1   From Micro to Macro: Avian Chromosome Evolution is Dominated by Natural Selection

2

3

4

5

6   James M. Alfieri[1, 2], Kevin Bolwerk[1], Zhaobo Hu[1, 3], Heath Blackmon[1, 2]

7

8   1, Department of Biology, Texas A&M University
9   2, Ecology and Evolutionary Biology Program, Texas A&M University
10   3, Department of Biology and Biochemistry, University of Houston

11

12   **Abstract**

13   Birds display striking variation in chromosome number, defying the traditional view of highly
14   conserved avian karyotypes. However, the evolutionary drivers of this variability remain unclear.
15   To address this, we fit probabilistic models of chromosome number evolution across birds,
16   enabling us to estimate rates of evolution for total chromosome number and the number of
17   microchromosomes and macrochromosomes while simultaneously accounting for the impact of
18   other evolving traits. Our analyses revealed higher rates of chromosome fusion than fission
19   across all bird lineages. Notably, much of this signal was driven by Passeriformes, where
20   migratory species showed a particularly strong bias towards fusions compared to sedentary
21   counterparts. Furthermore, a robust correlation between the rearrangement rates of
22   microchromosomes and macrochromosomes suggests that genome-wide processes drive rates
23   of structural evolution. Additionally, we found that lineages with larger population sizes exhibited
24   higher rates of both fusion and fission, indicating that positive selection plays a dominant role in
25   driving divergence in chromosome number. Our findings illuminate the evolutionary dynamics of
26   avian karyotypes and highlight that, while the fitness effects of random structural mutations are
27   often deleterious, beneficial mutations may dominate karyotype divergence in some clades.

28

29

Introduction

Historically, avian karyotypes have been characterized as highly conserved with few exceptions, as most birds have a diploid number around 80, consisting of both microchromosomes and macrochromosomes (Griffin et al. 2007; Ellegren 2010). Indeed, bird genomes have been shown to have strikingly fewer intrachromosomal rearrangements than is typical in other clades (e.g., mammals and insects) (Li et al. 2021; Alfieri et al. 2023). However, recent work challenges the view that avian karyotypes are largely static. Degrandi et al. (2020) reported a wide range of diploid counts, from as few as 40 to over 140 chromosomes (**Figure 1**), highlighting substantial variation in chromosome number. This diversity is potentially significant, as chromosome architecture can influence recombination frequency, genetic linkage, hybrid compatibility, sex-determining mechanisms, and evolutionary rates (Stebbins 1958; White 1973; Britton-Davidian et al. 1990; Blackmon and Demuth 2015; Blackmon et al. 2015). Given the profound impacts that karyotype evolution can have and the vast dataset available (i.e., over 1,087 reported avian karyotypes), it is surprising that we have yet to determine whether chromosome number evolution in birds is primarily driven by natural selection or genetic drift and by extension whether extant variation in chromosome number has been generated through the fixation of deleterious, neutral, or beneficial mutations.
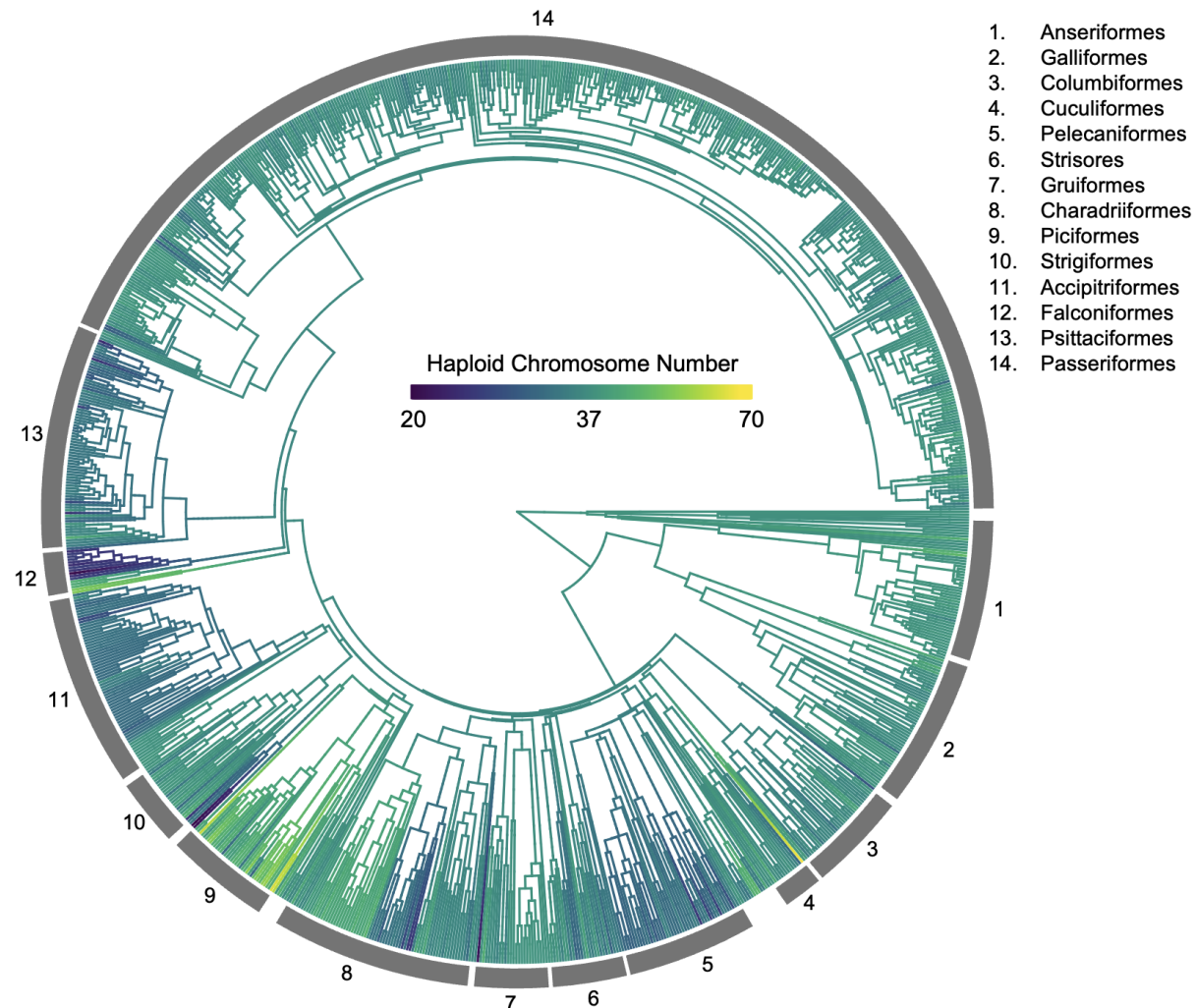
49

Figure 1. Haploid Chromosome Numbers Across Birds. Each branch on the phylogeny is painted to indicate chromosome number and the color ramp is on a log scale to ease visualization. The bands on the outside of the phylogeny indicate major orders.

Theoretical investigations have played a crucial role in shaping our understanding of chromosome number evolution. Early models, such as those proposed by Lande (1979), explored the conditions under which chromosomal rearrangements could become fixed in populations. These models emphasized the role of genetic drift, particularly in small populations, where the fixation of deleterious chromosomal mutations might occur despite their initial fitness costs. However, these theoretical approaches also have significant weaknesses. The assumption that chromosomal mutations are largely underdominant (i.e., deleterious when heterozygous) limits the applicability of these models, particularly in cases where chromosomal changes may have neutral or even beneficial effects.

