



**HAL**  
open science

## Searching for genetic evidence of demographic decline in an arctic seabird: beware of overlapping generations

Emeline Charbonnel, Claire Daguin-Thiébaud, Lucille Caradec, Eléonore Moittié, Olivier Gilg, Maria V. Gavrilov, Hallvard Strøm, Mark L. Mallory, R. I. Guy Morrison, H. Grant Gilchrist, et al.

### ► To cite this version:

Emeline Charbonnel, Claire Daguin-Thiébaud, Lucille Caradec, Eléonore Moittié, Olivier Gilg, et al.. Searching for genetic evidence of demographic decline in an arctic seabird: beware of overlapping generations. *Heredity*, 2022, 128 (5), pp.364-376. 10.1038/s41437-022-00515-3 . hal-03658284

**HAL Id: hal-03658284**

**<https://hal.science/hal-03658284v1>**

Submitted on 26 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1  
2  
3 **Searching for genetic evidence of demographic decline in an**  
4 **arctic seabird: beware of overlapping generations**

5  
6 Emeline Charbonnel<sup>1,4</sup>, Claire Daguin-Thiébaud<sup>2</sup>, Lucille Caradec<sup>2</sup>, Eléonore Moittié<sup>2</sup>,  
7 Olivier Gilg<sup>3,4</sup>, Maria V. Gavrilov<sup>5</sup>, Hallvard Strøm<sup>6</sup>, Mark L. Mallory<sup>7</sup>, R. I. Guy Morrison<sup>8</sup>,  
8 H. Grant Gilchrist<sup>8,9</sup>, Raphael Leblois<sup>10</sup>, Camille Roux<sup>11</sup>, Jonathan M. Yearsley<sup>12</sup>, Glenn  
9 Yannic<sup>1,4a\*</sup>, Thomas Broquet<sup>2a</sup>

10  
11  
12 <sup>1</sup> Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, 38000 Grenoble, France

13 <sup>2</sup> CNRS, Sorbonne Université, UMR 7144, Station Biologique de Roscoff, Place Georges  
14 Teissier, 29680 Roscoff, France

15 <sup>3</sup> Laboratoire Biogéosciences, UMR CNRS 6282, Equipe Ecologie Evolutive, Université  
16 de Bourgogne, Boulevard Gabriel, 21000 Dijon, France

17 <sup>4</sup> Groupe de Recherche en Ecologie Arctique (GREA), 21440 Francheville, France

18 <sup>5</sup> Arctic and Antarctic Research Institute (AARI), 198397 Saint-Petersburg, Russia

19 <sup>6</sup> Norwegian Polar Institute, Fram Centre, 9296 Tromsø, Norway

20 <sup>7</sup> Department of Biology, Acadia University, 33 Westwood Avenue, Wolfville, Nova  
21 Scotia B4P 2R6, Canada

22 <sup>8</sup> Environment Canada, National Wildlife Research Centre, Ottawa, Ontario, K1A0H3,  
23 Canada

24 <sup>9</sup> Department of Biology, Carleton University, Ottawa, Ontario, K1S5B6, Canada

25 <sup>10</sup> CBGP, INRAE, CIRAD, IRD, Institut Agro, Montpellier SupAgro, Univ. Montpellier,

26 Montferrier-sur-Lez Cedex, France

27 <sup>11</sup> Univ. Lille, CNRS, UMR 8198 - Evo-Eco-Paleo, F-59000 Lille, France

28 <sup>12</sup> School of Biology and Environmental Science, University College Dublin, Belfield,

29 Dublin, Ireland

30

31 <sup>a</sup> shared senior authorship

32 \* Corresponding author: Glenn Yannic, [glenn.yannic@univ-smb.fr](mailto:glenn.yannic@univ-smb.fr)

