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Polygamy and purifying selection in birds

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26 **AUTHOR CONTRIBUTIONS**

27 KW & AOU conceptualised the study. GC, SF, GZ, & KW processed and filtered the genome
28 sequence data to extract the dependent variables. TS, ZV & KW compiled the phenotypic data.
29 GC created the phylogeny used in analysis. GE & KW collected the six newly sequenced
30 shorebird samples. KW performed all statistical analyses and modelling, created figures and
31 tables, and wrote the paper. All authors discussed results and commented on the manuscript.

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48 **CONFLICT OF INTEREST STATEMENT**

49 The authors have no conflict of interest to declare.

50

51 **ABSTRACT**

52 Good genes theories of sexual selection predict that polygamy will be associated with more
53 efficient removal of deleterious alleles (purifying selection), due to the alignment of sexual
54 selection with natural selection. On the other hand, runaway selection theories expect no such
55 alignment of natural and sexual selection, and may instead predict less efficient purifying
56 selection in polygamous species due to higher reproductive variance. In an analysis of
57 polymorphism data extracted from 150 bird genome assemblies, we show that polygamous
58 species carry significantly fewer non-synonymous polymorphisms, relative to synonymous
59 polymorphisms, than monogamous bird species ($p=0.0005$). We also show that this effect is
60 independent of effective population size, consistent with the alignment of natural selection with
61 sexual selection and ‘good genes’ theories of sexual selection. Further analyses found no impact
62 of polygamy on genetic diversity, whilst polygamy in females (polyandry) had a marginally
63 significant impact ($p=0.045$). We also recapitulate previous findings that smaller body mass and
64 greater geographic range size are associated with more efficient purifying selection, more intense
65 GC-biased gene conversion (gBGC), and greater genetic diversity.

66 **KEYWORDS:** Sexual selection, natural selection, polymorphism, evolutionary genomics,
67 molecular evolution, mating systems

68

69

70 INTRODUCTION

71 Birds exhibit a broad range of mating systems, including monogamous, polyandrous, and
72 polygynous strategies, making them an ideal system to study the evolutionary consequences of
73 mating system (Pitelka et al. 1974). Polygamy has been predicted to influence evolution in a
74 number of ways, primarily due to the association between greater levels of polygamy and more
75 intense sexual selection. In particular, the extent to which sexual selection aligns or interferes
76 with natural selection has been the subject of much debate, and remains a controversial area of
77 evolutionary biology (Whitlock and Agrawal 2009; Rowe and Rundle 2021).

78 Elaborate morphological characteristics associated with polygamous mating systems, such as the
79 peacock's tail feathers, are clearly detrimental to individual survival. If the action of sexual
80 selection is restricted to the small subset of genes directly associated with such morphological
81 characteristics, as predicted by runaway selection theories of sexual selection, it will act in
82 opposition to natural selection (Fisher 1958; Arnold 1985; Kirkpatrick and Ryan 1991). On the
83 other hand, if sexual selection favours individuals that are healthier in general, as predicted by
84 'good genes' theories of sexual selection, it may act in concert with natural selection to remove
85 harmful alleles and promote adaptation (Whitlock and Agrawal 2009; Andersson 1982; Agrawal
86 2001; Siller 2001; Jennions et al. 2001). Sexual selection may also result in an increased
87 mutation rate, due to a trade-off between investment in DNA repair and investment in
88 reproduction (Dowling and Simmons 2009), selection for rare beneficial mutations when
89 variance in reproductive success is high (Bartosch-Harlid et al. 2003; Petrie and Roberts 2007;
90 Petrie 2021), and/or as a result of post-copulatory sperm competition, as greater sperm
91 production requires additional rounds of replication in the male germline (Møller and Cuervo
92 2003). Aside from the processes underlying sexual selection, the greater reproductive variance

93 associated with polygamy is expected to reduce the effective size of a polygamous population
94 (Nunney 1993). Polygamous species are therefore expected to be more affected by genetic drift,
95 which results in less stringent purifying natural selection and reduced genetic diversity (Wright
96 1931; Kimura 1969; Charlesworth 2001; Charlesworth 2009). Polygamy is also associated with
97 greater sexual dimorphism, increasing the possibility that alleles harmful to one sex are
98 maintained through their benefit to the other sex (Arnqvist and Rowe 2013). Finally, recent
99 comparative work in plovers has suggested that polygamous species may exhibit greater gene
100 flow between populations, which could result in an increase in the effective size of a given
101 population (D'Urban Jackson et al. 2017).

102 Empirical studies of laboratory invertebrate populations have provided evidence for some of
103 these theories, e.g. that the combination of sexual selection and natural selection improves
104 population fitness relative to natural selection alone (Baur and Berger 2020; Lumley et al. 2015;
105 Jarzebowska and Radwan 2010; Calley et al. 2019), and that increasing mate competition can
106 increase mutation rates (Baur and Berger 2020). However, the question of which processes are
107 most influential remains contentious (Whitlock and Agrawal 2009; Rowe and Rundle 2021).

108 Comparative analyses of non-model species can provide insight to this question, and here we
109 analyse the consequences of polygamy on molecular evolution in birds using the largest dataset
110 to date, including single nucleotide polymorphism (SNP) data from 150 species with sequenced
111 genomes. We focus on four hypotheses that make clear predictions for genome-wide signatures
112 of molecular evolution (summarised in Table 1): (1) Sexual selection acts in concert with natural
113 selection, by ensuring only the healthiest individuals breed ('good genes' theory of sexual
114 selection), (2) Sexual selection is limited to a small number of genes associated with secondary
115 sexual characteristics and preferences, and is unrelated to the efficacy of natural selection

116 ('runaway' theory of sexual selection), (3) Polygamy acts against natural selection, by lowering
 117 the effective population size of a population and thereby increasing the impact of genetic drift,
 118 (4) greater levels of polygamy are associated with a higher mutation rate, either due to a trade-off
 119 against DNA repair, selection for a higher mutation rate, or post-copulatory sperm competition.
 120 To tease apart the predictions of these hypotheses, we make use of three independent genomic
 121 signatures, which reflect the efficiency of purifying selection, the level of genetic diversity, and
 122 the intensity of GC-biased gene conversion (gBGC, a fixation bias thought to affect the majority
 123 of eukaryotes (Bolívar et al. 2016; Duret and Galtier 2009; Pessia et al. 2012), although perhaps
 124 not *Drosophila* (Robinson et al. 2014)). The predictions of each hypothesis for these separate
 125 genomic measures are summarised in Table 1.

126 **Table 1. Summary of hypotheses linking polygamy and genome-wide molecular evolution,**
 127 **with predictions for the signatures of three evolutionary processes.**

Hypothesis	Prediction for Purifying Selection efficiency (P_n/P_s)[†]	Prediction for neutral genetic diversity (Heterozygosity)[§]	Prediction for GC-biased gene conversion intensity (P_{sw+ws}/P_{ss+ww})[‡]
(1) Polygamy enhances natural selection via sexual selection (Whitlock and Agrawal 2009; Andersson	Polygamous species have more efficient purifying	No predicted effect	No predicted effect

1982; Agrawal 2001; Siller 2001; Jennions et al. 2001)	selection (lower P_n/P_s)		
(2) Polygamy does not enhance natural selection, being limited to the evolution of secondary sexual characteristics and preferences (Fisher 1958; Arnold 1985; Kirkpatrick and Ryan 1991)	No predicted effect	No predicted effect	No predicted effect
(3) Polygamy reduces effective population size (Nunney 1993; Charlesworth 2009)	Polygamous species have less efficient purifying selection (higher P_n/P_s)	Polygamous species show reduced heterozygosity	Polygamous species have less intense GC-biased gene conversion (higher P_{sw+ws}/P_{ss+ww})
(4a) Polygamy increases mutation rate via selection for rare beneficial mutations (Bartosch-Harlid et al. 2003; Petrie and Roberts 2007; Petrie 2021) or via a trade-	No predicted effect	Polygamous species show greater heterozygosity	No predicted effect

off between reproduction and DNA repair (Dowling and Simmons 2009)			
(4b) Polyandry increases mutation rate via sperm competition (Møller and Cuervo 2003)	No predicted effect	Polyandrous species show greater heterozygosity	No predicted effect

128

129 † P_n/P_s = Ratio of GC-conservative non-synonymous SNPs to GC-conservative synonymous
130 SNPs

131 § Heterozygosity = Proportion of intergenic loci that contain a GC-conservative SNP in a single
132 genome

133 ‡ P_{SW+WS}/P_{SS+WW} = Ratio of intergenic SNPs affected by GC-biased gene conversion to
134 intergenic SNPs unaffected by GC-biased gene conversion

135 **MATERIALS & METHODS: Overview of the genomic dataset**

136 Single whole genomes for a total of 150 species were used in this study, including 144 collated
137 as part of the 10,000 bird genomes project (B10k; Feng et al. 2020), and six newly sequenced
138 Arctic shorebird species (*Charadrius hiaticula*, *Pluvialis squatarola*, *Calidris alpina*, *Calidris*
139 *temmincki*, *Calidris minutus*, *Phalaropus lobatus*). Species were selected based on the
140 availability of genomes and the relevant life-history variables, after excluding flightless birds on
141 the basis that the relationship between geographic range size and body mass and effective
142 population size may be very different in flightless birds, due to reduced constraints on body mass

143 and reduced dispersal ability. The B10k project has deliberately set out to sequence examples
144 from each avian family, and the set of genomes therefore includes some particularly long
145 branches leading to families with only one sequenced individual. Long branch lengths cause
146 issues for comparative analyses based on substitutions, such as dN/dS (the ratio of non-
147 synonymous to synonymous substitutions) and GC₄ (the GC proportion at fourfold degenerate
148 sites), as differences between species are accumulated along evolutionary periods that might not
149 reflect current phenotypes. This is particularly problematic for fast-evolving behavioural traits
150 such as mating system, for which the entire spectrum of phenotypes can be identified among
151 species of a single family (Pitelka et al. 1974). To avoid these issues, we detect evolutionary
152 signatures in the pattern of polymorphisms, as these reflect more recent evolutionary pressures
153 (McDonald and Kreitman 1991; Müller et al. 2020). Signatures of genetic diversity and purifying
154 selection efficiency based on single genomes have previously been analysed using
155 polymorphism data from single genomes (e.g., Figuet et al. 2016), and our analyses of these
156 traits follow established methods: GC-conservative P_N/P_S (the ratio of non-synonymous to
157 synonymous SNPs) was used for analysing purifying selection, and intergenic GC-conservative
158 heterozygosity was used for analysing genetic diversity. In contrast, to our knowledge, previous
159 analyses of gBGC have either relied on substitution data (e.g. Romiguier et al. 2010) or have
160 required multiple genomes with polymorphism data (e.g. Muyle et al. 2011; Robinson et al.
161 2014; Glémin et al. 2015). Here we present a novel measure of the intensity of GC-biased gene
162 conversion (gBGC), which makes use of polymorphism data from a single genome: the
163 P_{sw+ws}/P_{ss+ww} ratio. This can be defined as the ratio of intergenic heterozygous sites affected by
164 gBGC to intergenic heterozygous sites unaffected by gBGC.