Empirical studies have provided valuable, albeit limited, insights into the fitness effects of structural mutations. Radiation induced chromosomal mutations in both *Drosophila melanogaster* and *Tribolium castaneum* have allowed us to measure the fitness effect of a

67 range of structural mutations but it is unclear whether the fitness effects of induced mutations is
68 similar to the fitness effects of naturally occurring mutations (Muller 1930; Sokoloff 1977).
69 Similarly, controlled crosses between karyotypically distinct strains or species have provided
70 additional measures of the fitness of individuals heterozygous for chromosomal mutations
71 (Ratomponirina 1988; Britton-Davidian et al. 1990). However, these crosses typically also
72 involve genic divergence between the strains or species being crossed making it at best difficult
73 to isolate the effects of structural variation alone. Despite these limitations, a consensus
74 emerged that most chromosomal mutations are likely deleterious, particularly in heterozygous
75 individuals (King 1995).
76
77 With this base assumption that most chromosomal mutations are deleterious, researchers
78 began attempting to understand the characteristics that could lead to accelerated rates of
79 chromosome number evolution in lineages. Although formal phylogenetic comparative methods
80 were unavailable at the time, studies comparing evolutionary rates across placental mammals,
81 other vertebrates, and mollusks (Wilson et al. 1975), among mammalian clades (Bush et al.
82 1977), and among beetle families (Petitpierre 1987) all suggested that clades with smaller
83 effective population sizes had higher rates of evolution. Recent advances in comparative
84 phylogenetic methods have provided new opportunities to investigate the forces shaping
85 chromosome number evolution and for statistical tests of a range of hypotheses (Mayrose et al.
86 2010; Glick and Mayrose 2014; Zenil-Ferguson et al. 2017; Freyman and Höhna 2018;
87 Blackmon et al. 2019). These tools allow researchers to explicitly test hypotheses while
88 controlling for phylogenetic history. Empirical studies leveraging these comparative approaches
89 have begun to reveal patterns across large clades that were not accessible in the past.
90
91 Some clades (i.e., Carnivores and Coleoptera) show strong support for a pattern where lineages
92 with reduced effective population size have increased rates of chromosome number evolution
93 (Blackmon et al. 2024; Jonika et al. 2024). These results suggest that karyotype diversity in
94 these lineages may be generated primarily through the fixation of deleterious mutations that are
95 fixed by genetic drift. Outside of Carnivores most mammalian lineages show a pattern that is
96 consistent with female meiotic drive as the primary driving force in generating karyotypic
97 diversity (Blackmon et al. 2019). While probabilistic models of chromosome number evolution
98 have not (to our knowledge) been applied to birds as a whole, some studies have looked at
99 rates of structural evolution in subclades. For instance, the rate of inversions in island and
100 mainland Estrildid finches was compared and demonstrated that inversions fix more frequently
101 in mainland species suggesting that these inversion are fixed by natural selection (Hooper and
102 Price 2015).
103
104 Bird karyotypes are notable for their combination of macrochromosomes and
105 microchromosomes. This bimodal structure has been largely conserved since the emergence of
106 modern birds, with cytogenetic and comparative genomic evidence suggesting that the
107 ancestral avian karyotype resembled that of present-day species, such as chickens (Griffin et al.
108 2007; O'Connor et al. 2018; Zhang 2018). Microchromosomes differ from macrochromosomes
109 in several key ways: they are much smaller (Pichugin et al. 2001), typically more gene-dense
110 (Smith et al. 2000), and exhibit higher rates of recombination (Rodionov et al. 1992). These

111   characteristics lead to an expectation that microchromosomes should have higher evolutionary
112   rates of rearrangements than macrochromosomes. However, microchromosomes show
113   remarkable conservation in gene content and organization across extremely diverged avian
114   species (Damas et al. 2018; Waters et al. 2021), suggesting that rates of microchromosomes
115   and macrochromosomes are decoupled. This conservation suggests purifying selection against
116   structural rearrangements in microchromosomes. In contrast, macrochromosomes exhibit
117   greater variability, suggesting a higher rate of structural rearrangements, particularly in some
118   specific lineages (O'Connor et al. 2019, 2024; Kretschmer et al. 2021).
119
120   In this study, we aim to clarify whether chromosome number evolution in birds is primarily driven
121   by genetic drift or natural selection and determine if the tempo and mode of evolution in
122   microchromosomes and macrochromosomes is coupled or decoupled. By leveraging extensive
123   comparative analyses of avian karyotypes, we demonstrate that species with larger population
124   sizes have elevated rates of chromosome fission and fusions, suggesting natural selection as a
125   key force in shaping karyotype dynamics. Additionally, our results indicate that
126   microchromosomes and macrochromosomes have coupled evolutionary rates of fissions and
127   fusions. These findings provide insights into the balance of adaptive and stochastic processes
128   driving genomic features in birds.
129
130   **Methods**
131
132   *Population Size*
133
134   We had two approaches for investigating the role of population size on chromosome number
135   evolution. The first approach was to characterize orders (with greater than 20 species) into
136   population size groups (small, medium, and large) based on three traits hypothesized to be
137   associated with effective population size (N_e). The traits used were body mass; small body mass
138   associated with lower N_e (Eo et al. 2011), range size; small range size associated with lower N_e
139   (Gaston and Blackburn 1996), and trophic level; with expected N_e lowest in carnivores,
140   intermediate in omnivores and highest in herbivores (Brüniche-Olsen et al. 2021). We obtained
141   this trait data from Tobias et al. (2022). For each of these traits we separated species into the
142   lowest quartile, the middle two quartiles, or the upper quartile. Each order was then assigned a
143   value of -1, 0, or 1 respectively based on which of these quartiles most species fell into.
144
145   For body mass, the lowest quartile of masses was less than 21.40 grams. The second group
146   contained the middle two quartiles, and the third group contained the highest quartile of masses
147   that were greater than 460 grams. For range size, the lowest quartile of range sizes was less
148   than 1.5 million sq km. The second group contained the middle two quartiles, and the third
149   group contained the highest quartile of range sizes greater than 10.35 million sq km. We
150   classified the trophic levels of species into 3 groups (i.e., herbivores, omnivores, and
151   carnivores). Similar to the previous two traits each order was assigned a value of -1, 0, or 1
152   depending on which trophic level was most common in the order. These values were then
153   summed for each order leading to population size indices that ranged from -1 to 2 (**Table 1**). We

154 treated orders as small N_e if their indices were 1 or 2. Orders with indices of 0 were considered

155 medium N_e. Finally, orders with indices of -1 were treated as large N_e.

156

157 **Table 1. Effective population size scoring scheme.** Positive values represent small

158 population size species, while negative numbers represent large population size species.

	Range Size	Trophic Level	Mass	Total
Accipitriformes	0	+1	+1	2
Anseriformes	-1	-1	+1	-1
Charadriiformes	0	+1	0	1
Columbiformes	0	-1	0	-1
Galliformes	+1	-1	+1	1
Gruiformes	0	-1	+1	0
Passeriformes	0	+1	-1	0
Pelecaniformes	-1	+1	+1	1
Piciformes	0	+1	0	1
Psittaciformes	+1	-1	0	0
Strigiformes	0	+1	0	1
Strisores	0	+1	0	1

159

160 We used the R package ChromePlus to construct Markov models of chromosome evolution

161 allowing for two mechanisms of chromosome number change: fusions and fissions (Blackmon

162 et al. 2019, 2023; R Core Team 2024). We fit a model for each order with more than 20 species.

163 We used chromosome number data from the Bird Chromosome Database (Degrandi et al.

164 2020). We used a recent phylogeny containing most bird species (McTavish et al. 2024). We fit

165 the models in a Bayesian framework using R package diversitree (FitzJohn 2012). Each Markov

166 Chain Monte Carlo (MCMC) run was initialized with parameter values drawn from a uniform

167 distribution from 0 to 1 and uniform priors. MCMCs were run for 4000 generations. Although all

168 MCMCs appeared to reach convergence at 1000 generations, we conservatively discarded the

169 first 2000 generations as burn-in. All reported rates are lambda parameters for exponential

170 distributions describing the expected waiting time for a transition to occur and are in units of

171 millions of years. From these estimated rates, we tested for phylogenetic signal and performed

172 a Spearman rank correlation analysis between the population size score and the mean rate of

173 fissions and fusions (Revell 2012).

174

175 Our second approach for investigating the impact of population size on rates of chromosome

176 number evolution was to utilize global abundance estimates (species census population size)

177 for every species in our dataset, regardless of order representation. We obtained global

178 abundance estimates from Callaghan et al. (2021). Species in the lowest quartile of abundance

179 estimates (< 1395806) were considered small population size species, while species in the

180 highest quartile of abundance estimates (> 16082707) were considered large population size

181 species. After subsetting for species in these quartiles, we incorporated the presence of this

182 binary trait (population size) and transitions between states into our model of chromosome

183 evolution (Blackmon et al. 2023). Each MCMC run was initialized with parameter values drawn

184 from a uniform distribution from 0 to 1 and uniform priors. The MCMC was run for 10000

185 generations. Although the MCMC appeared to reach convergence at 1000 generations, we

186 conservatively discarded the first 2000 generations as burn-in.

