

1 **The pace of mitochondrial molecular evolution varies with seasonal migration distance**

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10

11 **Abstract**

12 Animals that engage in long-distance seasonal migration experience strong selective pressures on  
13 their metabolic performance and life history, with potential consequences for molecular  
14 evolution. Species with slow life histories typically show lower rates of synonymous substitution  
15 (dS) than “fast” species. Previous work has suggested that long-distance seasonal migrants have  
16 a slower life history strategy than short-distance migrants, raising the possibility that rates of  
17 molecular evolution may covary with migration distance. Additionally, long-distance migrants  
18 may face strong selection on metabolically important mitochondrial genes owing to their long-  
19 distance flights. Using over 1000 mitochondrial genomes, we assessed the relationship between  
20 migration distance and mitochondrial molecular evolution in 39 boreal-breeding migratory bird  
21 species. We show that migration distance correlates negatively with dS, suggesting that the slow  
22 life history associated with long-distance migration is reflected in rates of molecular evolution.  
23 Mitochondrial genes in every study species exhibited evidence of purifying selection, but the  
24 strength of selection was greater in short-distance migrants, contrary to our predictions. This  
25 result may indicate selection for cold tolerance on mitochondrial evolution among species  
26 overwintering at high latitudes. Our study demonstrates that the pervasive correlation between  
27 life history and molecular evolutionary rates exists in the context of differential adaptations to  
28 seasonality.

29

30

## 31 **Introduction**

32

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

44           In this study, we investigate how patterns of mitochondrial molecular evolution are  
45 related to variation in seasonal migration distance. Migratory animals survive harsh seasonal  
46 conditions on their breeding grounds by temporarily departing until conditions improve (Winger  
47 et al. 2019). Migration distance varies across species, ranging from short-distance movements  
48 within an ecoregion to hemisphere-crossing journeys. Long-distance seasonal migration requires  
49 high metabolic performance (Weber 2009), with potential implications for the dynamics of  
50 selection on the metabolically-important mitochondrial genes (Shen et al. 2009; Strohm et al.  
51 2015). Migration distance has also been recognized as an important axis of life history variation  
52 (the balance between annual survival and reproduction) in birds (Greenberg 1980; Møller 2007;  
53 Bruderer and Salewski 2009; Winger and Pegan 2021). Through effects on life history

54 (Bromham 2020), migration distance may therefore also influence molecular evolutionary rates,  
55 but this relationship has not been directly assessed. Here, we assess how migration distance  
56 correlates with mitochondrial molecular evolution within the community of migratory birds  
57 breeding in the highly seasonal North American boreal region, and we explore the roles of life  
58 history and metabolic adaptation in mediating a relationship between molecular evolution and  
59 seasonal migration.

60

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

62 Reliance on locomotion (migration) for adaptation to seasonality may influence selection  
63 on mitochondrial genes, which play an important role in metabolism. Mitochondria typically  
64 experience purifying selection (i.e. selection that reduces genetic variation) because most  
65 mutations in these genes are deleterious to fitness (Nei et al. 2010; Nabholz et al. 2013; Popadin  
66 et al. 2013). Prior studies have shown that purifying selection tends to be stronger in the  
67 mitochondria of mobile animal species compared with less-mobile relatives. This pattern has  
68 been demonstrated in comparisons between flighted and flightless birds (Shen et al. 2009) and  
69 insects (Mitterboeck et al. 2017; Chang et al. 2020), between migratory and nonmigratory fishes  
70 (Strohm et al. 2015), and between amphibians (Chong and Mueller 2013) and mollusks (Sun et  
71 al. 2017) with different locomotory modes. Within flighted birds, species with slow flight and  
72 those that rely on soaring (versus flapping) have been shown to experience relaxed mitochondrial  
73 purifying selection compared with faster-flying species (Shen et al. 2009; De Panis et al. 2021).  
74 These studies suggest that mitochondrial genotype plays an especially important role in fitness  
75 for organisms that rely on high-energy locomotion, including migratory birds. Metabolic demand  
76 may be strongest in long-distance migrating species if these demands primarily arise from

77 locomotion. However, species that migrate short distances within seasonal high latitude regions  
78 may require alternative metabolic adaptations for dealing with harsh seasonal conditions since  
79 their shorter migrations do not allow them to fully escape cold, resource depleted winters  
80 (Winger et al. 2019). The effect of variation in seasonal migration distance on mitochondrial  
81 purifying selection is unknown.

82         A second and distinct way in which seasonal migration may influence molecular  
83 evolution is through its effect on life history and, consequently, molecular evolutionary rate. The  
84 slow-fast continuum of life history is commonly characterized by “life history traits” that underly  
85 or correlate with differing rates of growth, survival, and reproduction (Read and Harvey 1989;  
86 White et al. 2022). Within major lineages of plants, bacteria, vertebrates, and invertebrates,  
87 species with “slow” life history (i.e., long generation time, low annual fecundity, large size;  
88 Stearns 1983) also exhibit slower molecular substitution rate than “fast” species (i.e., shorter  
89 generation time, higher annual fecundity, and smaller size; Nabholz et al. 2008a; Smith and  
90 Donoghue 2008; Thomas et al. 2010; Weller and Wu 2015). Within migratory birds breeding in  
91 the temperate zone, seasonal migration distance covaries with annual fecundity and survival such  
92 that long-distance migrants show “slower” life history (i.e., higher annual survival, lower annual  
93 fecundity) than short-distance migrants (Greenberg 1980; Bruderer and Salewski 2009; Winger  
94 and Pegan 2021). As such, variation in migration distance across species may have consequences  
95 for molecular evolutionary rates. Specifically, the synonymous substitution rate “dS” is often  
96 correlated with the slow-fast life history continuum (Nikolaev et al. 2007, Bromham et al. 2015,  
97 Hua et al. 2015; Table 1). Prior studies suggest that life history may influence dS through effects  
98 on DNA replication rate or selection for mutation avoidance (reviewed in Bromham 2020),  
99 because dS is thought to primarily reflect the underlying mutation rate when synonymous

100 mutations are selectively neutral (Kimura 1983; Nei et al. 2010; Lanfear et al. 2014). Direct  
101 estimates of nuclear germline mutation rates support the hypothesis that species-level variation in  
102 mutation rate correlates with life history traits (Bergeron et al. 2023).

103

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

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

115 To test these hypotheses, we examined the relationship between migration distance and  
116 rates of molecular evolution of the mitochondrial coding genes in a community of small-bodied  
117 migratory songbirds breeding in the boreal forests of North America. The 39 co-distributed  
118 species we studied are ideal for investigating the effects of migration distance on molecular  
119 evolution because they vary greatly in migration distance (e.g., Fig. 1, Table S1), yet they  
120 otherwise share similar breeding habitat, population history, and body mass (Winger and Pegan  
121 2021). This system allows us to test hypotheses about migration distance while minimizing  
122 variation in other traits that could influence molecular evolution. We assessed effects of

123 migration distance on dS (synonymous substitution rate) and dN/dS (purifying selection) in a  
124 phylogenetic framework (Lartillot and Poujol 2011) with full mitochondrial gene sets we  
125 sequenced for 39 species. Further, we used population genetic datasets from all mitochondrial  
126 genes that we generated for 30 of the species (for a total of 1008 samples used across all  
127 analyses) to assess effects of migration distance on purifying selection within populations  
128 ( $\pi N/\pi S$ ) and to account for the potentially confounding influence of effective population size  
129 ( $N_e$ ) on molecular evolution.

130

## 131 **Methods**

132

### 133 *Study system*

134 We focused on 39 species of migratory birds breeding in the North American boreal  
135 forest, representing 11 families (Table S1). These are the same species for which a correlation  
136 between migration distance and the slow-fast life history continuum, independent of body size,  
137 was demonstrated using data on annual fecundity and survivorship (Winger and Pegan 2021).  
138 We focus our analyses on co-distributed populations of the eastern boreal belt of North America  
139 (Omernik 1987, Fig. S1). Some species' breeding ranges extend into other ecoregions (e.g., the  
140 mountain west or the temperate forests south of the boreal zone), but in these cases we only  
141 analyze samples from the boreal portion of the range so as to assess sympatric populations. The  
142 species in the dataset exhibit broad variation in migration distance, with their geographic range  
143 centroids shifting between 1048 km and 7600 km between the breeding and non-breeding  
144 periods (Fig. 2, Table S1; Winger and Pegan 2021). These centroid shifts represent migratory  
145 strategies ranging from short-distance movements within the temperate region to the movement

146 of an entire population across ocean and land barriers from North America to South America. All  
147 species are less than 100 g in mass (range of mean mass across species is 6-87 grams; Table S1)  
148 and are broadly similar in habitat use. They are all territorial species with socially monogamous  
149 breeding systems, which suggests that they probably do not vary substantially in population sex  
150 ratio (which can affect  $N_e$ ), although empirical sex ratio data is not available for these species.  
151 Small songbirds are typically capable of breeding in their second year, and this is true of all  
152 species in our study that have been assessed (Billerman et al. 2022). Additionally, the species  
153 share relatively similar demographic histories, with population expansions estimated to have  
154 mostly occurred during the period of glacial retreat that preceded the Last Glacial Maximum  
155 (~57,000 years before present; Kimmitt et al. 2023).