165 **MATERIALS & METHODS: Explanation and modelling of the P_{sw+ws}/P_{ss+ww} ratio**

166 GC-biased gene conversion (gBGC) results from a meiotic repair bias that favours G and C
167 nucleotides over A and T nucleotides, and acts to increase the frequency of ‘strong’ alleles (‘S’,
168 e.g. G:C) and reduce the frequency of ‘weak’ alleles (‘W’, e.g. A:T) in a population (Duret and
169 Galtier 2009; Webster and Hurst 2012). When a new mutation occurs that introduces a ‘weak’
170 nucleotide pair in the place of an existing ‘strong’ nucleotide pair ($S \rightarrow W$ mutation, e.g.
171 $G:C \rightarrow A:T$), gBGC reduces the chance of this mutation spreading through the population,
172 analogous to how selection acts on a weakly deleterious allele (Nagylaki, 1983; Capra et al.
173 2013). However, when a mutation occurs in the opposite direction ($W \rightarrow S$, e.g. $A:T \rightarrow G:C$),
174 gBGC increases the chance of this mutation spreading through the population, analogous to the
175 effect of selection on a weakly beneficial allele. In contrast, gBGC has no effect on GC
176 conservative mutations, which are rarer mutations that replace ‘strong’ alleles with other ‘strong’
177 alleles ($S \rightarrow S$, e.g., $G:C \rightarrow C:G$), or replace ‘weak’ alleles with other ‘weak’ alleles ($W \rightarrow W$, e.g.,
178 $A:T \rightarrow T:A$). GBGC acts in a consistent direction, and is expected to be more intense in larger
179 populations (Wright 1931; Nagylaki 1983). The effects of gBGC are more pronounced in areas
180 of the genome with high recombination, where the intensity of gBGC is greatest, but
181 nevertheless they have a significant effect on overall SNP frequencies and genomic GC content
182 (Bolívar et al. 2016). Previous research comparing GC content within the avian clade has found
183 evidence of stronger gBGC in larger populations (Weber et al. 2014), although evidence for this
184 relationship is more mixed in mammals (Romiguier et al. 2010; Kessler and Dean 2014), and no
185 such relationship has been found across more distantly related animal groups or plants (Galtier et
186 al., 2018; Clément et al., 2017).

187 Typically, when gBGC strength is measured using polymorphism data, the frequency spectrum
188 of $W \rightarrow S$ polymorphisms is compared to the frequency of $S \rightarrow W$ polymorphisms (e.g. Muyle et

189 al. 2011; Robinson et al. 2014; Glémin et al. 2015). However, here we use single whole genomes
190 in a dataset where divergence times between species are often very long, ancestral states cannot
191 be reliably inferred (Hernandez et al., 2007), and $S \rightarrow W$ and $W \rightarrow S$ polymorphisms cannot be
192 separated. By modelling the expected heterozygosity levels for the four different SNP categories
193 ($S \rightarrow W$, $W \rightarrow S$, $S \rightarrow S$ and $W \rightarrow W$), we show that as long as $W \rightarrow S$ SNPs are generated more
194 frequently than the reverse, gBGC reduces the total combined number of $W \rightarrow S$ and $S \rightarrow W$ SNPs
195 (these can be described as SNPs affected by gBGC). Research into germline mutation rates in
196 eukaryotes has shown consistently that $S \rightarrow W$ mutations occur more often than the reverse
197 (Bolívar et al. 2016, Lynch 2010, Smeds et al. 2016, Zhang and Gerstein 2003, Ossowski et al.
198 2010, Hwang and Green 2004) and so the overall frequency of SNPs affected by gBGC is
199 reduced by the action of gBGC. To control for mutation rate differences between species, we
200 divide the total number of $S \rightarrow W$ and $W \rightarrow S$ intergenic heterozygous sites with the total number
201 of $S \rightarrow S$ and $W \rightarrow W$ intergenic heterozygous sites, to create a measure of gBGC intensity:
202 P_{sw+ws}/P_{ss+ww} . As with all genomic correlates of gBGC intensity, this measure is affected by
203 variation in recombination rates and mutation biases, and assumes that such variation is not
204 correlated with the life history traits being compared.

205 The effect of GC-biased gene conversion (gBGC) on the frequency of $W \rightarrow S$ and $S \rightarrow W$
206 mutations is typically modelled by noting that the rate of gene conversion b is equivalent to a
207 selection coefficient promoting the ‘strong’ allele (e.g. Bolívar et al. 2015; Lartillot 2013; Mugal
208 et al. 2013). In this approach, $W \rightarrow S$ mutations are considered weakly beneficial, and $S \rightarrow W$
209 mutations are considered weakly deleterious, whilst GC-conservative mutations are neutral.
210 Kimura (1969b) provided equations for estimating the expected amount of heterozygosity in an

211 individual genome for sites under selection (formula 1a), and for selectively neutral sites
 212 (formula 1b).

$$213 \quad H(p) = \frac{4N_e v_m}{N_e s} \left(\frac{1 - e^{-2N_e s p}}{1 - e^{-2N_e s}} - p \right) \quad (1a)$$

$$214 \quad H(p) = 4N_e v_m p(1 - p) \quad (1b)$$

215 Where $H(p)$ = the number of heterozygous sites (per individual), N = the total population size,
 216 N_e = the variance effective population size, s = the selection coefficient, v_m = the total number
 217 of mutations appearing in the population each generation, and $p = 1/2N$

218 By substituting the selection coefficient and mutation rate parameters used by Kimura (1969b)
 219 with parameters relevant for $S \rightarrow W$, $W \rightarrow S$, $S \rightarrow S$ and $W \rightarrow W$ mutations, the effect of population
 220 size on the relative proportions of different categories of heterozygous sites can be modelled.
 221 These substitutions are summarised in formulas 2a and 2b, where formula 2a applies to
 222 mutations affected by gBGC ($S \rightarrow W$ and $W \rightarrow S$) and formula 2b applies to mutations unaffected
 223 by gBGC ($S \rightarrow S$ and $W \rightarrow W$). The ratio of polymorphisms affected by gBGC to those unaffected
 224 by gBGC (P_{SW+WS}/P_{SS+WW}) is then provided by formula 3.

$$225 \quad H_{x \rightarrow y} = \frac{8N_e^2 \mu_{x \rightarrow y} g_x}{N_e b_{x \rightarrow y}} \left(\frac{1 - e^{-2N_e b_{x \rightarrow y} p}}{1 - e^{-2N_e b_{x \rightarrow y}}} - p \right) \quad (2a)$$

$$226 \quad H_{x \rightarrow x} = 8N_e^2 \mu_{x \rightarrow x} g_x p(1 - p) \quad (2b)$$

227 Where x = ancestral nucleotide type (strong or weak), y = derived nucleotide type (strong or
 228 weak), μ = mutation rate per site (dependent on x and y), b = gBGC selection coefficient
 229 (dependent on x and y), and g = number of sites available for mutation per haploid genome
 230 (dependent on x).

231
$$P_{SW+WS}/P_{SS+WW} = \frac{H_{S \rightarrow W} + H_{W \rightarrow S}}{H_{S \rightarrow S} + H_{W \rightarrow W}} \quad (3)$$

232

233 Values for the parameters in formulas 2a and 2b were taken from the literature where possible,
 234 so that the impact of varying N_e could be modelled in a plausible setting (parameter values
 235 summarised in Table 2). Kessler & Dean (2014) noted that N_e estimates in mammals have varied
 236 from ~10,000 in humans to ~780,000 in rabbits. Assuming a similar amount of variation in birds,
 237 the impact of a 100-fold change in N_e (from 2,000 to 200,000) was modelled, and showed a
 238 negative relationship between N_e and P_{SW+WS}/P_{SS+WW} for the full range of intergenic GC-content
 239 in the genomic dataset (Figure 1). Consistent with these predictions, PGLS analysis showed a
 240 significant negative correlation between P_{SW+WS}/P_{SS+WW} and intergenic heterozygosity (Table 3).
 241 It should be noted that if GC content is sufficiently low, or the mutation rate bias towards $S \rightarrow W$
 242 is sufficiently weak, so that more $W \rightarrow S$ mutations are generated than $S \rightarrow W$ mutations, the
 243 predictions of the model are reversed and increasing N_e will increase the predicted
 244 P_{SW+WS}/P_{SS+WW} ratio. This switch occurs at $GC \sim 0.35$ for the parameters defined in Table 2.

245 **Table 2: Parameter values used in modelling the formulas 2a and 2b**

Parameter	Value	Justification
g_S	$4.14e^8$	1Gb = approx. size of a typical bird genome Average intergenic GC content = 0.414
g_W	$5.86e^8$	1Gb - g_S
N_e	50,000	N_e arbitrarily estimated as 50,000

		This is smaller than N_e estimated for the mouse, and greater than N_e estimated for the chimp†
N	50,000	Equal to N_e to simplify analysis
$\mu_{S \rightarrow W}$	$2.51e^{-9}$	Estimated germline mutation rate in the flycatcher§
$\mu_{W \rightarrow S}$	$1.42e^{-9}$	(as above)
$\mu_{W \rightarrow W}$	$2.51e^{-10}$	(as above)
$\mu_{S \rightarrow S}$	$4.18e^{-10}$	(as above)
$b_{S \rightarrow W}$	$-5e^{-6}$	In mammals, average strength of gBGC $4N_e b = \sim 1$ ‡ b estimated as $1/4N_e$ where $N_e=50,000$
$b_{W \rightarrow S}$	$5e^{-6}$	(as above)

246

247 † Geraldès et al. 2011; Won and Hey 2005

248 § Smeds et al. 2016

249 ‡ Lartillot 2013

250 **Table 3: P_{SW+WS}/P_{SS+WW} vs. GC-conservative intergenic heterozygosity + Intergenic GC**
251 **content + Genome Quality (L50), in a PGLS model†**

Model Term	β (SE) ‡	t-value	N	p-value
Heterozygosity : GC content	0.046 (0.045)	1.01	150	0.31
Heterozygosity	-0.16 (0.042)	-3.82	150	0.0002
GC content	-0.031 (0.047)	0.65	150	0.51

Genome Quality (L50) 0.082 (0.042) 1.93 150 0.0252

253

254 † PGLS was implemented using Pagel's correlation structure, *Pagel's* $\lambda = 0.93$

255 ‡ β = slope (coefficient), t-value = slope/standard error, N = number of species

256 Figure 1 also highlights a further complexity to the relationship between GC content, N_e and

257 $P_{\text{sw+ws}}/P_{\text{ss+ww}}$: when N_e is low and gBGC intensity is therefore very weak, both $\text{S} \rightarrow \text{W}$ and

258 $\text{W} \rightarrow \text{S}$ mutation contribute approximately equally to heterozygosity, and so the greater rate of

259 $\text{S} \rightarrow \text{W}$ mutations leads to $P_{\text{sw+ws}}/P_{\text{ss+ww}}$ increasing in line with the proportion of GC sites. In

260 contrast, when N_e is high and gBGC is having a meaningful impact, $\text{S} \rightarrow \text{W}$ mutations are quickly

261 removed and $\text{W} \rightarrow \text{S}$ mutations contribute more to heterozygosity, and so an increase in GC sites

262 reduces $P_{\text{sw+ws}}/P_{\text{ss+ww}}$. Such an effect did not appear to influence the current analysis, as PGLS

263 models found no interaction between intergenic GC content and heterozygosity, as well as no

264 main effect of intergenic GC content (Table 3). This may reflect the lack of variation in

265 intergenic GC content between species (ranging from 0.4 to 0.44, variance = $3.3e^{-5}$), especially

266 relative to the potentially 100-fold range in effective population size. The complexity of the

267 relationship between $P_{\text{sw+ws}}/P_{\text{ss+ww}}$, N_e , and GC content are a limitation of the $P_{\text{sw+ws}}/P_{\text{ss+ww}}$

268 measure, and may make it unsuitable for certain datasets. Nevertheless, for the current analysis,

269 predictions for the impact of N_e on $P_{\text{sw+ws}}/P_{\text{ss+ww}}$ are clear, and the measure can provide some

270 insight into the effect of polygamy on molecular evolution.

271 **MATERIALS & METHODS: Genomic variables**

272 Single Nucleotide Polymorphisms (SNPs) were called using GATK (version 3.4-46-gbc02625)

273 (DePristo et al. 2011), and filtered to include only those meeting the following quality criteria:

274 SNPs must be more than 10bp (base pairs) from another SNP, SNP coverage must be more than
275 1/3 mean coverage and less than 2x mean coverage, SNP root-mean-square mapping quality
276 must be at least 25 (as in Nadachowska-Brzyska et al. 2015).