187
188 *Microchromosome vs. Macrochromosome Rates of Evolution*
189
190 We searched the literature compiled in the Bird Chromosome Database for instances where
191 microchromosomes were differentiated from macrochromosomes (Degrandi et al. 2020). This
192 involved searching 353 references containing 1,562 karyotype descriptions of 1086 species. We
193 only included data in which the authors had explicitly provided the number of
194 microchromosomes and macrochromosomes in the karyotype. Authors were not consistent in
195 their definitions of microchromosomes or the heuristics they chose to use. Some authors
196 defined microchromosomes with relative length cut-offs (Hassan 1998; Ebied et al. 2005), while
197 others defined them using cytological and staining techniques (Kaul and Ansari 1978; de
198 Oliveira Furo et al. 2017), and still others differentiate them based on a notable size difference
199 (Goldschmidt et al. 1997). Despite these variations in definitions, the categorization into
200 microchromosomes and macrochromosomes has been strongly supported by the genomics
201 revolution. Specifically, microchromosomes have been shown to have a size distribution
202 independent of macrochromosomes (Burt 2002; International Chicken Genome Sequencing
203 Consortium 2004), elevated GC and gene content (International Chicken Genome Sequencing
204 Consortium 2004; Ellegren 2010), elevated recombination rates (even after controlling for their
205 small size) (Griffin et al. 2007; Skinner and Griffin 2012), and evolutionary conservation across
206 broad lineages (Ellegren 2010; Romanov et al. 2014).
207
208 We estimated the rates of fissions and fusions for microchromosomes and macrochromosomes
209 separately. We ran models of chromosome evolution separately for orders with more than 10
210 species with both microchromosome and macrochromosome counts (N = 8 orders). Each
211 MCMC run was initialized with parameter values drawn from a uniform distribution from 0 to 1.
212 Initial runs showed that the MCMC occasionally failed to mix well and instead explored regions
213 of parameter space that were characterized by unrealistically high rates. To remedy this we
214 used a weak broad exponential prior with a rate parameter of 0.1. We then ran each MCMC for
215 4000 generations, and discarded the first 2000 as burn-in. We used R package phytools to test
216 for phylogenetic signal (Revell 2012). We then performed a Spearman Rank Correlation test to
217 determine if the mean rate of microchromosome evolution was correlated with the mean rate of
218 macrochromosome evolution.
219
220 **Results**
221
222 We first estimated fission and fusion rates on all birds which had chromosome number and
223 phylogenetic information (N=1027). We found that fusion rates were slightly higher than fission
224 rates. The mean of the fission rate estimates was 0.12 (95% credible interval 0.09 to 0.15). The
225 mean of the fusion rate estimates was 0.20 (95% credible interval 0.16 to 0.23).
226
227 *Population Size*
228
229 Our first approach to investigate the impact of population size on rates of evolution was to
230 characterize orders (with greater than 20 species) into population size groups (small, medium,

231 and large) based on traits hypothesized to affect effective population size (body mass, range
232 size, and trophic level). We had 12 orders with species counts greater than 20: Accipitriformes,
233 Anseriformes, Charadriiformes, Columbiformes, Galliformes, Gruiformes, Passeriformes,
234 Pelecaniformes, Piciformes, Psittaciformes, Strigiformes, and Strisores. In total, 892 species
235 were included in these order-level models (**Table 2; Figure 2; Supplemental 1**). Our original
236 models for Piciformes yielded extremely variable rates (mean rate = 2.43/MY, 95% Credible
237 Interval: 0.71 - 6.21). It has been well documented that many comparative methods can be
238 heavily influenced by extreme signals in a very small portion of the phylogeny (Rabosky and
239 Goldberg 2015). To determine if this was the case, we used the tipRate function in the R
240 package evobiR (Jonika et al. 2023) and found one species, *Pteroglossus aracari*, that exhibited
241 recent, drastic reduction in chromosome number (Haploid chromosome number = 31) compared
242 to its closest relative diverged 5.76 MYA, *Pterroglossus castanotis* (Haploid chromosome
243 number = 43). To determine if this tip was leading to our elevated rate estimate in Piciformes,
244 we removed this outlier and repeated our analysis. Even after removal of this taxa Piciformes
245 still had the highest rates of fission and fusion across all examined orders. However, we found
246 that removal of this species led to a 3-fold reduction in the mean rate estimate for this order. In
247 an effort to focus on broadly supported rates of evolution we pruned this taxa from further
248 analyses.

249 **Table 2. Order level rates of chromosome evolution.** For each of 12 orders columns indicate
250 the fission, fusion, and mean rates as well as credible intervals for each.

|	Fissions (MY)	95% Credible Interval	Fusions (MY)	95% Credible Interval	Mean (MY)	95% Credible Interval
Accipitriformes	0.03	0.02 - 0.06	0.03	0.01 - 0.05	0.03	0.02 - 0.05
Anseriformes	0.05	0.02 - 0.09	0.03	0.01 - 0.07	0.04	0.02 - 0.07
Charadriiformes	0.16	0.05 - 0.29	0.22	0.08 - 0.33	0.19	0.12 - 0.28
Columbiformes	0.09	0.00 - 0.19	0.09	0.00 - 0.20	0.09	0.03 - 0.15
Galliformes	0.12	0.03 - 0.22	0.13	0.04 - 0.23	0.13	0.06 - 0.19
Gruiformes	0.05	0.02 - 0.09	0.02	0.00 - 0.05	0.03	0.02 - 0.06
Passeriformes	0.05	0.03 - 0.06	0.10	0.08 - 0.12	0.07	0.06 - 0.09
Pelecaniformes	0.12	0.03 - 0.23	0.17	0.08 - 0.27	0.14	0.07 - 0.22
Piciformes	0.84	0.33 - 1.46	0.84	0.03 - 1.53	0.84	0.34 - 1.43
Psittaciformes	0.32	0.10 - 0.64	0.58	0.35 - 0.77	0.45	0.29 - 0.62
Strigiformes	0.27	0.04 - 0.56	0.33	0.08 - 0.59	0.30	0.11 - 0.54
Strisores	0.16	0.01 - 0.33	0.15	0.00 - 0.32	0.15	0.05 - 0.31
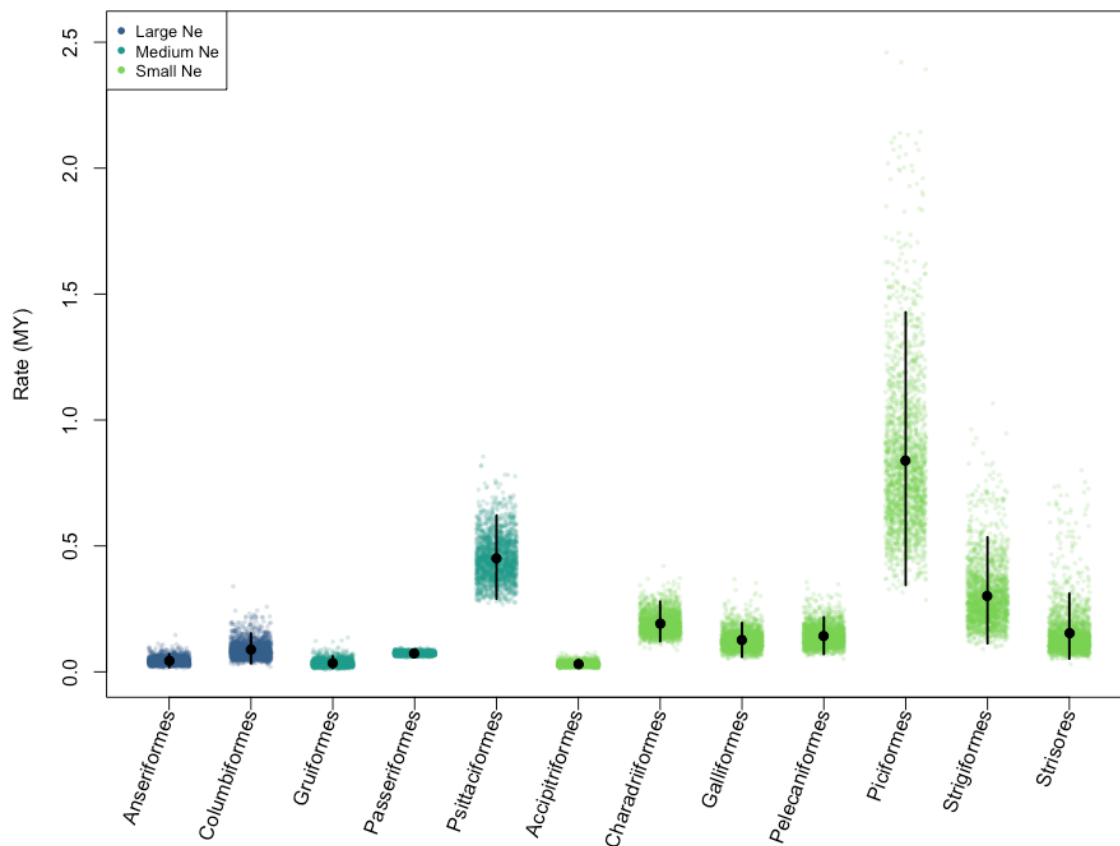
Figure 2. Mean rates of chromosome evolution across bird orders. Each clade displays 2000 posterior samples of mean rate estimates. The posterior mean is marked with a black circle, and the 95% credible interval is shown with a vertical black line. Colors represent the N_e class for each clade. The orders are in order of decreasing population size score.

