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Genomic diversity and differentiation between island and mainland populations of white-tailed eagles (*Haliaeetus albicilla*)

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Abstract

Divergence in the face of high dispersal capabilities is a documented but poorly understood phenomenon. The white-tailed eagle (*Haliaeetus albicilla*) has a large geographic dispersal capability and should theoretically be able to maintain genetic homogeneity across its dispersal range. However, following analysis of the genomic variation of white-tailed eagles, from both historical and contemporary samples, clear signatures of ancient biogeographic substructure across Europe and the North-East Atlantic is observed. The greatest genomic differentiation was observed between island (Greenland and Iceland) and mainland (Denmark, Norway and Estonia) populations. The two island populations share a common ancestry from a single mainland population, distinct from the other sampled mainland populations, and despite the potential for high connectivity between Iceland and Greenland they are well separated

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from each other and are characterized by inbreeding and little variation. Temporal differences also highlight a pattern of regional populations persisting despite the potential for admixture. All sampled populations generally showed a decline in effective population size over time, which may have been shaped by four historical events: (1) Isolation of refugia during the last glacial period 110–115,000 years ago, (2) population divergence following the colonization of the deglaciated areas ~10,000 years ago, (3) human population expansion, which led to the settlement in Iceland ~1100 years ago, and (4) human persecution and exposure to toxic pollutants during the last two centuries.

KEYWORDS
conservation genetics, dispersal, inbreeding, phylogeography, population size, temporal changes

1 | INTRODUCTION

Genetic signatures of connectivity between island and mainland populations have been the subject of numerous studies in evolutionary and conservation biology. In fact, foundational evolutionary models of the new modern synthesis were formulated specifically considering islands and island-mainland scenarios (Wright, 1931, 1932). Due to geographical isolation and reduced migration, island populations can often evolve independently from other island and mainland populations, and are thus often characterized by unique traits, or even recent speciation events (e.g., Amouret et al., 2016; Gross, 2006). However, island populations may suffer from low genetic variation, and thus be more prone to inbreeding depression or extinction, both due to "founder effect" bottlenecks during colonization and as a result of increased genetic drift in small isolated populations (e.g., Frankham, 1995; James et al., 2016). The reduced genetic variation may result in less evolvability, and the populations may not be able to adapt to local conditions (Sgrò et al., 2011).

Variation within both island and mainland species at high latitudes have been shaped by Ice Age glacial periods, with the last glacial maximum 20–25 thousand years ago seeing glacial sheets covering large region of northern Europe and the islands in the North Atlantic, such as Iceland and Greenland (Geirsdóttir et al., 2007). Genetic variation in many terrestrial species in this region reflect these historical changes, where populations diverged in allopatry at southern refugia during the glacial periods and after expansion following the retreat of glaciations. As a result, there may be little variation within population but sharp boundaries at secondary contact zones (e.g., Hewitt, 2001), and even subspecies which are frequent at high latitudes (Botero et al., 2014). This is true for several species of birds despite high dispersal capacity and migratory behaviour. However, birds often exhibit strong philopatry that greatly shapes realized dispersal (Greenwood & Harvey, 1982). For example, several subspecies of birds, characterized by morphological differences are restricted solely to Iceland or to the island and its immediate neighbouring countries (Petersen, 1998). Whether observed differentiation has resulted from historical changes prior to island colonization (e.g., allopatric divergence in glacial refugia followed by dispersal), or whether selection to new island conditions have been the key driving force is unclear, but insights can be gained by analysis of contemporary and historical genomic variation.

The white-tailed eagle (*Haliaeetus albicilla*, Linnaeus, 1758) is a large and long-lived raptor with an average lifespan of 17–25 years (del Hoyo et al., 1992; Hailer et al., 2006). The species range spans central and northern Asia, as well as most of Europe, extending out to the islands of Iceland and southwestern Greenland. It is considered to be a habitat generalist with a high dispersal potential (c.f. Hailer et al., 2007) and being an apex raptor it is an important indicator of environmental health (Badry et al., 2022). Habitat destruction and the expansion of humans into their territories during the last millennia (Kremer, 1993) may have restricted the population sizes of eagles via, for example, settlement of coastal sites and islands in the North Atlantic (Batt et al., 2015; Jackson et al., 2018). During the 19th and 20th centuries the white-tailed eagle experienced severe population declines and became locally extinct in several countries in western Europe, including Denmark and the British Isles (Ehmsen et al., 2011; Langguth et al., 2013; Love & Ball, 1979; Treinys et al., 2016). These population declines resulted primarily from human persecution (Bijleveld, 1974; Love & Ball, 1979) and the toxic effects of organochlorines and neurotoxins during the 20th century (Helander et al., 1982, 2002; Skarphéðinsson, 2003). Some of these negative effects have been mitigated by conservation programmes introduced in the late 20th century to help reintroduce the species to its former areas, for example as implemented in the UK (e.g., Royal Society for the Protection of Birds, 2023), and by the reduction of harmful substances in the environment such as persistent organic pollutants (POPs) (EPA, 2001). A decline in human densities in more rural areas, i.e., urbanization, in the late twentieth century may also have favoured the re-establishment of eagles in now less impacted coastal territories. Currently the species is categorized as a least-concern species by the International Union for Conservation of Nature (IUCN), and its census population size has been reported to

be growing (Birdlife International, 2020), although still endangered in some countries.

Despite recent increases in census counts, the reduction in genetic variation and population size may have reduced the potential of white-tailed eagles to adapt to environmental changes, and decreased the efficacy of selection to purge deleterious mutations from the collective gene pool (e.g., Hoban et al., 2020). Recent studies have shown that genetic diversity has been decreasing globally since the industrial revolution (Leigh et al., 2019), and concerns have been raised that this has been neglected in management and conservation policy (Hoban et al., 2021). Prior studies on genetic variation in mitochondria and microsatellites have not assessed temporal variation in white-tailed eagles. Assessment of spatial variation among the white-tailed eagle populations in northern Europe revealed no obvious signs of bottlenecks, possibly as the span of individual population bottleneck events has been short in comparison to the bird's lifespan (Hailer et al., 2006). Similarly, mitogenomic variation in the white-tailed eagle samples studied here, revealed two distinct lineages within countries, despite small population sizes and low haplotypic diversity (Hansen et al., 2022). However, a full genome analysis of a single white-tailed eagle individual from Greenland suggested that a severe reduction in N_e coincided with the beginning of the last glacial period, around 110 thousand years (kyr) ago, and that the population size was small and stable from last glacial maximum (c. 25–30 kyr) to end of the last glacial period (10 kyr ago) (Nadachowska-Brzyska et al., 2015).

Previous demographic studies based on the mitochondrial control region of the white-tailed eagle (Hailer et al., 2007; Honnen et al., 2010; Langguth et al., 2013), as well as the entire mitogenome (Hansen et al., 2022), have revealed two major genetic clusters within the species range, one in western Europe and the other in eastern Europe and Asia, which were shaped by refugia during the last glacial period of the present Ice Age. The mtDNA study by Hailer et al. (2007) reported minor variation in the populations in Greenland and Iceland and suggested a shared recent matrilineal origin with populations in north-western Europe, with high diversity in Estonia. A more recent analysis of the mitogenome data set revealed a more complex pattern, with polyphyletic lineages in Iceland, Greenland, and Norway, and where the recently established population in Denmark showed signs of admixture between the two main clusters (Hansen et al., 2022).