277 SNPs located within regions of tandem repeats and transposable elements (TEs) were excluded
278 to avoid inclusion of spurious SNPs from such regions. Tandem repeats were identified using
279 Tandem Repeats Finder v4.07b41 (Benson 1999). TEs were identified through homology-based
280 annotation by RepeatMasker (open-4.0.7) with parameters “-nolow -no_is -norna -engine ncbi -
281 parallel 1”) at the DNA level based on the Repbase library (v20170127). De novo repeat
282 annotation was completed using RepeatModeler (open-1-0-8) with default parameters to build a
283 de novo repeat library for each assembly (Smit et al. 2015), and this library was also used with
284 RepeatMasker (open-4.0.7) to predict repeats for each species (as in Feng et al. 2020). SNPs
285 located on sex chromosomes were also removed to reduce the noise generated by including a
286 mixture of male and female samples (these SNPs were identified by alignment to chicken sex
287 chromosomes, given the high conservation of synteny among avian species; Griffin et al. 2007;
288 Ellegren 2010). Locations of SNPs (exonic, intronic, or intergenic), were detected using the
289 protein coding gene annotation for each species.

290 The total number of GC-conservative autosomal SNPs passing these quality criteria and located
291 in exons were then identified as synonymous or non-synonymous, and extracted for analysis of
292 P_n/P_s . Only GC-conservative polymorphisms were included, as GC-biased gene conversion can
293 interfere with signatures of selection (Bolívar et al. 2018). 156 species were initially identified
294 for use in the study, however three were removed due to a low number of GC-conservative
295 exonic SNPs passing quality control criteria (<200), resulting in a final dataset of 153 species
296 with suitable genomic data. P_n/P_s was calculated by summing the number of GC-conservative

297 non-synonymous heterozygous sites, and dividing this number by 3 times the number of GC-
298 conservative synonymous heterozygous sites (this approximately controls for the greater
299 frequency of new non-synonymous mutations, as in Figuet et al. 2016). P_n/P_s ratios were natural
300 log (Ln)-transformed prior to statistical analysis to reduce the impact of extreme values.

301 Heterozygosity was calculated for each genome as the number of GC-conservative intergenic
302 SNPs passing quality control criteria, divided by the number of intergenic sites meeting quality
303 control criteria in that genome (as in Figuet et al. 2016). This measure can be defined as the
304 proportion of intergenic sites in a single genome containing GC-conservative SNPs.

305 Heterozygosity was square root transformed before analysis to reduce the impact of extreme
306 values. PGLS analysis revealed no effect of intergenic GC-content on heterozygosity ($p>0.5$).

307 To calculate P_{SW+WS}/P_{SS+WW} , autosomal SNPs passing quality criteria and located in intergenic
308 regions were extracted, and the number of SNPs identified as G:T, T:G, G:A, A:G, C:T, T:C,
309 C:A or A:C was simply divided by the number of SNPs identified as A:T, T:A, C:G or G:C. No
310 transformation was required for this variable.

311 **MATERIALS & METHODS: Life history data**

312 Effective population size is predicted to have a large impact on all the genomic measures
313 analysed, as purifying selection, GC-biased gene conversion (gBGC), and genetic diversity are
314 all affected by genetic drift (Charlesworth, 2009). Body mass and geographic range size were
315 therefore included in all models to reduce the unexplained variance, as these variables have
316 previously been found to correlate with population size, and thus may also correlate with
317 effective population size (Damuth 1981; Greenwood et al. 2006; Gaston and Blackburn 1996).
318 Body mass estimates were initially collated from the literature by Székely et al. 2022. Where

319 possible, average estimates for males and females were used, but if data was available for just
320 one sex, this was included without adjustment. Body mass was Ln-transformed to reduce the
321 impact of extreme values. Distribution ranges were downloaded for all study species as
322 shapefiles from Birdlife.org. Polygons of wintering ranges were then excluded, as these are
323 unrelated to population size when breeding ranges are accounted for. Breeding ranges and year-
324 round resident ranges were retained, and total geographic range size was calculated using the
325 'areaPolygon' function in the R package 'geosphere' (Hijmans 2012). For all analyses
326 geographic range size was Box-Cox transformed $((\text{Geographic Range Size (km}^2)^{0.2} - 1) / 0.2)$
327 to reduce the impact of extreme values. Three of the 153 species with suitable genomic data
328 exhibited outlying phenotypes for geographic range size or body mass (leverage $> 2 \times$ [number
329 of variables] / N), and these species were excluded from the analysis to avoid spurious
330 associations (as in Thomas et al. 2015). The final sample size for analyses was therefore 150
331 species.

332 For 149 of 150 species, estimates of the extent of polygamy were available from the literature for
333 both sexes, and for the remaining one species *Cuculus canorus* the extent of polygamy was
334 known for females only (collated by Székely et al. 2022). For the majority of hypotheses
335 outlined in Table 1, the predicted impacts of polygamy in males (polygyny) and polygamy in
336 females (polyandry) are alike, as polygamy in either sex increases the variance in reproductive
337 success and the intensity of sexual selection. Data on the extent of polygyny and polyandry was
338 therefore combined for most analyses in order to increase statistical power: species where $>5\%$
339 of breeding individuals from the more polygamous sex mated multiple times in a season were
340 considered polygamous (N = 29 species), with the rest considered monogamous (N = 121
341 species) (as in D'Urban Jackson et al. 2017). In contrast, the hypothesis that sperm competition

342 increases germline mutation rates predicts an impact of polyandry specifically, as sperm
343 competition is linked to polygamy in females (Cally et al. 2019; Møller 1991). To test for an
344 effect of sperm competition, heterozygosity was also analysed in a model comparing
345 polyandrous species (species where >5% of breeding females mate multiple times in a season, N
346 = 11) with all other species (N = 139 species).

347 Phylogenetic Generalised Least Squares (PGLS) models showed that there was no significant
348 association between any of the explanatory variables of polygamy, body mass, and geographic
349 range size (Table 4). A separate PGLS model for the 78 species with available census population
350 estimates found that smaller body mass and greater geographic range size were significantly
351 associated with larger census population size (Supplementary table 1). The lack of a significant
352 correlation between polygamy and census population size suggests there is no severe
353 confounding effect on the dataset, however only 12 polygamous species had census data
354 available, and so the power to detect an association in this analysis was low. Census population
355 size estimates were taken from three sources – IUCN (2020), BirdlifeInt (2020), and Birds of the
356 World (Billerman et al. 2020), and averages of the extremes were taken when estimates were
357 given as a likely range. This measure was Ln-transformed before analysis, to reduce the impact
358 of extreme values (averages of census minimum and maximum estimates were taken after
359 natural log transformation, as these estimates generally followed a logarithmic scale, e.g.
360 ‘10,000-100,000 individuals’).

361 **Table 4: PGLS analyses showing life history variable associations. Note that the**
362 **explanatory variable in each of these pairwise models was selected as the variable with the**
363 **weakest phylogenetic signal, to avoid conflating phylogenetic signal with correlation**
364 **(Supplementary Table 2)**

Model	β (SE) †	t-value	N	p-value	Page's λ ³⁶⁵ 366
Polygamy ~ Geographic Range Size	-0.0083 (0.031)	0.27	150	0.79	0.54 367 368
Body Mass ~ Polygamy	-0.043 (0.12)	-0.36	150	0.72	1.00 369 370
Body Mass ~ Geographic Range Size	0.082 (0.044)	1.86	150	0.064	1 371 372

373

374 † β = slope (coefficient), t-value = slope/standard error, N = number of species, λ = phylogenetic
375 signal

376 MATERIALS & METHODS: Software and analysis

377 All analysis was completed in R version 4.0.1 (R core team 2020). PGLS analyses were run
378 using the 'pgls' function of the caper package, with Page's λ estimated by maximum likelihood
379 (Orme et al. 2013). PGLS models were used for all species comparisons, and are a form of linear
380 model that controls for phylogenetic relatedness, in order to avoid issues regarding non-
381 independence of data from related species (Symonds & Blomberg, 2014). Statistical assumptions
382 of the models (normality of residuals, no heteroscedasticity) were checked visually by plotting
383 the data, and no issues were detected once variables were appropriately transformed to follow
384 normal distributions, and the three high leverage species were excluded. Interactions between

385 polygamy/polyandry and the model covariates were checked for in each model, and non-
386 significant interactions were removed sequentially to produce the final models (Ernqvist, 2005).
387 Body mass and geographic range size were centred and scaled, and the categorical variable of
388 polygamy was also centred, so that main effects could be interpreted in the presence of
389 interactions, and so that slope estimates were comparable among predictor variables (Schielzeth,
390 2010).

391 Genome quality, measured by contig L50, varied widely across species (910-46,581), however
392 PGLS analysis showed that this measure was not significantly associated with the life history
393 variables studied (Supplementary Table 3). Since a nonsignificant trend towards higher quality
394 genomes in polygamous species was found, all PGLS models involving polygamy were rerun
395 with L50 as a covariate (following square root transformation of L50 to reduce the impact of
396 extreme values). Interpretations from these models were unchanged, suggesting genome quality
397 was not confounding results. Collinearity between independent variables was tested by rerunning
398 all models using the ‘gls’ function of the nlme package (Pinheiro et al. 2017), along with the
399 ‘corPagel’ function of the ape package (Fox et al. 2007), and then applying the ‘vif’ function of
400 the car package (Paradis 2012). Variance Inflation factors (VIFs) for all variables in all models
401 were below 1.3, suggesting minimal impact of collinearity. Cohen’s D was calculated using the
402 ‘cohen.d’ function of the ‘effsize’ R package after life history variables were split into binary
403 groups (Torchiano 2017). Polygamy and polyandry were already binary variables, whereas body
404 mass and geographic range group were simply split around the mean (after the above-mentioned
405 transformation to normal distributions).

406 **MATERIALS & METHODS: Phylogeny**

407 The fourfold-degenerate (4d) site sequences for all 469 1:1 ortholog genes for the initially
408 identified 156 species were used to infer the highest-scoring maximum likelihood tree using a
409 GTRCAT substitution model by RAxML version 8.2.4 (Stamatakis 2014) and branch lengths
410 were estimated using a GTR substitution model by the phyloFit program in the PHAST package
411 (Siepel and Haussler 2004).

412 **MATERIALS & METHODS** *Data and code availability*

413 Genome sequencing data and genome assemblies of 6 newly sequenced species generated in this
414 study have been deposited in the NCBI SRA and GenBank (accession PRJNA739535) and
415 CNGBdb (accession CNP0001928). The trait and genomic datasets, as well as all original code,
416 have been deposited at Zenodo, and are publicly available (10.5281/zenodo.7043094). Any
417 additional information required is available from the lead author upon request.

418 **MATERIALS & METHODS: Fieldwork**

419 Blood samples for the six newly sequenced shorebird species (*Charadrius hiaticula*, *Pluvialis*
420 *squatarola*, *Calidris alpina*, *Calidris temmincki*, *Calidris minutus*, *Phalaropus lobatus*), were
421 collected from Kolokolkova Bay (68°35'N, 52°20'E) in Russia. Blood was taken from the
422 brachial vein of adult breeding birds, following established methods that were approved by the
423 University of Bath's Animal Welfare and Ethical Review Body (Székely et al. 2008). No
424 additional permissions were required according to §44 and §6 of the Federal Law of the Russian
425 Federation No. 52 from 24.04.1995 (last update 18.02.2020).