We did not find any phylogenetic signal in the residuals of the relationship between mean rate and the N_e classification (K = 0.87, p = 0.767). There was no linear relationship between N_e classification and mean rate (p = 0.41), and there was no correlation (Spearman's Rho = 0.39, S = 173.26, p = 0.205).

Our second approach was to utilize global abundance estimates (species census population size) for every species in our dataset regardless of order. We had 480 birds in our analysis (240 in the small population size state and 240 in the large population size state). Several small orders were monomorphic for population size, 10 orders were completely classified as small pop size (N = 29) while 6 orders were completely classified as large pop size (N = 8).

The mean fission and fusion rates for large population size lineages was 0.36 and 0.35 respectively (95% Credible Interval for both parameters was: 0.28 - 0.43). The mean fission and fusion rates for small population size lineages was 0.02 and 0.01 respectively (95% Credible Intervals of 0.01 - 0.03 and 0.002 - 0.03 respectively). We computed a mean rate difference statistic, denoted as ΔR_χ, where χ is the model parameter of interest (fissions or fusions). For

273   each post-burnin sample, we calculated ΔR_χ as follows: $\Delta R_\chi = \chi_{\text{small population}} - \chi_{\text{large population}}$. We

274   then assessed the 95% credible interval for ΔR_χ. If this interval is entirely below zero it provides

275   strong evidence that the fission and/or fusion rates are higher in clades with large population

276   sizes. Conversely, if the credible interval includes zero, it suggests little to no evidence for a

277   difference in rates between clades with small and large population sizes. We estimated the

278   mean ΔR for fissions to be -0.34 (95% Credible Interval: -0.41 to -0.26), and the mean ΔR for

279   fusions to be -0.34 (95% Credible Interval: -0.42 to -0.26). As the credible intervals do not

280   overlap zero, we infer that large population size species have higher rates of chromosomal
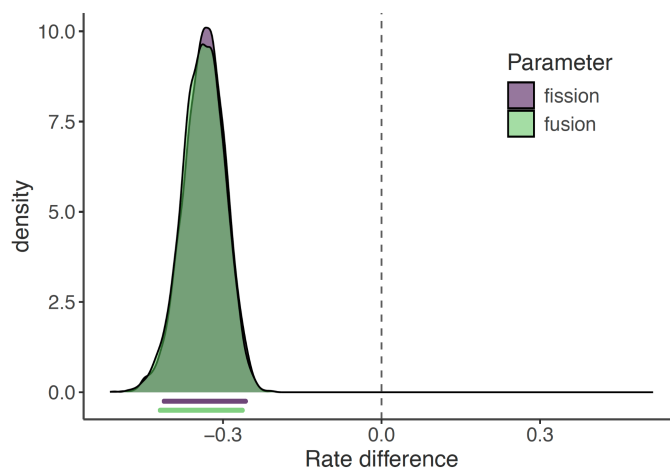
281   fissions and fusions (**Figure 3**).

282

283

284

285   **Figure 3. Chromosome evolution in small population size and large population size**

286   **lineages.** Each curve shows the distribution of the rate difference in small population size and

287   large population size lineages. The bars below the curve indicate the 95% credible interval for

288   the rate differences.

289

290   To assess whether our findings were disproportionately influenced by a few species with

291   extreme chromosome counts, we conducted a secondary analysis. Following Jonika et al.

292   (2024), we used the GetTipRates function in R package evobiR (Jonika et al. 2023) to calculate

293   the difference between an extant species' chromosome number and the most probable

294   chromosome number of the immediate ancestor. Species with exceptionally high tip rates could

295   reduce the generalizability of our results to broader clades. To address this, we repeated our

296   analysis–rate estimation with MCMCs and ΔR_χ calculation–excluding species with tip rates

297   greater than 0. This analysis revealed a similar pattern, with large population species exhibiting

298   faster rates than small population species, although the magnitude of the difference was

299   somewhat reduced (**Supplemental 2**).

300

301   _Post-hoc Analysis on Passeriformes Migratory Behavior_

302

303   We were surprised by how Passeriformes was the only bird order that had elevated rates of

304   fusions relative to fissions. We hypothesized that this rate difference might ultimately be due to

305   differences in migratory behavior among Passeriformes. Specifically, we reasoned that

306   migratory birds may more frequently be under selection for coadapted gene complexes
307   important in adaptation to diverse environments (Stebbins 1971). To investigate this we
308   performed a post-hoc analysis. We obtained migration data from Tobias et al. (2022). We
309   incorporated the presence of this binary trait (migratory or sedentary) and transitions between
310   states into our model of chromosome evolution (Blackmon et al. 2023). Each MCMC run was
311   initialized with parameter values drawn from a uniform distribution from 0 to 1 and uniform
312   priors. The MCMC was run for 10000 generations. Although the MCMC appeared to reach
313   convergence at 1000 generations, we conservatively discarded the first 2000 generations as
314   burn-in. We estimated ΔR statistics for fusion and fission rates by subtracting the rate inferred in
315   migratory lineages from the rate inferred in sedentary lineages. The mean of the ΔR_{fusion} statistic
316   was -0.08 with a credible interval of -0.14 to -0.03. The mean of the $\Delta R_{fission}$ statistic was -0.02
317   with a credible interval of -0.07 to 0.02. These results suggest that migratory Passeriformes
318   have higher rates of fusions than sedentary Passeriformes, but equal rates of fissions
319   (**Supplemental 3**).
320
321   *Microchromosome vs. Macrochromosome Rates*
322
323   We had 345 species which had both phylogenetic and microchromosome information. These
324   345 species represented 24 orders. Across these orders, we found a significant negative
325   relationship between the number of microchromosomes and number of macrochromosomes
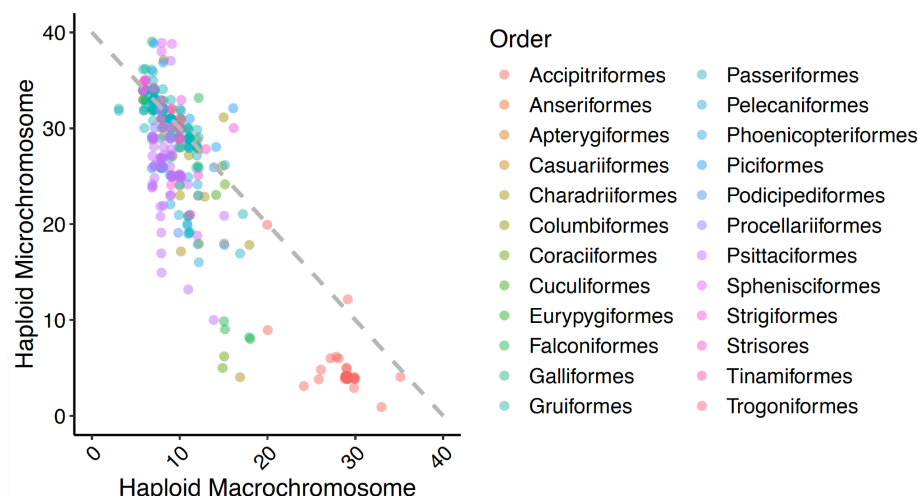326   (**Figure 4**).
327
328

329
330   **Figure 4. Haploid count of microchromosomes and macrochromosomes.** The data plotted
331   are all 339 species for which both microchromosome and macrochromosome counts have been
332   reported. The points are slightly jittered to minimize complete overlaps, and colors represent the
333   order in which the species belongs. The grey dashed diagonal indicates a sum of 40 which is
334   the modal value for our dataset.
335
336   We were interested in determining whether rates of microchromosomes and
337   macrochromosomes evolution were coupled. For our analysis, we retained orders with more