The white-tailed eagle has strong intergenerational site fidelity, and there are no reports of migrants between Greenland, Iceland, and mainland Europe (Birdlife International, 2020; Lyngs, 2003). The population in Greenland is classified as a subspecies, (*H. albicilla groenlandicus* Brehm, CL, 1831) due to its comparatively larger body size (Salomonsen, 1979). The number of breeding pairs in Greenland has increased in recent decades, from less than 75 pairs (Hansen, 1979) to around 200 pairs today (Boertmann & Bay, 2018). Similarly, the population in Iceland plummeted to around 20 pairs before conservation efforts were enacted in 1914, but did not increase in numbers until a ban on fox poisoning was introduced in 1964 although at a slow rate (Petersen, 1998; Skarphéðinsson, 2013). In a 2022 study of 92 territorial pairs only 38 chicks fledged (Náttúrufræðistofnun Íslands, 2022). The Norwegian population, presently the largest in Europe, consists of around 2000 breeding pairs (Jais, 2020). As in several other countries in Europe, the Danish population became extinct at the beginning of the 20th century but was re-established in 1995 from expanding neighbouring areas, and by 2020 it numbered 133 breeding pairs (Skelmose & Larsen, 2021). In Estonia, prior to the 19th century there was a large population consisting of c. 400–500 breeding pairs (Lõhmus, 1998), but by the end of the 19th century, it had declined to only 20 pairs (Randla & Õun, 1980). Today however, it has recovered to an estimated 290–330 pairs (Elts et al., 2019).

In this study, whole genomes from historical (up to 130 years old) and contemporary samples from the two isolated island populations in Greenland and Iceland are studied and compared with samples from the mainland populations in Norway and Denmark. Three contemporary samples from Estonia, and a single historical specimen from Turkey are also included. We specifically evaluate the impact of population size and bottlenecks on the Iceland and Greenland populations in comparison with the large mainland population in Norway and the recently established population in Denmark as well as the historical samples. The historical samples coincide with the onset of a reported reduction in population size (~100–200 years ago). Our key aims are to examine population structure across the sampled region, as well as to evaluate the differences between the island and mainland populations with respect to diversity, inbreeding, and historical demography of these populations back to the last interglacial period.

2 | MATERIALS AND METHODS

Tissue was obtained from 92 specimens, of which 63 were contemporary samples (42 blood, 12 toepads and 9 skin/muscle tissue samples), and 29 were historical toepad samples from the north Atlantic islands, Greenland ($N = 20$) and Iceland ($N = 27$), as well as from two sites in mainland Europe (Denmark, $N = 16$ and Norway, $N = 25$) (Table 1, Table S1, and Figure 1). Furthermore, three contemporary samples from Estonia and one historical sample from Turkey were included for reference. The historical specimens were originally sampled between 1885 and 1937, except for two Icelandic specimens that were from 1950, while all contemporary individuals were sampled post-1990 (full individual sampling information presented in Table S1). A description of specimen origin, handling, as well as DNA extraction and sequencing can be found in the Supporting Information.

The base quality in all raw sequence data was checked using FastQC (Babraham Bioinformatics, 2010). Adapters were removed using AdapterRemoval version 2 with standard settings, providing adapter sequences for samples and the arguments --collapse and –trimns (Schubert et al., 2016). The trimmed sequences were mapped to the confamilial golden eagle (*Aquila chrysaetos*) genome (GCA_900496995.3) using bwa aln, samse, and sampe, with the flags -q 15 and -k 1 (Li & Durbin, 2009). The golden eagle was deliberately chosen as the reference to minimize the potential of mapping biases (Gopalakrishnan et al., 2017) and because its assembly

TABLE 1 Molecular diversity per country and overall, for contemporary (C) and historical (H) samples.

Country	Time	*N*	H_E	H_O	F_{IS}	F_H	F_{ROH}	Mean IBS-distance
Greenland	C	12	0.1537 (0.191)	0.1649 (0.220)	−0.0729	0.362 (0.027)	0.349 (0.016)	0.124 (0.004)
	H	8	0.1407 (0.190)	0.1609 (0.245)	−0.1436	0.386 (0.016)	0.459 (0.226)	0.116 (0.007)
Iceland	C	25	0.1351 (0.185)	0.1407 (0.202)	−0.0415	0.456 (0.049)	0.435 (0.050)	0.108 (0.008)
	H	2	0.0789 (0.179)	0.1482 (0.346)	−0.8783	0.410 (0.064)	NA[a]	0.094 (NA)
Norway	C	12	0.3014 (0.156)	0.317 (0.201)	−0.0518	−0.22 (0.042)	0.081 (0.225)	0.256 (0.014)
	H	13	0.2855 (0.169)	0.3072 (0.234)	−0.0760	−0.21 (0.047)	0.066 (0.406)	0.239 (0.050)
Denmark	C	11	0.2718 (0.172)	0.281 (0.211)	−0.0339	−0.089 (0.089)	0.145 (0.065)	0.232 (0.032)
	H	5	0.2406 (0.205)	0.3091 (0.329)	−0.2847	−0.23 (0.14)	0.090 (0.418)	0.238 (0.017)
Estonia	C	3	0.2539 (0.196)	0.3052 (0.297)	−0.2021	−0.19 (0.033)	0.070 (0.018)	0.249 (0.0039)
Turkey	H	1	0.1458 (0.227)	0.2916 (0.455)	NA	−0.14	69	NA
Overall	–	92	0.2501 (0.145)	0.2112 (0.136)	0.1555	0.13 (0.31)	0.196 (0.262)	0.230 (0.058)

Note: Sample size (*N*). Expected and observed heterozygosity (H_E and H_O, respectively) calculated per SNP per population, which were used to calculate the inbreeding coefficient (F_{IS}). The inbreeding coefficient based on overall proportions of heterozygous sites (F_H) and mean IBS-distance between individuals are also reported. Standard deviations are displayed in parentheses.

[a]F_{ROH} for Historical Iceland were not included since those samples had >50% missing SNP data.

is more complete than that of the white-tailed eagle. The available white-tailed eagle genomes originate from Greenland, UK, and Germany, and thus are not equally related to all populations studied here and might introduce errors in the analyses, that is, reference bias (Gopalakrishnan et al., 2017, 2022). The golden eagle genome has also been assembled to chromosome-level completeness and annotated, thus enabling us both to identify and exclude sex chromosomes in some downstream analyses and to identify the genes present in regions under selection. Picard Tools (Broad Institute, 2020) was used to remove duplicate reads. To account for probably damaged bases, the base quality score was rescaled with mapDamage 2.0 (Jónsson et al., 2013). Genotypes were called using GraphTyper2 (Eggertsson et al., 2019) with standard settings. The VCF file for the 92 individuals was filtered using VCFtools, BCFtools, and VCF-annotate: SNPs had to have a minor allele count of one, quality of 1000, genotype quality 20, mapping quality 30, max. missingness of 25%, and an allelic heterozygosity balance between 0.2 and 0.8. Individuals had to have a sequencing depth of eight for a particular SNP to have it called. Only the known autosomes 1–26 (LR606181.1-LR606206.1) from the golden eagle genome were analysed in this study (these make up 84.43% of the full published genome). Linkage filtration was accomplished with PLINK 1.9 (Purcell et al., 2007).