156

157 *Life history covariates: Migration distance and mass*

158 Direct measurements of migration distance of individuals are lacking for most of the species in  
159 our system, so we used the distance between the centroid of a species' breeding range and the  
160 centroid of its nonbreeding range to represent the migration distance of the species. Although the  
161 distance between centroids does not represent individual variation in migration distance within a  
162 species, this metric captures broad differences in migratory strategies between species. Our  
163 method for calculating the distance between range centroids is described in detail in Winger and  
164 Pegan (2021). We included mass as a covariate in our analyses because body mass and rates of  
165 molecular evolution are often associated (Figueroa et al. 2014; Nabholz et al. 2016), and the  
166 relationships between survival and fecundity and migration distance demonstrated by Winger  
167 and Pegan (2021) were recovered after controlling for the effect of mass. We obtained mass data  
168 from Dunning (2008) and Billerman et al. (2022).



169

170 *Sampling and DNA sequencing*

171 Our analysis of the relationship between migration distance and dS requires one  
172 mitochondrial genome for each species in the study, while analyses of  $N_e$  and  $\pi_N/\pi_S$  require  
173 population-level sampling. For our analysis of dS, we obtained whole mitochondrial genomes  
174 from one individual of each of the 39 species in our study by sequencing DNA as described  
175 below. Samples were collected during the breeding season from near the longitudinal center of  
176 the boreal forest (Manitoba, Minnesota, or Michigan; S2). For two species (*Contopus cooperi*  
177 and *Euphagus carolinus*), we sequenced DNA from individuals sampled during migration in  
178 Michigan from collision mortalities.

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

187 We obtained high-depth mitochondrial genomes captured as a byproduct from low-  
188 coverage whole genome sequencing, as described in detail in Kimmitt et al. (2023). Briefly,  
189 sequencing libraries were prepared using a modified Illumina Nextera library preparation  
190 protocol (Schweizer et al. 2021) and sequenced on HiSeq or NovaSeq machines using services  
191 provided by Novogene and the University of Michigan Advanced Genomics Core. We used

192 NOVOPlasty v4.3.1 (Dierckxsens et al. 2016) to assemble mitochondrial contigs, specifying a  
193 target genome size of 20-30 kb and using a k-mer of 21. We provided NOVOPlasty with a  
194 conspecific mitochondrial seed sequence (Table S1) for each species. We annotated the contigs  
195 built by NOVOPlasty using Geneious Prime 2020.2.2 (<https://www.geneious.com>) with copies  
196 of mitochondrial genes from GenBank (Table S1). Whenever applicable in the filtering and  
197 analysis steps described below, we used options specifying the vertebrate mitochondrial code.

198         Our initial dataset across all species contained mitochondrial sequences from 1229 total  
199 individuals. To ensure data quality, we used BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to  
200 check species identity and we removed samples with evidence of species misidentification,  
201 chimerism, or introgression from related species (14 samples removed). We aligned and  
202 translated sequences with the R package DECIPHER v2.18.1 (Wright 2016) and we visually  
203 inspected each alignment, ensuring that sequences contained no premature stop codons or other  
204 alignment issues. We used DECIPHER to remove partial stop codons and to remove the  
205 untranslated C in the ND3 sequence of woodpecker (Picidae) species (Mindell et al. 1998). As  
206 our population analyses require complete data matrices, we excluded individuals with incomplete  
207 datasets (those with assemblies that were missing genes and/or with ambiguous base calls; 202  
208 samples removed) and we concatenated the 13 genes for each remaining individual. An  
209 additional 5 individuals were removed during populational structure analysis, described below.  
210 This data filtering resulted in 1008 complete mitochondrial coding sequences: 999 individuals  
211 across 30 species used in the population genomic analyses plus one sequence for each of the 9  
212 additional species we used only in the interspecific Coevol analyses. The full list of samples,  
213 including those that were removed from the analyses, can be found in Table S2.

214

215 *Accounting for effects of  $N_e$  on substitution rates using  $\theta$*

216 Many parameters of molecular evolution are fundamentally associated with effective  
217 population size ( $N_e$ ), so estimating  $N_e$  provides important context for our analyses. Variation in  
218  $N_e$  can cause variation in substitution rates because in theory, the efficiency of natural selection  
219 in purging deleterious mutations is determined by the balance between strength of selection and  
220 strength of drift, which is reflected by  $N_e$  (Ohta 1992). Specifically, studies on empirical  
221 populations have demonstrated that populations with small  $N_e$  typically show weaker purifying  
222 selection (i.e., higher  $dN/dS$ , e.g., Popadin et al. 2007, Leroy et al. 2021; and higher  $\pi N/\pi S$ , e.g.,  
223 Chen et al 2017). Similarly, nearly neutral theory suggests that  $N_e$  can influence  $dS$  when  
224 synonymous sites are not selectively neutral (as in Chamary et al. 2006). That is, under nearly-  
225 neutral theory, synonymous sites with weak influence on fitness may be under purifying  
226 selection in populations with large  $N_e$  and not in populations with small  $N_e$ , which could result in  
227 lower  $dS$  for species with high  $N_e$ . Several recent studies find correlations between traits  
228 associated with life history and genetic diversity, suggesting that species with “slow” life  
229 histories often have low  $N_e$  (Romiguier et al. 2014; Brüniche-Olsen et al. 2021; De Kort et al.  
230 2021). For these reasons, when assessing the relationship between seasonal migration and  
231 molecular evolution, we tested whether molecular rate variation across species could  
232 alternatively be explained by confounding variation in  $N_e$ .

233 We used genetic diversity ( $\theta$ ) as a proxy for  $N_e$ . Effective population size ( $N_e$ ) can be  
234 calculated based on  $\theta$  and mutation rate (Watterson 1975, Nabholz et al. 2008b; Table 1), but  
235 accurate estimates of mitochondrial mutation rate are lacking for most non-model organisms.  
236 Accordingly, many empirical studies interested in  $N_e$  focus on genetic diversity, which is thought  
237 to reflect the harmonic mean of  $N_e$  over time and which does not require mutation rate

238 information to calculate (e.g., Ellegren and Galtier 2016; Hague and Routman 2016). We  
239 hereafter use the genetic diversity parameter  $\theta$  as a proxy for  $N_e$ . We used LAMARC v2.1.10  
240 (Kuhner 2006) to estimate  $\theta$  for each species. We imported our sequence data into LAMARC  
241 after converting our concatenated fasta files into the phylip format for each species. We used the  
242 program's likelihood-based method in 10 initial chains (samples = 500, discard = 1000, interval  
243 = 20) and 2 final chains (samples = 10,000, discard = 1000, interval = 20). We used the F84  
244 model of molecular evolution and we provided a separate transition/transversion ratio for each  
245 species using values we calculated from population sequence datasets using the R package  
246 'spider' (Brown et al. 2012). We examined the output for each species to check for chain  
247 convergence and we ran two replicate chains for each species to make sure they produced  
248 consistent results. For 5 species (*Leiothlypis ruficapilla*, *Setophaga castanea*, *Setophaga*  
249 *coronata*, *Setophaga fusca*, and *Vireo olivaceus*), we repeated LAMARC for 25 initial chains  
250 instead of 10 to improve convergence and used the values from these longer runs.