338   than 10 species (N = 8): Accipitriformes, Anseriformes, Charadriiformes, Galliformes,
339   Gruiformes, Passeriformes, Pelecaniformes, and Psittaciformes. For each order we fit a simple
340   model of chromosome evolution where the chromosome number was the haploid count of
341   macrochromosomes or microchromosomes. Each MCMC was initiated with parameter values
342   drawn from a uniform distribution and run for 4000 generations with 2000 generations discarded
343   as burnin. We did not find a significant phylogenetic signal of the relationship between
344   microchromosome and macrochromosome evolutionary rates across these orders (K = 0.859, p
345   = 0.643). To determine whether rates for microchromosomes and macrochromosomes were
346   coupled we calculated Spearman's correlation coefficient for the mean rate of
347   microchromosome and macrochromosome evolution across the eight included orders. This
348   process was repeated for each of our 2000 samples from the posterior distribution of rate
349   estimates. The credible interval of the correlation coefficient ranged from 0.02 to 0.88. This
350   suggests that rates of microchromosome and macrochromosome evolution are coupled (**Figure**
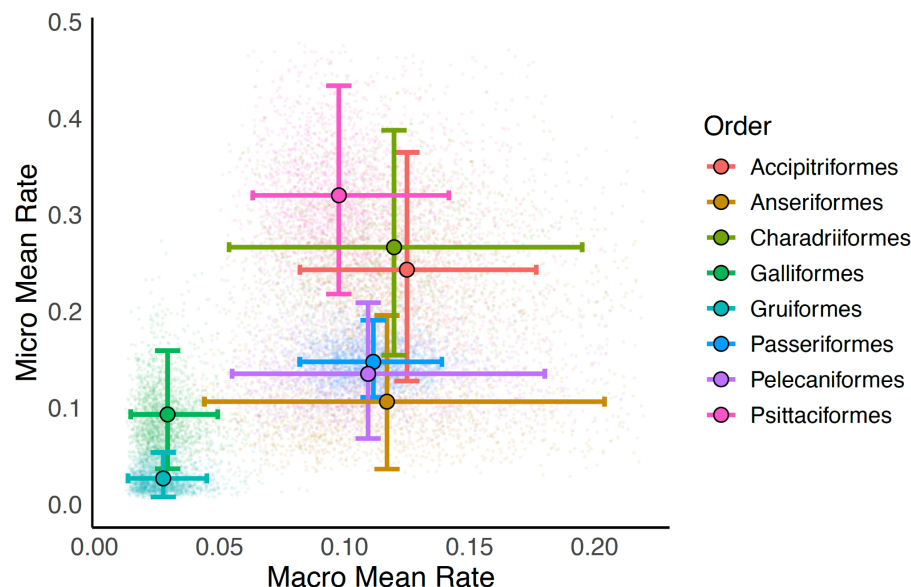351   **5**).
352
353

354
355   **Figure 5. Rates of evolution in microchromosomes and macrochromosomes.** The
356   horizontal dimension indicates the mean of the fusion and fission rate in macrochromosomes
357   while the vertical dimension indicates the mean of the fission and fusion rate in
358   microchromosomes. Error bars indicate the 95% credible interval. All elements are colored to
359   reflect the order. The plot is cropped for visualization purposes and fails to include 165
360   (approximately 1%) of the sampled macrochromosome rates that are well beyond the credible
361   interval.
362
363   **Discussion**
364
365   *Selection vs Drift*
366

367   A central goal of our study was to determine whether natural selection or genetic drift dominates
368   the evolution of chromosome number in birds. By extension this also allows us to determine the
369   average fitness effect of mutations that have led to divergence in chromosome number among
370   birds (Mackintosh et al. 2023). Briefly, if lineages that have large N_e have higher rates of
371   evolution than those with small N_e, we can infer that selection dominates chromosome number
372   evolution and that these mutations must be on average beneficial (Qumsiyeh and Handal 2022).
373   In contrast, if lineages that have small N_e have higher rates of evolution than those with large N_e
374   we can infer that drift must dominate chromosome number evolution and that these mutations
375   must be on average deleterious (Wilson et al. 1975; Bush et al. 1977). Finally, if we find no
376   significant differences in the rates of evolution among small and large N_e lineages then we
377   would infer that on average these mutations are effectively neutral.
378
379   We did not find a significant relationship between population size–measured using the proxies
380   body mass, range size, and trophic level–and chromosomal fission and fusion rates when
381   categorizing entire orders into homogenous population size groups. This lack of a relationship
382   could stem from several factors. First, our proxies may not accurately reflect effective population
383   size. Additionally, characterizing an entire order as having uniformly "small" or "large" population
384   sizes may oversimplify the substantial variation within orders. It is also possible that our dataset,
385   comprising only 12 orders, lacks the statistical power to detect such a relationship. Finally, there
386   may truly be no connection between population size and chromosomal rearrangement rates. Of
387   these possibilities, the oversimplification of population size within orders seems the most likely
388   explanation, as we observed strong, consistent patterns when accounting for finer-scale
389   variation.
390
391   We did find a significant relationship between population size and chromosomal fission and
392   fusion rates when analyzing all bird species collectively. Specifically, we found that larger
393   populations exhibited higher rates of fissions and fusions compared to smaller populations,
394   suggesting that chromosomal fissions and fusions are significant evolutionary mechanisms that
395   contribute to the adaptation of birds, and that selection is the dominant driver of chromosome
396   number changes across birds. Our results align with findings by Hooper and Price (2015), who
397   observed higher fixation rates of chromosomal inversions in genetically diverse, larger mainland
398   Estrildid finches compared to smaller island populations, suggesting positive selection as the
399   dominant mechanism driving fixation of intrachromosomal variants in birds. Our finding does
400   conflict or add nuance to a previous hypothesis that natural selection acted primarily to maintain
401   stasis in bird karyotypes (Zhang 2018). Instead, we would argue that while natural selection
402   certainly does act as a sieve and limit the number and type of structural mutations that can fix in
403   populations, it also serves as the primary force in driving disparification of chromosome number
404   across birds.
405
406   *Fusions vs Fissions*
407
408   We found that in our analysis of all birds rates of fusions were higher than rates of fissions. This
409   pattern appears to be strongly driven by the signal from Passeriformes. In our order level
410   analyses we found that Passeriformes was the only order of birds to exhibit unequal rates of

411 chromosomal fissions and fusions, with significantly higher rates of fusions than fissions. Within
412 passerines migratory lineages had higher fusion rates than sedentary lineages. This pattern
413 may be explained by several factors. First, migratory species often maintain larger effective
414 population sizes (Winker and Delmore 2023), which reduces the effects of drift. In such
415 populations, chromosomal fusions that might otherwise be lost to drift in smaller, more isolated
416 sedentary populations can persist, potentially accumulating over time. Second, selective
417 pressures associated with long-distance navigation could favor chromosomal fusions if they
418 enhance gene regulation or create co-adapted gene complexes that are advantageous in
419 divergent environments (Stebbins 1971; Liu et al. 2022). Finally, fusions may confer energy
420 efficiency by reducing the total number of chromosomes, which could lower the energetic costs
421 of cellular processes, a potential advantage for species engaged in metabolically-intense
422 activities like long-distance flight for migration (Waltari and Edwards 2002; Wright et al. 2014;
423 Gregory 2018).
424
425 _Microchromosomes vs Macrochromosomes_
426
427 Our findings show that the number of microchromosomes is negatively correlated with the
428 number of macrochromosomes (**Figure 4**). This suggests that to some extent chromosome
429 number can stay constant with only the partitioning between microchromosomes and
430 macrochromosomes changing. This difference in partitioning could be due to biological reality
431 (e.g., size expansion of microchromosome to such an extent that cytogeneticists classify them
432 as microchromosomes) or due to differences in the heuristics that cytogeneticists use to classify
433 chromosomes as microchromosomes or macrochromosomes as discussed in the introduction.
434 In contrast if we look at the species that occur off the primary diagonal in **Figure 4**, we see
435 species where fusions or fissions have changed the count of one or both size classes of
436 chromosomes.
437
438 We observed a significant positive correlation between the evolutionary rates of
439 microchromosomes and macrochromosomes across birds. This finding challenges the
440 traditional view that microchromosomes evolve independently of macrochromosomes in birds
441 (Damas et al. 2018; O'Connor et al. 2019). Instead, it suggests that shared mechanisms drive
442 chromosomal rearrangements regardless of chromosome size. This could be mediated by the
443 genomic architecture of evolutionary breakpoint regions (EBRs) that can span both
444 microchromosomes and macrochromosomes (Claeys et al. 2023).
445
446 _Conclusion_
447
448 Our study has several important limitations that should be acknowledged. First, the distinction
449 between germline-restricted chromosomes (GRCs) and standard chromosomes was not
450 explicitly addressed. GRCs are unique chromosomes found in germline cells but absent in
451 somatic cells. They are known to occur in all Passeriformes but not other birds (Torgasheva et
452 al. 2019). Not accounting for this variation could influence our rate calculations in
453 Passeriformes. Secondly, the definition of microchromosomes is not universally standardized,

454 and differences across studies biases among clades could lead to inaccuracies in our rate
455 estimates.
456
457 The part of the Wright-Fisher debate in evolutionary biology centers around the relative
458 importance of genetic drift and natural selection in shaping genetic variation and evolutionary
459 trajectories (Provine 1992). This debate can be extended to the evolution of chromosome
460 number across different clades, where some groups exhibit patterns consistent with Wrightian
461 dynamics, dominated by genetic drift, while others show Fisherian characteristics, where
462 selection plays a predominant role. In clades such as Coleoptera and Carnivora chromosome
463 number evolution appears to be dominated by genetic drift (Blackmon et al. 2019, 2024; Jonika
464 et al. 2024). Conversely, in birds chromosome number evolution appears to be dominated by
465 natural selection.
466

472 **Data Availability Statement**
473 The data as well all analysis code underlying this article are available in GitHub repository, at
474 https://github.com/JamieAlfieri/bird.chroms.
475

479 **References**

480 Alfieri, J. M., M. M. Jonika, J. N. Dulin, and H. Blackmon. 2023. Tempo and mode of genome

481   structure evolution in insects. Genes 14:336. MDPI AG.