The *Ts/Tv* ratio was examined using VCFtools TsTv-summary (Danecek et al., 2011) to evaluate whether a bias is observed in the historical samples and whether the transitions should be excluded. MapDamage 2.0 (Jónsson et al., 2013) was used to investigate nucleotide misincorporation (polymerase incorporation of nonendogenous nucleotides in a DNA sequence) between the historical and contemporary samples, as historical and ancient samples are expected to have G-to-A and C-to-T substitutions at the 3′- and 5′end due to post-mortem DNA damage (fragmentation and base modification) (Jónsson et al., 2013).

As nest origin was known for the contemporary Icelandic specimens, all specimens selected originated from different nests. To estimate relatedness, KING (Manichaikul et al., 2010) was run with the settings *--unrelated* and *--degree 3*. No pair of individuals were found to be related at third degree or higher, and thus all 92 individuals were kept.

Diversity within each sample was evaluated based on the observed and expected heterozygosity calculated on a per-individual basis using VCFtools *--het*, and per-SNP per-population using VCFtools *--hardy* (Danecek et al., 2011). Euclidean distances between individuals within populations were also calculated as 1-identity by state proportions (IBS distance) obtained with SNPrelate in R (Zheng et al., 2012). The heterozygosity along the genome for each population, obtained with VCFtools *--hardy* was inspected visually to assess whether its distribution varied over the whole genome.

To look for signals of inbreeding in each population, the coefficient *F* was calculated by comparing the observed and expected estimates per loci averaged over the genome (from VCFtools *--hardy*, for populations with a sample size of five or more to ensure adequate power when calculating expected heterozygosity) as F_{IS} (Nei, 1977). The inbreeding was also estimated based on the proportions of heterozygous sites (F_H) (from VCFtools --het) as in PLINK, and by runs of homozygosity (ROH) using PLINK homozyg (F_{ROH}), defined as the sum of ROH length for an individual divided by the total length of the autosomes (McQuillan et al., 2008). The settings for ROH were: *homozyg-window-snp 10*, *homozyg-window-missing 10*, *homozyg-window-het 1*, *homozyg-snp 30*, *homozyg-kb 500*, *homozyg-gap 1000*, *homozyg-density 200*. It has been shown that F_H and especially F_{ROH} can reflect true inbreeding signatures (Forutan et al., 2018; Kardos et al., 2015).

The population structure, admixture, and divergence were further examined with a principal component analysis (PCA), admixture plot, Weir and Cockerham's F_{ST} (Weir & Cockerham, 1984), and net

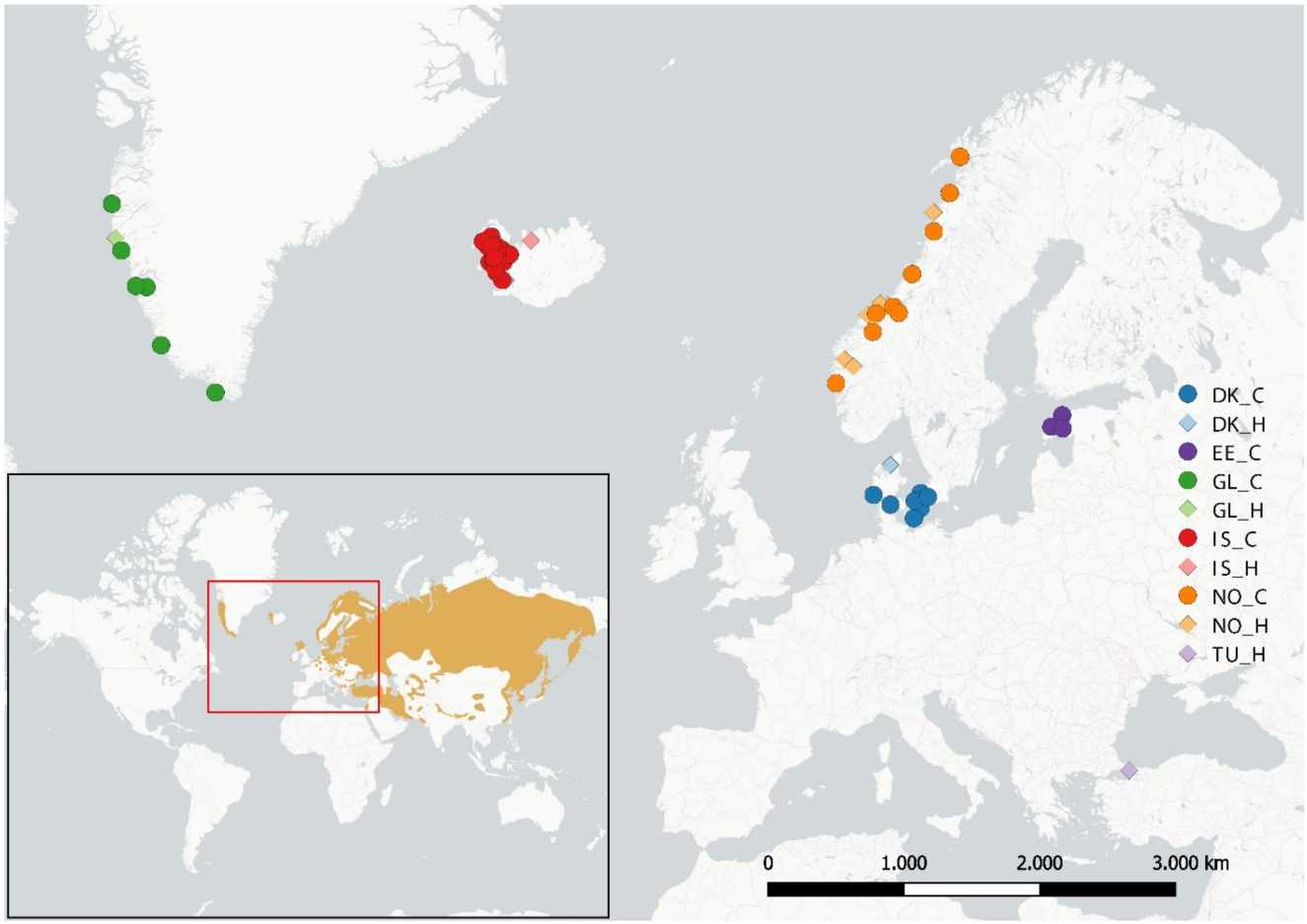

FIGURE 1 Maps of the sample sites and the species range. Locations of known sampling sites for the 92 white-tailed eagle individuals are marked with specific colours per country. Dots represent contemporary samples, and diamonds represent historical samples. The map in the corner shows the species distribution in orange. The red square is the part that makes up the larger map. GL_C, contemporary Greenland; GL_H, historical Greenland; IS_C, contemporary Iceland; IS_H, historical Iceland; NO_C, contemporary Norway; NO_H, historical Norway; DK_C, contemporary Denmark; DK_H, historical Denmark; EE_C, contemporary Estonia; TU_H, historical Turkey.

