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3 **Searching for genetic evidence of demographic decline in an**
4 **arctic seabird: beware of overlapping generations**

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48

49 **Abstract**

50 Genetic data are useful for detecting sudden population declines in species that are
51 difficult to study in the field. Yet this indirect approach has its own drawbacks,
52 including population structure, mutation patterns, and generation overlap. The ivory
53 gull (*Pagophila eburnea*), a long-lived Arctic seabird, is currently suffering from rapid
54 alteration of its primary habitat (i.e., sea ice), and dramatic climatic events affecting
55 reproduction and recruitment. However, ivory gulls live in remote areas, and it is
56 difficult to assess the population trend of the species across its distribution. Here we
57 present complementary microsatellite- and SNP-based genetic analyses to test a
58 recent bottleneck genetic signal in ivory gulls over a large portion of their distribution.
59 With attention to the potential effects of population structure, mutation patterns, and
60 sample size, we found no significant signatures of population decline worldwide. At a
61 finer scale, we found a significant bottleneck signal at one location in Canada. These
62 results were compared with predictions from simulations showing how generation
63 time and generation overlap can delay and reduce the bottleneck microsatellite
64 heterozygosity excess signal. The consistency of the results obtained with independent
65 methods strongly indicates that the species shows no genetic evidence of an overall
66 decline in population size. However, drawing conclusions related to the species'
67 population trends will require a better understanding of the effect of age structure in
68 long-lived species. In addition, estimates of the effective global population size of ivory
69 gulls were surprisingly low (approximately 1000 ind.), suggesting that the evolutionary
70 potential of the species is not assured.

71

72 **Keywords:** ivory gull, *Pagophila eburnea*, conservation, bottleneck, past population

73 size variation, coalescence, effective population size, microsatellites, SNPs.

74

75 **INTRODUCTION**

76 Unraveling the recent demographic trajectory of threatened species using genetic data
77 may complement observational studies and help detect recent population declines,
78 which is particularly useful when a species is hard to monitor in the field. In addition,
79 the genetic data will also provide estimates of the current levels of genetic diversity
80 and effective size of populations, which may alert us to changes in the adaptive
81 evolvability of species facing rapid environmental change.

82 Environmental changes are particularly acute in the Arctic, a sensitive region that
83 experiences large, negative effects from climate change compared to elsewhere
84 globally, including cryosphere change, air and water temperatures increase, ocean
85 acidification, change in precipitation levels, and sea-level increase (ACIA, 2004; Box *et*
86 *al.*, 2019; Meredith *et al.*, 2020). Species might cope with these changes by shifting
87 their distribution to track suitable conditions, acclimating through phenotypic
88 plasticity, or evolving adaptations to the new local conditions (Parmesan, 2006;
89 Gienapp *et al.*, 2008; Chen *et al.*, 2011; Hoffmann and Sgrò, 2011). Depending on the
90 speed and extent of environmental changes, range shift can be accompanied by a
91 range contraction (Arenas *et al.*, 2011). Such a contraction usually implies a decline in
92 population size that can be followed by an increased level of genetic drift that may
93 erode genetic diversity within populations (McInerny *et al.*, 2009; Arenas *et al.*, 2011;
94 Collevatti *et al.*, 2011; Alsos *et al.*, 2012; Rubidge *et al.*, 2012; Garnier and Lewis, 2016).
95 Evaluating trends in population abundance is thus critical to understand how arctic
96 species are reacting to threats (McRae *et al.*, 2012). This difficult task may benefit from

97 indirect genetic approaches, which will also provide information directly relevant to
98 the evolutionary potential of populations.

99 Microsatellite markers have been widely used in conservation genetic studies
100 testing for recent bottlenecks, using a variety of methods (Nyström *et al.*, 2006; Peery
101 *et al.*, 2012; Xenikoudakis *et al.*, 2015). One of the most widely used so far has been
102 the heterozygosity-excess test, which compares gene diversity (expected
103 heterozygosity, H_E) in a focal population to the heterozygosity expected (H_{Eeq}) in a
104 population at mutation-drift equilibrium (Cornuet and Luikart, 1996). More detailed
105 information (e.g., strength and timing of the contraction) can be obtained by
106 calculating the likelihood of the observed genetic data given a coalescent model
107 allowing for variations in population size (Beaumont, 1999; Leblois *et al.*, 2014;
108 Rousset *et al.*, 2018). These methods, however, suffer from the limitations of
109 microsatellites (low number of markers and thus limited information in the genetic
110 data, and relatively complex mutation process).

111 New powerful tools have emerged following the next-generation sequencing
112 (NGS) technical revolution (Shafer *et al.*, 2015; Andrews *et al.*, 2016), including
113 methods designed to infer current and past population size (e.g., Li and Durbin, 2011;
114 Liu and Fu, 2015; Boitard *et al.*, 2016). These methods aim to make the best of the
115 information contained in genome-wide data (e.g., genome-wide single nucleotide
116 polymorphism (SNP) variants or complete genomes) to infer complex demographic
117 histories.

118 All genetic inference methods make several simplifying assumptions. For
119 instance, the effects of mutation process (Williamson-Natesan, 2005; Peery *et al.*,

120 2012; Leblois *et al.*, 2014), extreme variance in reproductive success (Hoban *et al.*,
121 2013), and internal population structure and connectivity (Broquet *et al.*, 2010; Chikhi
122 *et al.*, 2010) are important to take into account. The complexity induced by
123 overlapping generations in iteroparous species could also affect genetic signals of
124 bottleneck, but this effect has, to the best of our knowledge, never been specifically
125 examined - but see, e.g., Storz *et al.*, (2002) for a relevant discussion and Parreira *et*
126 *al.*, (2015; 2020) for an example of model including overlapping generations to
127 investigate the genetic consequences of social structure in mammals.

128 The effective size N_E of a population for an iteroparous species with overlapping
129 generations depends on generation time T_G and lifetime variance in reproductive
130 success among individuals $V_{k\bullet}$ (following notation from Waples, 2016). Assuming
131 constant population size and a stable age distribution, Hill (1972) obtained $N_E =$
132 $4N_1T_G/(V_{k\bullet} + 2)$, where N_1 is the number of new individuals entering the population
133 at each time step. The parameters in this equation result from the variation in
134 individual survival and fertility among age classes (Felsenstein, 1971; Hill, 1972; see
135 also Rousset, 1999; Laporte and Charlesworth, 2002, and ; Yannic, Yearsley, *et al.*, 2016
136 for models of structured populations). Although it was shown that the ratio of
137 effective to census population size is mainly determined by age at maturity and adult
138 lifespan (Waples *et al.*, 2013) or adult survival (Waples, 2016), precisely predicting the
139 effective population size of a long-lived iteroparous species is difficult since it requires
140 detailed demographic data on the species' vital rates. Considering the simplest
141 situation where newborns become reproductively mature within one breeding cycle
142 (say one year) and adults have constant fecundity and survive to the next breeding

143 cycle with probability ν (constant across age classes), then $T_G = 1/(1 - \nu)$ and $N_E =$
144 $N/(1 + \nu)$ or $N_E = N/(2 - 1/T_G)$ where N is the number of adults in the population
145 (Nunney, 1993; Nunney and Elam, 1994; Yannic, Yearsley, *et al.*, 2016, and in
146 agreement with ; Felsenstein, 1971; Orive, 1993; Waples, 2016). With this simplistic
147 model (detailed in Yannic, Yearsley, *et al.*, 2016), we see that iteroparity and
148 overlapping generations reduce effective population size by a factor that approaches 2
149 as adult survival increases. Regarding the heterozygosity-excess method to detect
150 bottlenecks mentioned above, the disequilibrium signal was found to peak around N_E
151 generations past population size reduction, with N_E the post-bottleneck effective
152 population size (Cornuet and Luikart, 1996). Hence, a demographic bottleneck in a
153 population with overlapping generations should produce a signal of heterozygosity
154 excess earlier (with time expressed in generations) than in a population with discrete
155 non-overlapping generations. Of course, in units of absolute time, the whole dynamics
156 of the bottleneck signature will appear scaled up by a factor equal to generation length
157 T_G .

158 Yet the intensity and temporal dynamics of bottleneck signatures are in fact
159 complex functions of the severity of the demographic decline, mutation models and
160 rates, and even sample size (Cornuet and Luikart, 1996). Moreover, more realistic age
161 structures (accounting for delayed maturity and variation in survival and fecundity
162 with age) will bring additional complexity that makes it difficult to obtain explicit
163 predictions about the behavior of bottleneck detection methods applied to long-lived
164 iteroparous species.