251

## 252 *Population Structure*

253 Our population-level analyses (estimation of  $\theta$  and  $\pi N/\pi S$ ) assume that there is no  
254 geographic population genetic structure within the samples used. To check this assumption, we  
255 calculated mitochondrial genetic distance between all individuals within each species using  
256 "nei.dist()" from the R package poppr v2.9.3 (Kamvar et al. 2014) and created a neighbor-joining  
257 tree with "nj()" from the R package ape v5.6-2 (Paradis and Schliep 2019). We identified and  
258 removed 4 individuals from *Regulus satrapa* and one individual from *Oporornis agilis*, all from  
259 Alberta in the far western part of our sampling area, that were clearly genetically distinct from all

260 other samples in their respective species. Otherwise, there was little evidence of geographic  
261 genetic structure in the mitochondrial genome in these species.

262

263 *Estimating dS and dN/dS and their correlations with traits associated with life history*

264 We used Coevol v1.6 (Lartillot and Poujol 2011) to evaluate associations between  
265 migration distance and molecular evolutionary rates using a single representative of each species.  
266 Coevol uses a Bayesian phylogenetic framework to estimate dS and dN/dS and to simultaneously  
267 measure the relationship between these traits and covariates of interest (migration distance, mass,  
268 and  $\theta$ ). We included mass to account for the expected relationship between mass and molecular  
269 rates (Nabholz et al. 2016). Models with mass also provide a useful point of comparison,  
270 allowing us to ask whether migration distance correlates with dS and dN/dS to the same extent as  
271 (or more or less than) this well-studied life history trait. Similarly, including  $\theta$  in the models  
272 allows us to assess whether variation in  $N_e$  accounts for differences in molecular evolutionary  
273 rates.

274 We provided Coevol with one complete mitochondrial coding sequence from each  
275 species and a phylogenetic tree (Fig. 2) we built with data from birdtree.org (Jetz et al. 2012) as  
276 described in Pegan and Winger (2020). In brief, we sampled 2000 trees comprising all North  
277 American bird species from the Jetz et al. dataset and we used the python package “DendroPy”  
278 (Sukumaran and Holder 2010) to generate a consensus tree. We then trimmed this tree to include  
279 only the 39 species used in this study. Importantly, Coevol only uses the phylogenetic tree for  
280 topological information, and the program estimates branch lengths from the separate sequence  
281 data during modeling (Lartillot and Poujol 2021). Coevol does not require information about  
282 mutation rates. Because Coevol does not take topological uncertainty into account, we

283 investigated potential effects of phylogenetic tree topology on our results by sampling 10 random  
284 marginal trees from the original tree dataset (trimmed to include only relevant species) and  
285 running a Coevol model on each, which we found to produce consistent results (Table S3).

286 We created two data subsets for Coevol models: one subset contained all species in the  
287 study and included mass and migration distance as covariates. The other subset included the 30  
288 species for which we had population-level data available; for these we included  $\theta$  as a covariate  
289 in addition to mass and migration distance. For each data subset, we ran Coevol in 4 chains: two  
290 replicate chains with the option “dnds” (estimating dS; models 1 and 2, Table 2) and two with  
291 “dsom” (estimating dN/dS; models 3 and 4, Table 2). We let each chain run for approximately  
292 20000 steps and examined the resulting trace files to ensure convergence and evaluate estimated  
293 sample sizes (ESS). All models converged and all parameters had ESS > 300. We removed the  
294 first 500 steps of each chain and thinned the chain to retain every 10<sup>th</sup> step to reduce  
295 autocorrelation. Replicate chains produced highly similar estimates, and the values we report  
296 here represent the mean value of estimates made by each replicate chain. Full Coevol model  
297 output for each chain is presented in Tables S4 and S5.

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

304

305

306  $\pi N/\pi S$

307  $\pi N/\pi S$  is a population genetic summary statistic representing the amount of  
308 nonsynonymous vs synonymous polymorphism within a population. This value is measured by  
309 comparing individuals within a species rather than by comparing between species in a  
310 phylogenetic framework (and thus cannot be estimated by Coevol). We estimated  $\pi N/\pi S$  from  
311 each species with population-level fasta alignments, using the python package egglib v3.1.0 (De  
312 Mita and Siol 2012) to create a “CodingDiversity” class with attributes describing the number of  
313 codons with synonymous or nonsynonymous polymorphisms. Predictions about the effect of  
314 purifying selection on polymorphisms are more complex than predictions about substitution rates  
315 because within-population variation can be purged by strong directional selective sweeps in  
316 addition to purifying selection (Kryazhimskiy and Plotkin 2008). Nevertheless, we predict a  
317 negative relationship between migration distance and the  $\pi N/\pi S$  ratio, which could result from  
318 either stronger purifying or stronger positive selection in long-distance migrants on  
319 mitochondrial function. In either case, such a relationship would broadly support the hypothesis  
320 that migration distance covaries with the dynamics of selection on the mitochondrial genome.  
321 We used linear modeling to test for an effect of migration distance, mass and  $\theta$  on  $\pi N/\pi S$  (Tables  
322 S6, S7). Prior to linear modeling, we centered and standardized our predictors using the function  
323 “standardize” from the R package “robustHD” (Alfons 2021) with the mean value of each  
324 predictor as the center. We used a similar linear modeling approach to test whether  $\theta$  exhibits a  
325 relationship with mass or migration distance to ensure that apparent relationships between these  
326 traits and molecular rates are not confounded by correlation with  $\theta$ .

327 For each response variable ( $\theta$  and  $\pi N/\pi S$ ; Tables S6, S7), we first created a model with  
328 all covariates of interest. We then used the function “phylosig()” from the R package phytools

329 v0.7-70 (Revell 2010) to test for phylogenetic signal in the model's residuals (Revell 2012). For  
330 both response variables, the estimate of lambda (phylogenetic signal) was low ( $< 0.2$ ) and the p-  
331 value for evidence of phylogenetic signal was  $> 0.8$ , so we proceeded with linear modeling rather  
332 than using models with phylogenetic covariance matrices. For each response variable, we created  
333 a null (intercept-only) model with no predictors and models with all possible combinations of our  
334 predictors of interest, and we used the function "model.sel()" from the R package MuMIn  
335 v1.43.17 (Bartón 2019) to compare the models' AICc.

336

## 337 **Results**

338

339 For each model, we report correlation coefficients between traits of interest (migration  
340 distance, mass, or  $\theta$ ) and molecular evolutionary rates (dS or dN/dS). We assess the strength of  
341 evidence for correlations using posterior probabilities (*pp*), which are close to 0 in the case of a  
342 strong negative correlation and close to 1 in the case of a strong positive correlation. We also  
343 report partial correlation coefficients and their posterior probabilities, which indicate the  
344 relationship between variables of interest after accounting for effects of all other covariates.

345

### 346 *Correlations between migration distance and molecular evolutionary rates (dS and dN/dS)*

347 Our analyses with Coevol show that migration distance has a negative relationship with  
348 dS across the 39 species we studied, conforming to our initial predictions (Fig. 2, Fig. S2). For  
349 Coevol models with the full species set, the correlation coefficient between migration distance  
350 and dS was -0.39 with a posterior probability (*pp*) of 0.018, indicating strong support for a



351 negative relationship. The partial correlation coefficient (which controls for the effects of mass)  
352 between migration distance and dS was  $-0.47$  ( $pp = 0.0090$ ).

353 We did not detect evidence of a relationship between migration distance and dN/dS  
354 (correlation coefficient =  $0.096$ ,  $pp = 0.63$ ). The partial correlation coefficient (accounting for  
355 effects of mass) between migration distance and dN/dS showed weak support for a relationship  
356 (partial correlation coefficient =  $0.26$ ,  $pp = 0.82$ ).

357 Results from the Coevol models of the subset of 30 species for which we had estimates of  
358  $\theta$  were consistent with results produced by the full subset (39 species) models, although support  
359 for the correlation between dS and migration distance was slightly weaker. In the model  
360 estimating dS, migration distance had a correlation coefficient of  $-0.43$  ( $pp = 0.02$ ) and a partial  
361 correlation coefficient of  $-0.31$  ( $pp = 0.11$ ). In the model estimating dN/dS, we did not find  
362 support for a relationship with migration distance, as this variable had a correlation coefficient of  
363  $-0.15$  ( $pp = 0.32$ ) with dN/dS and a partial correlation coefficient of  $-0.010$  ( $pp = 0.52$ ) with  
364 dN/dS.