pairwise IBS distances between populations. To generate the PCA, EIGENSOFT (Patterson et al., 2006; Price et al., 2006) was used with standard settings, except the option numoutlieriter was set to 0, and it was run with lsqproject. For lsqproject, 61 individuals were used to make the initial PCA which the remaining 31 individuals were projected on. The 61 individuals had a maximum missingness of 13% (see Table S1), which meant all sample groups (locality/time), except historical Icelandic, were represented in the initial PCA using 61 individuals. A total of 218,115 variable sites were used in the analysis. The ADMIXTURE (Alexander et al., 2009) analysis was run with 100-fold cross-validation (*–cv = 100*) and with 200 iterations for $K = 1$–15. The program was considered to have converged to a given *K*-value if delta was below 10^{-4} for five iterations in a row. Weighted F_{ST} was calculated using standard settings in VCFtools. Pairwise Euclidean distances of nonidentical-by-state (1-IBS) genotypes were calculated from IBS values obtained with SNPrelate (Zheng et al., 2012) in R. The difference in IBS distances between temporal samples within localities was tested with a Wilcoxon test (Sokal & Rohlf, 2012). Net IBS distances (D_{IBS}) were calculated separately between the historical and the contemporary samples as $D = d_{ij}\text{-}(d_{ii} + d_{jj})/2$ (Nei & Li, 1979), where d_{ij} is the average distance between individuals from samples *i* and *j* and d_{ii} and d_{jj} are the average distances within samples. The probability of the observed outcome (*p*-value) was estimated by permuting the distances 100 times among each pair of samples, and adjusted using the sequential Holms- Bonferroni procedure (Holm, 1977).

Following the analyses of structure and divergence, potential selection or deviation from neutral equilibrium was examined by estimating Tajima's *D* (Tajima, 1989) per population, both for the contemporary and historical samples, using VCFtools in nonoverlapping windows of 50 K bases and calculating the mean and standard deviations. Additional signatures of possible selection driving differentiation at individual SNPs and/or gene regions were investigated by looking for spikes or throughs in differentiation along the genome. Weir and Cockerham's F_{ST} was calculated along the genome in nonoverlapping windows of 100 K bases between all pairs of population samples, using VCFtools. The Estonia and Turkey samples were not included as they were only represented by one temporal sample.

Outlier estimation across all sampled populations, per SNP, was performed with OutFLANK version 0.2 (Whitlock & Lotterhos, 2015).

Demographic changes over time were estimated with, SMC++ (Terhorst et al., 2017) for all autosomal data. First, by evaluating the changes in effective population size using the estimate command, and second to date the divergence of the populations using the split command. Fifty expectation maximization steps were enough to reach convergence for all populations, and each population analysis and divergence estimation was repeated 10 times. A generation time of eight years was chosen to allow for comparison to the work performed in Nadachowska-Brzyska et al. (2015), although generation time may vary over time within species depending on population density (Araya-Ajoy et al., 2021). A mutation rate of 3.2×10^{-9} per site per year (Nadachowska-Brzyska et al., 2015) was assumed. The changes in N_e were also analysed using stairway plot version 2 (Liu & Fu, 2015, 2020), with options ninput set at 200 and pct_training at 0.67. The stairway plot method has been shown to most accurately reflect recent changes in effective population size, with SMC++ generally performing better over longer time periods (Patton et al., 2019). Unfolded site-frequency spectra (using the golden eagle reference genome [GCA_900496995.3]) for contemporary Iceland, contemporary and historical Greenland, and contemporary and historical mainland populations were calculated using easySFS (Overcast, 2022).

3 | RESULTS

3.1 | Quality of sequences

The ratios of transitions to transversions (*Ts*/*Tv*) were similar for the contemporary (2.85) and historical individuals (2.9), suggesting depurination was not significant across the historical samples. As low damage was observed with the MapDamage recalculated quality scores (see below), and as we applied a minimum depth filter of 8, we elected to keep both transitions and transversions for further analyses. After filtering was complete, the data set included 210,322 total SNPs. The mean depth including missing sites per individual over all SNPs was 16.2 for the contemporary specimens (ranging from 4.6 and 41.39) and 10.3 for the historical specimen (ranging from 3.9 to 35.8) (Table S1). The total number of SNPs and mean depth (without missing sites) for the analysed SNPs per individual, with individual heritage information for the 92 individuals are summarized in Table S1. The historical samples had, as expected due to post-mortem damage, slightly more substitutions than the contemporary samples for both the 3′ and 5′ ends which could lead to overestimates of diversity, but it is limited just to the very end of the reads. At the 3′ ends the historical samples had a mean substitution rate of 0.031 at the first site and 0.024 at the tenth site. For the contemporary samples the corresponding numbers were 0.017 at the first site and 0.023 at the tenth. The same pattern was seen at the 5′ ends for the first and tenth sites, respectively, in the historical sample (0.031 and 0.023) and the contemporary sample (0.015 and 0.023) (Figure S1). The substitution rate is increased by 0.01 on average for the five bases at the end, so its impact on the overall heterozygosity is small or about 0.1% (e.g., expected to be 2*0.01*0.99 *10/150) due to those errors.

3.2 | Diversity within samples

The island populations (Greenland and Iceland), had substantially lower diversity than the mainland populations: the average observed heterozygosity per SNP for the island populations ranged from 0.14 to 0.17, while the mainland averages are almost double that, ranging from 0.28–0.32 (Table 1). The observed heterozygosity per individual was 0.16 to 0.17, and in the mainland populations range from 0.22–0.28 (Wilcox exact test, $p < 2.2 \times 10^{-16}$, Figure 2). A similar difference between the island and mainland populations was observed for the average IBS distance between individuals, which ranged from 0.1–0.12 and from 0.23–0.26 for the island and mainland populations, respectively (Table 1). The lowest diversity in the contemporary populations was found in Iceland (0.1407 average observed heterozygosity and 0.108 IBS-distance, Table 1). The largest temporal change in proportions of heterozygous sites across individuals was observed within Iceland, where it decreased from 0.156 ($SD = 0.017$) to 0.139 ($SD = 0.013$) (Figure 2). Observed heterozygosity per site only showed a temporal reduction in Denmark and Iceland (Table 1). IBS-distances were significantly larger within the contemporary samples than within the historical samples, both when comparing the averages and the ranks, again except for the Danish and Icelandic samples (Wilcoxon test *p*-values: GL = 3.94×10^{-8}, IS = 5.81×10^{-2}, NO = 5.69×10^{-5}, DK = 8.64×10^{-1}).

Variation at each locus within populations showed little deviation between the observed and expected values or deviation from random mating in the mainland populations, and thus no evidence of inbreeding (F_{IS}). When considering the proportion of expected heterozygous sites per individual similar values were observed (0.252–0.268) for all populations and sample ages. However, for the island populations, a strong signature of inbreeding was observed. F_H values for the island populations indicate large inbreeding, ranging from 0.362–0.456 (Table 1), with individual F_{IS} values in the contemporary Icelandic population ranging from 0.384 to 0.595, which had the highest observed mean F_{IS} (0.456), with the second highest mean F_{IS} seen in the historical Icelandic sample (0.410). Contemporary Greenland, conversely, had a lower mean F_{IS} (0.362) than historical Greenland (0.386). Little or no inbreeding was found in the mainland populations, ranging from −0.32–0.06 (Figure 2).

F_{ROH} displayed the same pattern of inbreeding as F_H ($r = 0.98$, $p < 2.2 \times 10^{-16}$, Figure S2) when 14 individuals with >50% missing data were excluded (reducing the data to 78 individuals), as this was shown to affect the ROH length ($r = .39$, $p = 1 \times 10^{-4}$, Figure S3). The smaller populations of Iceland and Greenland exhibited higher values (0.349 and 0.435) than mainland samples (0.07–0.145) (Table 1).