165 A case in point, the ivory gull (*Pagophila eburnea*) is a long-lived high-arctic bird
166 that has delayed sexual maturity and high annual adult survival (estimated to
167 0.86 ± 0.04 , Stenhouse *et al.*, 2004; and maximum observed life span of 28 years
168 Mallory *et al.*, 2012). The ivory gull is closely associated with sea ice throughout the
169 year (Spencer *et al.*, 2014; Gilg *et al.*, 2016), and has a patchy breeding range at the
170 high latitudes of Atlantic Arctic, *i.e.*, in Canada, Greenland, Norway (Svalbard), and the
171 Western Russian Arctic. The global population size was estimated at 38,000-52,000
172 mature individuals (BirdLife International, 2018) and has been revised downward to
173 14,400-19,900 mature individuals by the most recent surveys (see Table 1). The Ivory
174 gull drew attention following reports suggesting that the species had declined by 70%
175 since the 1980s in Canada (Gilchrist and Mallory, 2005) and by 40% in Svalbard
176 (Norway) from 2009 to 2019 (Strøm *et al.*, 2020). Now, the global population is
177 considered declining (BirdLife International, 2018), although trends are contrasted
178 across breeding regions. Populations are supposed to be stable in Greenland (Gilg *et*
179 *al.*, 2009; Boertmann *et al.*, 2020) and Russia (Gavrilo and Martynova, 2017). The three
180 main identified threats that support its "Near Threatened" status according to the
181 IUCN (BirdLife International, 2018) are: (i) global warming inducing sea ice decline, *i.e.*
182 decline of ivory gulls' main habitat; (ii) exposure to contaminants such as persistent
183 organic pollutants and heavy metals in the environment (Braune *et al.*, 2006; Miljeteig
184 *et al.*, 2009; Lucia *et al.*, 2015); and (iii) extensive development of human activities,
185 *e.g.*, resources exploitation (oil, gas, mineral), or shipping lanes (Gilg *et al.*, 2012; Fort
186 *et al.*, 2013; Yurkowski *et al.*, 2019).

187 The primary objective of this study was to investigate the recent demographic
188 trajectory of ivory gull populations worldwide. To achieve this goal, we analyzed
189 microsatellite and SNP data obtained from a large part of the species' range, predicting
190 that a decline in population size should have produced a genetic bottleneck signature.
191 Before analyzing such signatures, we first explored population structure to check if the
192 genetic homogeneity reported by Yannic et al. (2016) using microsatellites would
193 withstand SNP examination. We then applied three bottleneck inference methods with
194 the microsatellites (heterozygosity-excess, *M*-ratio, and coalescent-based maximum
195 likelihood inference) and one Approximate Bayesian computation (ABC) method with
196 the SNPs. Our second objective was to explore the effect of age structure on genetic
197 signatures of a bottleneck. Focusing on the microsatellite heterozygosity excess
198 method (Cornuet and Luikart, 1996), we used stochastic simulations to look at the
199 dynamics of genetic signals in a long-lived species experiencing a demographic
200 bottleneck. Finally, our third objective was to evaluate the current effective size (N_e) of
201 the ivory gull, a key evolutionary parameter in conservation biology (Hohenlohe *et al.*,
202 2021). We approached this objective using the linkage disequilibrium method (Waples
203 and Do, 2010), both with microsatellite and SNP data.

204

205 **MATERIALS AND METHODS**

206 **Sample collection and genotyping**

207 We analyzed genetic data obtained for individuals sampled from the global breeding
208 range of the species in summers 2006 to 2012 (Table 1, Fig. 1; Yannic, Yearsley, *et al.*,
209 2016). Full sampling protocols are described in Yannic et al. (2011; 2016). Three non-

210 destructive DNA sampling methods (buccal swabs, plucked feathers, and blood) and a
211 non-invasive sampling method (shed feathers) were used. Here we considered data
212 from adult birds only ($n= 271$), but we distinguished individuals that were seen to be
213 incubating eggs or feeding chicks (hereafter called "breeding" individuals) and
214 individuals for which we had no information on their breeding status (*i.e.*, "unknown
215 status", possibly including visiting breeding birds from other colonies, or prospectors,
216 Volkov and de Korte (2000)). Then, we characterized each sampling site by a single
217 type of birds (*i.e.*, "breeding" or "unknown" status), except Station Nord, where both
218 categories of birds were sampled (Table 1). Thus, we considered 15 distinct samples,
219 which we hereafter consistently called "populations" for the sake of simplicity.

220 Our microsatellite dataset was a subset of the multilocus genotypes obtained
221 using 22 microsatellites previously published in Yannic et al. (2016). Because the
222 demographic inference methods that we use assume typical microsatellite mutation
223 models, we selected the 15 loci (4 dinucleotides: loci A111, A115, A129 and A132 and
224 11 tetranucleotides: loci B103, B125, C6, C7, D1, D103, D110, D126, D5, D6, and D9),
225 that presented allele size distribution expected for dinucleotides (*i.e.*, multiple of 2-bp
226 units) or tetranucleotides (*i.e.*, multiple of 4-bp units). The number of alleles observed
227 and heterozygosity for each of the 15-microsatellite loci are provided in Table S1.

228 We used a second set of data consisting of SNP genotypes obtained for a subset
229 of individuals, which were generated specifically for this study using a ddRAD-seq
230 approach (see Supplementary Materials). This method was used only with high quality
231 DNA extracts and thus limited to 6 of our populations (circled in red in Fig. 1).

232

233 **SNPs genotyping**

234 At the end of the de novo SNP calling and filtering procedure, the data set
235 encompassed 5912 SNPs distributed over 3,490 RAD loci genotyped for 87 adult birds
236 (9-20 ind. per population, Table 1). We discarded 9 birds with > 10% of missing data.
237 Error rates estimated on the 5,912 SNPs obtained from replicated samples were 0.009
238 +/- 0.003 and dropped to 0.001 +/- 0.003 when we considered a single SNP per locus
239 (n=3490 SNPs). The characteristics of this dataset (number of individuals, number of
240 SNPs, proportion of missing data, and error rates) should be well suited to analyze
241 variations in population size and current effective population size (Nunziata and
242 Weisrock, 2018; see e.g., Marandel *et al.*, 2020).

243

244 **Genetic structure**

245 We estimated the genetic differentiation among all populations by estimating global
246 and pairwise F_{ST} at two spatial scales: among populations ($n = 15$) or among regions
247 (see Fig. 1). We also estimated F_{ST} considering only the "breeding" birds to test
248 whether spatial genetic structure could have been affected by including "visiting" birds
249 (see Yannic, Yearsley, *et al.*, 2016). For microsatellite loci, we used the online *Genepop*
250 v.4.7 software (Raymond and Rousset, 1995; Rousset, 2008) to estimate F_{ST} according
251 to Weir and Cockerham (1984), among all populations and regions. We tested
252 genotypic differentiation using Markov chain algorithms with default parameters in
253 *Genepop*. With the SNP data, the same analysis was performed on the subset of
254 populations for which SNPs were available (6 populations and 3 regions; Fig. 1, see also

255 Table 1), using *Genepop* v.4.6 called through the R package *strataG* (Archer *et al.*,
256 2017).

257

258 **Demographic inference**

259 To test for a recent population genetic bottleneck we combined different approaches,
260 using three alternative methods for the microsatellite dataset and a single approach
261 for the SNP dataset.

262

263 *Microsatellites: heterozygosity excess*

264 First, we tested for a recent genetic bottleneck using the heterozygosity-excess
265 method presented in Luikart and Cornuet (1998), and implemented in the software
266 *Bottleneck* v.1.2.02 (Cornuet and Luikart, 1996; Piry *et al.*, 1999). This method
267 compares expected heterozygosity H_E in an empirical sample to the heterozygosity H_{Eq}
268 that is expected in a population at mutation-drift equilibrium given the number of
269 alleles observed in the sample. Strong reductions in N_E are followed by a sharp
270 decrease in the number of alleles (rare alleles being quickly lost) while heterozygosity
271 H_E decreases less rapidly. A transitory excess in H_E (measured as $\Delta H = H_E - H_{Eq}$) is
272 therefore expected in recently bottlenecked populations (and a transitory deficit in H_E
273 is expected in case of population expansion). Microsatellites generally evolve under
274 the classic stepwise mutation model (SMM; by the gain or loss of a single repeat unit),
275 but mutations of several repeat units may occasionally occur. Therefore, we used a
276 mixed Two-Phase mutational Model (TPM), which fits best the mutation processes in
277 microsatellites (Di Rienzo *et al.*, 1994). But because the heterozygosity-excess method

278 is very sensitive to mutation models, we ran the analyses assuming that the probability
279 of single-step mutations is 0.70 (TPM70) or 0.95 (TPM95), as suggested by Miller *et al.*
280 (2012) and Piry *et al.* (1999), respectively. The variance of the geometric distribution
281 for the multi-steps mutations was set to 12 in both cases. We used 1,000 coalescent
282 simulations and one-tailed Wilcoxon sign rank tests to test for an excess of
283 heterozygosity considering the whole population ($n = 271$ ind.), each region ($n = 23$ -
284 106 ind.) and each population (but only the 9 populations with $n \geq 10$ individuals, Table
285 1).