33 **ORCID number:**

34 Emeline Charbonnel : 0000-0002-8561-7610

35 Claire Daguin Thiébaud : 0000-0003-4665-6411

36 Olivier Gilg : 0000-0002-9083-4492

37 Maria Gavriilo : 0000-0002-3500-9617

38 Hallvard Strøm : 0000-0002-4823-0409

39 Mark Mallory : 0000-0003-2744-3437

40 Guy Morrison : 0000-0003-3964-2118

41 Grant Gilchrist : 0000-0001-5031-5092

42 Raphael Leblois : 0000-0002-3051-4497

43 Camille Roux : 0000-0001-9497-1446

44 Jon Yearsley : 0000-0003-1838-0454

45 Glenn Yannic : 0000-0002-6477-2312

46 Thomas Broquet : 0000-0002-2986-2822

47

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  $\nu$  (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  $0.86\pm 0.04$ , 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  $\Delta H$ . 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  $T_\mu$  (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  $\Delta H$  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  $\Delta H$  every 10  
389 years and we averaged these values over 100 simulation replicates. To produce as  
390 many  $\Delta H$  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  $\Delta H$  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  $\leq 0.02$  (*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  $\Delta H$  estimates and associated  $p$ -values are  
448 sensitive to sample size. Under TPM70,  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\nu$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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,  $p_{\text{GSM}}$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\Delta H$  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  $\sim 0.022$  [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

829 **References**

- 830 ACIA (2004). *Impacts of a Warming Arctic: Arctic Climate Impact Assessment*. Cambridge  
831 University Press, Cambridge, UK.
- 832 Alsos IG, Ehrich D, Thuiller W, Eidesen PB, Tribsch A, Schonswetter P, *et al.* (2012).  
833 Genetic consequences of climate change for northern plants. *Proceedings of the*  
834 *Royal Society B-Biological Sciences* **279**: 2042–2051.
- 835 Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA (2016). Harnessing the  
836 power of RADseq for ecological and evolutionary genomics. *Nature Reviews*  
837 *Genetics* **17**: 81–92.
- 838 Archer FI, Adams PE, Schneiders BB (2017). strataG: An R package for manipulating,  
839 summarizing and analysing population genetic data. *Molecular Ecology*  
840 *Resources* **17**: 5–11.
- 841 Arenas M, Ray N, Currat M, Excoffier L (2011). Consequences of range contractions and  
842 range shifts on molecular diversity. *Molecular Biology and Evolution* **29**: 207–  
843 218.
- 844 Beaumont MA (1999). Detecting population expansion and decline using microsatellites.  
845 *Genetics* **153**: 2013–2029.
- 846 Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and  
847 powerful approach to multiple testing. *Journal of the Royal Statistical Society,*  
848 *Series B*: 289–300.
- 849 BirdLife International (2018). *Pagophila eburnea*. The IUCN Red List of Threatened  
850 Species 2018: e.T22694473A132555020.  
851 <https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T22694473A132555020.en>.  
852 Downloaded on 11 June 2020.
- 853 Boertmann D, Petersen IK, Nielsen HH (2020). Ivory Gull population status in Greenland  
854 2019. *Dansk Orn Foren Tidsskr* **114**: 141–150.
- 855 Boitard S, Rodríguez W, Jay F, Mona S, Austerlitz F (2016). Inferring population size  
856 history from large samples of genome-wide molecular data - an approximate  
857 Bayesian computation approach. *PLOS Genetics* **12**: 1–36.
- 858 Box JE, Colgan WT, Christensen TR, Schmidt NM, Lund M, Parmentier F-JW, *et al.* (2019).  
859 Key indicators of Arctic climate change: 1971–2017. *Environmental Research*  
860 *Letters* **14**: 045010.
- 861 Braune BM, Mallory ML, Gilchrist HG (2006). Elevated mercury levels in a declining  
862 population of ivory gulls in the Canadian Arctic. *Marine Pollution Bulletin* **52**:  
863 978–982.
- 864 Broquet T, Angelone S, Jaquière J, Joly P, Léna JP, Lengagne T, *et al.* (2010). Genetic  
865 bottlenecks driven by population disconnection. *Conservation Biology* **24**: 1596–  
866 1605.
- 867 Canada E (2014). Recovery Strategy for the Ivory Gull (*Pagophila eburnea*) in Canada.