426 **RESULTS AND DISCUSSION: Purifying Selection Efficiency**

427 Polygamy may increase purifying selection efficiency due to the alignment of natural and sexual
428 selection (Whitlock and Agrawal 2009; Andersson 1982; Agrawal 2001; Siller 2001; Jennions et

429 al. 2001), or it may reduce purifying selection efficiency due to a reduction in effective
430 population size (Nunney 1993; Charlesworth 2009). A PGLS model analysing the effect of
431 polygamy, body mass and geographic range size on purifying selection efficiency (P_n/P_s , the
432 ratio of GC-conservative non-synonymous to synonymous SNPs), found that polygamous
433 species had a significantly lower P_n/P_s than monogamous species, consistent with polygamy
434 enhancing purifying selection through the alignment of sexual selection and natural selection
435 (Table 5; Figure 2). Significant effects of body mass and range size were also found, which
436 reflect previous studies in a range of taxa showing larger effective population sizes are associated
437 with more efficient purifying selection (Romiguier et al. 2014; Figuet et al. 2016; Botero-Castro
438 et al. 2017; Corcoran et al. 2017; Bolívar et al. 2019; Rolland et al. 2020; Kutschera et al. 2020;
439 Leroy et al. 2021). Effect sizes were calculated independently for each variable using Cohen’s D
440 (Cohen 1988). Body mass and polygamy both had a ‘large’ effect size ($D = 0.88$ and 0.84
441 respectively), whilst geographic range size had a ‘small’ effect size ($D = 0.15$). Variance
442 inflation factor analysis suggested very weak internal correlation between the covariates
443 ($VIF < 1.3$). The relatively small effect of geographic range size may reflect the noise introduced
444 by recent demographic changes in populations, as whilst polymorphism-based measures of
445 effective population size reflect the average population size of many past generations (Müller et
446 al. 2020; Charlesworth 2009), current geographic range size will be more closely linked to the
447 current effective population size.

448 **Table 5: Purifying selection efficiency (GC-conservative P_n/P_s) vs. polygamy**
449 **(presence/absence), geographic range size (km^2), and body mass (g) in a PGLS model**

Model Term	β (SE) †	t-value	N	p-value
------------	----------------	---------	---	---------

Polygamy	-0.14 (0.038)	-3.57	150	0.0005
Body Mass	0.10 (0.015)	6.70	150	0.0001 451
Geographic Range Size	-0.039 (0.015)	-2.61	150	0.0099 452

453

454 † β = slope (coefficient), t-value = slope/standard error, N = number of species, *Pagel's* $\lambda = 0.00$

455 To confirm that the effect of polygamy on purifying selection did not reflect a correlation
456 between polygamy and effective population size, intergenic GC-conservative heterozygosity was
457 added as a covariate to the model (Supplementary Table 4). Comparison of t-values following
458 the addition of the heterozygosity covariate revealed greatly reduced explanatory power of body
459 mass (64% reduction in t-value) and geographic range size (72% reduction in t-value), consistent
460 with effective population size underlying the effect of these variables. However, the explanatory
461 power of polygamy was mostly unaffected (11% reduction in t-value), consistent with sexual
462 selection strength underlying the impact of polygamy on purifying selection efficiency. Previous
463 research comparing the efficiency of genome-wide purifying selection with mating systems
464 failed to find this effect (Nadeau et al. 2007; Harrison et al. 2015; Iglesias-Carrasco et al. 2019).
465 The difference in results may reflect a lack of power in the previous studies, resulting from fewer
466 variable genetic sites and/or fewer species. The largest previous study into the question (Iglesias-
467 Carrasco et al. 2019) also included some key methodological differences that may affect the
468 results, such as the use of substitution data (dN/dS) to measure purifying selection strength,
469 which is more influenced by positive selection than the polymorphism data used here (Smith and
470 Eyre-Walker 2002), and a focus on polygyny rather than polygamy in general.

471

472

473 **RESULTS AND DISCUSSION: Genetic Diversity**

474 Various theories have suggested that polygamy, or polyandry specifically, may lead to increased
475 mutation rates and greater genetic diversity (summarised in Table 1). In contrast, genetic
476 diversity may be reduced if polygamous species have smaller effective population sizes (Nunney
477 1993; Charlesworth 2009). Table 6 shows the results of PGLS models analysing the effect of
478 body mass, geographic range size, and either polygamy or polyandry, on the response variable
479 genetic diversity (intergenic heterozygosity). Significant effects of body mass and geographic
480 range size were found, consistent with many previous studies showing that greater population
481 size is associated with greater genetic diversity (reviewed in Charlesworth 2009). Body mass had
482 a large effect on heterozygosity (Cohen's $D = 1.15$), and geographic range size again had a small
483 effect ($D = 0.27$) (Cohen 1988). No effect of overall polygamy was found in this model;
484 however, a marginally significant effect of polyandry was detected, with greater genetic diversity
485 in polyandrous species (Figure 3; Cohen's $D = 0.67$). Greater genetic diversity in polyandrous
486 species is consistent with previous comparative analyses in birds, which have found evidence
487 that higher rates of extra pair paternity are associated with a greater male mutation bias
488 (Bartosch-Harlid et al. 2003), greater genetic diversity (Petrie et al. 1998; Gohli et al. 2013;
489 Møller et al. 2008), and higher mutation rates (Møller and Cuervo 2003; 2009), although this was
490 not replicated in swallows (Anmarkrud et al. 2011). Overall, this result provides weak evidence
491 in favour of the hypothesis that sperm competition leads to a greater mutation rate, although it
492 should be treated with caution due to the low number of polyandrous species in the dataset ($N =$
493 11 species).

494 **Table 6: GC-conservative intergenic heterozygosity vs. geographic range size (km²), body**
 495 **mass (g), and either polygamy (presence/absence) or polyandry (presence/absence) in a**
 496 **PGLS model.**

Model Term	β (SE) †	t-value	N	p-value ⁴⁹⁷
Polygamy	0.0011 (0.0013)	0.87	150	0.398
Polyandry	0.0043 (0.0021)	2.02	150	0.045 499
Body Mass‡	-0.0031 (0.00072)	-4.27	150	< 0.0001 500
Geographic Range Size‡	0.0014 (0.00047)	2.98	150	0.0034 501

502

503 † β = slope (coefficient), t-value = slope/standard error, N = number of species, *Pagel's* $\lambda = 0.63$

504 ‡ Results for the body mass and geographic range size variables were almost identical for the
 505 two models, and estimates from the polyandry model are presented

506 **RESULTS AND DISCUSSION: GC-biased gene conversion (gBGC) intensity**

507 The process of GC-biased gene conversion provides an opportunity to understand the impact of
 508 polygamy on directional selection without the influence of sexual selection, which is not
 509 expected to affect gBGC. Polygamy is therefore hypothesised to be associated with reduced
 510 gBGC if polygamous species have smaller effective population sizes (Nunney 1993;
 511 Charlesworth 2009; Nagylaki 1983) but no other association is predicted (Table 1). Table 7
 512 shows the results of a PGLS analysis comparing the polymorphism-based measure of GC-biased
 513 gene conversion, P_{sw+ws}/P_{ss+ww} , with body mass, geographic range size, and polygamy. In this
 514 analysis, significant main effects of both body mass and geographic range size were found,

515 consistent with more intense GC-biased gene conversion in larger populations and previous
516 research in birds (Weber et al. 2014). In line with the previous models of purifying selection
517 efficiency and heterozygosity, body mass had a large effect on GC-biased gene conversion
518 (Cohen's $D = 1.59$), whereas geographic range size had a small effect ($D = 0.19$) (Cohen 1988).
519 Whilst no main effect of polygamy was found in this model, a significant interaction with
520 geographic range size was found. Post-hoc PGLS analyses revealed that in polygamous species,
521 gBGC was significantly more intense for species with greater geographic range sizes ($p=0.005$),
522 whilst in monogamous species, this trend was much weaker and failed to reach significance
523 ($p=0.22$) (Figure 4; Supplementary Table 5). This result may reflect greater gene flow in
524 polygamous species, which would connect disparate parts of a species' range and result in a
525 stronger connection between geographic range size and effective population size (an extension of
526 the 'Dispersal to Mate' hypothesis (D'Urban-Jackson et al. 2017); illustrated in Supplementary
527 Figure 1). However, greater gene flow in polygamous species should also moderate the impact of
528 geographic range size on purifying selection efficiency and heterozygosity, whereas no such
529 interactions were detected ($p>0.5$). It is possible that purifying selection, gBGC, and genetic
530 diversity respond differently to gene flow between populations, however the necessary modelling
531 to make such predictions has not been completed to our knowledge. A more direct test of the
532 'Dispersal to Mate' theory would compare measurements of gene flow between species, and the
533 interaction between polygamy and geographic range size should be treated with caution until
534 such research is completed.

535 **Table 7: GC-biased gene conversion (gBGC) intensity (intergenic P_{sw+ws}/P_{ss+ww}) vs.**
536 **polygamy (presence/absence), geographic range size (km^2), body mass (g), in a PGLS**
537 **model, retaining a significant interaction term for polygamy : geographic range size.**

Model Term	β (SE) †	t-value	N	p-value ⁵³⁸
Polygamy : Geographic	-0.30 (0.092)	-3.20	150	0.0017 ⁵³⁹
Range Size				540
Polygamy	-0.021 (0.091)	-0.24	150	0.814 ⁵⁴¹
Body Mass	0.26 (0.062)	4.16	150	<0.0001 ⁵⁴²
Geographic Range Size	-0.10 (0.035)	-3.01	150	0.0031 ⁵⁴³

544

545 † β = slope (coefficient), t-value = slope/standard error, N = number of species, Pagel's λ = 0.95

546 **RESULTS AND DISCUSSION: Summary and conclusions**

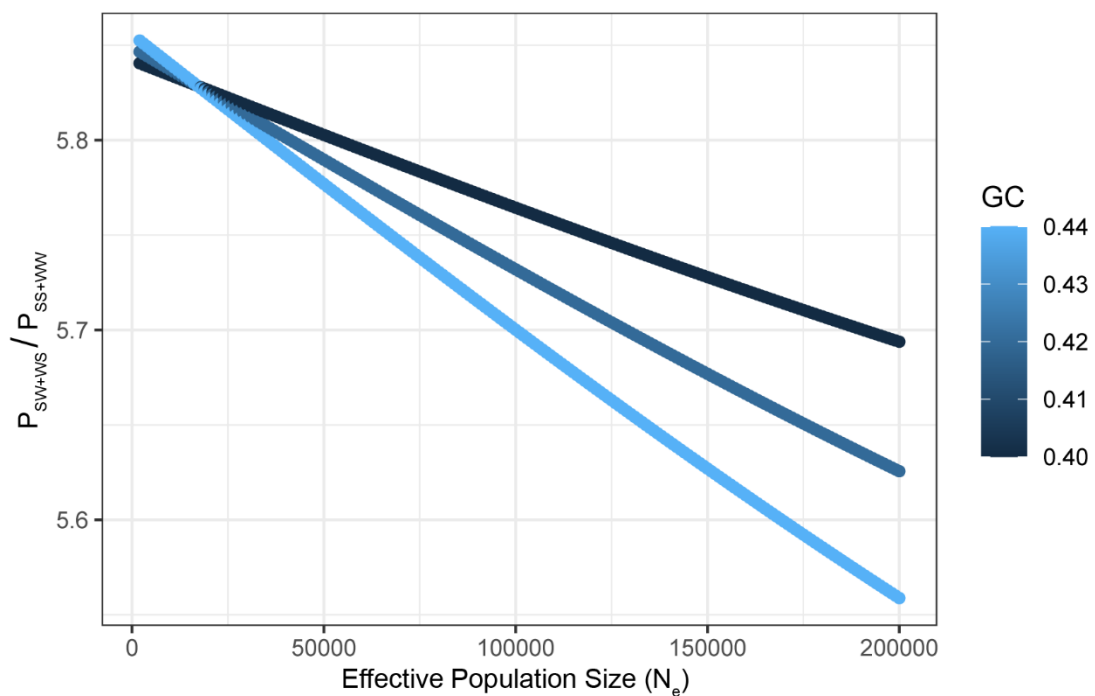
547 Polygamy was strongly associated with more efficient purifying selection, whereas no direct
548 effect of polygamy was detected on the signatures of genetic diversity or GC-biased gene
549 conversion (gBGC) intensity (PGLS model results are summarised in Supplementary Figure 2).
550 This pattern contrasts with the effects of geographic range size and body mass, which were
551 consistent across all genomic signatures and highlight the large impact of effective population
552 size on genome-wide evolutionary processes. Referring to the predictions in Table 1, the pattern
553 of results for polygamy is consistent with sexual selection enhancing purifying selection for
554 polygamous species, as predicted by 'good genes' theories of sexual selection. Wider
555 implications of 'good genes' theories of sexual selection include a reduced vulnerability to
556 inbreeding for polygamous species (Jarzebowska et al. 2010), and more efficient adaptation in
557 polygamous species (Lorch et al. 2003), which may in turn underlie the link between sexual
558 selection and diversification (Iglesias-Carrasco et al. 2019; Cally et al. 2021). Furthermore, the

559 pattern of results suggests that the increased variance in reproductive success associated with
560 polygamy does not have a sizeable impact on effective population size, at least relative to the
561 effects of geographic range size and body mass.