286 Although we observed a genetic homogeneity across the species distribution
287 range (see Yannic, Yearsley, *et al.*, 2016 and the results below), we considered that the
288 observed disparity in demographic trends among regions called for an investigation of
289 genetic bottleneck inference at the regional and population scales too. However, to
290 ensure the detection of genetic bottlenecks is not sensitive to sample size according to
291 the spatial scale considered, we randomly generated sets of individuals of various size
292 ($11 \leq n \leq 250$), sampled among the 271 ivory gull genotypes. These sample sizes
293 roughly correspond to the size of the different sets of individuals considered in this
294 study at different spatial scales (i.e., populations, regions, and whole population).
295 Sampling was replicated 10 times for each sample size to estimate standard errors (se)
296 around ΔH . The effect of sample size on bottleneck detection was then estimated with
297 the software *Bottleneck* considering the two mutation models: A) TPM70 and B)
298 TPM95. We report mean $\Delta H \pm$ se as well as the probability (p -value for one tailed
299 Wilcoxon test) of detecting an excess in H_E for each sample size and each mutation
300 model.

301 Because we tested the same hypothesis several times on different data sets (*i.e.*,
302 at different scales) and using different mutation models (TPM70 et TPM95), we also
303 provided in addition of the original *p-values*, the adjusted *p-values* for multiple
304 comparisons obtained using the Benjamini and Hochberg (1995) false discovery rate
305 procedure with initial $\alpha = 0.05$ (later denoted *fdr.p-values*).

306

307 *Microsatellites: M-ratio*

308 Second, we used an alternative method based on the ratio (*M*) of the number of
309 microsatellite alleles (*k*) to the range in allelic size (*r*) (Garza and Williamson, 2001). It is
310 expected that *M* is smaller in populations that have experienced a reduction in size (M
311 < 0.68 ; Garza and Williamson, 2001). We ran this analysis in R using the *mRatio*
312 function implemented in the package *strataG* (Archer *et al.*, 2017). The method was
313 applied to the same sets of individuals and spatial scales as described above.

314

315 *Microsatellites: Migraine*

316 Third, we used a more general method described in Leblois *et al.* (2014), that aims to
317 detect and date past changes in population size. This method implemented in the
318 software *Migraine* (Leblois *et al.*, 2014; Rousset *et al.*, 2018) uses simulations to
319 estimate the likelihood of demographic parameters given observed genetic data. We
320 used the demographic model considering one population with a single past variation in
321 population size (*OnePopVarSize*), which considers a single population of ancestral size
322 $N_{E,past}$ that instantly changed to the current size N_E some *T* generations in the past. The
323 population may have experienced a bottleneck ($N_{ratio} = N_{E,past}/N_E < 1$) or an expansion

324 ($N_{ratio} > 1$). Contrary to the two methods presented above, *Migraine* allows
325 microsatellites to be characterized by different mutation models within the same
326 dataset. In addition, it considers a generalized stepwise mutation model (GSM) where
327 the number of repeats added or removed by a mutation follows a geometric
328 distribution with parameter p_{GSM} , which is estimated by the method. We ran
329 preliminary analyses where we allowed dinucleotides ($n=4$) and tetranucleotides
330 ($n=11$) to follow either one of two models (SMM or GSM). We concluded that the
331 method gave the most reliable results when considering only the tetranucleotides,
332 under GSM (see discussion). We then ran *Migraine* using 4 iterations of 10 000 points
333 and 10 000 trees per point (detailed settings in supplementary materials) to infer
334 parameters $4N_E\mu$, $4N_{E,past}\mu$ and the composite parameter N_{ratio} . *Migraine* also
335 estimated p_{GSM} and T_μ (noted $T_{g\mu}$ in *Migraine*).

336

337 *SNPs: Demographic Inference with Linked Selection (DILS)*

338 Finally, we analyzed our ddRAD-seq genotypes (filtered dataset: 3490 loci) with a
339 general method (*DILS: Demographic Inferences with Linked Selection*) newly
340 developed by Fraïsse *et al.* (2021) that compares the genetic properties of the data at
341 hand to that of a large number of simulated demographic scenarios to identify what
342 historical scenario is most likely to have produced the observed data (ABC framework).
343 We considered a single population of ancestral size $N_{E,past}$ that is allowed to vary in size
344 instantaneously at some time T in the past to reach its current size N_E . We ran three
345 runs where we allowed $N_{E,past}$ and N_E to take any value from 0 to 10^5 individuals, and
346 population size change could have happened at any time between 0 (i.e. present) to

347 10^5 generations in the past. The three runs differed only in the minimum allelic
348 frequency (*maf*) used to generate the dataset used in *DILS* (no *maf*, *maf*= 0.01, and
349 *maf* = 0.02). Other parameters followed the authors' recommendations: mutation rate
350 set to 10^{-8} , ratio of recombination (intra-RAD locus) and mutation set to 0.8 (Fraïsse *et*
351 *al.*, 2021). Since our filtered dataset contained at least 77 out of 87 individuals
352 genotyped per SNP, we set the minimum number of haploid copies required to process
353 a locus (*Nmin*) to 154. Similarly, since our smallest RAD sequence was 150 bp long, we
354 set the minimum sequence length to *Lmin* = 150 bp (complete settings file in
355 supplementary material). We retained results from the optimized posterior and using
356 the random-forest method implemented in *DILS*.

357

358 **Simulation of microsatellite heterozygosity excess when generations overlap**

359 We used simulations to explore the effect of age structure on genetic signatures of a
360 demographic decline. With the ivory gull case in mind, we designed simplified
361 simulations that would help us interpret the signal of heterozygosity excess observed
362 with our microsatellite dataset.

363 We used a modified version of *Nemo* (Guillaume and Rougemont, 2006) , called
364 *Nemo-age* (Cotto *et al.*, 2020), an individual-based, genetically explicit and stochastic
365 population computer program where populations are age-structured. This tool creates
366 stochastic forward-in-time simulations of population demography and genetic
367 markers. We designed a life cycle where chicks become adults after one-time unit
368 (year) and adults survive from one year to the next with probability v . We simulated
369 two bottleneck scenarios for a population that took one of three age structures.

370 Simulation parameters were chosen so that diversity $\theta = 4N_E\mu$ would be not too small
371 even at the new equilibrium after bottleneck (so that microsatellite H_E and k remain
372 meaningful) and not too large ($\theta \leq 1$) so that analytical expressions for ΔH are not
373 biased (especially under SMM, see below).

374 In the first scenario, the population was reduced from 1000 to 250 individuals
375 within a single time unit (and then remained at that size), while it was reduced from
376 1000 to 500 in our second scenario. In each case we ran simulations with adult survival
377 v set to 0 (no overlapping generations), 0.8 (*i.e.*, a value like that expected for ivory
378 gull) or 0.95 (allowing us to look at the effect of a very pronounced age structure, see
379 Fig. S3 for an example of resulting age distributions). Increasing adult survival has two
380 consequences. First, it will increase generation time (T_G), defined as the mean age of
381 adults (given that all adults have the same fecundity and there is no sex-biased survival
382 in the simulations). We obtained $T_G = 1$ year when $v = 0$, $T_G = 4.5$ years when $v = 0.8$,
383 and $T_G = 13.4$ years with $v = 0.95$ (these values estimated empirically from the
384 simulations agreed with theoretical predictions, see supplementary material). Second,
385 increasing v also means that generations will be overlapping.