- 868 Species at Risk Act Recovery Strategy Series. *Recovery Planning, Environment*  
869 *Canada*.
- 870 Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011). Rapid range shifts of species  
871 associated with high levels of climate warming. *Science* **333**: 1024–1026.
- 872 Chikhi L, Sousa VC, Luisi P, Goossens B, Beaumont MA (2010). The confounding effects  
873 of population structure, genetic diversity and the sampling scheme on the  
874 detection and quantification of population size changes. *Genetics* **186**: 983–995.
- 875 Collevatti RG, Nabout JC, Diniz-Filho JAF (2011). Range shift and loss of genetic diversity  
876 under climate change in *Caryocar brasiliense*, a Neotropical tree species. *Tree*  
877 *Genetics & Genomes* **7**: 1237–1247.
- 878 Cornuet JM, Luikart G (1996). Description and power analysis of two tests for detecting  
879 recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–  
880 2014.
- 881 Cotto O, Schmid M, Guillaume F (2020). Nemo-age: spatially explicit simulations of eco-  
882 evolutionary dynamics in stage-structured populations under changing  
883 environments. *Methods in Ecology and Evolution* **11**: 1227–1236.
- 884 Cubaynes S, Doherty PF, Schreiber EA, Gimenez O (2011). To breed or not to breed: a  
885 seabird's response to extreme climatic events. *Biology Letters* **7**: 303–306.
- 886 Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994). Mutational  
887 processes of simple-sequence repeat loci in human populations. *Proceedings of*  
888 *the National Academy of Sciences of the United States of America* **91**: 3166–  
889 3170.
- 890 Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014). NeEstimator V2: re-  
891 implementation of software for the estimation of contemporary effective  
892 population size ( $N_e$ ) from genetic data. *Molecular Ecology Resources* **14**: 209–  
893 214.
- 894 Ellegren H (2004). Microsatellites: simple sequences with complex evolution. *Nature*  
895 *Reviews Genetics* **5**: 435–445.
- 896 England PR, Cornuet J-M, Berthier P, Tallmon DA, Luikart G (2006). Estimating effective  
897 population size from linkage disequilibrium: severe bias in small samples.  
898 *Conservation Genetics* **7**: 303.
- 899 Engler JO, Secondi J, Dawson DA, Elle O, Hochkirch A (2016). Range expansion and  
900 retraction along a moving contact zone has no effect on the genetic diversity of  
901 two passerine birds. *Ecography* **39**: 884–893.
- 902 Estoup A, Angers B (1998). Microsatellites and minisatellites for molecular ecology:  
903 theoretical and empirical considerations. *Advances in Molecular Ecology* **306**.
- 904 Ewens WJ (1972). The sampling theory of selectively neutral alleles. *Theoretical*  
905 *Population Biology* **3**: 87–112.
- 906 Felsenstein J (1971). Inbreeding and variance effective numbers in populations with  
907 overlapping generations. *Genetics* **168**: 581–597.