482 Blackmon, H., M. Chin, and M. Jonika. 2023. chromePlus: Analysis of Chromosome Number

483   Evolution and Binary Traits.

484 Blackmon, H., and J. P. Demuth. 2015. The fragile Y hypothesis: Y chromosome aneuploidy as

485   a selective pressure in sex chromosome and meiotic mechanism evolution. Bioessays

486   37:942–950.

487 Blackmon, H., N. B. Hardy, and L. Ross. 2015. The evolutionary dynamics of haplodiploidy:

488   Genome architecture and haploid viability. Evolution 69:2971–2978.

489 Blackmon, H., M. M. Jonika, J. M. Alfieri, L. Fardoun, and J. P. Demuth. 2024. Drift drives the

490   evolution of chromosome number I: The impact of trait transitions on genome evolution in

491   Coleoptera. J. Hered. 115:173–182. Oxford University Press (OUP).

492   Blackmon, H., J. Justison, I. Mayrose, and E. E. Goldberg. 2019. Meiotic drive shapes rates of

493   karyotype evolution in mammals. Evolution 73:511–523. Wiley.

494   Britton-Davidian, J., H. Sonjaya, J. Catalan, and G. Cattaneo-Berrebi. 1990. Robertsonian

495   heterozygosity in wild mice: fertility and transmission rates in Rb(16.17) translocation

496   heterozygotes. Genetica 80:171–174. Springer Nature.

497   Brüniche-Olsen, A., K. F. Kellner, J. L. Belant, and J. A. DeWoody. 2021. Life-history traits and

498   habitat availability shape genomic diversity in birds: implications for conservation. Proc.

499   Biol. Sci. 288:20211441.

500   Burt, D. W. 2002. Origin and evolution of avian microchromosomes. Cytogenet. Genome Res.

501   96:97–112. S. Karger AG.

502   Bush, G. L., S. M. Case, A. C. Wilson, and J. L. Patton. 1977. Rapid speciation and

503   chromosomal evolution in mammals. Proc. Natl. Acad. Sci. U. S. A. 74:3942–3946.

504   Callaghan, C. T., S. Nakagawa, and W. K. Cornwell. 2021. Global abundance estimates for

505   9,700 bird species. Proc. Natl. Acad. Sci. U. S. A. 118:e2023170118. Proceedings of the

506   National Academy of Sciences.

507   Claeys, J., M. N. Romanov, and D. K. Griffin. 2023. Integrative comparative analysis of avian

508   chromosome evolution by in-silico mapping of the gene ontology of homologous synteny

509   blocks and evolutionary breakpoint regions. Genetica 151:167–178. Springer Science and

510   Business Media LLC.

511   Damas, J., J. Kim, M. Farré, D. K. Griffin, and D. M. Larkin. 2018. Reconstruction of avian

512   ancestral karyotypes reveals differences in the evolutionary history of macro- and

513   microchromosomes. Genome Biol. 19:155. Springer Science and Business Media LLC.

514   Degrandi, T. M., S. A. Barcellos, A. L. Costa, A. D. V. Garnero, I. Hass, and R. J. Gunski. 2020.

515   Introducing the Bird Chromosome Database: An overview of cytogenetic studies in birds.

516   Cytogenet. Genome Res. 160:199–205. S. Karger AG.

517 de Oliveira Furo, I., R. Kretschmer, M. S. Dos Santos, C. A. de Lima Carvalho, R. J. Gunski, P.

518   C. M. O'Brien, M. A. Ferguson-Smith, M. B. Cioffi, and E. H. C. de Oliveira. 2017.

519   Chromosomal Mapping of Repetitive DNAs in Myiopsitta monachus and Amazona aestiva

520   (Psittaciformes, Psittacidae) with Emphasis on the Sex Chromosomes. Cytogenet. Genome

521   Res. 151:151–160. S. Karger AG.

522 Ebied, A. M., H. A. Hassan, A. H. Abu Almaaty, and A. E. Yaseen. 2005. Karyotypic

523   characterization of ten species of birds. Cytologia (Tokyo) 70:181–194. International

524   Society of Cytology.

525 Ellegren, H. 2010. Evolutionary stasis: the stable chromosomes of birds. Trends Ecol. Evol.

526   25:283–291. Elsevier BV.

527 Eo, S. H., J. M. Doyle, and J. A. DeWoody. 2011. Genetic diversity in birds is associated with

528   body mass and habitat type: Microsatellite diversity in birds. J. Zool. (1987) 283:220–226.

529   Wiley.

530 FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R.

531   Methods in Ecology and Evolution 3:1084–1092. John Wiley & Sons, Ltd.

532 Freyman, W. A., and S. Höhna. 2018. Cladogenetic and anagenetic models of chromosome

533   number evolution: A Bayesian model averaging approach. Syst. Biol. 67:195–215.

534 Gaston, K. J., and T. M. Blackburn. 1996. Global scale macroecology: Interactions between

535   population size, geographic range size and body size in the Anseriformes. J. Anim. Ecol.

536   65:701. JSTOR.

537 Glick, L., and I. Mayrose. 2014. ChromEvol: assessing the pattern of chromosome number

538   evolution and the inference of polyploidy along a phylogeny. Molecular biology and

539   evolution 31:1914–1922. academic.oup.com.

540 Goldschmidt, B., D. M. Nogueira, D. W. Monsores, and L. M. Souza. 1997. Chromosome study

541   in two Aratinga species (A. guarouba and A. acuticaudata) (Psittaciformes). Rev. Bras.

542   Genet. 20:659–662. FapUNIFESP (SciELO).

543   Gregory, T. R. 2018. Genome size and brain cell density in birds. Can. J. Zool. 96:379–382.

544   Canadian Science Publishing.

545   Griffin, D. K., L. B. W. Robertson, H. G. Tempest, and B. M. Skinner. 2007. The evolution of the

546   avian genome as revealed by comparative molecular cytogenetics. Cytogenet. Genome

547   Res. 117:64–77. Cytogenet Genome Res.

548   Hassan, H. A. 1998. Karyological studies on six species of birds. Cytologia (Tokyo) 63:349–363.

549   International Society of Cytology.

550   Hooper, D. M., and T. D. Price. 2015. Rates of karyotypic evolution in Estrildid finches differ

551   between island and continental clades. Evolution 69:890–903. Wiley.

552   International Chicken Genome Sequencing Consortium. 2004. Sequence and comparative

553   analysis of the chicken genome provide unique perspectives on vertebrate evolution.

554   Nature 432:695–716. Springer Science and Business Media LLC.

555   Jonika, M. M., M. Chin, N. Anderson, R. H. Adams, J. P. Demuth, and B. H. 2023. EvoBiR:

556   Tools for comparative analyses and teaching evolutionary biology.

557   Jonika, M. M., K. T. Wilhoit, M. Chin, A. Arekere, and H. Blackmon. 2024. Drift drives the

558   evolution of chromosome number II: The impact of range size on genome evolution in

559   Carnivora. J. Hered. 115:524–531. Oxford University Press (OUP).

560   Kaul, D., and H. A. Ansari. 1978. Chromosome studies in three species of Piciformes (Aves).

561   Genetica 48:193–196. Springer Nature.

562   King, M. 1995. Species evolution: The role of chromosome change. Cambridge University

563   Press, Cambridge, England.

564   Kretschmer, R., M. S. de Souza, I. de O. Furo, M. N. Romanov, R. J. Gunski, A. D. V. Garnero,

565   T. R. O. de Freitas, E. H. C. de Oliveira, R. E. O'Connor, and D. K. Griffin. 2021.

566   Interspecies chromosome mapping in Caprimulgiformes, Piciformes, Suliformes, and

567   Trogoniformes (Aves): Cytogenomic insight into microchromosome organization and

568   karyotype evolution in birds. Cells 10:826. MDPI AG.

569   Lande, R. 1979. Effective Deme sizes during long-term evolution estimated from rates of

570   chromosomal rearrangement. Evolution 33:234–251. Wiley.

571   Li, J., J. Zhang, J. Liu, Y. Zhou, C. Cai, L. Xu, X. Dai, S. Feng, C. Guo, J. Rao, K. Wei, E. D.

572   Jarvis, Y. Jiang, Z. Zhou, G. Zhang, and Q. Zhou. 2021. A new duck genome reveals

573   conserved and convergently evolved chromosome architectures of birds and mammals.