386 We simulated 20 microsatellite markers neutrally evolving under the conditions
387 of IAM or SMM with mutation rate $\mu = 2.5 \cdot 10^{-4}$ per locus per generation (Estoup and
388 Angers, 1998). Using these markers, we estimated heterozygosity excess ΔH every 10
389 years and we averaged these values over 100 simulation replicates. To produce as
390 many ΔH estimates, we used the number of alleles k and gene diversity H_E reported by
391 *Nemo-age* and estimated H_{Eq} analytically from the number of alleles k observed in a
392 sample of size n (number of gene copies sampled). Under IAM, the relationship is $k =$

393 $\sum_{i=0}^{n-1} \theta / (\theta + i)$ with $\theta = H_{\text{Eq}} / (1 - H_{\text{Eq}})$ (Ewens, 1972), and for SMM it is (Kimura and
394 Ohta, 1978): $k = (\theta + \beta) / \beta [1 - \prod_{i=0}^{n-1} (\theta + i) / (\theta + \beta + i)]$, where $\beta =$
395 $\theta(1 - H_{\text{Eq}}) / H_{\text{Eq}} - 1$ and $\theta = 1/2 [1 / (1 - H_{\text{Eq}})^2 - 1]$.

396 Because these relationships may be slightly biased in some conditions, for 10
397 additional replicates, we also asked *Nemo-age* to create *Genepop* files from the
398 simulated markers 20 years before the bottleneck (*i.e.*, at mutation-drift equilibrium)
399 and 20 years after the bottleneck (*i.e.*, imitating the situation suspected for the ivory
400 gull). These files were then used to estimate ΔH and test for its significance in the
401 software *Bottleneck*. Additional technical details for the simulation settings are given
402 in supplementary material.

403

404 **Effective population size**

405 Effective population size (N_E) was estimated with microsatellites for each population (n
406 = 15), each region (Canada, Greenland, Norway and Russia), and the whole population,
407 using *NeEstimator* v.2.1 software (Do *et al.*, 2014). We used the linkage disequilibrium
408 method considering a random mating model. Following Waples and Do (2010), we
409 excluded rare alleles to limit estimation bias. To do so, we used two methods, first
410 excluding singletons only (*i.e.*, alleles represented by a single copy in the population),
411 and second excluding alleles with a frequency ≤ 0.02 (*maf*, called *Pcrit* in *NeEstimator*).
412 We assumed that all loci are physically unlinked (as verified in Yannic *et al.* 2016). The
413 same analyses were performed using SNP data but considering only the 6 populations
414 or 3 regions for which we had such data (Table 1) and using *maf* = 0.05 (Nunziata and
415 Weisrock, 2018; see Marandel *et al.*, 2020).

416

417 **RESULTS**

418 **Genetic structure**

419 A low level of genetic differentiation was observed among populations, both with
420 microsatellites (15 populations, $F_{ST,\mu sat} = 0.0044$, $p < 0.05$) and SNPs (6 populations,
421 $F_{ST,snp} = 0.0043$; $p < 0.001$). These results confirm the findings of Yannic *et al.* (2016)
422 and suggest that bottleneck analyses can be run considering a single global population
423 of ivory gulls. Similar conclusions were obtained when measuring the differentiation
424 using breeding individuals only ($F_{ST,\mu sat} = 0.0006$, NS ; $F_{ST,snp} = 0.0024$, NS) or at the scale
425 of regions ($F_{ST,\mu sat} = 0.0035$, $p < 0.001$; $F_{ST,snp} = 0.0057$, $p < 0.001$). Pairwise F_{ST} between
426 populations or regions varied between -0.0036 and 0.0092 (Tables S2 and S3).

427

428 **Demographic inference**

429 *Microsatellites: heterozygosity excess*

430 Assuming a TPM95 model, we did not detect any departure from mutation-drift
431 equilibrium in the whole ivory gull population ($\Delta H = -0.016$; one-tailed Wilcoxon test, p
432 = 0.93 and $fdr-p = 0.97$; Fig. 2 and Table S4). If we considered a TPM70 model, we
433 found a significant but slight excess of H_E ($H = 0.027$; one-tailed Wilcoxon test, $p =$
434 0.04), which became non-significant after correction for multiple tests ($fdr-p = 0.13$;
435 Table S4).

436 At the scale of regions (Fig. 3), we did not detect any excess of heterozygosity
437 under TPM95 (all one-tailed Wilcoxon tests, $p > 0.05$ and $fdr-p > 0.05$; Table S4). Using

438 TPM70, Greenland was the only region with a significant signal of bottleneck ($\Delta H =$
439 0.021; one-tailed Wilcoxon test, $p = 0.01$, but $\text{fdr-}p = 0.07$; Table S4).

440 At the population scale, we found a significant heterozygosity excess under
441 TPM70 in four populations (1_StNo, 2_StBr, 8_Rudo and 15_AIEI, $\Delta H > 0$; one-tailed
442 Wilcoxon tests, $p < 0.05$; Fig. S1), but this signal was significant with TPM95 for the
443 population of Alert on Ellesmere Island, Canada only (15_AIEI, $\Delta H = 0.015$; one-tailed
444 Wilcoxon test, $p = 0.001$). After correction for multiple tests, a significant excess of
445 heterozygosity was still observed in the Alert population with TPM70 ($\text{fdr-}p = 0.02$) but
446 not with TPM95 ($\text{fdr-}p = 0.13$).

447 Resampling analyses indicated that ΔH estimates and associated p -values are
448 sensitive to sample size. Under TPM70, ΔH estimates became slightly underestimated
449 as sample sizes decreased (Fig. 4A). The estimates also became more variable for the
450 smallest sample sizes (e.g., $n \leq 25$) but remain underestimated in all cases. Under
451 TPM95, sample size had essentially no impact on ΔH estimates (Fig. 4A). Sub-sampling
452 had more contrasted effects on the results of the Wilcoxon test used by *Bottleneck*
453 (Fig. 4B). Increasing sample size increased power under the TPM70 but we see in Fig.
454 4B that increasing sample size moved the p -value away from significance under
455 TPM95.

456

457 *Microsatellites: M-ratio*

458 M -ratio was 0.956 for the whole population, and regardless of the scale of analysis, M
459 values were always higher than the critical value defined by Garza and Williamson

460 (2001), i.e., $M = 0.68$ (Fig. S2), suggesting no genetic bottleneck at the considered
461 scales for ivory gull.

462

463 *Microsatellites: Migraine*

464 *Migraine* estimated N_{ratio} at 3.45 (95%CI [1.88- 6.17]), with current effective
465 population size $4N_E\mu = 50.92$ (95%CI [35.76- 94.47]), about three times larger than
466 ancestral population size $4N_{E,past}\mu = 14.76$ (95%CI [7.64- 25.37]) (see Fig. 5). The value
467 of $T\mu$ was estimated to 0.76 (95%CI [0.21- 2.21]), suggesting that the expansion
468 occurred a very long time ago (even considering high microsatellite mutation rates, see
469 discussion).

470 Finally, p_{GSM} was estimated at 0.0319 (95%CI [0.00- 0.129]), indicating that the
471 mutation process of the microsatellites is very close to SMM and TPM95 (with this
472 parameter value, the probability of a single-step mutation is $g(1)\approx 0.968$, while a two-
473 step mutation will happen with probability $g(2)\approx 0.031$, and $g(3)\approx 0.001$).

474

475 *SNPs: DILS*

476 The analysis of SNP data with *DILS* consistently found that the most likely demographic
477 scenario was an ancient expansion, regardless of the *maf* used in the analysis. While
478 *DILS* provides absolute values for each demographic parameter (N_E , $N_{E,past}$, T), these
479 values depend directly on the mutation rate assumed. Hence here we report the ratio
480 of $N_E/N_{E,past}$, which was found to be 6.13 (95%CI [4.16-8.76]) with *maf* = 0, 3.67 (95%CI
481 [2.56-5.60]) with *maf* = 0.01, and 10.60 (95%CI [6.76-19]) with *maf* = 0.02. The lower
482 limit for this ratio (estimated from 95% highest posterior density intervals) was never

483 below 3. These results, obtained with the optimized posterior, random forest options
484 of *DILS*, did not differ much when using alternative computation methods ($N_E/N_{E,past}$
485 for each *maf* was always in [3.63-8.80] when using non-optimized posterior, and/or
486 neural network estimation).

487 The timing of the change in population size was very sensitive to the choice of
488 *maf* ($T = 7290$ generations with *maf* = 0, $T = 20338$ gen. with *maf* = 0.01, and $T = 67601$
489 gen. with *maf* = 0.02). Moreover, these estimates are directly impacted by the value
490 chosen for mutation rate (here 10^{-8}). But even if this value is over- or underestimated
491 10 times, all results from *DILS* point to an ancient population expansion (>900
492 generations ago).