365

#### 366 *Correlations between mass and molecular evolutionary rates (dS and dN/dS)*

367 Our Coevol models with the full species set support the expected negative relationship  
368 between mass and dS (correlation coefficient =  $-0.28$ ,  $pp = 0.065$ ; Fig. 2). This relationship  
369 weakens when effects of migration distance are accounted for (i.e., with partial correlation  
370 coefficient =  $-0.18$ ,  $pp = 0.20$ ). We did not find a strong correlation between mass and dN/dS  
371 (correlation coefficient =  $-0.25$ ,  $pp = 0.19$ ; partial correlation coefficient =  $-0.072$ ,  $pp = 0.41$ ). In  
372 models of dS with the subset of 30 species that included  $\theta$  as a predictor, mass had a correlation  
373 coefficient of  $-0.30$  ( $pp = 0.10$ ) and a partial correlation coefficient (which controls for the

374 effects of migration distance) of -0.40 ( $pp = 0.039$ ). In models of dN/dS from this subset, mass  
375 had a correlation coefficient of 0.25 ( $pp = 0.8$ ) and a partial correlation coefficient of 0.23 ( $pp =$   
376 0.79).

377

### 378 *The influence of $N_e$ on molecular rates and their correlation with traits of interest*

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

387

### 388 *Linear modeling of $\pi N/\pi S$*

389 In comparison of AICc, the highest-ranked model of  $\pi N/\pi S$  showed a strongly supported  
390 negative relationship between  $\theta$  and  $\pi N/\pi S$  (Fig. 4, Table S6, model weight 0.55), as predicted if  
391 purifying selection is stronger in species with higher  $N_e$ . Compared to a model with  $\theta$  alone, a  
392 model with both  $\theta$  and migration distance shows an increase in multiple  $r^2$  from 0.15 to 0.28 and  
393 a decrease in AICc by more than two units, suggesting the inclusion of migration distance  
394 improves model fit. However, contrary to our prediction, migration distance has a weak positive  
395 relationship with  $\pi N/\pi S$  (Fig. 4). The estimated coefficient relating  $\theta$  and  $\pi N/\pi S$  in the best-fit  
396 model is -0.027 (std error = 0.01) and the estimated effect of migration distance from the best-fit

397 model is 0.022 (std error = 0.01). Model comparison did not support the inclusion of mass as a  
398 predictor of  $\pi_N/\pi_S$  (Table S6).

399

400 *N<sub>e</sub> is unlikely a confounding factor in inferred relationships*

401 We used linear modeling to test whether migration distance or mass show a relationship  
402 with  $\theta$ , our proxy of  $N_e$ . We did not find strong evidence that body mass or migration distance  
403 are correlated with  $\theta$  among the 30 species we studied. The null model for  $\theta$  (an intercept-only  
404 model with no predictors) showed the lowest AICc, suggesting that the addition of mass and  
405 migration distance as predictors did not improve model fit (Table S7, model weight 0.45).  
406 However, the model with migration distance as a predictor was within 2 AICc units of the null  
407 model and showed a model weights of 0.30, indicating considerable model uncertainty. The  
408 estimated effect of migration distance on  $\theta$  was positive but had a negligible effect size in the  
409 second-best model (estimate = 0.0017, std error = 0.0013 model multiple  $r^2 = 0.054$ ).

410

## 411 **Discussion**

412

413 *Seasonal migration distance correlates with mitochondrial dS*

414 We examined the relationship between life history and patterns of mitochondrial  
415 sequence evolution within North American boreal birds. These species occupy a region where  
416 strong seasonality demands specialized adaptations that carry life history tradeoffs (Varpe 2017;  
417 Winger and Pegan 2021). Our results implicate the life history axis of seasonal migration  
418 distance as a novel correlate of mitochondrial synonymous substitution rate (dS). Previous work  
419 demonstrates that, even after accounting for body size, long-distance migrants in this system

420 have slower life history strategies than short-distance migrants, showing higher annual adult  
421 survival and lower fecundity (Winger and Pegan 2021). Here, we find that the slow life history  
422 of long-distance migrants is accompanied by a slower rate of neutral molecular evolution in the  
423 mitochondria of these species compared with that of shorter-migrating species in the region.  
424 Indeed, among the 39 species we studied, the correlation between migration distance and dS is  
425 stronger than the correlation between mass and dS, which is notable given that the relationship  
426 between mass and substitution rate has been documented in previous work (Nabholz et al. 2016).  
427 As such, we suggest that the association between migration distance and the slow-fast life history  
428 continuum extends to effects on dS.

429

430 *What evolutionary processes link migration distance with mitochondrial dS?*

431 Substitution rates are fundamentally influenced by mutation rate, which provides new  
432 molecular variants with potential to become substitutions, and by natural selection, which  
433 influences whether variants are fixed as substitutions or lost. The correlation between migration  
434 distance and dS therefore reflects one or both processes. dS is often treated as a proxy for  
435 mutation rate alone based on the assumption that natural selection does not operate on  
436 synonymous sites (Nei et al. 2010), but in some cases synonymous sites are known to evolve  
437 non-neutrally (Chamary et al. 2006). If synonymous sites are not evolving neutrally, nearly  
438 neutral theory suggests that the relationship we find between dS and migration distance could  
439 hypothetically be explained by larger  $N_e$  in long-distance migrants (Ohta 1992). However, we  
440 did not find evidence for a correlation between dS and our proxy for  $N_e$  ( $\theta$ ) (Table S4) nor  
441 correlation between  $\theta$  and migration distance (Table S7). Together, these results suggest that  
442 synonymous sites are evolving neutrally in our system and that variation in dS among species

443 with different migration distances is not well explained by variation in demographic parameters  
444 that influence substitution rate. Rather, we suggest that the negative relationship we found  
445 between migration distance and dS may reflect a negative relationship between migration  
446 distance and mutation rate.

447

448 *Why might long-distance migrants have lower mitochondrial mutation rate?*

449 We predicted that migration distance would correlate with dS because of its relationship  
450 with the slow-fast continuum of life history in these species independent of body size (Winger  
451 and Pegan 2021). In turn, a species' position on the slow-fast life history continuum is  
452 hypothesized to affect mutation rate (Bromham 2020). There are several potential mechanisms to  
453 explain the link between life history and mutation rate, and the relative importance of each is not  
454 clear (Bromham 2020). The "copy error effect" hypothesis suggests that the explanation is  
455 related to generation time, assuming that "fast" species with short generation times and young  
456 age at first reproduction experience higher rates of germline replication (and thus replication-  
457 induced mutation) than species with "slow" life histories (Li et al. 1996; Thomas et al. 2010;  
458 Lehtonen and Lanfear 2014). However, recent studies comparing cell division rates with  
459 directly-measured mutation rates suggest that replication-induced copy errors may not be the  
460 only driver of differences in mutation rate between lineages (Wu et al. 2020; Wang et al. 2022).  
461 The "mutation avoidance" hypothesis offers another non-exclusive explanation for lower dS in  
462 organisms with slow life history based on higher costs of mutation in longer-lived species  
463 (Bromham 2020). Under this hypothesis, organisms with slow life history are predicted to have  
464 adaptations that reduce the introduction of mutations from DNA damage or from DNA  
465 replication and repair processes (Galtier et al. 2009; Tian et al. 2019; Zhang et al. 2021; Cagan et

466 al. 2022). Long-distance migrants may be especially sensitive to costs of mitochondrial mutation,  
467 which may cause mitochondrial senescence (Galtier et al. 2009; Hua et al. 2015), because of the  
468 high physical performance demanded by their migratory behavior across their entire lifespans  
469 (Møller 2007; Conklin et al. 2017). Further research is necessary to understand what processes  
470 contribute to the apparent reduction of mutation rate in species at the slow end of the slow-fast  
471 continuum of life history.

472 Another possible link between migration distance and mutation rate is oxidative damage  
473 from metabolism, which is recognized as a potential source of mutation rate variation (Martin  
474 and Palumbi 1993, Gillooly et al. 2005, Berv and Field 2018; but see Lanfear et al. 2007, Galtier  
475 et al. 2009). Thus, a potential explanation for our results—lower mitochondrial dS in long-  
476 distance migrants—is that long-distance migrants incur less metabolically-induced DNA damage  
477 than do short-distance migrants. This explanation is initially surprising in light of studies  
478 showing that migratory birds experience oxidative damage from endurance flight (Jenni-  
479 Eiermann et al. 2014; Skrip and McWilliams 2016). However, we suggest that there are three  
480 plausible and non-exclusive scenarios that could lead to lower metabolically-induced DNA  
481 damage in long-distance compared to short-distance migrants. First, long-distance migrants may  
482 have better adaptations for flight efficiency (Weber 2009; Elowe et al. 2023), reducing the  
483 amount of oxidative damage they experience per mile traveled. Second, the mutation avoidance  
484 hypothesis predicts that long-distance migrants may have more efficient DNA repair  
485 mechanisms than short-distance migrants, which could reduce metabolically-induced mutation  
486 rate even when long-distance flight does induce high oxidative stress. Last, short-distance  
487 migrants in our boreal study system may experience greater oxidative damage arising from their  
488 increased need for winter cold tolerance than long-distance migrants that winter in the tropics.