- 908 Fort J, Moe B, Strøm H, Grémillet D, Welcker J, Schultner J, *et al.* (2013). Multicolony  
909 tracking reveals potential threats to little auks wintering in the North Atlantic  
910 from marine pollution and shrinking sea ice cover. *Diversity and Distributions* **19**:  
911 1322–1332.
- 912 Fraïsse C, Popovic I, Mazoyer C, Spataro B, Delmotte S, Romiguier J, *et al.* (2021). DILS:  
913 Demographic inferences with linked selection by using ABC. *Molecular Ecology*  
914 *Resources* **in press**.
- 915 Frankham R (1995). Effective population size/adult population size ratios in wildlife: a  
916 review. *Genetical Research* **66**: 95–107.
- 917 Frankham R, Bradshaw CJA, Brook BW (2014). Genetics in conservation management:  
918 Revised recommendations for the 50/500 rules, Red List criteria and population  
919 viability analyses. *Biological Conservation* **170**: 56–63.
- 920 Garnier J, Lewis MA (2016). Expansion Under Climate Change: The Genetic  
921 Consequences. *Bulletin of Mathematical Biology* **78**: 2165–2185.
- 922 Garza JC, Williamson EG (2001). Detection of reduction in population size using data  
923 from microsatellite loci. *Molecular Ecology* **10**: 305–318.
- 924 Gavrilov M, Martynova D (2017). Conservation of rare species of marine flora and fauna  
925 of the Russian Arctic National Park, included in the Red Data Book of the Russian  
926 Federation and in the IUCN Red List. *Nature Conservation Research* **2**: 10–42.
- 927 Gienapp P, Teplitsky C, Alho JS, Mills JA, Merila J (2008). Climate change and evolution:  
928 disentangling environmental and genetic responses. *Molecular Ecology* **17**: 167–  
929 178.
- 930 Gilchrist HG, Mallory ML (2005). Declines in abundance and distribution of the ivory gull  
931 (*Pagophila eburnea*) in Arctic Canada. *Biological Conservation* **121**: 303–309.
- 932 Gilchrist HG, Strøm H, Gavrilov MV, Mosbech A (2008). *International ivory gull*  
933 *conservation strategy and action plan*. Conservation of Arctic Flora and Fauna  
934 International Secretariat, Circumpolar Seabird Group.
- 935 Gilg O, Boertmann D, Merkel F, Aebischer A, Sabard B (2009). Status of the endangered  
936 ivory gull, *Pagophila eburnea*, in Greenland. *Polar Biology* **32**: 1275–1286.
- 937 Gilg O, Istomina L, Heygster G, Strøm H, Gavrilov M, Mallory ML, *et al.* (2016). Living on  
938 the edge of a shrinking habitat: the ivory gull, *Pagophila eburnea*, an endangered  
939 sea-ice specialist. *Biology Letters* **12**: 20160277.
- 940 Gilg O, Kovacs KM, Aars J, Fort J, Gauthier G, Grémillet D, *et al.* (2012). Climate change  
941 and the ecology and evolution of Arctic vertebrates. *Annals of the New York*  
942 *Academy of Sciences* **1249**: 166–190.
- 943 Goutte A, Kriloff M, Weimerskirch H, Chastel O (2011). Why do some adult birds skip  
944 breeding? A hormonal investigation in a long-lived bird. *Biology Letters* **7**: 790–  
945 792.
- 946 Guillaume F, Rougemont J (2006). Nemo: an evolutionary and population genetics  
947 programming framework. *Bioinformatics* **22**: 2556–2557.