493

494 **Simulation of microsatellite heterozygosity excess when generations overlap**

495 The dynamics of ΔH obtained from simulations are presented in Figure 6 (strong
496 bottleneck scenario, 1000 --> 250) and Figure S4 (mild bottleneck 1000 --> 500). To
497 understand the effect of age structure, the results are presented with time expressed
498 in years (top panels in Figs. 6 and S4) and generations (bottom panels).

499 Increasing adult survival resulted in a delayed genetic bottleneck signal (in
500 absolute time units), but the delay was not simply proportional to the increase in
501 generation time. For instance, under IAM in the strong bottleneck scenario (Figs. 6A
502 and C), the maximum ΔH value was observed 80 years after the bottleneck in the case
503 of a semelparous, annual species (*i.e.*, $\nu = 0$, max signal 80 generations post-
504 bottleneck), while it was 220 years post-bottleneck (49 generations) with $\nu = 0.8$, and
505 670 years (50 generations) with $\nu = 0.95$. Hence in a long-lived species with

506 overlapping generations, the signal peaked later in absolute time, but still much
507 sooner than predicted by generation time alone. This is at least in part because the
508 dynamics of the heterozygosity excess depends on the post-bottleneck N_E , which is
509 reduced in case of an iteroparous life cycle where generations overlap. Although our
510 stochastic simulations depart slightly from the simplistic conditions where $N_E =$
511 $N/(1 + \nu)$ (see introduction and supplementary material), in the example above the
512 difference in the timing of the ΔH peak ($80 / 49 = 1.6$) appears relatively well predicted
513 by the difference in post-bottleneck N_E predicted by $N/(2 - 1/T_G)$: with $N = 250$, $\nu =$
514 0.8 and $T_G = 4.5$, we have $N_E \approx 140$, that is, 1.8 times smaller than the post-bottleneck
515 N_E when $\nu = 0$ and $T_G = 1$. Increasing ν to 0.95 had, as it should, not a strong effect on
516 this timing since it the effect on N_E is asymptotic (with $\nu = 0.95$ and $T_G = 13.4$, $N_E \approx$
517 130).

518 The strength of the bottleneck signal was also affected by age structure. Using
519 the same example as above (strong bottleneck, IAM, Figs. 6A and C), the maximum
520 values for ΔH were 0.12, 0.10, and 0.09 with $\nu = 0$, $\nu = 0.8$, and $\nu = 0.95$, respectively.

521 The same consequences were observed under SMM, although the effect of age
522 structure on heterozygosity excess was less visible because the analytical estimation of
523 ΔH was slightly biased (see discussion, $\Delta H \neq 0$ even when gene diversity and the number
524 of alleles are at mutation-drift equilibrium in our simulations, Figs. 6B and D, and
525 S4B,D).

526 These simulations also showed that the maximum signal of bottleneck was
527 rather low (between 0.045 and 0.12 depending on simulation scenario) and took time
528 to be reached (80 to 1040 years after bottleneck). Using the software *Bottleneck* to

529 analyze simulated datasets, we found that a significant signal of bottleneck was
530 correctly detected 20 years after the decline in 0 to 9 replicates out of 10, depending
531 on the simulation scenario, *i.e.*, the combined effect of mutation, survival, and
532 strength of bottleneck (Fig. S4 and Table S5). In particular, the probability to detect a
533 significant genetic bottleneck 20 years after the simulated decline with *Bottleneck* was
534 globally higher under IAM than under SMM, and was also higher for a strong
535 bottleneck, *i.e.*, 75% of decline (1000 -> 250) than for a lower bottleneck, *i.e.*, 50% of
536 decline (1000 -> 500). In addition, these analyses also allowed us to compare our
537 analytical estimates of ΔH against the values estimated by *Bottleneck*. These two
538 methods produced identical values under IAM, but the analytical estimates appeared
539 slightly overestimated under SMM (Fig. S6).

540

541 **Effective population size**

542 With microsatellite markers, the effective size of the whole population was estimated
543 to $N_E = 1138.6$ individuals (95%CI [754.0-2204.3]) with *maf* set to 0.02. When
544 considering all alleles but singletons, N_E was estimated to 1487.9 individuals (95%CI
545 [990.5-2871.4]). With SNPs we obtained $N_E = 729.5$ (95%CI [689.4-774.3]) and 861
546 (95%CI [828.9-895.6]) individuals, considering a minimum allele frequency of 0.05 and
547 no singleton, respectively.

548 At the regional scale, estimates ranged from 250.6 individuals in Canada to
549 3288.5 individuals in Norway (microsatellites, Table 2A) with *maf* set to 0.02, and
550 similar results were obtained when removing only the singletons from the calculations
551 (Table 2A). However, at that scale, precise confidence intervals could not be obtained

552 with the microsatellites. The SNPs again returned lower N_E estimates (from 191.0 in
553 Greenland to 368.9 in Norway with $maf = 0.05$) with narrower confidence intervals
554 (Table 2B). At the population scale, estimates of N_E are imprecise, for both
555 microsatellites and SNPs, with large confidence intervals that span to infinity.

556

557 **DISCUSSION**

558 *No genetic signature of demographic decline globally*

559 We found no genetic signature of a recent population decline in the ivory gull
560 population as a whole. This result was consistent across three independent methods
561 using microsatellites and one method using SNPs. According to the heterozygosity-
562 excess approach, we did not find a departure from mutation-drift that would indicate a
563 recent genetic bottleneck in the ivory gull population at the global scale. The
564 heterozygosity excess was globally close to zero (Fig. 2) and only significant before
565 correction for multiple tests under the two-phase mutational model TPM70 ($p = 0.036$;
566 Table S4), which as discussed further below, is not an appropriate model in this case.
567 We therefore conclude that there was no evidence for biologically relevant
568 heterozygosity excess that would indicate that the population has suffered a recent
569 genetic bottleneck (but see below for some limitations associated with this
570 conclusion). Results from the M -ratio approach led to the same conclusion. There was
571 no loss of rare alleles in ivory gull populations, because M was always much larger than
572 critical value 0.68 (Fig. S2).

573 The two methods that yield quantitative parameter estimates (*Migraine* and
574 *DILS*) even found that the ivory gull population has experienced an ancient expansion,

575 but not a recent decline. The $N_E/N_{E,past}$ ratios estimated by these two methods were
576 remarkably similar given that they used independent datasets (microsatellites with
577 *Migraine* : $N_E/N_{E,past} = 3.45$; SNPs with *DILS*: $N_E/N_{E,past}$ between 3.67 and 10.60
578 depending on the minimum allelic frequency retained for data filtering). Estimates of
579 the timing of the past changes in population size are more elusive because they are
580 directly proportional to mutation rates, but all results point towards an ancient
581 expansion event even considering conservative mutation rates (e.g., $T \geq 757$
582 generations if $\mu_{microsat} \leq 10^{-3}$, $T \geq 927$ gen. if $\mu_{SNP} \leq 10^{-7}$). We cannot date that expansion
583 more precisely because the number of generations since the expansion varies widely
584 depending on the data set (microsatellites or SNPs), choice of minimum allele
585 frequency, mutation rate, and adult annual survival ($v = 0.86 \pm 0.04$; Stenhouse *et al.*,
586 2004).

587 The heterozygosity-excess method is known to be highly sensitive to the choice
588 of mutation model. The TPM is thought to provide a better approximation of
589 microsatellite mutation than either a strict IAM or strict SMM (Di Rienzo *et al.*, 1994)
590 but the expected number of alleles at mutation-drift equilibrium still depends directly
591 on two unknown parameters that define the proportion and distribution of multi-steps
592 mutations within the TPM. The *Migraine* method uses a slightly simpler model (GSM),
593 where a geometric distribution alone defines the number of repeats that each
594 mutation removes or adds to the ancestral state of the allele. Importantly, *Migraine*
595 infers this distribution from the data by estimating the geometric distribution
596 parameter p_{GSM} . Here we found that *Migraine* could not correctly infer p_{GSM} when
597 using the mixture of di- and tetra-nucleotides composing our original dataset (flat

598 likelihood profiles even when we allowed di- and tetra-nucleotides to follow different
599 mutation models, data not shown). Using only tetranucleotides, p_{GSM} was estimated to
600 0.0319 (95%CI [0.00 - 0.129]), indicating that the mutation process of the
601 microsatellites is very close to TPM95 and SMM (probability of a single-step mutation
602 $g(1) \approx 0.968$). This means that the results obtained from software *Bottleneck* with
603 TPM95 (no bottleneck signal) are more relevant than that obtained with TPM70
604 (contrary to Ellegren, 2004; Engler, Secondi, Dawson, Elle, & Hochkirch, 2016; Peery et
605 al., 2012; Wogan, Voelker, Oatley, & Bowie, 2020). We can also parameterize a TPM in
606 *Bottleneck* that follows exactly the GSM inferred by *Migraine* (0% SMM and variance =
607 $p_{\text{GSM}}/(1 - p_{\text{GSM}}^2) = 0.0319$). Using these parameters with the tetranucleotides, we
608 found no heterozygosity excess, and even a significant heterozygosity deficit (one-
609 tailed Wilcoxon test, $p = 0.034$, indicating a possible expansion). All things considered,
610 our analyses using alternative approaches based on microsatellites (*Bottleneck*,
611 *Migraine* and *M-ratio*) and SNPs (*DILS*) converged towards an absence of recent
612 genetic bottleneck in the global ivory gull population.