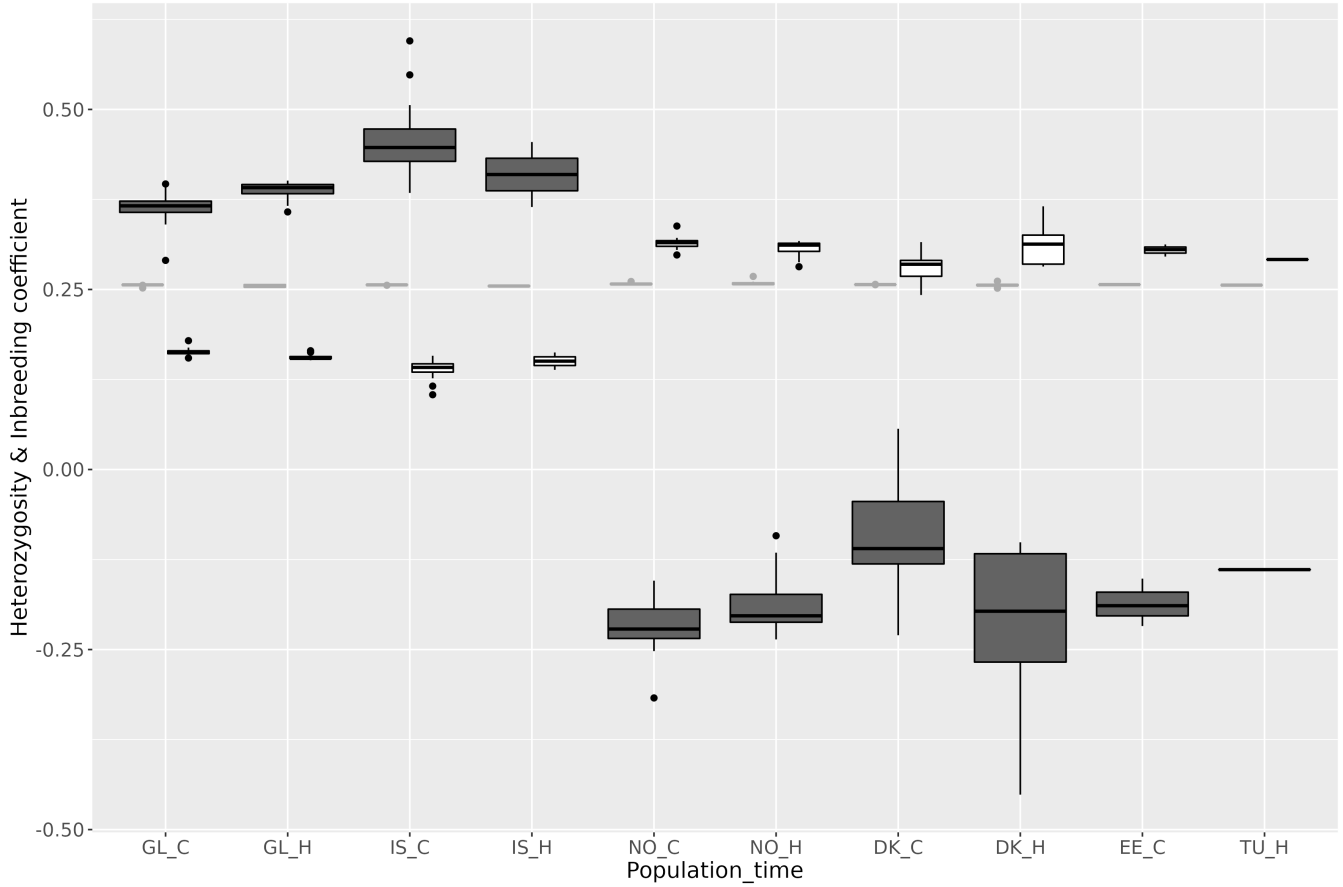

FIGURE 2 Deviation from random mating within samples. Three boxes are shown for each temporal sample per country: Narrow boxes present expected (shaded grey) and observed (white filled) heterozygosity per individual. Wide boxes (dark grey filled) present the inbreeding coefficient per individual "C" refers to contemporary samples and "H" to historical samples. DK, Denmark; EE, Estonia; GL, Greenland; IS, Iceland; NO, Norway; TU, Turkey.

Contemporary Denmark showed the highest F_{ROH} of the mainland populations, which makes sense given its much more recent formation. The number of heterozygotic sites was independent of coverage ($r = -.08$), but showed a weak correlation with missingness ($r = .31$, $p = .003$).

The contemporary island populations both had elevated levels of ROH, as did the historical population from Greenland (Figure 3). A few historical individuals from all localities and one contemporary Norwegian individual displayed extreme values and a large spread of ROH. ROH length and depth of coverage for those 78 individuals were uncorrelated ($r = -.039$, $p = .73$, Figure S4). Furthermore, both contemporary and historical samples from Greenland and the contemporary Icelandic sample show signs of having experienced an older bottleneck as they have many ROH segments and long segments. The Icelandic and the contemporary Danish samples are also located below the diagonal between the number and length of ROH segments, further indicating recent consanguinity (Figure 3). Most historical specimens from Norway and Denmark as well as the contemporary Norwegian and Estonian samples all show low levels of number and length of ROH segments, ranging from 28–160 and 25–265 Mb, respectively (Figure 3).

3.3 | Population structure and historical demographics

The assessment of divergence of samples from the different localities, based on F_{ST}, net IBS-distances (D_{IBS}) between the contemporary and the historical samples separately, as well as the principal component analysis (PCA, described below), revealed clear differentiation. In accordance with the PCA plot in Figure 4, the largest contemporary F_{ST} values are between the island and mainland samples (0.172–0.351, Table 2). The F_{ST} values between the contemporary mainland samples are smaller (0.027–0.065) than between the two island populations (0.16). The temporal comparisons within locality display lower differentiation, with the largest temporal difference found in Iceland and Denmark (F_{ST}, D_{IBS}), with p-value in parentheses: Greenland 0.007, 0.002 (0); Iceland 0.096, 0.033 (0.01); Norway 0.014, 0.010 (0); and Denmark 0.069, 0.028 (0). The distance metrics for the historical populations follow the same pattern as the contemporary ones, except for F_{ST} for the small Icelandic population which shows the same distance to Norway and Denmark as it does to Greenland (~0.190), possibly due to the small size of the historical Icelandic sample. Both pairwise F_{ST} and D_{IBS} show larger