574   Gigascience 10:giaa142. Oxford University Press (OUP).

575   Liu, Z., M. Roesti, D. Marques, M. Hiltbrunner, V. Saladin, and C. L. Peichel. 2022.

576   Chromosomal fusions facilitate adaptation to divergent environments in threespine

577   stickleback. Mol. Biol. Evol. 39:msab358. Oxford University Press (OUP).

578   Mackintosh, A., R. Vila, S. H. Martin, D. Setter, and K. Lohse. 2023. Do chromosome

579   rearrangements fix by genetic drift or natural selection? Insights from Brenthis butterflies.

580   Mol. Ecol., doi: 10.1111/mec.17146. Wiley.

581   Mayrose, I., M. S. Barker, and S. P. Otto. 2010. Probabilistic models of chromosome number

582   evolution and the inference of polyploidy. Syst. Biol. 59:132–144.

583   McTavish, E. J., J. A. Gerbracht, M. T. Holder, M. J. Iliff, D. Lepage, P. Rasmussen, B.

584   Redelings, L. L. Sanchez Reyes, and E. T. Miller. 2024. A complete and dynamic tree of

585   birds.

586   Muller, H. J. 1930. Types of visible variations induced by X-rays in Drosophila. Journal of

587   genetics 22:299–334.

588   O'Connor, R. E., L. Kiazim, B. Skinner, G. Fonseka, S. Joseph, R. Jennings, D. M. Larkin, and

589   D. K. Griffin. 2019. Patterns of microchromosome organization remain highly conserved

590   throughout avian evolution. Chromosoma 128:21–29. Springer Science and Business

591   Media LLC.

592   O'Connor, R. E., R. Kretschmer, M. N. Romanov, and D. K. Griffin. 2024. A bird's-eye view of

593   chromosomic evolution in the Class Aves. Cells 13:310. MDPI AG.

594   O'Connor, R. E., M. N. Romanov, L. G. Kiazim, P. M. Barrett, M. Farré, J. Damas, M. Ferguson-

595   Smith, N. Valenzuela, D. M. Larkin, and D. K. Griffin. 2018. Reconstruction of the diapsid

596   ancestral genome permits chromosome evolution tracing in avian and non-avian dinosaurs.

597   Nat. Commun. 9:1883. Nature Publishing Group.

598   Petitpierre, E. 1987. Why beetles have strikingly different rates of chromosomal evolution.

599   Elytron 1:25–32.

600   Pichugin, A. M., S. A. Galkina, A. A. Potekhin, E. O. Punina, M. S. Rautian, and A. V. Rodionov.

601   2001. Estimation of the Minimal Size of Chicken Gallus gallus

602   domesticusMicrochromosomes via Pulsed-Field Electrophoresis. Russ. J. Genet. 37:535–

603   538. Springer Nature.

604   Provine, W. B. 1992. The R. a. fisher—Sewall wright controversy. Pp. 201–229 in Boston

605   Studies in the Philosophy of Science. Springer Netherlands, Dordrecht.

606   Qumsiyeh, M., and E. Handal. 2022. Adaptive nature of chromosome variation in placental

607   mammals and applicability to domestication and invasiveness. Hystrix It. J. Mamm. 33:103–

608   107. Associazione Teriologica Italiana.

609   Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-

610   dependent speciation. Syst. Biol. 64:340–355.

611   Ratomponirina, C. 1988. Synaptonemal complexes in Robertsonian translocation heterozygous

612   in lemurs. Kew Chromosomes. HMSO Publ. Centre.

613   R Core Team. 2024. R: A Language and Environment for Statistical Computing.

614   Revell, L. J. 2012. phytools: An R package for phylogenetic comparative biology (and other

615   things).

616   Rodionov, A. V., L. A. Chel'sheva, I. V. Soloveĭ, and I. A. Miakoshina. 1992. Chiasma

617   distribution in the lampbrush chromosomes of the chicken Gallus gallus domesticus: hot

618   spots of recombination and their possible role in proper dysjunction of homologous

619   chromosomes at the first meiotic division. Genetika 28:151–160. Genetika.

620   Romanov, M. N., M. Farré, P. E. Lithgow, K. E. Fowler, B. M. Skinner, R. O'Connor, G.

621   Fonseka, N. Backström, Y. Matsuda, C. Nishida, P. Houde, E. D. Jarvis, H. Ellegren, D. W.

622   Burt, D. M. Larkin, and D. K. Griffin. 2014. Reconstruction of gross avian genome structure,

623   organization and evolution suggests that the chicken lineage most closely resembles the

624   dinosaur avian ancestor. BMC Genomics 15:1060. Springer Science and Business Media

625   LLC.

626   Skinner, B. M., and D. K. Griffin. 2012. Intrachromosomal rearrangements in avian genome

627   evolution: evidence for regions prone to breakpoints. Heredity (Edinb.) 108:37–41. Springer

628   Science and Business Media LLC.

629   Smith, J., C. K. Bruley, I. R. Paton, I. Dunn, C. T. Jones, D. Windsor, D. R. Morrice, A. S. Law,

630   J. Masabanda, A. Sazanov, D. Waddington, R. Fries, and D. W. Burt. 2000. Differences in

631   gene density on chicken macrochromosomes and microchromosomes. Anim. Genet.

632   31:96–103. Wiley.

633   Sokoloff, A. 1977. The biology of Tribolium with special emphasis on genetic aspects. Volume 3.

634   cabidigitallibrary.org.

635   Stebbins, G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.

636   Stebbins, G. L. 1958. *Longevity, habitat, and release of genetic variability in the higher plants*.

637   Cold Spring Harb. Symp. Quant. Biol. 23:365–378.

638   Tobias, J. A., C. Sheard, A. L. Pigot, A. J. M. Devenish, J. Yang, F. Sayol, M. H. C. Neate-

639   Clegg, N. Alioravainen, T. L. Weeks, R. A. Barber, P. A. Walkden, H. E. A. MacGregor, S.

640   E. I. Jones, C. Vincent, A. G. Phillips, N. M. Marples, F. A. Montaño-Centellas, V. Leandro-

641   Silva, S. Claramunt, B. Darski, B. G. Freeman, T. P. Bregman, C. R. Cooney, E. C.

642   Hughes, E. J. R. Capp, Z. K. Varley, N. R. Friedman, H. Korntheuer, A. Corrales-Vargas, C.

643   H. Trisos, B. C. Weeks, D. M. Hanz, T. Töpfer, G. A. Bravo, V. Remeš, L. Nowak, L. S.

644   Carneiro, A. J. Moncada R, B. Matysioková, D. T. Baldassarre, A. Martínez-Salinas, J. D.

645   Wolfe, P. M. Chapman, B. G. Daly, M. C. Sorensen, A. Neu, M. A. Ford, R. J. Mayhew, L.

646   Fabio Silveira, D. J. Kelly, N. N. D. Annorbah, H. S. Pollock, A. M. Grabowska-Zhang, J. P.

647   McEntee, J. Carlos T Gonzalez, C. G. Meneses, M. C. Muñoz, L. L. Powell, G. A. Jamie, T.

648   J. Matthews, O. Johnson, G. R. R. Brito, K. Zyskowski, R. Crates, M. G. Harvey, M. Jurado

649   Zevallos, P. A. Hosner, T. Bradfer-Lawrence, J. M. Maley, F. G. Stiles, H. S. Lima, K. L.

650   Provost, M. Chibesa, M. Mashao, J. T. Howard, E. Mlamba, M. A. H. Chua, B. Li, M. I.

651   Gómez, N. C. García, M. Päckert, J. Fuchs, J. R. Ali, E. P. Derryberry, M. L. Carlson, R. C.

652   Urriza, K. E. Brzeski, D. M. Prawiradilaga, M. J. Rayner, E. T. Miller, R. C. K. Bowie, R.-M.

653   Lafontaine, R. P. Scofield, Y. Lou, L. Somarathna, D. Lepage, M. Illif, E. L. Neuschulz, M.

654   Templin, D. M. Dehling, J. C. Cooper, O. S. G. Pauwels, K. Analuddin, J. Fjeldså, N.

655   Seddon, P. R. Sweet, F. A. J. DeClerck, L. N. Naka, J. D. Brawn, A. Aleixo, K. Böhning-

656   Gaese, C. Rahbek, S. A. Fritz, G. H. Thomas, and M. Schleuning. 2022. AVONET:

657   morphological, ecological and geographical data for all birds. Ecol. Lett. 25:581–597.

658 Torgasheva, A. A., L. P. Malinovskaya, K. S. Zadesenets, T. V. Karamysheva, E. A. Kizilova, E.

659   A. Akberdina, I. E. Pristyazhnyuk, E. P. Shnaider, V. A. Volodkina, A. F. Saifitdinova, S. A.

660   Galkina, D. M. Larkin, N. B. Rubtsov, and P. M. Borodin. 2019. Germline-restricted

661   chromosome (GRC) is widespread among songbirds. Proc. Natl. Acad. Sci. U. S. A.