489 The mitochondria also play an important role in the metabolic challenge of maintaining  
490 homeostasis during cold weather and resource shortages (Bicudo et al. 2001; Chen et al. 2018).  
491 Short-distance boreal migrants likely face more of these kinds of challenges than long-distance  
492 migrants during both migration and winter (Winger and Pegan 2021). Despite the view that long-  
493 distance migration is an extreme performance challenge, its alternative—spending the winter  
494 within the temperate zone—is also an extreme metabolic challenge for small-bodied  
495 homoeothermic endotherms that do not hibernate (Dawson and Yacoe 1983; Winger et al. 2019).  
496 Further investigation of the comparative metabolic challenges faced by short versus long  
497 distance boreal migrants is needed to clarify whether and how migration distance influences  
498 metabolically-induced mutation in the mitochondria.

499

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

501       Whereas evolutionary rate at synonymous sites (dS) may primarily reflect mutation rate,  
502 evolution at nonsynonymous sites is expected to strongly reflect natural selection because  
503 nonsynonymous mutations alter the amino acid sequence of a gene's protein product. We found  
504 that the ratio of nonsynonymous to synonymous substitutions (dN/dS) among our species is  
505 universally much less than 1 (Fig. 3), indicating that the mitochondrial genes we studied are  
506 under purifying selection in all species in the system. We similarly found low ratios of  
507 nonsynonymous to synonymous polymorphisms within each population ( $\pi_N/\pi_S$ ; Fig. 4), which is  
508 also consistent with purifying selection. Moreover, both dN/dS and the  $\pi_N/\pi_S$  ratio are strongly  
509 correlated with  $\theta$ , our proxy for  $N_e$  (Fig. 3, 4), as expected under nearly neutral theory (Ohta  
510 1992). A complexity of our results is that dN/dS reflects the accumulation of substitutions across  
511 the entire history of a lineage, whereas population parameters such as  $\theta$  and  $\pi_N/\pi_S$  may be more

512 strongly influenced by recent demographic processes. However, that we and others (e.g.,  
513 Popadin et al. 2007; Leroy et al. 2021) find empirical evidence for the relationship between  $\theta$   
514 and dN/dS predicted by nearly-neutral theory, despite this potential mismatch in evolutionary  
515 timescales, suggests that empirical estimates of genetic diversity and molecular evolutionary  
516 rates may be shaped by similar demographic processes.

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

529

### 530 *Migration distance and the costs of mitochondrial mutations*

531 In this study, we based our predictions on several complementary hypotheses about the  
532 costs of mutation in species with slow life history and high demand for physiological  
533 performance, such as long-distance migrants. From the perspective of molecular evolution, the  
534 mutation avoidance hypothesis (Bromham 2020) and studies on the relationship between lifespan



535 and mutation rate (Nabholz et al. 2008a; Galtier et al. 2009; Tian et al. 2019; Zhang et al. 2021)  
536 predict that phenotype-altering genetic variation is harmful enough to induce selection for  
537 mutation avoidance in organisms with slow life history. From the perspective of population  
538 biology, the hypothesis proposed by Conklin et al. (2017) predicts that “slow” species with high  
539 performance demands experience a strong selective filter on phenotypic performance in early  
540 life, reducing phenotypic variation in these populations. While Conklin et al. (2017) frame their  
541 hypothesis around reduction of phenotypic variation, a similar prediction about reduction of  
542 genetic variation emerges from a series of studies showing that mitochondrial purifying selection  
543 is stronger in species with higher locomotory metabolic demands (Shen et al. 2009; Chong and  
544 Mueller 2013; Strohm et al. 2015; Mitterboeck et al. 2017; Sun et al. 2017; Chang et al. 2020; De  
545 Panis et al. 2021). Together, these hypotheses led us to predict that costs of mitochondrial  
546 mutation in long-distance migrants, which have slow life history, would cause them to exhibit  
547 slower mitochondrial mutation rate and stronger mitochondrial purifying selection than short-  
548 distance migrants.

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

558 genome and which reflect more general interactions between life history and molecular  
559 evolution.

560

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

563         Adaptation to seasonality entails life history tradeoffs (Varpe 2017). Organisms balance  
564 these tradeoffs in different ways, creating variation in life history strategy within communities  
565 that inhabit seasonal environments (e.g., Winger and Pegan 2021). Our study demonstrates that  
566 life history variation related to seasonality can influence molecular evolutionary rate, which has  
567 potential implications for accurate reconstruction of evolutionary history (Shafir et al. 2020;  
568 Ritchie et al. 2022). More broadly, we suggest that communities adapted to seasonal habitats  
569 provide an interesting context in which to investigate potential drivers of the relationship  
570 between life history and molecular evolution. Co-distributed species show varying adaptations to  
571 seasonality—e.g., cold tolerance, migration, hibernation—and they express these strategies to  
572 different degrees (Auteri 2022). Cold adaptations can influence biological processes  
573 hypothesized to be relevant for germline replication rate or mutation rate (e.g., Wang et al.  
574 2022), even among species that show little variation in commonly-studied life history proxies  
575 such as body mass. Comparative studies using seasonal communities can therefore allow us to  
576 draw new insights into how life history tradeoffs affect mutation rate, one of the most  
577 fundamental processes in evolution.