613

614 *Local signature of population declines in Canada?*

615 To search for genetic evidence of population decline, we focused primarily on global
616 analyses, considering all ivory gulls as belonging to a single Arctic-wide population. We
617 did so because the large-scale genetic homogeneity reported in Yannic et al. (2016)
618 was essentially confirmed by the new analyses presented here either with a subset of
619 microsatellites ($n = 15$, $F_{\text{ST}} = 0.0044$, $p < 0.05$) or a new SNP dataset ($n = 3490$, $F_{\text{ST}} =$
620 0.0043 ; $p < 0.001$). Using a general model for coalescence times where age structure is

621 entirely defined by a constant rate of adult survival and dispersal rates may differ in
622 juveniles and adults, we suggested in Yannic et al. (2016) that the large-scale genetic
623 homogeneity found in ivory gulls most likely implies massive movements of individuals
624 among colonies, occurring possibly both at the juvenile and adult stages. However,
625 although we did find genetic differentiation to be low, it was not strictly as low as
626 reported previously: $F_{ST} = 0.001$, 95%CI [-0.002- 0.005] (Yannic, Yearsley, *et al.*, 2016).
627 The difference may come from the selection of a slightly different marker set (see
628 methods, but 11 markers were common to the two studies), and the previous inclusion
629 of markers that presented complex mutation patterns (*i.e.*, involving insertions or
630 deletions of some base pair numbers that are not multiples of the repeated motif
631 length). Some slight differentiation was also detected using SNPs, leading to the
632 question of sensitivity of our bottleneck detection results to sampling scale. Moreover,
633 the disparity in demographic trends reported across regions (declines were reported
634 especially in Canada and in Norway) prompted us to examine genetic signals at
635 different spatial scales. Looking at the scale of regions or populations, we found no
636 signature of demographic decline except in one case: in the population of Alert (site 15
637 in Fig. 1), the hypothesis of a local decline cannot be excluded, in line with field
638 observations in Canada (Gilchrist and Mallory, 2005). *Bottleneck* analyses concluded to
639 a significant or nearly significant excess of heterozygosity for this population
640 considering the most relevant (and conservative) mutation model tested (TPM95; $p=$
641 0.032, $fdr-p = 0.132$, see Fig. S1). This result was also supported when reanalyzing the
642 data with TPM parameters designed to follow the GSM inferred by *Migraine* (with

643 tetranucleotides only, noted TPM00 in Table S4) as explained above ($p=0.006$, and still
644 marginally significant after correction for multiple tests, $fdr-p=0.059$).

645 However, this Canadian population showed no clear sign of disconnection from
646 any other population (all pairwise $F_{ST} \leq 0.007$ with microsatellites or SNPs). It is
647 therefore not trivial to understand why a single sample could show a different genetic
648 signal of population decline. One possibility is the presence in this sample of recent
649 immigrants, but again this hypothesis seems at odds with the low genetic
650 differentiation among populations. Another possibility is that it could be an artifact
651 due to the small size of the sample used ($n = 12$ individuals). To test this idea, we
652 investigated the sensitivity of *Bottleneck* to sampling size. A resampling analysis
653 showed that small sample sizes did not result in overestimated heterozygosity excess
654 (Fig. 4A) using either model (although we note a strong difference in behavior between
655 TPM70 and TPM95). Moreover, as expected, small sample size did not produce any
656 false positive result (Fig. 4B), although here again with a notable difference between
657 models. We conclude that the result observed for the Canadian population at Alert is
658 unlikely to be an artifact and thus indicates a significant heterozygosity excess,
659 signature of genetic bottleneck and thus demographic decline. We note, however, that
660 the ΔH value for that population is low ($\Delta H = 0.015$) and that *M-ratio* analysis did not
661 detect any signature of bottleneck. We suggest that it would be interesting to have
662 more samples from Alert population and perhaps, if at all feasible, from other
663 Canadian sites to test the hypothesis of a local bottleneck (but see below for
664 methodological limits linked with the lifespan of the species). Increasing sample size
665 can improve *Bottleneck's* analytical power and make accessible the genetic dataset to

666 other methods of genetic decline inference like *Migraine* or *DILS* (which were not
667 employed in this case because we had only 12 individuals from this population). It
668 would also be useful to investigate what local bottleneck signatures are theoretically
669 expected in strongly connected metapopulations.

670

671 *Life-history and signature of bottleneck*

672 One important aspect of our work was to evaluate the impact of life-history on the
673 genetic inference of demographic declines. This is not an easy task, as it requires to
674 run complex simulations and analyze multiple datasets with resource-demanding
675 inference methods. To tackle this problem, we focused here on the heterozygosity
676 excess method, which we could investigate using analytical approximations on many
677 microsatellite datasets simulated with two extreme mutation models. Although we
678 view this analysis as a first step toward understanding the applicability of genetic
679 bottleneck inference in long-lived species (in particular, pending the development and
680 testing of methods specifically designed to study recent demography using genome-
681 wide data), the simulations provided useful observations. Most importantly, we found
682 that increasing adult survival (and thus introducing longer generation time and
683 generation overlap) resulted in a decreased and delayed signal of genetic bottleneck
684 (Figs. 6 and S4). The ΔH estimates under SMM appeared to be overestimated in the
685 conditions of our simulations (Fig. S6), meaning that the effect of age structure on the
686 intensity of the bottleneck signature was less easily examined under SMM vs IAM (Fig.
687 6). Under IAM, the peak ΔH in a population where adults breed once a year and
688 survive to the next year with a probability $v = 0.8$ was ca. 17% lower than in a

689 population with a semelparous and annual life-history subject to the same
690 demographic decline (1000 --> 250). The decrease was 11% in the less severe
691 bottleneck scenario (1000 --> 500, Figs. S4A,C).

692 The temporal effect was clear regardless of mutation models and corresponded
693 relatively well to what would be expected from the consequences of adult survival on
694 generation time and N_E (Figs. 6 and S4). According to our stochastic simulations, a
695 population that went from 1000 to 250 individuals with adult survival $v = 0.8$ would
696 show a maximum ΔH only ca. 50 generations (220 years) after the event, as compared
697 to 80 generations when $v = 0$. This effect on the timing of the bottleneck signal roughly
698 corresponded to the ratio of post-bottleneck N_E estimated with vs without generation
699 overlap.

700 The question of practical importance is thus, how many generations of drift are
701 required before the ΔH signal starts being detectable? Of course, we cannot answer
702 that question precisely for the ivory gull case (which would require the simulation of
703 larger metapopulations and less caricatured life-cycle and demographic scenarios), but
704 applying the *Bottleneck* software on simulated datasets, we found that genetic
705 bottlenecks were almost never detected in any SMM model regardless of the strength
706 of the bottleneck, even when the peak of ΔH has been reached (1000 \rightarrow 250 or 1000
707 \rightarrow 500; Table S5 and Fig. S4). The results obtained in IAM are more contrasted. Genetic
708 bottlenecks were significantly detected by *Bottleneck* in several simulation scenarios,
709 but this is highly dependent on the strength of the decline and the adult survival rate
710 (Table S5 and Fig. S5).

711 If ivory gulls begin breeding at two years old and adult survival is ca. 0.86
712 (Stenhouse *et al.*, 2004), generation time for the species can be approximated by $T_G =$
713 $a + \left(\frac{v}{1-v}\right) = 8.1$ years (see supplementary material), meaning that a 20-year-old
714 decline corresponds to only 2 to 3 generations. Although our simulations were
715 simplistic, they suggest that it is potentially difficult to detect such a recent event. For
716 example, in the strong bottleneck scenario under TPM and $v = 0.8$ (Fig. 1B and D, pale
717 blue curve), the bottleneck signal after 20 years (2.2 generations in this scenario) was
718 half its maximum value (which is seen after generation 55). The difficulty could even
719 be increased if ivory gulls start breeding at a later age than what is currently thought
720 based on plumage color (because T_G would then be higher).