562 A significant effect of polyandry on heterozygosity was also detected, consistent with a
563 mutagenic effect of sperm competition. However more research is required to corroborate the
564 link between polyandry and genetic diversity, as the current dataset included just 11 polyandrous
565 species. The effect of geographic range size on gBGC intensity was stronger in polygamous
566 species, which is hypothetically consistent with greater gene flow between polygamous
567 populations (D'Urban-Jackson et al. 2017), however the lack of such a moderating effect on
568 purifying selection efficiency or heterozygosity provides evidence against this theory. It should
569 be noted that life-history traits and strategies vary widely in the avian class, and whilst a
570 confounding correlation between effective population size and polygamy was ruled out, it is
571 difficult to exclude the possibility of more complex confounds (e.g. ecological generalism;
572 Tobias and Seddon 2009). The theories tested in this paper would therefore benefit from further
573 comparative work on a more closely related group of species.

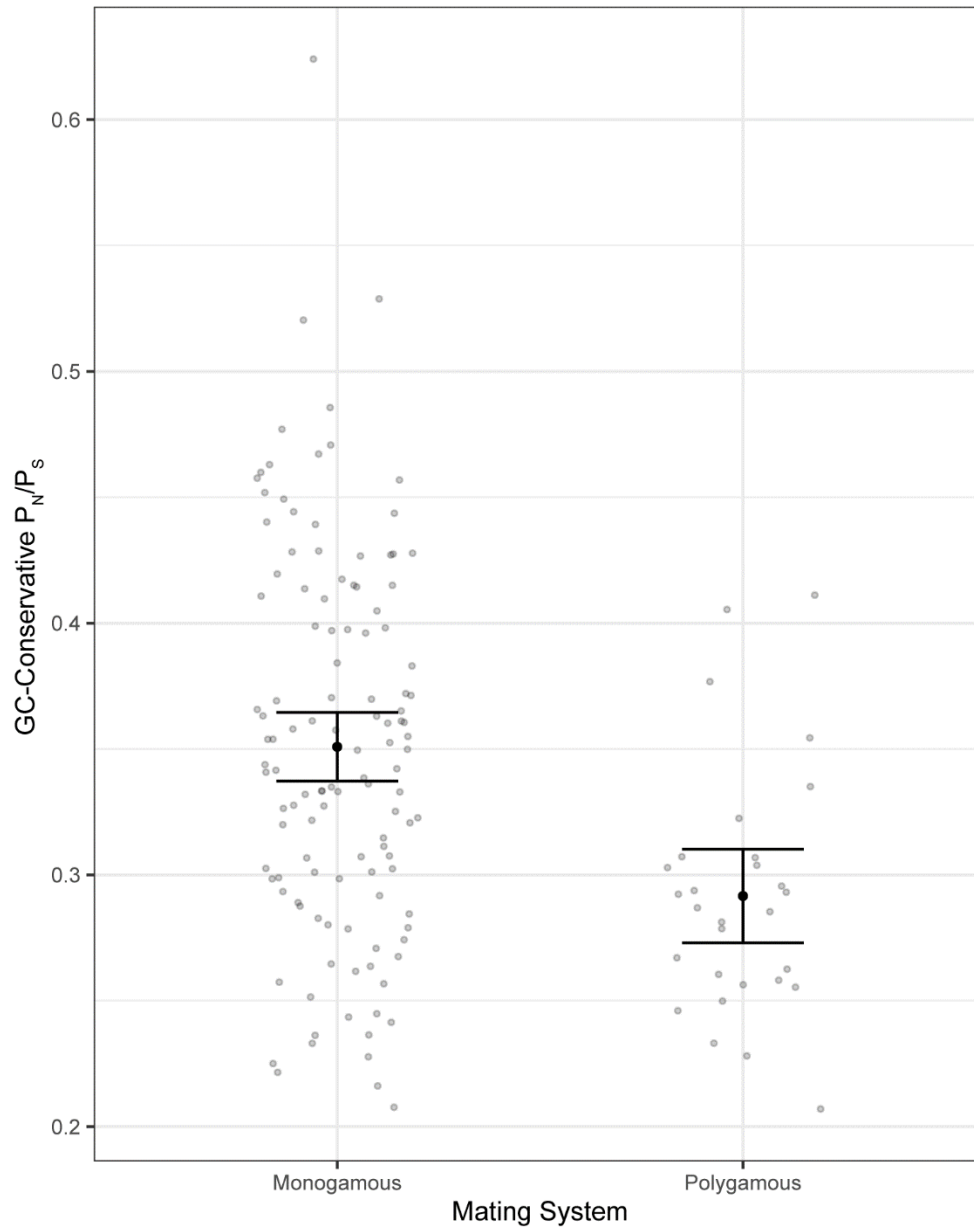
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575 **FIGURES**



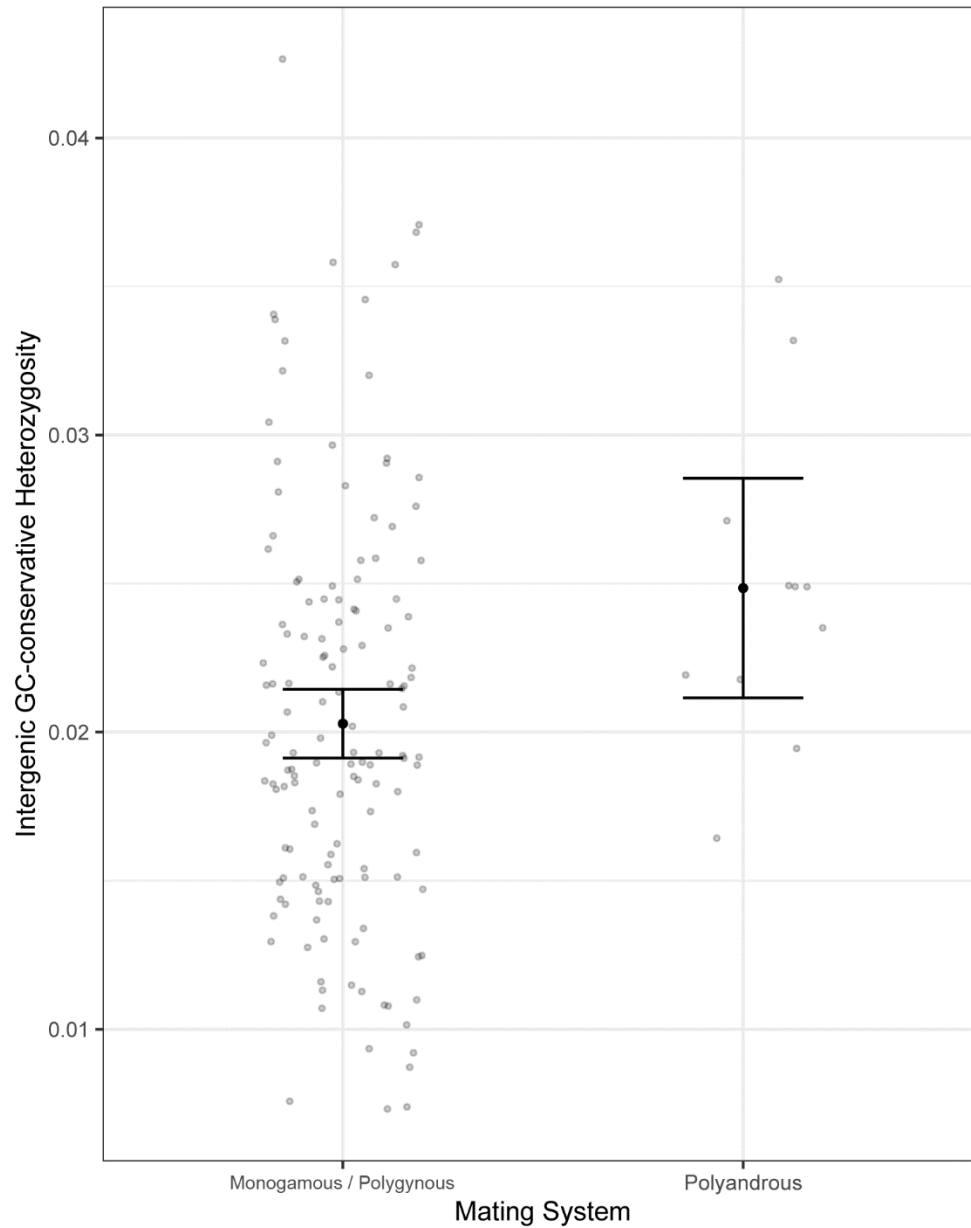
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577 **Figure 1. Predicting the impact of N_e (effective population size) on P_{SW+WS}/P_{SS+WW} under**
578 **plausible parameter values. Predictions are based on calculations using formulas 2a, 2b,**
579 **and 3, and Table 2 for parameter values.**



580

581 **Figure 2. Purifying selection is more efficient in polygamous species (N=29) than**
 582 **monogamous species (N=121), as shown by a lower GC-conservative non-synonymous to**
 583 **synonymous SNP ratio (P_n/P_s) ($p=0.0005$). Grey dots represent species, black dots represent**
 584 **means, error bars represent 95% confidence intervals.**