- 948 Hill WG (1972). Effective size of populations with overlapping generations. *Theoretical*  
949 *Population Biology* **3**: 278–289.
- 950 Hoban SM, Mezzavilla M, Gaggiotti OE, Benazzo A, van Oosterhout C, Bertorelle G  
951 (2013). High variance in reproductive success generates a false signature of a  
952 genetic bottleneck in populations of constant size: a simulation study. *BMC*  
953 *Bioinformatics* **14**: 309.
- 954 Hoffmann AA, Sgrò CM (2011). Climate change and evolutionary adaptation. *Nature*  
955 **470**: 479–485.
- 956 Hohenlohe PA, Funk WC, Rajora OP (2021). Population genomics for wildlife  
957 conservation and management. *Molecular Ecology* **30**: 62–82.
- 958 Jamieson IG, Allendorf FW (2012). How does the 50/500 rule apply to MVPs? *Trends in*  
959 *Ecology & Evolution* **27**: 578–584.
- 960 Kimura M, Ohta T (1978). Stepwise mutation model and distribution of allelic  
961 frequencies in a finite population. *Proc Natl Acad Sci U S A* **75**: 2868–2872.
- 962 Laporte V, Charlesworth B (2002). Effective population size and population subdivision  
963 in demographically structured populations. *Genetics* **162**: 501–519.
- 964 Leblois R, Pudlo P, Néron J, Bertaux F, Reddy Beeravolu C, Vitalis R, *et al.* (2014).  
965 Maximum-Likelihood Inference of Population Size Contractions from  
966 Microsatellite Data. *Molecular Biology and Evolution* **31**: 2805–2823.
- 967 Li H, Durbin R (2011). Inference of human population history from individual whole-  
968 genome sequences. *Nature* **475**: 493–496.
- 969 Liu X, Fu Y-X (2015). Exploring population size changes using SNP frequency spectra.  
970 *Nature Genetics* **47**: 555–559.
- 971 Lucia M, Verboven N, Strom H, Miljeteig C, Gavrilo MV, Braune BM, *et al.* (2015).  
972 Circumpolar contamination in eggs of the high-arctic ivory gull *Pagophila*  
973 *eburnea*. *Environmental Toxicology and Chemistry* **34**: 1552–1561.
- 974 Luikart G, Cornuet J-M (1998). Empirical evaluation of a test for identifying recently  
975 bottlenecked populations from allele frequency data. *Conservation Biology* **12**:  
976 228–237.
- 977 Mallory ML, Allard KA, Braune BM, Gilchrist HG, Thomas VG (2012). New longevity  
978 record for ivory gulls (*Pagophila eburnea*) and evidence of natal philopatry. *Arctic*  
979 **65**: 98–101.
- 980 Marandel F, Charrier G, Lamy J-B, Le Cam S, Lorange P, Trenkel VM (2020). Estimating  
981 effective population size using RADseq: Effects of SNP selection and sample size.  
982 *Ecology and Evolution* **10**: 1929–1937.
- 983 McInerny GJ, Turner JRG, Wong HY, Travis JMJ, Benton TG (2009). How range shifts  
984 induced by climate change affect neutral evolution. *Proceedings of the Royal*  
985 *Society B: Biological Sciences* **276**: 1527–1534.
- 986 McRae L, Deinet S, Gill M, Collen B (2012). Arctic species trend index: tracking trends in

- 987 Arctic marine populations. CAFF Assessment Series No. 7. Conservation of Arctic  
988 Flora and Fauna, Iceland.
- 989 Meredith M, Sommerkorn M, Cassotta S, Derksen C, Ekaykin A, Hollowed A, *et al.*  
990 (2020). Polar Regions. In: IPCC Special Report on the Ocean and Cryosphere in a  
991 Changing Climate [H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M.  
992 Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold,  
993 B. Rama, N.M. Weyer (eds.)].
- 994 Miljeteig C, Strom H, Gavriilo MV, Volkov A, Jenssen BM, Gabrielsen GW (2009). High  
995 levels of contaminants in ivory gull *Pagophila eburnea* eggs from the Russian and  
996 Norwegian Arctic. *Environmental Science & Technology* **43**: 5521–5528.
- 997 Miller MP, Haig SM, Mullins TD, Popper KJ, Green M (2012). Evidence for population  
998 bottlenecks and subtle genetic structure in the yellow rail. *The Condor* **114**: 100–  
999 112.
- 1000 Nadachowska-Brzyska K, Li C, Smeds L, Zhang G, Ellegren H (2015). Temporal dynamics  
1001 of avian populations during Pleistocene revealed by whole-genome sequences.  
1002 *Current Biology* **25**: 1375–1380.
- 1003 Nunney L (1993). The influence of mating system and overlapping generations on  
1004 effective population size. *Evolution* **47**: 1329–1341.
- 1005 Nunney L, Elam DR (1994). Estimating the Effective Population Size of Conserved  
1006 Populations. *Conservation Biology* **8**: 175–184.
- 1007 Nunziata SO, Weisrock DW (2018). Estimation of contemporary effective population size  
1008 and population declines using RAD sequence data. *Heredity* **120**: 196–207.
- 1009 Nyström V, Angerbjörn A, Dalén L (2006). Genetic consequences of a demographic  
1010 bottleneck in the Scandinavian arctic fox. *Oikos* **114**: 84–94.
- 1011 Orive ME (1993). Effective population size in organisms with complex life-histories.  
1012 *Theor Popul Biol* **44**: 316–340.
- 1013 Parmesan C (2006). Ecological and evolutionary responses to recent climate change.  
1014 *Annual Review of Ecology, Evolution, and Systematics* **37**: 637–669.
- 1015 Parreira BR, Chikhi L (2015). On some genetic consequences of social structure, mating  
1016 systems, dispersal, and sampling. *Proc Natl Acad Sci USA* **112**: E3318.
- 1017 Parreira B, Quéméré E, Vanpé C, Carvalho I, Chikhi L (2020). Genetic consequences of  
1018 social structure in the golden-crowned sifaka. *Heredity* **125**: 328–339.
- 1019 Peart CR, Tusso S, Pophaly SD, Botero-Castro F, Wu C-C, Auriolles-Gamboa D, *et al.*  
1020 (2020). Determinants of genetic variation across eco-evolutionary scales in  
1021 pinnipeds. *Nature Ecology & Evolution* **4**: 1095–1104.
- 1022 Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Béer E, Robinson S, *et al.* (2012).  
1023 Reliability of genetic bottleneck tests for detecting recent population declines.  
1024 *Molecular Ecology* **21**: 3403–3418.
- 1025 Piry S, Luikart G, Cornuet J-M (1999). Computer note. BOTTLENECK: a computer program