721 The agreement between the different independent methods used here compels
722 us to conclude that there is no genetic bottleneck signal in ivory gulls, but this
723 conclusion has some limitations. First, according to the simulations, the heterozygosity
724 excess method may not be able to detect a signal of recent genetic bottleneck that is
725 reduced and delayed for long-lived species with overlapping generations and not very
726 powerful with microsatellite loci evolving under SMM (or nearly so). Second, there is
727 also some uncertainty in the capacity of the other methods used here to detect
728 bottleneck events that recent (2-3 generations).

729

730 *Genetic diversity and effective population size*

731 Estimates of effective size of the ivory gull meta-population were around 1,000
732 individuals, according to the results obtained on the two sets of genetic markers: N_E

733 ranged from 729.5 (95%CI [689.4-774.3], with SNPS, $maf = 0.05$) to 1487.9 (95%CI
734 [990.5-2871.4], with microsatellites, no singleton).

735 When we consider a finer scale (e.g., at the population level), confidence
736 intervals increase dramatically, spanning orders of magnitude, up to infinity. Such
737 uncertainties probably stem from the low sample size used to infer local N_E (England *et*
738 *al.*, 2006; Nunziata and Weisrock, 2018). However, because we found an extremely
739 low genetic differentiation among sampling sites (*i.e.*, implying a homogenization of
740 allele frequencies), we would expect similar estimates of N_E at regional and global
741 scales. On the contrary, we observed at least for SNP data a global estimation of N_E
742 being the sum of regional estimates (Table 2). In line with the previous discussion on
743 the "local signature of population decline in Canada", the observed discrepancies in N_E
744 estimated at global and regional scales suggests that high connectivity among samples
745 sites does not necessarily imply a common demographic trajectory as inferred with
746 genetic information.

747 An effective worldwide population size of 1,000 individuals remains extremely
748 low in comparison to the number of individuals estimated for the species. This result
749 suggests that genetic drift can occur, decreasing the adaptive evolvability of the
750 species, which can lead to an increased risk of extinction.

751 The low N_E estimated for ivory gull may result in part from the effect of this
752 species' life cycle. As explained in the introduction, with constant adult survival,
753 generation overlap reduces N_E following $N_E = N / (1 + \nu)$. Yet this effect will be
754 partially compensated in ivory gulls because juveniles do not reproduce until they are
755 2 years old. Following Nunney (1993, equation 22), we find that delayed maturity will

756 increase N_E by a factor equal to $1 + (a - 1)(1 - \nu)$. Taking $a = 2$ and $\nu = 0.86$, we find
757 $N_E/N = (2 - \nu)/(1 + \nu) \approx 0.6$.

758 Moreover, this estimate assumes an even sex ratio, while the ivory gull
759 population is known to be strongly male-biased (67.8 % males, Yannic, Broquet, *et al.*,
760 2016). The origin of this bias is unknown, but its effect on N_E will be different if it
761 stems from uneven primary sex ratio or reduced male survival at the juvenile or adult
762 stage (Nunney, 1993). Details of sex-specific life history traits are required to precisely
763 estimate the effect of this bias in sex ratio, but it will only reduce further the effective
764 population size of the ivory gull population. Finally, all the above calculations disregard
765 variations in fecundity across age classes. Yet intermittent breeding (*i.e.* the non-
766 reproduction of individuals that have already reproduced) is regularly observed in
767 ivory gulls. In seabirds, age appears to be the key to skipped breeding, as this behavior
768 is usually observed in the youngest and in the oldest adult birds (Cubaynes *et al.*, 2011;
769 Goutte *et al.*, 2011) as an adaptive response of birds to the trade-off between survival
770 and future reproduction considering environmental constraints. Skipped reproduction
771 as a response to harsh conditions of breeding in the Arctic might in turn markedly
772 reduce N_E in ivory gull and further decrease the N_E/N ratio.

773 From estimation of effective population size, we deduced a mean N_E/N ratio
774 value of ~ 0.022 [0.019-0.026] considering a worldwide population of 38,000-52,000
775 mature individuals (BirdLife International, 2018) and N_E of 1,000. Estimations of census
776 populations size for ivory gull remain however relatively uncertain, and N_E/N ratio will
777 be around 0.12 [0.087-0.158], considering 6,325-11,500 breeding pairs (Gilchrist *et al.*,
778 2008), and N_E of 1,000. In the case of ivory gull, it is particularly low in comparison with

779 N_E/N ratio generally observed in birds (Frankham, 1995). The 100/1000 rule, formerly
780 50/500, postulates that at least $N_E = 100$ is required to avoid inbreeding depression,
781 and $N_E = 1,000$ is necessary to maintain evolutionary potential in population
782 (Frankham *et al.*, 2014). This rule is often used in conservation biology to evaluate the
783 risk of extinction of the concerned species (e.g., Jamieson and Allendorf, 2012). Based
784 on these estimates, the global ivory gull population does not appear to be at imminent
785 risk of inbreeding depression, but N_E close to 1,000 should be of concern for the long-
786 term adaptability of the species. This is particularly problematic for species living in a
787 rapidly changing environment. However, it would be necessary to make a correction in
788 estimation (e.g., regarding confidence in N , or life-cycle characteristics) in N_E , N and
789 N_E/N ratio. Some assumptions underlying the LD method we used to estimate N_E were
790 not fulfilled here. First, while the method assumes discrete generations, it can still be
791 applied to age-structured species provided that the number of cohorts represented in
792 the sampling is close to the generation time (Waples *et al.*, 2014). Here, we randomly
793 sampled adult birds on colonies, without any indication on their specific age or cohort,
794 and for some colonies, sampled over a few consecutive breeding seasons. However, a
795 random sample of adults consistently underestimates the true N_E (Waples *et al.*, 2014).
796 Thus, our estimates of N_E close to 1,000 may be a slight underestimation of the true N_E
797 (depending on species life-history traits; Waples *et al.*, 2014). Second, the LD method
798 assumes the population is at the mutation-drift equilibrium (Waples and Do, 2010).
799 This is, however, precisely the hypothesis we want to test in this study, when inferring
800 the signature of genetic bottlenecks based on an excess of heterozygosity.
801

802 **Conclusion**

803 The ivory gull presents no signature of recent genetic bottleneck across its circumpolar
804 breeding range, while the hypothesis of a local decline cannot be definitively ruled out.
805 Our results, indeed, contradict field observations showing that the number of ivory
806 gulls has been severely declining at least in Canada (70% decline between 1970-early
807 1980 and 2004-2006; Gilchrist *et al.*, 2008) and Svalbard (40% of decline between 2009
808 and 2019; Strøm *et al.*, 2020). Two factors may contribute to this discrepancy between
809 bottleneck genetic inferences and regional demographic trends obtained from field
810 surveys: 1) a lowered and delayed response of genetic signal due to life-history
811 features (generation time and overlapping generations), and 2) differences among
812 regions in demographic trends, with a potential strong impact of larger colonies (e.g.,
813 in Russia) to maintain worldwide genetic diversity. Uncertainties on local demography
814 are related to the difficulties to obtain larger sample sizes from such remote areas that
815 would allow more accurate demographic reconstruction. These difficulties could be
816 circumvented by the use of complete genome –now accessible for several bird species
817 (*i.e.*, the Bird 10,000 Genomes (B10K) Project)– and individual resequencing to
818 reconstruct past demographic history (e.g., PSMC; Li and Durbin, 2011), see for some
819 notable examples, on pinnipeds (Peart *et al.*, 2020) or birds (Nadachowska-Brzyska *et*
820 *al.*, 2015). In addition, comparative genomic analyses studying simultaneously several
821 Arctic seabird species, coupled with species distribution modelling, could be highly
822 informative. Finally, we found a strong effect of age structure on the detection of
823 genetic bottleneck with a decreased and delayed signal of decline of several years,
824 even for a strong decline (*i.e.*, 75% decrease). This effect should not be overlooked in

825 the search for signals of population decline, particularly for long-lived species, because
826 a species could experience an even strong demographic decline, without being
827 genetically detectable.
828

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1095

1096 **Figure legends**

1097

1098 **Figure 1.** Map of the study area illustrating the high-Arctic distribution of ivory gull

1099 (*Pagophila eburnea*) colonies. Sampling localities are indicated by the numbers given in

1100 Table 1. Small orange dots depict known breeding sites (Gilchrist *et al.*, 2008). Dashed lines

1101 show wintering grounds (variable during the winter and among years according to the

1102 extension of the sea ice). The background map represents the maximum sea ice extent in

1103 July between 1979 and 2013 (light blue) and the sea ice extent in July 2013 (dark blue) (data

1104 from the National Snow and Ice Data Centre, Boulder, Colorado; <http://nsidc.org/>).