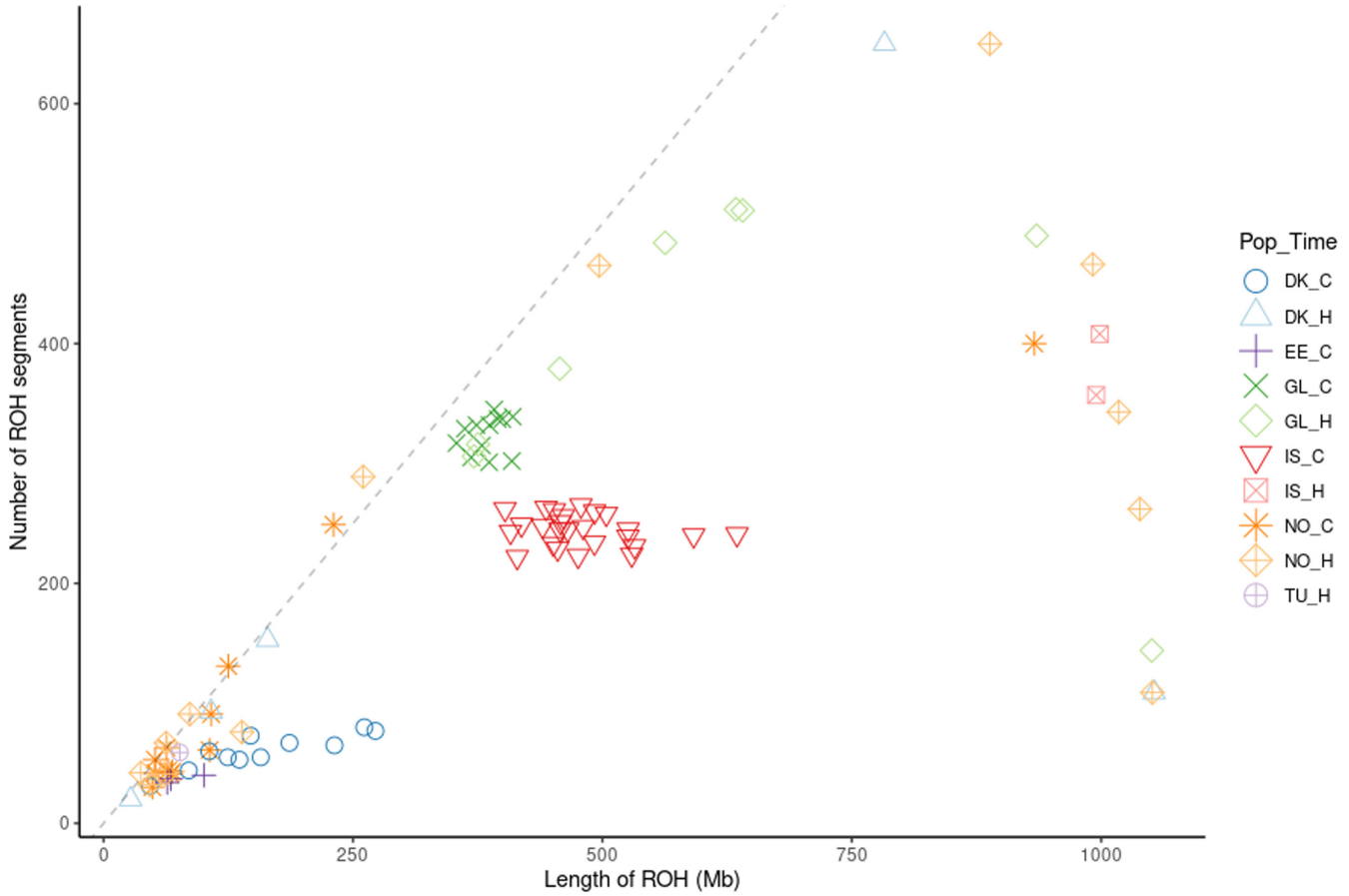

FIGURE 3 Runs of homozygosity (ROH) for all 92 individuals. The x-axis displays the length of ROHs in megabases (Mb), the y-axis shows the number of ROH segments. A dashed diagonal line shows a presumed 1:1 relationship between number and length of ROH segments. Colours indicate country and temporal category (Pop_Time). C, contemporary samples; H, historical samples. DK, Denmark; EE, Estonia; GL, Greenland; IS, Iceland; NO, Norway; TU, Turkey.

differentiation between the contemporary samples than between the historical samples.

The EIGENSOFT PCA analysis, based on 218,115 variable sites, resulted in a clear split between the mainland (Denmark and Norway) and the two island populations (Greenland and Iceland) on the first PCA-axis (explaining 13.55% of the total variation), but separated the two on the second PCA axis (explaining 4.58%) (Figure 4a). The contemporary Danish population (excepting one individual) is distinct from the rest of the mainland as well as the historical Danish samples, while only a minor distinction is observed between the contemporary and historical samples for all other populations (Figure 4). An additional analysis of only mainland samples showed similar clustering as when the second and third principal components were compared in the full analysis (Figure 4b), where both contemporary and historical Norwegian samples cluster together, contemporary Danish samples remain distinct, the historical Turkish sample clusters with the historical Danish samples, but one contemporary Danish sample clusters with the three contemporary Estonian samples.

All runs of ADMIXTURE (K = 2–15) converged per the criteria of delta being below 10^{-4} for five iterations in a row. Cross Validation (CV) errors were similar for K values between 2–6. Although the best supported value of K, according to a minimum CV error criteria, was K = 4, which split the populations up into the two distinct island populations of Iceland and Greenland, a Norwegian population, and then an admixed Danish, Estonian, and Turkish population (Figure 5a). Although the K = 2 (Figure 5b) separates the island and the mainland populations (similar to the first PCA-axis). At K = 3 (Figure 5c) Norway separates from the rest of the mainland populations. For K = 3–6 (Figure 5c–e), further splits are observed: the historical Icelandic individuals get a separate signature from the contemporary Icelandic, and the contemporary Danish population seems to be a mixed population (perhaps containing something from an unsampled origin) and contains one individual with a signature like the Estonian individuals. The historical Danish and Turkish individuals display the same signature.

A majority of the Tajima's D values in all populations are positive and skewed to the right, especially for the island populations and the historical Danish sample (Table 3 and Figure S5). A large fraction of the Tajima's D values in the island populations, and especially in Iceland, exceeds two standard deviations, indicating significant values, and the large proportion of positive Tajima D values reflects larger mean nucleotide diversities than expected based on the number of segregating sites.