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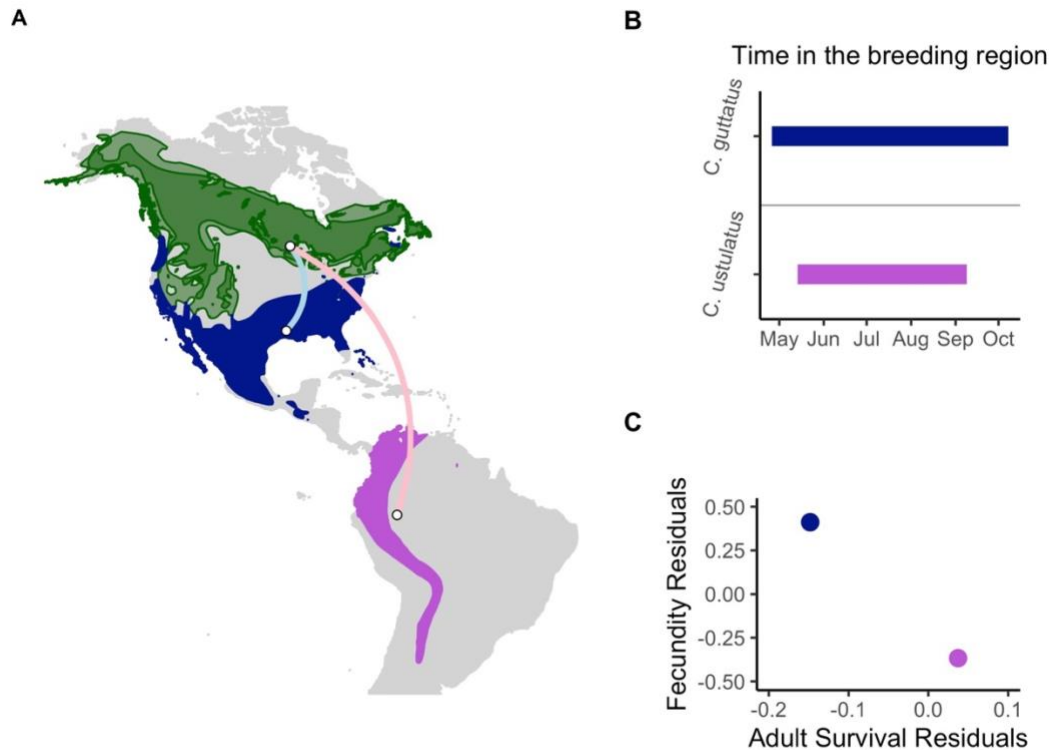
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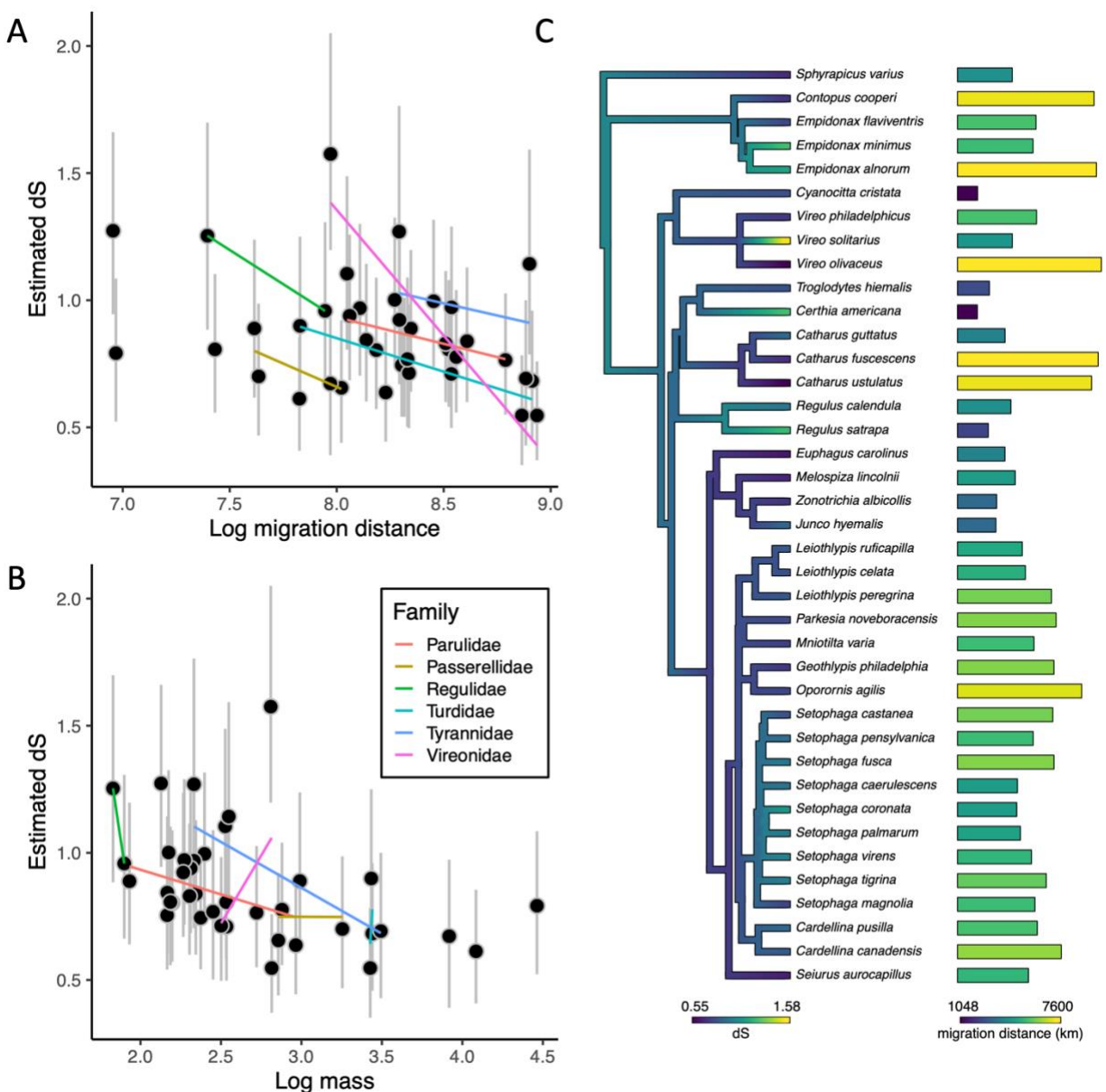
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853 **Figure 1.** An example contrast between a shorter-distance migrant *Catharus guttatus* and a  
854 closely related longer-distance migrant *Catharus ustulatus swainsoni* illustrates the relationship  
855 between migration distance and life history in our study system. Both species have broadly  
856 overlapping breeding ranges (green), but *C. guttatus* (dark blue nonbreeding range) migrates a  
857 shorter distance (blue migratory route) than *C. u. swainsoni* (purple nonbreeding range, pink  
858 migratory route) (panel A). Accordingly, *C. guttatus* spends more time in its breeding range than  
859 *C. u. swainsoni* (panel B). With more time in the breeding range and the possibility of raising a  
860 second brood, the short-distance migrant has higher fecundity but lower adult survival—i.e.,  
861 faster life history—than the long-distance migrant (panel C, showing model residuals from mass-  
862 corrected analysis of fecundity and survival). The short-distance migrant spends the winter in  
863 colder, more resource-depleted regions than the long-distance migrant. Figure and data adapted  
864 from Winger and Pegan (2021). Our sampling for this study occurred only within the eastern  
865 boreal belt (Fig. S1).





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868 **Figure 2.** dS vs. traits associated with life history (A, B) and a phylogenetic tree showing dS and

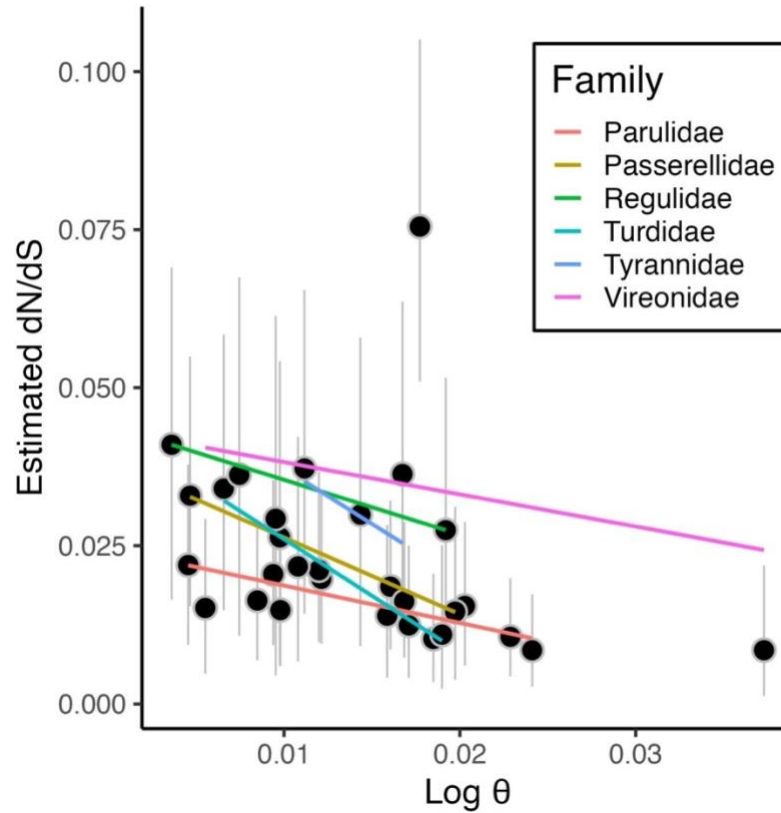
869 migration distance for each species (C). In panels A and B, posterior mean tip estimates of dS

870 (black dots) from Coevol are shown compared to migration distance (A), and mass (B) from

871 models using our full species set. Gray vertical bars indicate 95% credible intervals for each

872 estimate. These analyses reveal that both migration distance and mass have a negative