- 1026 for detecting recent reductions in the effective size using allele frequency data.  
1027 *Journal of Heredity* **90**: 502–503.
- 1028 Raymond M, Rousset F (1995). GENEPOP (version 1.2): population genetics software for  
1029 exact tests and ecumenicism. *Journal of Heredity* **86**: 248–249.
- 1030 Rousset F (1999). Genetic Differentiation in Populations with Different Classes of  
1031 Individuals. *Theoretical Population Biology* **55**: 297–308.
- 1032 Rousset F (2008). Genepop'007: a complete re-implementation of the Genepop  
1033 software for windows and linux. *Molecular Ecology Notes* **8**: 103–1006.
- 1034 Rousset F, Beeravolu CR, Leblois R (2018). Likelihood computation and inference of  
1035 demographic and mutational parameters from population genetic data under  
1036 coalescent approximations. *Journal de la Société Française de Statistique* **159**:  
1037 142–166.
- 1038 Rubidge EM, Patton JL, Lim M, Burton AC, Brashares JS, Moritz C (2012). Climate-  
1039 induced range contraction drives genetic erosion in an alpine mammal. *Nature*  
1040 *Climate Change* **2**: 285–288.
- 1041 Shafer ABA, Gattepaille LM, Stewart REA, Wolf JBW (2015). Demographic inferences  
1042 using short-read genomic data in an approximate Bayesian computation  
1043 framework: in silico evaluation of power, biases and proof of concept in Atlantic  
1044 walrus. *Molecular Ecology* **24**: 328–345.
- 1045 Spencer NC, Gilchrist HGrant, Mallory ML (2014). Annual movement patterns of  
1046 endangered ivory gulls: the importance of sea ice. *Plos One* **9**: e115231.
- 1047 Stenhouse IJ, Robertson GJ, Gilchrist HG (2004). Recoveries and survival rates of ivory  
1048 gulls (*Pagophila eburnea*) banded in Nunavut, Canada, 1971–1999. *Waterbirds*  
1049 **27**: 486–492.
- 1050 Storz J, Ramakrishnan U, Alberts S (2002). Genetic effective size of a wild primate  
1051 population: influence of current and historical demography. *Evolution* **56**: 817–  
1052 29.
- 1053 Strøm H, Bakken V, Skoglund, Descamps S, Fjeldheim VB, Steen H (2020). Population  
1054 status and trend of the threatened ivory gull *Pagophila eburnea* in Svalbard.  
1055 *Endangered Species Research* **43**: 435–445.
- 1056 Volkov AE, de Korte J (2000). Breeding ecology of the Ivory Gull (*Pagophila eburnea*) in  
1057 Sedov Archipelago, Severnaya Zemlya. Heritage of the Russian Arctic. Research,  
1058 conservation and international cooperation. *Moscow: Ecopros Publishers*: 483–  
1059 500.
- 1060 Waples RS (2016). Life-history traits and effective population size in species with  
1061 overlapping generations revisited: the importance of adult mortality. *Heredity*  
1062 **117**: 241–250.
- 1063 Waples RS, Antao T, Luikart G (2014). Effects of overlapping generations on linkage  
1064 disequilibrium estimates of effective population size. *Genetics* **197**: 769–780.
- 1065 Waples RS, Do C (2010). Linkage disequilibrium estimates of contemporary Ne using