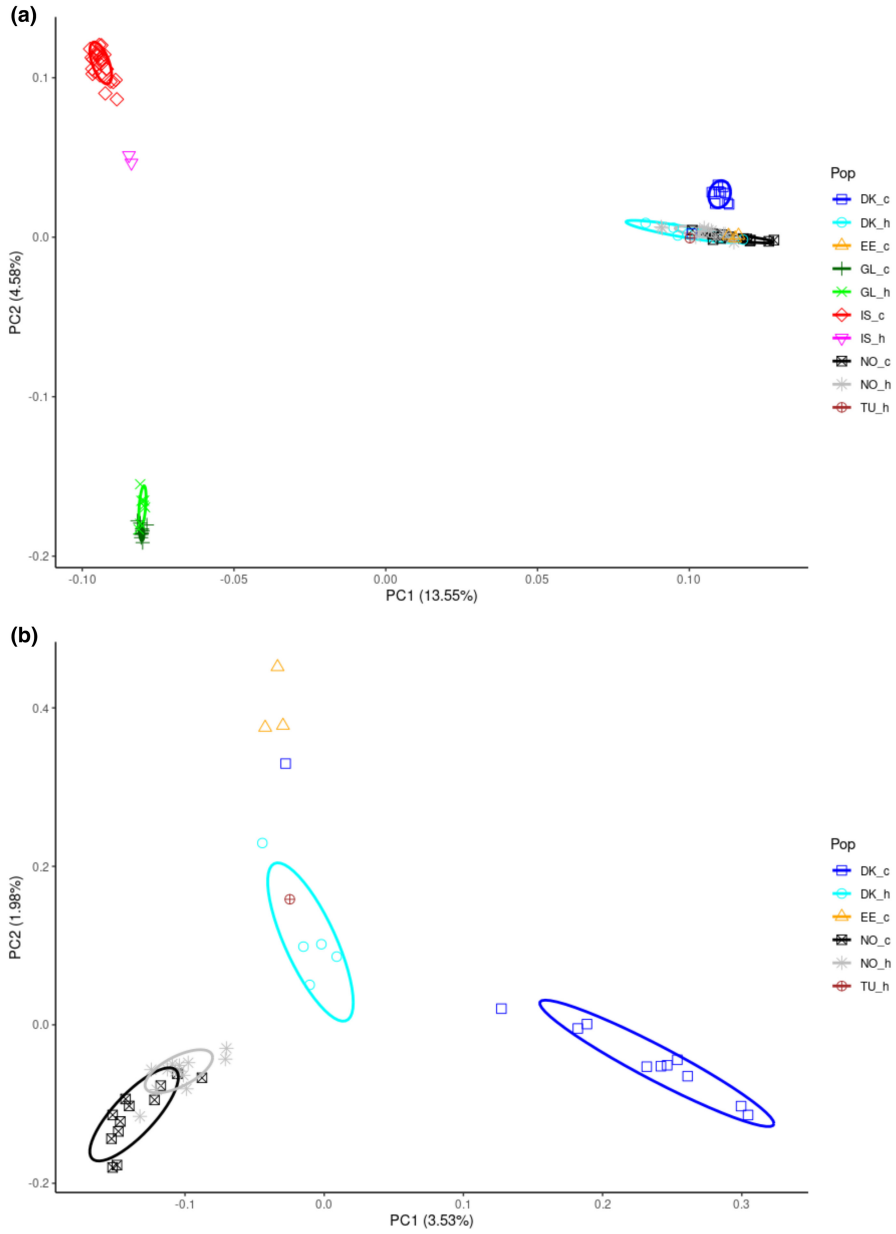

FIGURE 4 Clustering of white-tailed eagle individuals from contemporary and historical samples based on principal component analysis (PCA) of the genomic variation. The calculation was based on 210,322 SNPs using EIGENSOFT. Percentages given in the axis labels refer to the amount of variation explained by the respective axis. Colours indicate country and temporal category. D, Denmark; E, Estonia; G, Greenland; I, Iceland; N, Norway; T, Turkey. C suffix indicates contemporary samples, H suffix indicates historical samples. (a). Includes all sampled populations. (b) Only mainland populations included.

The divergence between populations, as measured by per SNP F_{ST} estimates, appeared homogeneous across all autosomal regions of the genome (Figure S6). No outliers were identified by OutFLANK, and the distribution of the F_{ST} showed a lack of high F_{ST} SNPs, that is, the observed frequencies of F_{ST} >0.3 were all fewer than expected (Figure S7).

Analysis of the changes in N_e over time with SMC++ and stairway plot showed similar fluctuations in population sizes since the last interglacial period, although SMC++ showed considerably lower population sizes (Figure 6). A large reduction in population size happened during the Eemian period 110–150 kyr ago, but if a slightly shorter generation time was used (seven years instead of eight) the drop occurred right at the onset of the glaciation at the end of the Eemian interglacial period. Following that drop, population sizes remained rather stable until about 10,000 to 20,000 years ago, during or just after the last glacial maximum, when the population sizes dropped again. This population reduction is not as evident in the SMC++ results as in the stairway plot output for the Danish population, which remained rather stable until around 1000 years ago when a drop was observed along with the Norwegian population. A further inspection of the recent changes in the stairway plot shows a drop around 1700–3000 years ago and a continual population decline during the last 500 years. Very low estimates of current N_e were reported for the contemporary populations. Both the island populations had a median N_e estimate of just two individuals, where Iceland had an upper (97.5%) confidence level threshold of 10 while Greenland had an upper threshold of 14. The mainland population estimates were similarly low, with a median N_e value of four in Denmark and five in Norway, although both had higher upper thresholds of 25 and 42, respectively. It should be noted that the effective recombination rate (rho), estimated with SMC++, was considerably higher than the effective mutation rate (theta) (0.0001, SE = 0) and varied between

TABLE 2 Mean F_{ST} above the horizontal line, and distance based on Identity-by-State below (D_{IBS}), with *p*-value above the diagonal.

Contemporary						Historical				
F_{ST}	GL	IS	NO	DK	EE	F_{ST}	GL	IS	NO	DK
GL						GL				
IS	.162					IS	.030			
NO	.173	.233				NO	.134	−.059		
DK	.172	.221	.065			DK	.187	−.063	.003	
EE	.260	.351	.027	.053		TU	.241	.292	−.081	−.096
D_{IBS}	GL	IS	NO	DK	EE	D_{IBS}	GL	IS	NO	DK
GL		.000	.000	.000	.000	GL		.010	.000	.000
IS	.047		.000	.000	.000	IS	.045		.050	.040
NO	.086	.084		.000	.000	NO	.085	.091		.040
DK	.089	.086	.035		.000	DK	.075	.079	.016	
EE	.086	.085	.023	.029						

Note: Left only comparison of contemporary samples, right only comparison of historical. *p*-value, given for the IBS-distances above the diagonal are based on 100 permutations. Abbreviations refer to country: GL, Greenland; IS, Iceland; NO, Norway; DK, Denmark; EE, Estonia; Tu, Turkey.

the populations. Estimates of rho were highest in the island populations of Greenland and Iceland (0.01, SE = 0), lower in Norway (0.007, SE = 6×10^{-5}), and lowest in Denmark and Estonia (0.006, SE = 5×10^{-5}).

Looking only at the populations as defined by the best supported ADMIXTURE results (Iceland, Greenland, Norway, Mainland), average dates of divergence estimates from the SMC++ split command showed that Iceland and Greenland had diverged from each other 4278 years ago (95% confidence interval [CI]: 3470–5085), while the two island populations together had diverged from Norway and the mainland 7835 years ago (95% CI: 5536–10,133). The Norwegian population became distinct from the other mainland populations 948 years ago (95% CI: 276–1620).

4 | DISCUSSION

Despite their possible dispersal capabilities, white-tailed eagles show clear population substructure in the North Atlantic, based on genome-wide analysis. The main split between populations is observed between the island populations (Iceland and Greenland) and the mainland (Norway, Denmark, Estonia, Turkey), with the island populations harbouring less genetic diversity than the mainland populations. A split of Iceland bird populations from mainland conspecifics has been found for other species, including species with limited dispersal abilities, such as wrens (*Troglodytes troglodytes*) (Amouret et al., 2016), and migratory birds, such as the black-tailed godwit (*Limosa limosa islandica*) (Trimbos et al., 2014). The two islands are also differentiated from each other, and the Norwegian population appears well diverged from the other sampled mainland populations. Interestingly, fine-scale clustering ($K = 6$, Figure 5e) in the structure analysis splits the small population within Iceland into two groups. The basis for this split is unclear and warrants further study.

Comparisons of contemporary and historical samples, on average dating back over 100 years, show no major temporal changes in population structure. The historical samples from the extinct Danish populations (Ehmsen et al., 2011), appears admixed throughout, showing the strongest association with the similarly admixed Estonian and Turkey samples, along with only three of the 11 contemporary individuals from Denmark. The newly established contemporary Danish population is somewhat divergent and less mixed. All analyses generally suggest that the connectivity between the islands and the mainland has consistently remained low at least over the last 10,000 years.