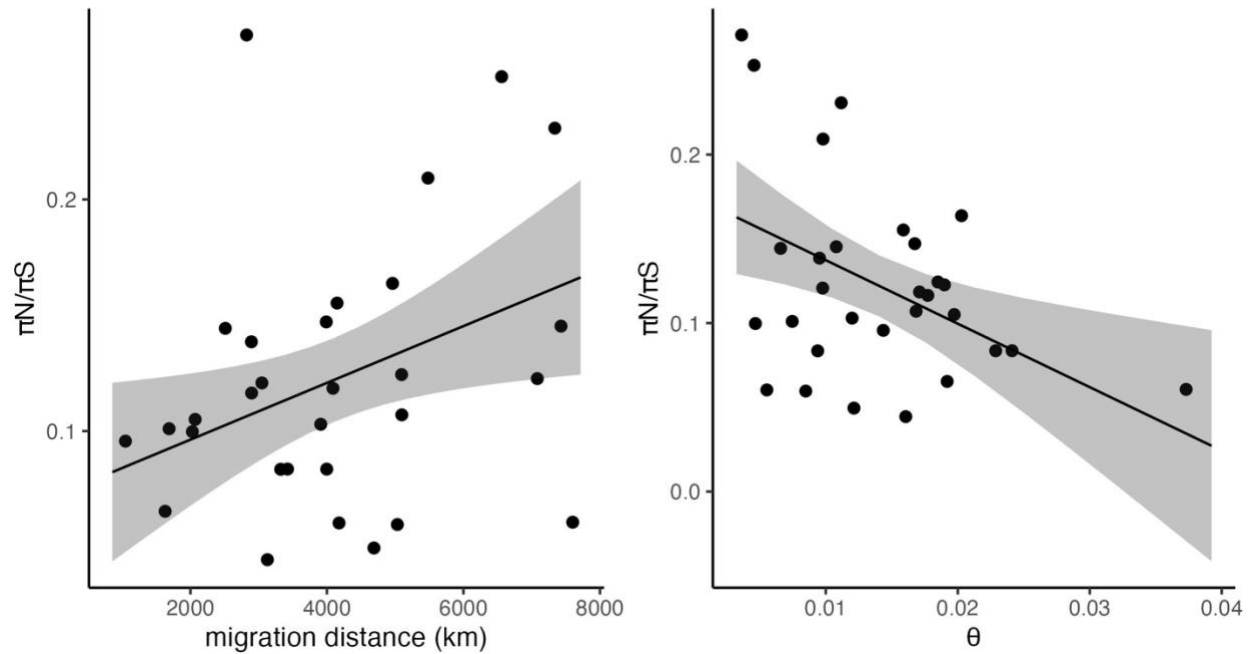
873 relationship with dS. Plotted lines use linear models to visualize the relationship between  
874 estimated tip dS and a given covariate within each family of birds (when represented in our  
875 dataset by two or more species), demonstrating a consistently negative relationship between dS  
876 and migration distance within and among major clades in our system. In panel C, the  
877 phylogenetic tree was created in phytools (Revell 2012) and is colored based on posterior mean  
878 tip and node estimates of dS from Coevol.  
879



880

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

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

895

896 **Table 1.** Definitions of abbreviations for molecular substitution rates and population genetic  
 897 parameters and predictions for their relationships with migration distance.  
 898

Concept	Abbr.	Description and assumptions	Predictions (this study)
Synonymous substitution rate	dS	Assuming synonymous sites evolve neutrally, dS primarily reflects $\mu$ (Nei et al. 2010; Lanfear et al. 2014)	Negative relationship between migration distance and dS
Nonsynonymous substitution rate	dN	Assuming nonsynonymous sites are generally deleterious, dN is influenced by both $\mu$ and $N_e$ (reviewed in Nei 2005)	NA
dN/dS ratio	dN/dS	Assuming nonsynonymous mutations are generally deleterious, dN/dS reflects strength of purifying selection on dN while accounting for variation in $\mu$ . Low dN/dS = strong purifying selection. (Nei 2005; Kryazhimskiy and Plotkin 2008)	Negative relationship between $\theta$ and dN/dS, reflecting the influence of $N_e$ on dN/dS. Negative relationship between migration distance and dN/dS, 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 $\theta / \mu$ . (Watterson 1975; Nabholz et al. 2008a)	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 dN/dS 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 in dN/dS	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.

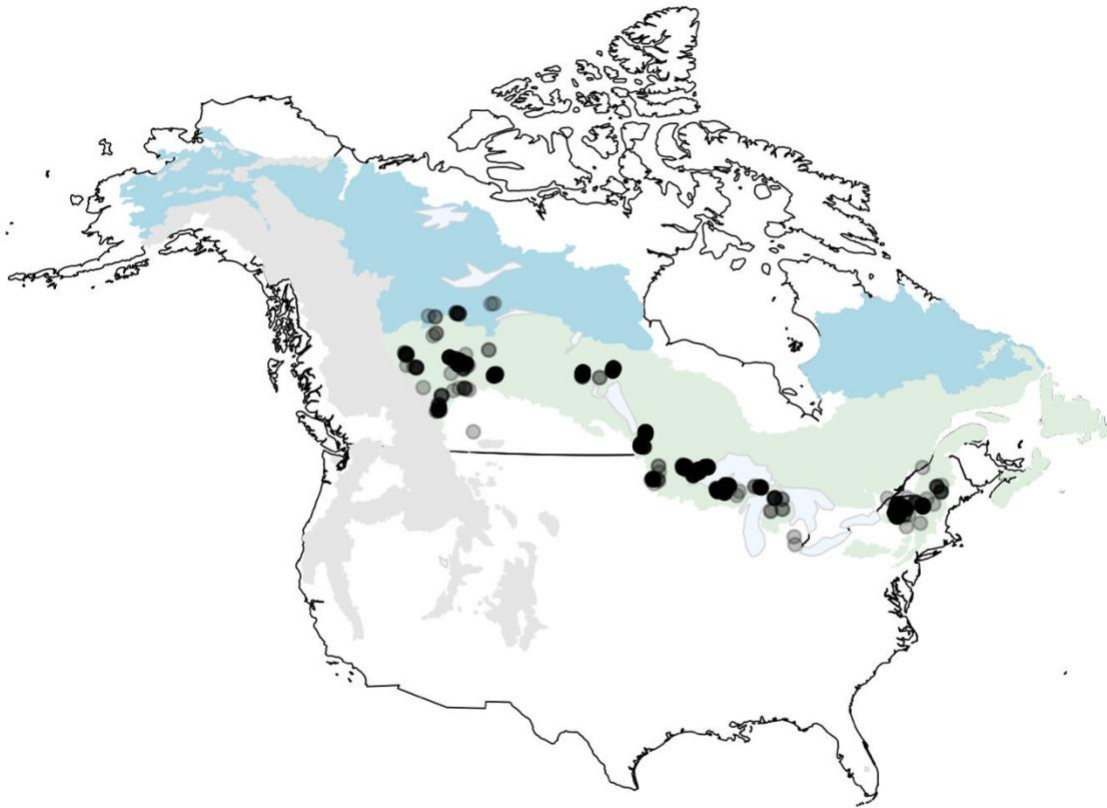
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901 **Table 2.** A summary of analyses. Models 1 and 2 use Coevol test our hypothesis that  
902 synonymous substitution rate (dS) is influenced by migration distance, with mass and  $\theta$  (model 2  
903 only) as additional covariates. Models 3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

		<b>Data subset</b>	<b>Method</b>
1	dS ~ migration distance + mass	full (39 species)	Coevol
2	dS ~ migration distance + mass + $\theta$	theta (30 species)	Coevol
3	dN/dS ~ migration distance + mass	full (39 species)	Coevol
4	dN/dS ~ migration distance + mass + $\theta$	theta (30 species)	Coevol
5	$\pi N/\pi S$ ~ migration distance + mass + $\theta$	theta (30 species)	linear modeling
6	$\theta$ ~ migration distance + mass	theta (30 species)	linear modeling