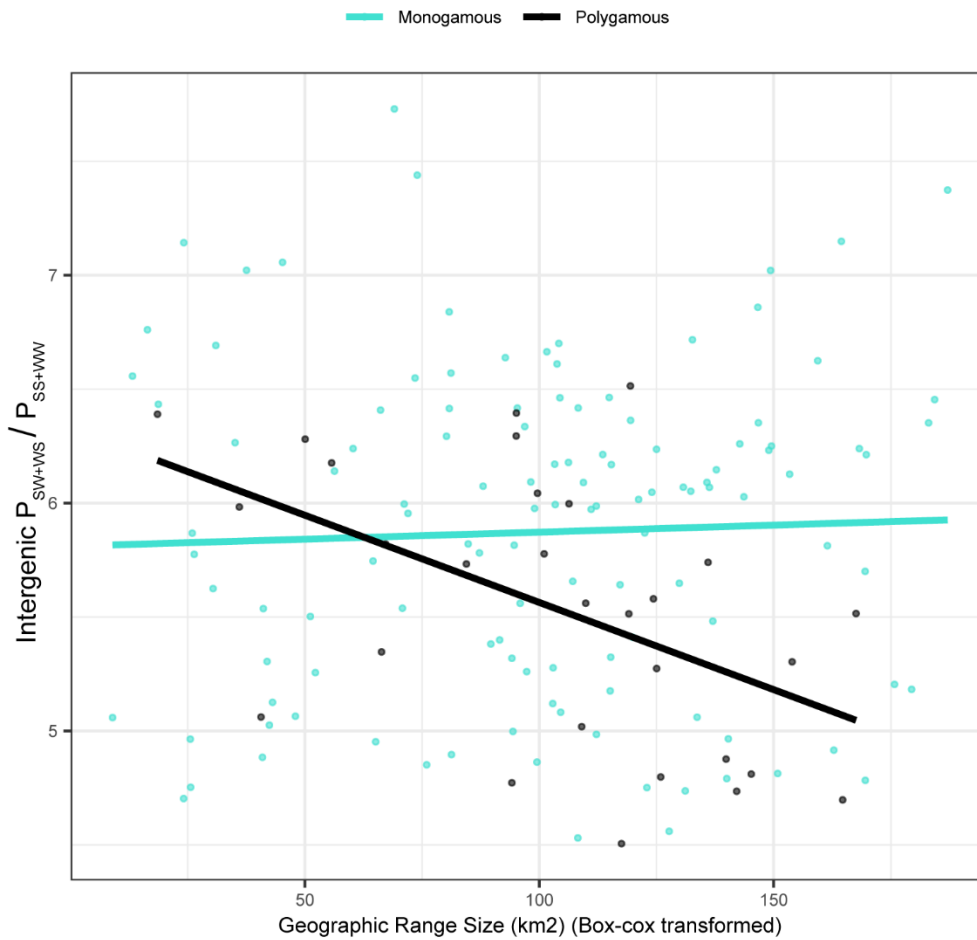


585

586 **Figure 3. GC-conservative intergenic heterozygosity is higher in polyandrous species**

587 **(N=11) than monogamous (and polygynous) (N=139) (p=0.045). Grey dots represent**

588 **species, black dots represent means, error bars represent 95% confidence intervals.**



589

590 **Figure 4. Greater geographic range size is associated with more intense GC-biased gene**
 591 **conversion (gBGC) ($p=0.0031$), measured by the ratio of SNPs affected by gBGC to those**
 592 **unaffected by gBGC (P_{sw+ws}/P_{ss+ww}), and this relationship is stronger for polygamous**
 593 **species ($N=29$) than monogamous species ($N=121$) (interaction effect: $p=0.0017$). Dots**
 594 **represent species, lines represent linear regressions.**

595

596 **SUPPLEMENTARY MATERIAL**

597 **SUPPLEMENTARY TABLES**

598 **Supplementary Table 1: Census population size vs. polygamy (presence/absence),**
 599 **geographic range size (km²), and body mass (g) in a PGLS model †**

Model Term	β (SE) ‡	t-value	N	p-value ⁶⁰⁰ †
Polygamy	1.51 (0.91)	1.66	78	0.10
Body Mass	-1.55 (0.29)	-5.30	78	<0.0001
Geographic Range Size	1.85 (0.24)	7.66	78	<0.0001

601 *Pagel's $\lambda = 0$*

602 ‡ β = slope (coefficient), t-value = slope/standard error, N = number of species

603

604 **Supplementary Table 2: Phylogenetic signal of Body Mass, Geographic Range Size, and**
 605 **Polygamy.**

Model Term	N (Number of Species)	Pagel's λ (95% CI) ⁶⁰⁶
Polygamy	150	0.54 (0.17-81) ⁶⁰⁷
Body Mass	150	1.00 (0.94-1) ⁶⁰⁸
Geographic Range Size	150	0.38 (0-0.76) ⁶⁰⁹

610

611

612 **Supplementary Table 3: PGLS analysis of Genome Quality (L50) vs. polygamy**
 613 **(presence/absence), geographic range size (km²), and body mass (g) in a PGLS model †**

Model Term	β (SE) ‡	t-value	N	p-value ⁶¹⁴ †
Polygamy	-11.95 (6.49)	-1.84	150	0.068
Body Mass	2.51 (3.84)	0.65	150	0.51
Geographic Range Size	-3.96 (2.44)	-1.62	150	0.11

615 *Pagel's* $\lambda = 0.69$

616 ‡ β = slope (coefficient), t-value = slope/standard error, N = number of species

617

618 **Supplementary Table 4: Purifying selection efficiency (GC-conservative P_n/P_s) vs.**
 619 **polygamy (presence/absence), geographic range size (km²), body mass (g) and GC-**
 620 **conservative heterozygosity in a PGLS model †**

Model Term	β (SE) ‡	t-value	N	p-value ⁶²¹ †
Polygamy	-0.089 (0.028)	-3.17	150	0.0018
Body Mass	0.030 (0.013)	2.42	150	0.017
Geographic Range Size	-0.008 (0.011)	-0.72	150	0.48
Heterozygosity §	-0.15 (0.013)	-11.61	150	<0.0001

622 *Pagel's* $\lambda = 0.00$

623 ‡ β = slope (coefficient), t-value = slope/standard error, N = number of species

624 § Heterozygosity was centred for this model, along with the other explanatory variables

625

626 **Supplementary Table 5: gBGC (intergenic P_{sw+ws}/P_{ss+ww}) vs. geographic range size (km²)**
 627 **and body mass (g) in a PGLS model of polygamous species[†], and in a PGLS model of**
 628 **monogamous species[‡].**

PGLS model	Model Term	β (SE) §	t-value	N	p-value
Polygamous	Geographic Range Size	-0.258 (0.084)	-3.06	29	0.0051
	Species	0.294 (0.11)	2.65	29	0.0135
Monogamous	Geographic Range Size	-0.046 (0.037)	-1.22	121	0.22
	Species	0.186 (0.064)	2.88	121	0.0046

629 [†] Pagel's $\lambda = 0.98$

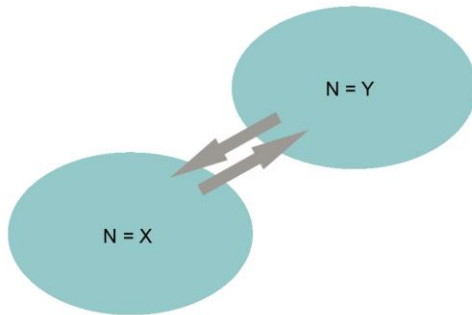
630 [‡] Pagel's $\lambda = 0.88$

631 § β = slope (coefficient), t-value = slope/standard error, N = number of species

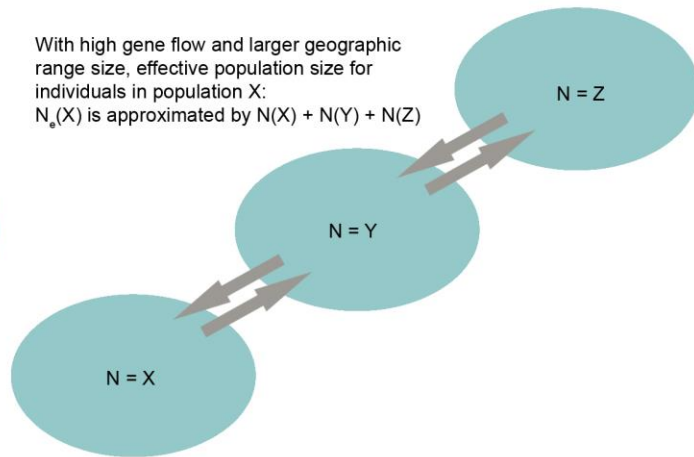
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A

With high gene flow and small geographic range size, effective population size for individuals in population X:
 $N_e(X)$ is approximated by $N(X) + N(Y)$

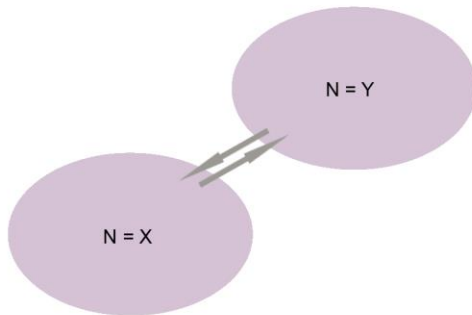


With high gene flow and larger geographic range size, effective population size for individuals in population X:
 $N_e(X)$ is approximated by $N(X) + N(Y) + N(Z)$

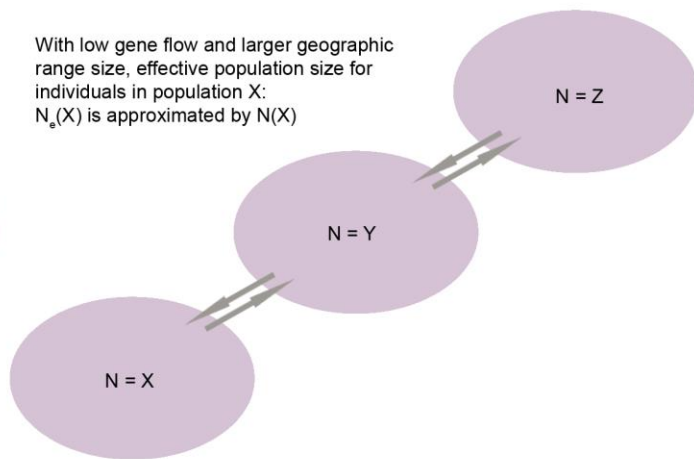


B

With low gene flow and small geographic range size, effective population size for individuals in population X:
 $N_e(X)$ is approximated by $N(X)$



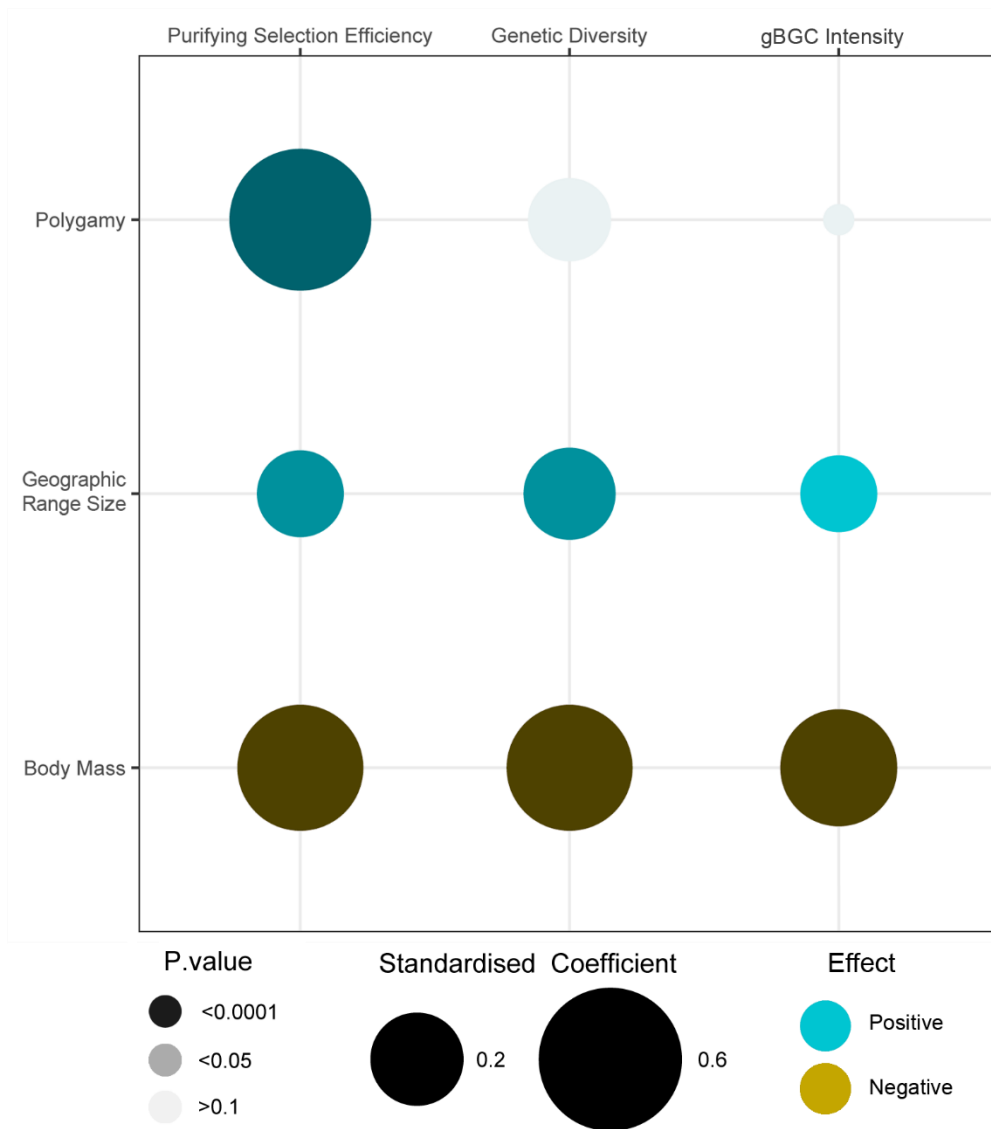
With low gene flow and larger geographic range size, effective population size for individuals in population X:
 $N_e(X)$ is approximated by $N(X)$