1066 highly variable genetic markers: a largely untapped resource for applied  
1067 conservation and evolution. *Evolutionary Applications* **3**: 244–262.

1068 Waples RS, Luikart G, Faulkner JR, Tallmon DA (2013). Simple life-history traits explain  
1069 key effective population size ratios across diverse taxa. *Proceedings of the Royal  
1070 Society B: Biological Sciences* **280**: 20131339.

1071 Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population  
1072 structure. *Evolution* **38**: 1358–1370.

1073 Williamson-Natesan EG (2005). Comparison of methods for detecting bottlenecks from  
1074 microsatellite loci. *Conservation Genetics* **6**: 551–562.

1075 Wogan GOU, Voelker G, Oatley G, Bowie RCK (2020). Biome stability predicts population  
1076 structure of a southern African aridland bird species. *Ecology and evolution* **10**:  
1077 4066–4081.

1078 Xenikoudakis G, Ersmark E, Tison J-L, Waits L, Kindberg J, Swenson JE, *et al.* (2015).  
1079 Consequences of a demographic bottleneck on genetic structure and variation in  
1080 the Scandinavian brown bear. *Molecular Ecology* **24**: 3441–3454.

1081 Yannic G, Broquet T, Strøm H, Aebischer A, Dufresnes C, Gavrilo MV, *et al.* (2016).  
1082 Genetic and morphological sex identification methods reveal a male-biased sex  
1083 ratio in the Ivory Gull *Pagophila eburnea*. *Journal of Ornithology* **157**: 861–873.

1084 Yannic G, Sermier R, Aebischer A, Gavrilo MV, Gilg O, Miljeteig C, *et al.* (2011).  
1085 Description of microsatellite markers and genotyping performances using  
1086 feathers and buccal swabs for the ivory gull (*Pagophila eburnea*). *Molecular  
1087 Ecology Resources* **11**: 877–889.

1088 Yannic G, Yearsley J, Sermier R, Dufresnes C, Gilg O, Aebischer A, *et al.* (2016). High  
1089 connectivity in a long-lived high-Arctic seabird, the ivory gull *Pagophila eburnea*.  
1090 *Polar Biology* **39**: 221–236.

1091 Yurkowski DJ, Auger-Méthé M, Mallory ML, Wong SNP, Gilchrist G, Derocher AE, *et al.*  
1092 (2019). Abundance and species diversity hotspots of tracked marine predators  
1093 across the North American Arctic. *Diversity and Distributions* **25**: 328–345.

1094

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 +/- se over loci.

1114

1115 **Figure 3.** Heterozygosity-excess ( $\Delta H$ ) 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  $\Delta H$  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  $\Delta H$  (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  $\Delta H$  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  $N_{\text{SNP}}$  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  $N_{\text{SNP}}$  give the number of sampled individuals used  
 1157 for microsatellites and SNPs analyses, respectively.