1105

1106 **Figure 2.** Pairwise comparison of expected heterozygosity (H_E) versus expected

1107 heterozygosity at mutation-drift equilibrium (H_{Eq}) in the whole ivory gull population

1108 obtained with *Bottleneck* using 15 microsatellites and considering two mutational models:

1109 A) TPM70 and B) TPM95. The diagonal represents $H_E - H_{Eq}$, equality, *i.e.*, population at

1110 mutation-drift equilibrium. Above the diagonal the population experiences a heterozygosity

1111 excess, *i.e.*, a signature of demographic decline, below a heterozygosity deficit, signature of

1112 demographic expansion. Grey dots represent each locus ($n = 15$) and the black dot is the

1113 mean \pm se over loci.

1114

1115 **Figure 3.** Heterozygosity-excess (ΔH) at 15 microsatellite loci in four breeding regions of

1116 ivory gull, obtained with *Bottleneck* software for two mutational models (A) TPM70 and B)

1117 TPM95). The average ΔH over all loci is represented by a black dot, and locus-specific values

1118 are represented by smaller grey dots. The dashed line represents equality between

1119 observed and expected heterozygosity, *i.e.*, $\Delta H = H_E - H_{Eq} = 0$. Symbols (*) and (NS) indicate

1120 that the one tail probability of $H_E > H_{Eq}$, *i.e.*, heterozygosity-excess, is significant (p-value <
1121 0.05) or not significant, using Wilcoxon's test. The p-values obtained after correction for
1122 multiple tests are provided in Table S4.

1123

1124 **Figure 4.** Effects of sample size on A) the estimation of ΔH (mean +/- standard error) and B)
1125 one-tailed Wilcoxon test p -values obtained with 2 different mutation models (TPM70 and
1126 TPM95), estimated with the software *Bottleneck*. On panels A) and B), dashed lines
1127 correspond to $H_E = H_{Eq}$ and p -value = 0.05, respectively.

1128

1129 **Figure 5.** Likelihood profile of parameters $4N_E\mu$ and $4N_{E,past}\mu$ estimated by *Migrate* (note
1130 the log scale on both axes). The color bar represents the likelihood of parameter values. The
1131 estimates that have the highest likelihood suggest that the ivory gull population has been
1132 increasing.

1133

1134 **Figure 6.** Temporal dynamics of ΔH calculated from stochastic simulations of microsatellite
1135 markers evolving under IAM (panels A and C) or SMM (panels B and D) in a single population
1136 that went from 1000 to 250 individuals within a single generation at time 0. Top and bottom
1137 figures show the same data with time expressed either in years (A and B) or generations (C
1138 and D). The dark blue curve corresponds to a population without overlapping generations
1139 (adult survival $\nu = 0$, generation time $T = 1$ year), while the light blue and grey curves
1140 correspond to adult survival $\nu = 0.8$ ($T = 4.5$ years) and $\nu = 0.95$ ($T = 13.4$ years). Increased
1141 generation time logically resulted in a delayed signal of genetic bottleneck (panels A, B). In
1142 addition to that effect, in the IAM case, age structure and overlapping generations also

1143 resulted in a reduced bottleneck signal and additional delay not explained by changes in

1144 generation time (panel C).

1145

1146 **Table legends**

1147

1148 **Table 1.** Sampling of ivory gull. $N_{\mu\text{sat.}}$ and N_{SNP} give the number of sampled individuals used
1149 for microsatellites and SNPs analyses, respectively.

1150 **Table 2.** Estimates of effective size N_E and 95% confidence interval for regional and global
1151 populations using the linkage disequilibrium method, based on A) 15 microsatellites and B)
1152 3,490 SNPs, considering a maf of 0.02 and 0.05 for microsatellites and SNPs, respectively,
1153 and no singleton (No S*).

1154

1155

1156 **Table 1.** Sampling of ivory gull. $N_{\mu\text{sat}}$ and N_{SNP} give the number of sampled individuals used
 1157 for microsatellites and SNPs analyses, respectively.

| Breeding region | Nb pairs estimated | Location | Site | Abbr. | Status | $N_{\mu\text{sat}}$ | N_{SNP} |
|--------------------------|--------------------------|------------------|--------------------|---------|----------------|---------------------|------------------|
| Greenland | 2000-2500 ¹ | National Park | Station Nord | 1_StNo | Unknown | 81 | 11 |
| | | | | 2_StBr | Breeding | 25 | 17 |
| Norway | 1500-2000 ² | Svalbard | Svenskoya | 3_Sven | Breeding | 9 | 9 |
| | | | Auga | 4_Auga | Breeding | 18 | 18 |
| | | | Hübnerbreen | 5_Hubn | Breeding | 7 | - |
| | | | Freemanbreen | 6_Free | Breeding | 36 | 20 |
| Russia | 10500-15000 ³ | Franz Josef Land | Nagurskoje | 7_Nagu | Unknown | 5 | - |
| | | | Rudolf Island | 8_Rudo | Unknown | 17 | - |
| | | | Eva-Liv Island | 9_EvLi | Unknown | 5 | - |
| | | Severnaya Zemlya | Schmidt Island | 10_Schl | Unknown | 12 | - |
| | | | Domashny Island | 11_Doma | Unknown | 23 | - |
| | | | Komsomalets Island | 12_Koms | Unknown | 6 | - |
| | | Kara Sea Islands | Heiberg Islands | 13_Heil | Unknown | 4 | - |
| | | Canada | 400 ⁴ | Nunavut | Seymour Island | 14_Seyl | Breeding |
| Alert (Ellesmere Island) | 15_AIEI | | | | Unknown | 12 | 12 |
| Total | 14400-19900 | | | | | 271 | 87 |

1158
 1159 ¹ Boertmann *et al.*, (2020), ² Strøm *et al.*, (2020), ³ Gavrilov and Martynova (2017), ⁴ Environment Canada (2014).

1160

1161

1162 **Table 2.** Estimates of effective size N_E and 95% confidence interval for regional and global
 1163 populations using the linkage disequilibrium method, based on A) 15 microsatellites and B)
 1164 3,490 SNPs, considering a maf of 0.02 and 0.05 for microsatellites and SNPs, respectively,
 1165 and no singleton (No S*).

| A) Microsatellites | | | | | | | |
|---------------------------|----------|--------|---------|--------------|--------|---------|--------------|
| | 0.02 | | | | No S* | | |
| | <i>N</i> | N_E | 95% CIs | | N_E | 95% CIs | |
| Greenland | 106 | 815.2 | 423.2 | 6479.9 | 845.8 | 460.7 | 4119.6 |
| Norway | 70 | 3288.5 | 496.2 | Infinit e | 801.9 | 355.0 | Infinit e |
| Russia | 72 | 728.2 | 313.1 | Infinit e | 1128.0 | 398.0 | Infinit e |
| Canada | 23 | 250.6 | 91.0 | Infinit e | 102.7 | 51.5 | 946.5 |
| World | 271 | 1138.6 | 754.0 | 2204.3 | 1487.9 | 990.5 | 2871.4 |

| B) SNPs | | | | | | | |
|----------------|----------|-------|---------|-------|-------|---------|-------|
| | 0.05 | | | | No S* | | |
| | <i>N</i> | N_E | 95% CIs | | N_E | 95% CIs | |
| Greenland | 28 | 191.0 | 181.1 | 201.9 | 221.8 | 210.9 | 233.8 |
| Norway | 47 | 368.9 | 349.6 | 390.4 | 420.1 | 403.0 | 438.7 |
| Canada | 12 | 210.6 | 179.3 | 254.8 | 200.2 | 171.5 | 240.2 |
| World | 87 | 729.5 | 689.4 | 774.3 | 861.0 | 828.9 | 895.6 |

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1167

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1192 **COMPETING INTERESTS**

1193 The authors declare that they do not have any competing financial interests in relation to
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1195

1196 **ADDITIONAL INFORMATION**

1197 **Supplementary information** The online version contains supplementary material available
1198 at XXXXXX

1199

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1202 **Data availability**

1203 Genotypic data are deposited in DRYAD : <https://doi.org/10.5061/dryad.j0zpc86gk>

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