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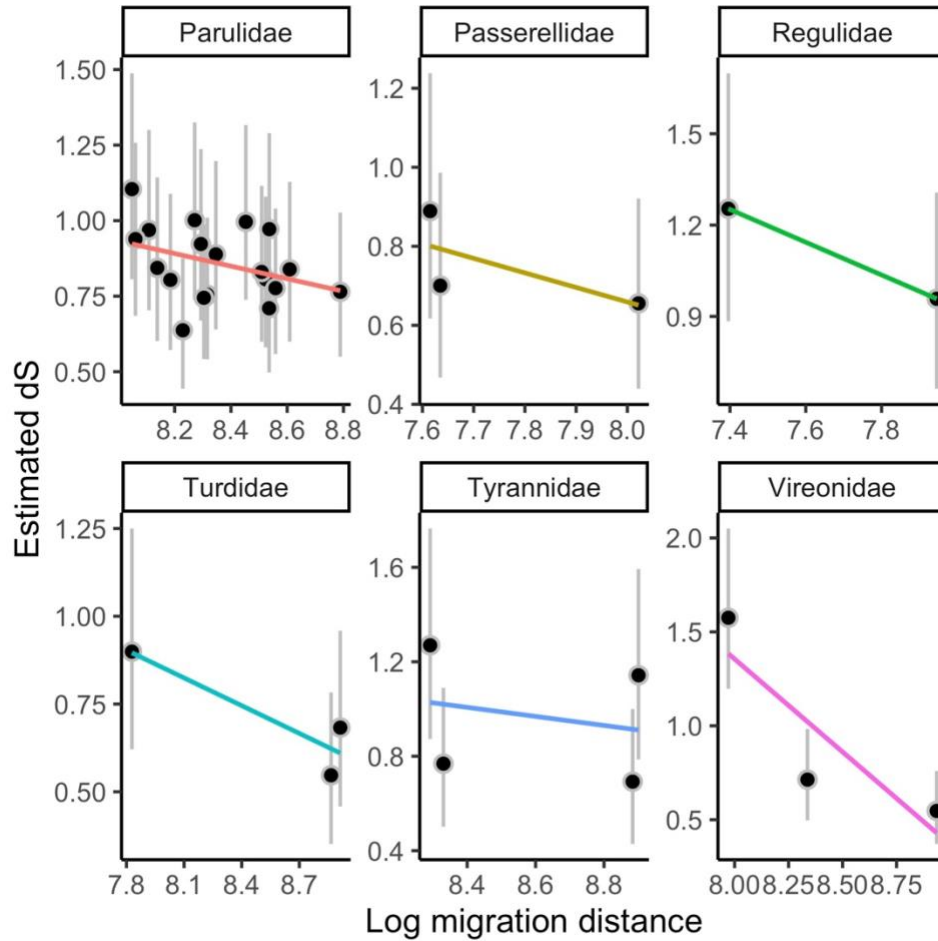
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913 **Figure S1.** Map of sampling locations for all 39 species. Each point represents an individual,  
914 such that darker shading indicates multiple individuals. The boreal forest (green), taiga (blue)  
915 and Rocky Mountains (grey) are designated following ‘level 1’ ecoregions defined by Omernik  
916 and Griffith (2014).

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918

919 **Figure S2.** The relationship between dS and migration distance within each family represented  
920 in our study by more than one species. Posterior mean tip estimates of dS (black dots) from  
921 Coevol are shown compared to migration distance (left), and mass (right) from models using our  
922 full species set. Gray vertical bars indicate 95% credible intervals for each estimate. Plotted lines  
923 use linear models to visualize the relationship between estimated tip dS and a given covariate  
924 within each family, demonstrating a consistently negative relationship between dS and migration  
925 distance within and among major clades in our system.

926



927 **Table S1.** [provided as a separate spreadsheet]. Species used in this study with information about  
928 each sequence used in Coevol analyses (sample catalog number); previously-published GenBank  
929 sequences used during sample processing for each species (seed sequence provided to  
930 NOVOPlasty and mitochondrial coding sequences used for annotation with Geneious); data used  
931 in this study (mass and migration distance from sources described in the methods,  $\theta$  as calculated  
932 in this study); the number of samples used to calculate population genetic summary statistics;  
933 posterior mean estimates of dS and dN/dS with upper and lower 95% credible intervals; and  
934  $\pi_N/\pi_S$  estimates. Estimates of dS and dN/dS (and credible intervals) each come from one  
935 replicate of a Coevol model using the entire 39-species dataset and serve as representative  
936 estimates. Detailed information about each sample, including all of those used in population  
937 genetic analyses, can be found in Table S2.  
938

939 **Table S2.** [provided as a separate spreadsheet]. Information about each sample used in this  
940 study, including its catalog number and institution, sex, date and locality of collection (state or  
941 province within the US or Canada and geographic coordinates), whether the sample was  
942 ultimately removed (retained = FALSE) from analysis because of low-quality data or other  
943 problems (1008 out of 1229 samples were retained), and the GenBank accession number for each  
944 mitochondrial gene. Museum abbreviations: AMNH = American Museum of Natural History;  
945 CMNH = Cleveland Museum of Natural History; CUMV = Cornell University Museum of  
946 Vertebrates; MMNH = Bell Museum of Natural History; MVZ = Museum of Vertebrate  
947 Zoology, UC Berkeley; NYSM = New York State Museum; RAM = Royal Alberta Museum;  
948 UMMZ = University of Michigan Museum of Zoology.  
949

950 **Table S3.** Coevol models using alternative topologies produce consistent results. This table  
951 summarizes Coevol's estimated relationship between dS and migration distance across models  
952 using 10 different marginal trees sampled from our phylogenetic tree dataset (see Methods). We  
953 show correlation coefficients and partial correlation coefficients and their associated posterior  
954 probabilities. Despite variation in topology across the marginal trees, these models each estimate  
955 similar correlation coefficients and similar levels support from posterior probabilities.

<b>Sampled tree</b>	<b>Cor. Coeff.</b>	<b>Cor. Coeff. Post. Prob.</b>	<b>Part. Cor. Coeff.</b>	<b>Part. Cor. Coeff. Post. Prob.</b>
1	-0.348	0.046	-0.379	0.03
2	-0.355	0.046	-0.405	0.023
3	-0.331	0.054	-0.371	0.029
4	-0.326	0.058	-0.303	0.067
5	-0.275	0.094	-0.274	0.089
6	-0.349	0.047	-0.379	0.029
7	-0.305	0.067	-0.297	0.072
8	-0.285	0.081	-0.28	0.079
9	-0.325	0.06	-0.321	0.057
10	-0.351	0.045	-0.406	0.021

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957

958 **Table S4.** [provided as a separate spreadsheet]. Full output from Coevol models with dS and dN  
959 as independent variables. Four models are summarized: two replicate models with the full dataset  
960 of 39 species, and two replicate models with the subset of 30 species with available estimates of  
961  $\theta$ . Each row contains a flattened pairwise matrix for each pair of variables (dS, dN, mass,  
962 migration distance, and  $\theta$ ), showing covariance, correlation coefficients, posterior probabilities  
963 of correlation coefficients, precisions, partial correlation coefficients, and posterior probabilities  
964 of partial correlation coefficients. Posterior probabilities near 0 indicate strong support for a  
965 negative relationship while posterior probabilities near 1 indicate strong support for a positive  
966 relationship. Values obtained from replicate models are highly similar.  
967

968 **Table S5.** [provided as a separate spreadsheet]. Full output from Coevol models with dS and  
969 dN/dS as independent variables. Four models are summarized: two replicate models with the full  
970 dataset of 39 species, and two replicate models with the subset of 30 species with available  
971 estimates of  $\theta$ . Each row contains a flattened pairwise matrix for each pair of variables (dS,  
972 dN/dS, mass, migration distance, and  $\theta$ ), showing covariance, correlation coefficients, posterior  
973 probabilities of correlation coefficients, precisions, partial correlation coefficients, and posterior  
974 probabilities of partial correlation coefficients. Posterior probabilities near 0 indicate strong  
975 support for a negative relationship while posterior probabilities near 1 indicate strong support for  
976 a positive relationship. Values obtained from replicate models are highly similar.  
977

978 **Table S6.** Full model selection results for models predicting  $\pi N/\pi S$ .

<b>Model formula</b>	<b>logLik</b>	<b>AICc</b>	<b>deltaAICc</b>	<b>weight</b>
$\pi N/\pi S \sim \theta + \text{migration distance}$	48.55	-87.5	0	0.55
$\pi N/\pi S \sim \theta + \text{mass} + \text{migration distance}$	48.68	-84.9	2.63	0.15
$\pi N/\pi S \sim \theta$	45.88	-84.8	2.65	0.15
$\pi N/\pi S \sim 1$ (null)	43.53	-82.6	4.89	0.048
$\pi N/\pi S \sim \text{migration distance}$	44.70	-82.5	5.02	0.045
$\pi N/\pi S \sim \theta + \text{mass}$	45.88	-82.2	5.33	0.038
$\pi N/\pi S \sim \text{mass}$	43.55	-80.2	7.31	0.014
$\pi N/\pi S \sim \text{mass} + \text{migration distance}$	44.70	-79.8	7.69	0.012

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981 **Table S7.** Full model selection results for models predicting  $\theta$ .

<b>Model formula</b>	<b>logLik</b>	<b>AICc</b>	<b>deltaAICc</b>	<b>weight</b>
$\theta \sim 1$ (null model)	105.96	-207.5	0	0.45
$\theta \sim$ migration distance	106.88	-206.6	0.82	0.30
$\theta \sim$ mass	106.08	-205.2	2.24	0.15
$\theta \sim$ mass + migration distance	107.10	-204.6	2.88	0.11

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