634

635 **Supplementary Figure 1: The ‘Dispersal to Mate’ theory (D’Urban-Jackson et al. 2017)**

636 **and geographic range size. (a) High gene flow in polygamous species may cause a strong**
 637 **association between geographic range size and effective population size (N_e), whereas (b) in**
 638 **monogamous species, reduced dispersal leads to partial reproductive isolation and a weak**
 639 **association between geographic range size and N_e**



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641

Supplementary Figure 2. Bubble plot summarising the main effects of polygamy,

642

geographic range size, and body mass, on purifying selection efficiency, genetic diversity,

643

and GC-biased gene conversion efficiency (gBGC). Larger bubbles represent larger effect

644

sizes (standardised coefficients (slopes) from PGLS models), darker colours represent more

645

statistically significant results. Blue bubbles represent positive associations (e.g. greater

646

geographic range size is associated with more effective purifying selection), yellow bubbles

647 **represent negative associations (e.g. greater body mass results is associated with less**
648 **effective purifying selection)**

649

650

651 **REFERENCES**

- 652 Agrawal, A. F. 2001. Sexual selection and the maintenance of sexual reproduction. *Nature*.
653 411(6838):692-5.
- 654 Andersson M. 1982. Sexual selection, natural selection and quality advertisement. *Biol. J. Linn.*
655 *Soc.* 17(4):375-93.
- 656 Anmarkrud, J. A., O. Kleven, J. Augustin, K. H. Bentz, D. Blomqvist, K. J. Fernie, M. J.
657 Magrath, H. Pärn, J. S. Quinn, R. J. Robertson, and T. Szép. 2011. Factors affecting germline
658 mutations in a hypervariable microsatellite: a comparative analysis of six species of swallows
659 (Aves: Hirundinidae). *Mutat. Res.* 15;708(1-2):37-43.
- 660 Arnold, S. J. 1985. Quantitative genetic models of sexual selection. *Experientia.* 41(10):1296-
661 310.
- 662 Arnqvist, G., and L. Rowe. 2013. *Sexual conflict*. Princeton university press, Princeton, NJ.
- 663 Bartosch-Harlid, A., S. Berlin, N. G. Smith, A. P. Mosler, and H. Ellegren. 2003. Life history
664 and the male mutation bias. *Evolution.* 57(10):2398-406.
- 665 Baur, J., and D. Berger. 2020. Experimental evidence for effects of sexual selection on
666 condition-dependent mutation rates. *Nat. Ecol. Evol.* 4(5):737-44.
- 667 Benson, G. 1999. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids*
668 *Res.* 27(2):573-80.
- 669 Billerman, S. M., B. K. Keeney, P. G. Rodewald, and T. S. Schulenberg. 2020. *Birds of the*
670 *World*. <https://birdsoftheworld.org/bow/home>. Accessed 09/09/2020.

671 BirdLife International. 2020. IUCN Red List for Birds. <http://www.birdlife.org>. Accessed
672 09/09/2020.

673 Bolívar, P., L. Guéguen, L. Duret, H. Ellegren, and C. F. Mugal. 2019. GC-biased gene
674 conversion conceals the prediction of the nearly neutral theory in avian genomes. *Genome Biol.*
675 20(1):1-3.

676 Bolívar, P., C. F. Mugal, A. Nater, and H. Ellegren. 2016. Recombination rate variation
677 modulates gene sequence evolution mainly via GC-biased gene conversion, not Hill–Robertson
678 interference, in an avian system. *Mol. Biol. Evol.* 33(1):216-27.

679 Botero-Castro, F., E. Figuet, M. K. Tilak, B. Nabholz, and N. Galtier. 2017. Avian genomes
680 revisited: hidden genes uncovered and the rates versus traits paradox in birds. *Mol. Biol. Evol.*
681 34(12):3123-31.

682 Cally, J. G., D. Stuart-Fox, and L. Holman. 2019. Meta-analytic evidence that sexual selection
683 improves population fitness. *Nat. Commun.* 10(1):1-0.

684 Cally, J. G., D. Stuart-Fox, L. Holman, J. Dale, and I. Medina. 2021. Male-biased sexual
685 selection, but not sexual dichromatism, predicts speciation in birds. *Evolution.* 75(4):931-944.

686 Capra, J. A., M. J. Hubisz, D. Kostka, K. S. Pollard, and A. Siepel. 2013. A model-based
687 analysis of GC-biased gene conversion in the human and chimpanzee genomes. *PLoS Genet.*
688 9(8):e1003684.

689 Charlesworth, B. 2001. The effect of life-history and mode of inheritance on neutral genetic
690 variability. *Genet. Res.* 77(2):153-66.

691 Charlesworth, B. 2009. Effective population size and patterns of molecular evolution and
692 variation. *Nat. Rev. Genet.* 10(3):195-205.

693 Clément, Y., G. Sarah, Y. Holtz, F. Homa, S. Pointet, S. Contreras, B. Nabholz, F. Sabot, L.
694 Saune, M. ardisson and R. Bacilieri. 2017. Evolutionary forces affecting synonymous variations
695 in plant genomes. *PLoS Genet.* 13(5):e1006799.

696 Cohen, J. 1988. The effect size index: d. *Statistical power analysis for the behavioral sciences.*
697 Abingdon-on-Thames: Routledge Academic.

698 Corcoran, P., T. I. Gossman, H. J. Barton, Great Tit HapMap Consortium, J. Slate, and K. Zeng.
699 2017. Determinants of the efficacy of natural selection on coding and noncoding variability in
700 two passerine species. *Genome Biol. Evol.* 9(11):2987-3007.

701 Damuth, J. 1981. Population density and body size in mammals. *Nature.* 290(5808):699-700.

702 DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire, C. Hartl, A. A.
703 Philippakis, G. Del Angel, M. A. Rivas, M. Hanna, and A. McKenna. 2011. A framework for
704 variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.*
705 43(5):491-8.

706 Dowling, D. K., and L. W. Simmons. 2009. Reactive oxygen species as universal constraints in
707 life-history evolution. *Proc. Royal Soc. B.* 276(1663):1737-1745.

708 D'Urban Jackson, J., N. Dos Remedios, K. H. Maher, S. Zefania, S. Haig, S. Oyler-McCance, D.
709 Blomqvist, T. Burke, M. W. Bruford, T. Székely, and C. Küpper. 2017. Polygamy slows down
710 population divergence in shorebirds. *Evolution.* 71(5):1313-26.

711 Duret, L. and N. Galtier. 2009. Biased gene conversion and the evolution of mammalian
712 genomic landscapes. *Annu. Rev. Genom. Hum. Genet.* 22;10:285-311.

713 Ellegren, H. 2010. Evolutionary stasis: the stable chromosomes of birds. *Trends Ecol. Evol.*
714 25(5):283-91.

715 Engqvist L. 2005. The mistreatment of covariate interaction terms in linear model analyses of
716 behavioural and evolutionary ecology studies. *Anim. Behav.* 70(4).

717 Feng, S., J. Stiller, Y. Deng, J. Armstrong, Q. Fang, A. H. Reeve, D. Xie, G. Chen, C. Guo, B. C.
718 Faircloth, and B. Petersen. 2020. Dense sampling of bird diversity increases power of
719 comparative genomics. *Nature.* 587(7833):252-7.

720 Figuet, E., B. Nabholz, M. Bonneau, E. Mas Carrio, K. Nadachowska-Brzyska, H. Ellegren, and
721 N. Galtier. 2016. Life history traits, protein evolution, and the nearly neutral theory in amniotes.
722 *Mol. Biol. Evol.* 33(6):1517-27.

723 Fisher, R. A. 1958. *The genetical theory of natural selection.* Рипол Классик.

724 Fox, J., G. G. Friendly, S. Graves, R. Heiberger, G. Monette, H. Nilsson, B. Ripley, S. Weisberg,
725 M. J. Fox and M.A.S.S Suggests. 2007. The car package. *R foundation for statistical computing,*
726 1109.

727 Galtier, N., C. Roux, M. Rousselle, J. Romiguier, E. Figuet, S. Glémin, N. Bierne, and L. Duret.
728 2018. Codon usage bias in animals: disentangling the effects of natural selection, effective
729 population size, and GC-biased gene conversion. *Mol. Biol. Evol.* 35(5):1092-1103.

730 Gaston, K. J., and T. M. Blackburn. 1996. Global scale macroecology: interactions between
731 population size, geographic range size and body size in the Anseriformes. *J. Anim. Ecol.* 1:701-
732 14.

733 Geraldès, A., P. Basset, K. L. Smith, and M. W. Nachman. 2011. Higher differentiation among
734 subspecies of the house mouse (*Mus musculus*) in genomic regions with low recombination.
735 *Mol. Ecol.* 20(22):4722-36.

736 Glémin, S., P. F. Arndt, P. W. Messer, D. Petrov, N. Galtier, and L. Duret. 2015. Quantification
737 of GC-biased gene conversion in the human genome. *Genome Res.* 25(8):1215-28.

738 Gohli, J., J.A. Anmarkrud, A. Johnsen, O. Kleven, T. Borge, and J. T. Lifjeld. 2013. Female
739 promiscuity is positively associated with neutral and selected genetic diversity in passerine birds.
740 *Evolution.* 67(5):1406-19.

741 Greenwood, J. J., R. D. Gregory, S. Harris, P. A. Morris, and D. W. Yalden. 1996. Relations
742 between abundance, body size and species number in British birds and mammals. *Philos. Trans.*
743 *R. Soc. Lond., B, Biol. Sci.* 351(1337):265-78.

744 Griffin, D.K., L. B. Robertson, H. G. Tempest, and B. M. Skinner. 2007. The evolution of the
745 avian genome as revealed by comparative molecular cytogenetics. *Cytogenet. Genome Res.*
746 117(1-4):64-77.

747 Harrison, P. W., A. E. Wright, F. Zimmer, R. Dean, S. H. Montgomery, M. A. Pointer, and J. E.
748 Mank. 2015. Sexual selection drives evolution and rapid turnover of male gene expression. *Proc.*
749 *Natl. Acad. Sci.* 112(14):4393-8.

750 Hernandez, R. D., S. H. Williamson, and C. D. Bustamante. 2007. Context dependence, ancestral
751 misidentification, and spurious signatures of natural selection. *Mol. Biol. Evol.* 24(8):1792-1800.

752 Hijmans R. J. 2012. Introduction to the "geosphere" package (Version 1.5-14).

753 Hwang, D. G., and P. Green. 2004. Bayesian Markov chain Monte Carlo sequence analysis
754 reveals varying neutral substitution patterns in mammalian evolution. *Proc. Natl. Acad. Sci.*
755 101(39):13994-14001.