Breeding region	Nb pairs estimated	Location	Site	Abbr.	Status	$N_{\mu\text{sat}}$	$N_{\text{SNP}}$
Greenland	2000-2500 <sup>1</sup>	National Park	Station Nord	1_StNo	Unknown	81	11
				2_StBr	Breeding	25	17
Norway	1500-2000 <sup>2</sup>	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 <sup>3</sup>	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 <sup>4</sup>	Nunavut	Seymour Island	14_Seyl	Breeding
Alert (Ellesmere Island)	15_AIEI				Unknown	12	12
<b>Total</b>	14400-19900					<b>271</b>	<b>87</b>

1158  
 1159 <sup>1</sup> Boertmann *et al.*, (2020), <sup>2</sup> Strøm *et al.*, (2020), <sup>3</sup> Gavrilov and Martynova (2017), <sup>4</sup> 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\*).

<b>A) Microsatellites</b>							
	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	Infinite	801.9	355.0	Infinite
Russia	72	728.2	313.1	Infinite	1128.0	398.0	Infinite
Canada	23	250.6	91.0	Infinite	102.7	51.5	946.5
World	271	1138.6	754.0	2204.3	1487.9	990.5	2871.4

  

<b>B) SNPs</b>							
	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

1166

1167

1168 **Acknowledgments**

1169 We thank Adrian Aebischer, Christophe Dufresnes, Emmanuelle Pouivé, Roberto Sermier,  
1170 and Brigitte Sabard for lab and field assistance. We wish to thank the two reviewers and the  
1171 subject editor for their constructive and challenging comments that improved the quality of  
1172 the manuscript. This work was supported by grants from the foundation Ellis Elliot  
1173 (Switzerland), Société vaudoise des Sciences naturelles (Switzerland) and Nos Oiseaux  
1174 (Switzerland) to G.Y., by a foundation Agassiz (Switzerland) grant to T.B. and by Nicolas  
1175 Perrin's research group, Department of Ecology and Evolution at University of Lausanne,  
1176 Switzerland. This work benefited from access to the Biogenouest genomic platform at  
1177 Station Biologique de Roscoff and we are grateful to the Roscoff Bioinformatics platform  
1178 ABiMS (<http://abims.sb-roscoff.fr>), the national INRA MIGALE (<http://migale.jouy.inra.fr>)  
1179 and GENOTOUL (Toulouse Midi-Pyrénées) bioinformatics HPC platforms, as well as the CBGP  
1180 and the local Montpellier Bioinformatics Biodiversity (MBB, supported by the LabEx CeMEB  
1181 ANR-10-LABX-04-01) HPC platform services for providing storage and computing resources.  
1182 RL was supported by the Agence Nationale de la Recherche (projects GENOSPACE ANR-16-  
1183 CE02-0008 and INTROSPEC ANR-19-CE02-0011). The sampling in Canada was funded by the  
1184 Department of Environment and Climate Change Canada. The sampling in Greenland was  
1185 supported by the Groupe de Recherche in Ecologie Arctique (GREA) and funded by the  
1186 French Polar Institute-IPEV (Program 'Ivory 1210'). The sampling in Svalbard was funded by  
1187 the Norwegian Polar Institute and the Norwegian seabird monitoring program SEAPOP  
1188 ([www.seapop.no](http://www.seapop.no), grant number 192141). The sampling in Russia was part of the work plan  
1189 of the Joint Norwegian-Russian Commission on Environmental Protection and funded by the  
1190 Norwegian Ministry of Environment, Arctic and Antarctic Research Institute and the Russian  
1191 IPY 2007/08 program.

1192 **COMPETING INTERESTS**

1193 The authors declare that they do not have any competing financial interests in relation to  
1194 the work described.

1195

1196 **ADDITIONAL INFORMATION**

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

1199

1200 **Correspondence** should be addressed to Glenn Yannic

1201

1202 **Data availability**

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

1204

1205

1206