that mitochondrial purifying selection is stronger in species with higher locomotory metabolic demands (Chang et al., 2020;

Chong & Mueller, 2013; De Panis et al., 2021; Mitterboeck et al., 2017; Shen et al., 2009; Strohm et al., 2015; Sun et al., 2017). Together, these hypotheses led us to predict that the costs of mitochondrial mutation in long-distance migrants, which have slow life histories, would cause them to exhibit slower mitochondrial mutation rates and stronger mitochondrial purifying selection than short-distance migrants.

Our predictions were only partially supported. The negative relationship we found between migration distance and  $d_s$  is consistent with lower mitochondrial mutation rate in long-distance migrants, but we did not find evidence that these species experience stronger mitochondrial purifying selection than do short-distance migrants. To reconcile these findings and advance our understanding of how long-distance migration influences molecular evolutionary dynamics, further research is needed on the relative metabolic demands of long-distance flight versus cold tolerance and on the consequences of mitochondrial genetic variation for migratory phenotype. Additionally, studying molecular rates across the nuclear genome will help clarify which dynamics we report here are related to selection on the mitochondrial genome and which reflect more general interactions between life history and molecular evolution.

### Conclusions: seasonal adaptation provides novel context for studying the links between life history and molecular evolutionary rates

Adaptation to seasonality entails life history tradeoffs (Varpe, 2017). Organisms balance these tradeoffs in different ways, creating variation in life history strategy within communities that inhabit seasonal environments (e.g., Winger & Pegan, 2021). Our study demonstrates that life history variation related to seasonality can influence molecular evolutionary rates, which has implications for the accurate reconstruction of evolutionary history (Berv & Field, 2018; Ritchie et al., 2022; Shafir et al., 2020). More broadly, communities adapted to seasonal habitats provide an important context to investigate potential drivers of the relationship between life history and molecular evolution. Codistributed species show varying adaptations to seasonality—for example, cold tolerance, migration, and hibernation—and they express these strategies to different degrees (Auteri, 2022). Cold adaptations can influence biological processes hypothesized to be relevant for germline replication rate or mutation rate (e.g., Wang et al., 2022), even among species that show little variation in commonly-studied life history proxies such as body mass. Comparative studies using seasonal communities can therefore allow us to draw new insights into how life history tradeoffs affect mutation rate, one of the most fundamental processes in evolution.

### Supplementary material

Supplementary material is available online at *Evolution*.

### Data availability

Sequence data are available on GenBank. Accession numbers may be found in Supplementary Table S2. Code and other associated data are available at Dryad DOI: 10.5061/dryad.cvdncjt99.

### Author contributions

Ideas conceived by T.M.P., J.S.B., E.R.G.C., and B.M.W.; data generated by T.M.P. and A.A.K.; data analyzed by T.M.P. and

J.S.B.; manuscript written by T.M.P. and B.M.W.; and manuscript revised by all authors.

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*Conflict of interest:* The authors have no conflict of interests to declare.

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