662   116:11845–11850. Proceedings of the National Academy of Sciences.

663 Waltari, E., and S. V. Edwards. 2002. Evolutionary dynamics of intron size, genome size, and

664   physiological correlates in archosaurs. Am. Nat. 160:539–552. University of Chicago Press.

665 Waters, P. D., H. R. Patel, A. Ruiz-Herrera, L. Álvarez-González, N. C. Lister, O. Simakov, T.

666   Ezaz, P. Kaur, C. Frere, F. Grützner, A. Georges, and J. A. M. Graves. 2021.

667   Microchromosomes are building blocks of bird, reptile, and mammal chromosomes. Proc.

668   Natl. Acad. Sci. U.S.A. 118:e2112494118. National Academy of Sciences.

669 White, M. J. D. 1973. Animal Cytology and Evolution. Cambridge University Press.

670 Wilson, A. C., G. L. Bush, S. M. Case, and M. C. King. 1975. Social structuring of mammalian

671   populations and rate of chromosomal evolution. Proc. Natl. Acad. Sci. U. S. A. 72:5061–

672   5065.

673   Winker, K., and K. Delmore. 2023. Seasonally migratory songbirds have different historic

674   population size characteristics than resident relatives.

675   Wright, N. A., T. R. Gregory, and C. C. Witt. 2014. Metabolic "engines" of flight drive genome

676   size reduction in birds. Proc. Biol. Sci. 281:20132780. The Royal Society.

677   Zenil-Ferguson, R., J. M. Ponciano, and J. G. Burleigh. 2017. Testing the association of

678   phenotypes with polyploidy: An example using herbaceous and woody eudicots. Evolution
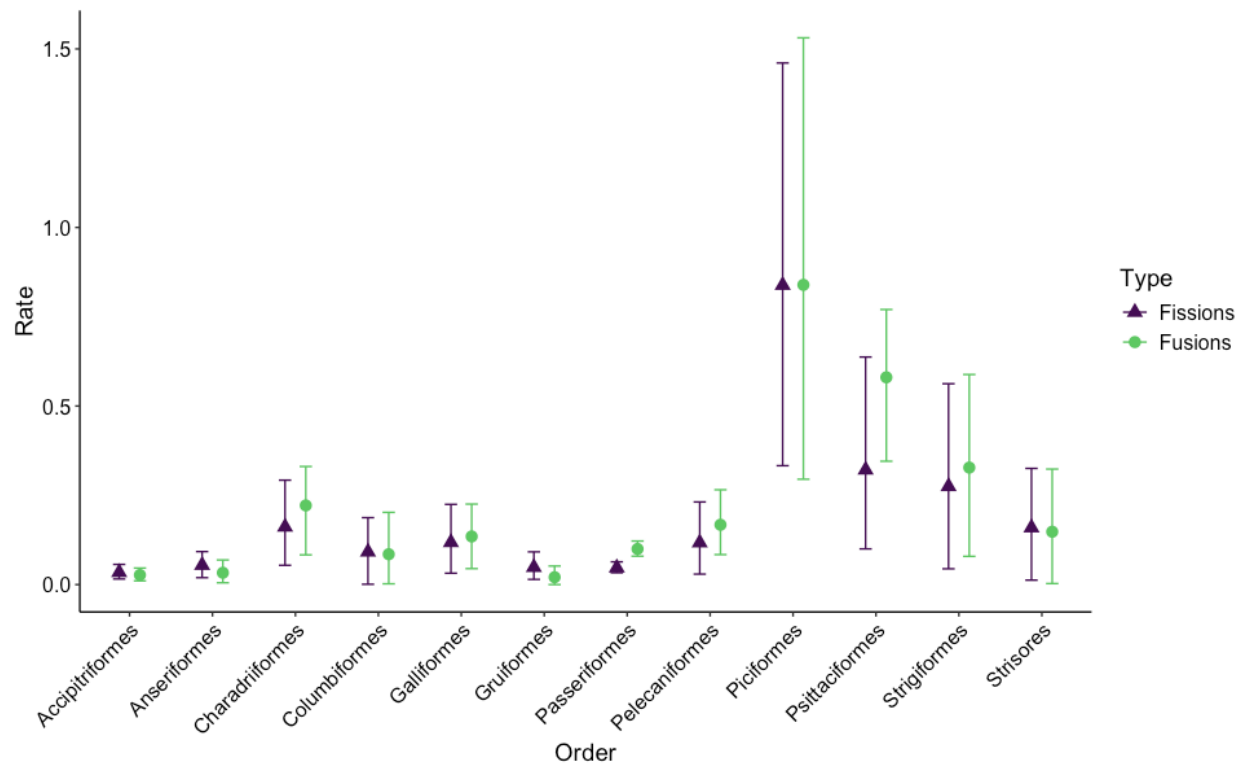
679   71:1138–1148.

680   Zhang, G. 2018. The bird's-eye view on chromosome evolution. Genome Biol. 19:201. Springer

681   Science and Business Media LLC.

682

683 **Supplemental Figure 1. Chromosome fission and fusion rates for 12 bird orders.** Bars

684 represent the 95% credible interval.

685

686

687   **Supplemental Figure 2. Chromosome evolution in small population size and large**
688   **population size lineages excluding high tip rate taxa.** Each curve shows the distribution of
689   the rate difference in small population size and large population size lineages. The bars below
690   the curve indicate the 95% credible interval for the rate differences.

691

692
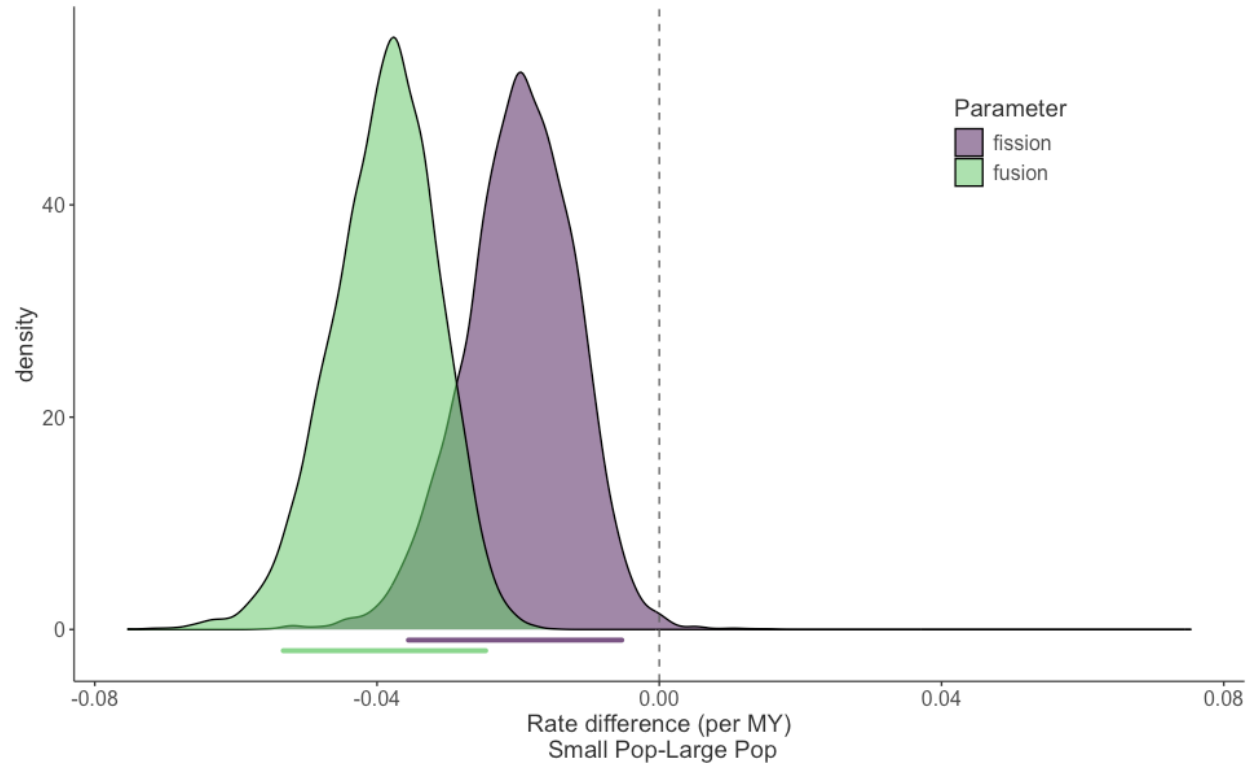
693   **Supplemental Figure 3. Chromosome evolution in migratory and sedentary**
694   **Passeriformes.** Each curve shows the distribution of the rate difference in sedentary and
695   migratory lineages within Passeriformes. The bars below the curve indicate the 95% credible
696   interval for the rate differences.
697

698