756 Iglesias-Carrasco, M, M. D. Jennions, S. Y. Ho, and D. A. Duchêne. 2019. Sexual selection,
757 body mass and molecular evolution interact to predict diversification in birds. *Proc. Royal Soc.*
758 B. 286(1899):20190172.

759 IUCN. 2020. The IUCN Red List of Threatened Species. <http://www.iucnredlist.org>. Accessed
760 09/09/2020.

761 Jarzebowska, M., and J. Radwan. 2010. Sexual selection counteracts extinction of small
762 populations of the bulb mites. *Evolution: Int. J. Org. Evol.* 64(5):1283-9.

763 Jennions, M. D, A. P. Møller, and M. Petrie. 2001. Sexually selected traits and adult survival: a
764 meta-analysis. *Q. Rev. Biol.* 76(1):3-6.

765 Kessler, M. D., and M. D. Dean. 2014. Effective population size does not predict codon usage
766 bias in mammals. *Ecol. Evol.* 4(20):3887-3900.

767 Kimura, M. 1969. The rate of molecular evolution considered from the standpoint of population
768 genetics. *Proc. Natl. Acad. Sci.* 63(4):1181-8.

769 Kimura, M. 1969b. The number of heterozygous nucleotide sites maintained in a finite
770 population due to steady flux of mutations. *Genetics.* 61(4):893.

771 Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of
772 the lek. *Nature*. 350(6313):33-8.

773 Kutschera, V. E., J. W. Poelstra, F. Botero-Castro, N. Dussex, N. J. Gemmell, G. R. Hunt, M. G.
774 Ritchie, C. Rutz, R. A. Wiberg, and J. B. Wolf. 2020. Purifying selection in corvids is less
775 efficient on islands. *Mol. Biol. Evol.* 37(2):469-74.

776 Lartillot, N. 2013. Phylogenetic patterns of GC-biased gene conversion in placental mammals
777 and the evolutionary dynamics of recombination landscapes. *Mol. Biol. Evol.* 30(3):489-502.

778 Leroy, T., M. Rousselle, M. K. Tilak, A. E. Caizergues, C. Scornavacca, M. Recuerda, J. Fuchs,
779 J. C. Illera, D. H. De Swardt, G. Blanco, and C. Thébaud. 2021. Island songbirds as windows
780 into evolution in small populations. *Curr. Biol.* 31(6):1303-10.

781 Lorch, P. D., S Proulx, L. Rowe, and T. Day. 2003. Condition-dependent sexual selection can
782 accelerate adaptation. *Evol. Ecol. Res.* 5(6):867-81.

783 Lumley, A. J., Ł. Michalczyk, J. J. Kitson, L. G. Spurgin, C. A. Morrison, J. L. Godwin, M. E.
784 Dickinson, O. Y. Martin, B. C. Emerson, T. Chapman, and M. J. Gage. 2015. Sexual selection
785 protects against extinction. *Nature*. 2015. 522(7557):470-3.

786 Lynch, M. 2010. Rate, molecular spectrum, and consequences of human mutation. *Proc. Natl.*
787 *Acad. Sci.* 107(3):961-8.

788 McDonald, J. H., and M. Kreitman. 1991. Adaptive protein evolution at the *Adh* locus in
789 *Drosophila*. *Nature*. 351(6328):652-4.

790 Møller, A. P. 1991. Sperm competition, sperm depletion, paternal care, and relative testis size in
791 birds. *Am. Nat.* 137(6):882-906.

792 Møller, A. P., and J. J. Cuervo. 2003. Sexual selection, germline mutation rate and sperm
793 competition. *BMC Evol. Biol.* 3(1):1-1.

794 Møller, A. P., and J. J. Cuervo. 2009. Minisatellite mutation rates increase with extra-pair
795 paternity among birds. *BMC Evol. Biol.* 9(1):1-5.

796 Møller, A. P., L. Z. Garamszegi, and C. N. Spottiswoode. 2008. Genetic similarity, breeding
797 distribution range and sexual selection. *J. Evol. Biol.* 21(1):213-25.

798 Mugal, C. F., P. F. Arndt, and H. Ellegren. 2013. Twisted signatures of GC-biased gene
799 conversion embedded in an evolutionary stable karyotype. *Mol. Biol. Evol.* 30(7):1700-12.

800 Müller, R., I. Kaj, C. F. Mugal. 2020. The Behavior of Molecular Measures of Natural Selection
801 after a Change in Population Size [PREPRINT]. *bioRxiv*.

802 Muyle, A., L. Serres-Giardi, A. Ressayre, J. Escobar, and S. Glémin. 2011. GC-biased gene
803 conversion and selection affect GC content in the *Oryza* genus (rice). *Mol. Biol. Evol.*
804 28(9):2695-706.

805 Nadachowska-Brzyska, K., C. Li, L. Smeds, G. Zhang, and H. Ellegren. 2015. Temporal
806 dynamics of avian populations during Pleistocene revealed by whole-genome sequences. *Curr.*
807 *Biol.* 25(10):1375-80.

808 Nadeau, N. J., T. Burke, and N. I. Mundy. 2007. Evolution of an avian pigmentation gene
809 correlates with a measure of sexual selection. *Proc. Royal Soc. B.* 274(1620):1807-13.

810 Nagylaki, T. 1983. Evolution of a finite population under gene conversion. *Proc. Natl. Acad. Sci.*
811 80(20):6278-81.

812 Nunney, L. 1993. The influence of mating system and overlapping generations on effective
813 population size. *Evolution*. 47(5):1329-41.

814 Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2013. *Caper:*
815 *Comparative Analyses of Phylogenetics and Evolution in R* (version 0.5. 2).

816 Ossowski, S., K. Schneeberger, J. I. Lucas-Lledó, N. Warthmann, R. M. Clark, R. G. Shaw, D.
817 Weigel, and M. Lynch. 2010. The rate and molecular spectrum of spontaneous mutations in
818 *Arabidopsis thaliana*. *Science*. 327(5961):92-4.

819 Paradis, E. 2012. *Analysis of Phylogenetics and Evolution with R* (Vol 2). New York: Springer.

820 Pessia, E., A. Popa, S. Mousset, C. Rezvoy, L. Duret, and G. A. Marais. 2012. Evidence for
821 widespread GC-biased gene conversion in eukaryotes. *Genome Biol. Evol.* 4(7):675-82.

822 Petrie, M. 2021. *Evolution by Sexual Selection*. *Front. Ecol. Evol.* 950.

823 Petrie, M., C. Doums, and A. P. Møller. 1998. The degree of extra-pair paternity increases with
824 genetic variability. *Proc. Natl. Acad. Sci.* 95(16):9390-5.

825 Petrie, M., and G. Roberts. 2007. Sexual selection and the evolution of evolvability. *Heredity*.
826 98(4):198-205.

827 Pinheiro, J. D. Bates, S. DebRoy, D. Sarkar, S. Heisterkamp, B. Van Willigen, and R.
828 Maintainer. 2017. Package ‘nlme’. *Linear and nonlinear mixed effects models*, version 3(1).

829 Pitelka, F. A., R. T. Holmes, and S. F. MacLean Jr. 1974. Ecology and evolution of social
830 organization in arctic sandpipers. *Am. Zool.* 14(1):185-204.

831 R Core Team R. 2020. *R: A language and environment for statistical computing*.

832 Robinson, M. C., E. A. Stone, and N. D. Singh. 2014. Population genomic analysis reveals no
833 evidence for GC-biased gene conversion in *Drosophila melanogaster*. *Mol. Biol. Evol.*
834 31(2):425-33.

835 Rolland, J., D. Schluter, and J. Romiguier. 2020. Vulnerability to fishing and life history traits
836 correlate with the load of deleterious mutations in teleosts. *Mol. Biol. Evol.* 37(8):2192-6.

837 Romiguier, J., P. Gayral, M. Ballenghien, A. Bernard, V. Cahais, A. Chenuil, Y. Chiari, R.
838 Dernet, L. Duret, N. Faivre, and E. Loire. 2014. Comparative population genomics in animals
839 uncovers the determinants of genetic diversity. *Nature*. 515(7526):261-3.

840 Romiguier, J., V. Ranwez, E. J. Douzery, and N. Galtier. 2010. Contrasting GC-content
841 dynamics across 33 mammalian genomes: relationship with life-history traits and chromosome
842 sizes. *Genome Res.* 20(8):1001-9.

843 Rowe, L., and H. D. Rundle. 2021. The alignment of natural and sexual selection. *Annu. Rev.*
844 *Ecol. Evol. Syst. and Systematics.* 52:499-517.

845 Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients.
846 *Methods Ecol. Evol.* 1(2):103-13.

847 Siepel A., and D. Haussler. 2004. Phylogenetic estimation of context-dependent substitution
848 rates by maximum likelihood. *Mol. Biol. Evol.* 21(3):468-88.

849 Siller, S. 2001. Sexual selection and the maintenance of sex. *Nature*. 411(6838):689-92.

850 Smeds, L., A. Qvarnström, H. Ellegren. 2016. Direct estimate of the rate of germline mutation in
851 a bird. *Genome Res.* 26(9):1211-8.

852 Smit, A. F., R. Hubley, and P. Green. 2015. RepeatMasker Open-4.0.

853 Smith, N. G., and A. Eyre-Walker. 2002. Adaptive protein evolution in *Drosophila*. *Nature*.
854 415(6875):1022-24.

855 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
856 large phylogenies. *Bioinformatics*. 30(9):1312-3.

857 Symonds, M. R., and S. P. Blomberg. 2014. A primer on phylogenetic generalised least squares,
858 in *Modern phylogenetic comparative methods and their application in evolutionary biology*.
859 Springer, Berlin.

860 Székely, T., A. Liker, G. H. Thomas, N. Brett, G. Brooks, E. Capp, N. Engel, S. Hodges, E.
861 Hughes, A. Krystalli, T. Lislevand, A. Mapp, I. Pipoly, R. Rice, L. Rossi, J. Komdeur, O.
862 Krüger, and A. Gonzalez-Voyer. 2022. Sex roles in birds: influence of climate, life histories and
863 social environment. Database: Dryad. Available from: <https://doi.org/10.5061/dryad.fbg79cnw7>

864 Székely et al. 2008. Practical guide for investigating breeding ecology of Kentish plover.
865 *Charadrius alexandrinus*.

866 Thomas, R. J. 2015. Data analysis with R statistical software: a guidebook for scientists. Eco-
867 explore.

868 Tobias, J. A., and N. Seddon. 2009. Sexual selection and ecological generalism are correlated in
869 antbirds. *J. Evol. Biol.* 22(3):623-36.

870 Torchiano, M. 2017. effsize: Efficient effect size computation. R package version 0.7. 1. 1.

871 Weber, C. C., B. Boussau, J. Romiguier, E. D. Jarvis, and H. Ellegren. 2014. Evidence for GC-
872 biased gene conversion as a driver of between-lineage differences in avian base composition.
873 *Genome Biol.* 15(12):1-6.

- 874 Webster, M. T. and L. D. Hurst. 2012. Direct and indirect consequences of meiotic
875 recombination: implications for genome evolution. *Trends Genet.* 28(3):101-9.
- 876 Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection: reducing
877 mutation load through selection on males. *Evol.; Int. J. Org. Evol.* 63(3):569-82.
- 878 Won, Y. J., and J. Hey. 2005. Divergence population genetics of chimpanzees. *Mol. Biol. Evol.*
879 22(2):297-307.
- 880 Wright, S. 1931. Evolution in Mendelian populations. *Genetics.* 16(2):97.
- 881 Zhang, Z. and M. Gerstein. 2003. Patterns of nucleotide substitution, insertion and deletion in the
882 human genome inferred from pseudogenes. *Nucleic. Acids. Res.* 31(18):5338-48.