The genome-wide pattern of the two island populations descending from a shared ancestral population, distinct from the remainder of the European mainland samples studied, contrasts with the mitogenomic lineages in Greenland, Iceland, and Norway, which are polyphyletic, each containing lineages from two distinct mitogenomic clades (Hailer et al., 2007; Hansen et al., 2022). The mitochondrial variation was shown to deviate from neutral expectation (Hansen et al., 2022), possibly owing to selection on the W-chromosome due to shared inheritance and linkage disequilibrium between the W-chromosome and mitochondrial DNA in birds. Despite clear divergence between the geographic populations, the distribution of F_{ST} values are on the smaller side (~0.2) with respect to their range. This, coupled with positive values of Tajima's *D*, indicates higher variation within populations than expected based on the number of segregating sites. It is unlikely that this is due to migration or admixture as migration has been shown to be limited, but this may reflect the impact of selection where heterozygous individuals could have higher fitness, as they are less likely to be homozygous for deleterious mutations, resulting in associative overdominance at linked neutral sites. Such effects have been shown to be more probable in small populations and would lead to overestimates of the effective population sizes (Charlesworth & Jensen, 2021; Pálsson & Pamilo, 1999). The lack of large F_{ST} values is furthermore noteworthy

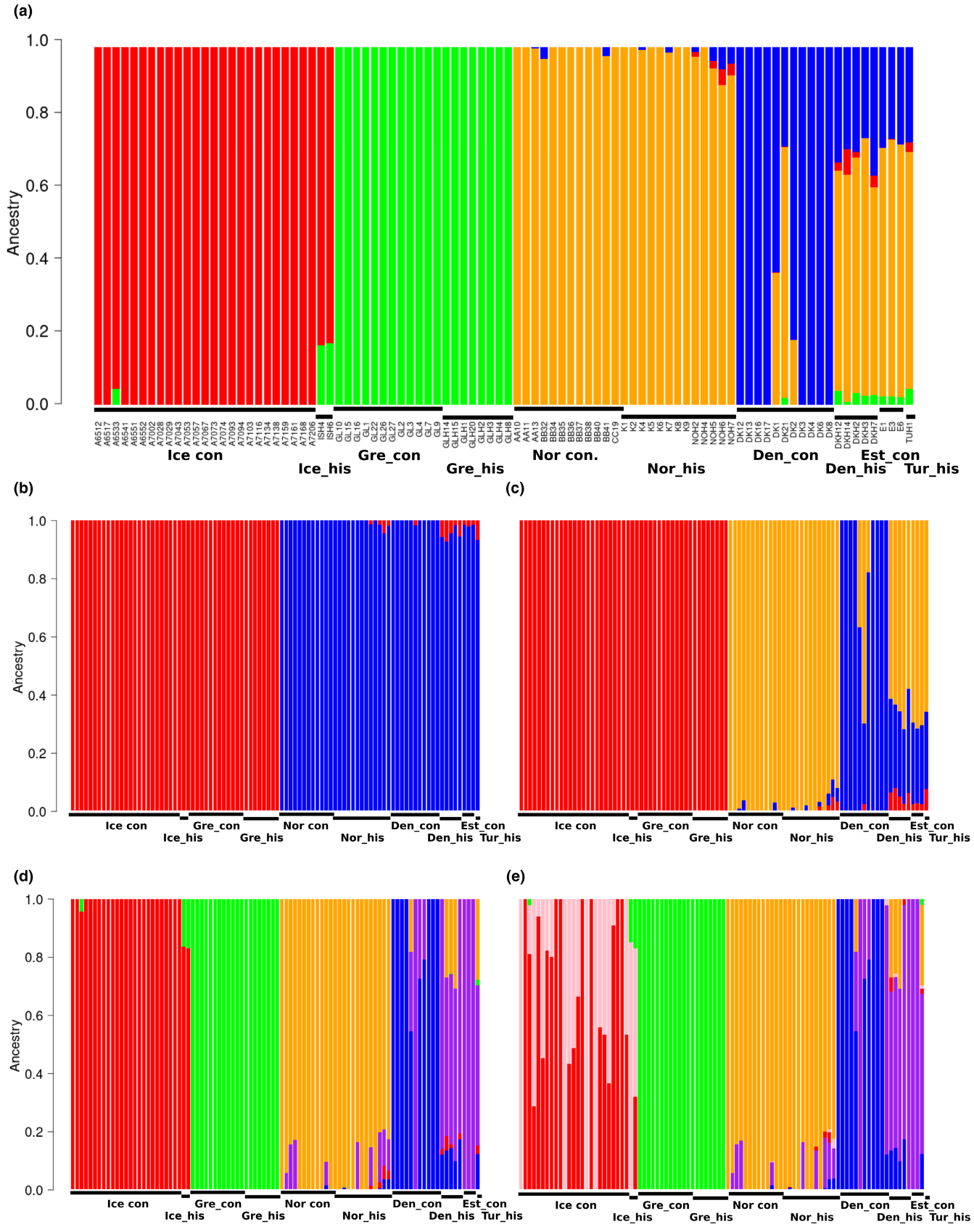

FIGURE 5 Admixture plots were obtained with ADMIXTURE for a different number of clusters (*K*). The best supported K, according to a minimum CV error criteria, was $K = 4$, shown in (a). (b) $K = 2$, (c) $K = 3$, (d) $K = 5$, (e) $K = 6$, (f) $K = 7$. The value on the y-axis shows the assignment probability of each individual to the genetic cluster (K_i). The first three letters in the labels refer to the countries. Gre, Greenland; Ice, Iceland; Nor, Norway; Den, Denmark; Est, Estonia; Tur, Turkey, and the last to the temporal sample: con, contemporary; his, historical.

TABLE 3 Results from the Tajima *D* test.

Country	Temporal sample	N_SNPs	TajD mean (SD)	*P* (<−2, >0, >2)
Iceland	C	18,220	0.951 (1.110)	0.001, 0.785, 0.181
Iceland	H	7814	1.374 (0.788)	0, 0.889, 0.105
Greenland	C	18,531	0.754 (1.050)	0.002, 0.763, 0.104
Greenland	H	17,697	0.776 (0.881)	0, 0.780, 0.058
Mainland	C	20,811	1.224 (0.613)	0, 0.960, 0.082
Mainland	H	20,756	1.114 (0.617)	0, 0.950, 0.057
Norway	C	20,714	0.898 (0.614)	0, 0.919, 0.022
Norway	H	20,619	1.052 (0.608)	0, 0.942, 0.041
Denmark	C	20,637	0.701 (0.681)	0, 0.846, 0.014
Denmark	H	20,162	0.924 (0.544)	0, 0.940, 0.009
Estonia	C	20,217	0.271 (0.665)	0, 0.667, 0.0006

Note: Mean Tajima's *D* (TajD) and sd in parentheses, and number of SNPs used in Tajima's *D* analysis (N_SNPs). Proportion of sites along the genome being less than −2, above 0, or above 2.

as it may reflect the limited evolvability of these small island populations, despite being isolated and living in such distinct environments as arctic Greenland and subarctic Iceland, in comparison with temperate mainland Europe.

The observed level of inbreeding varied depending on the estimation approach used, whether it was based on per-SNP within the population (F_{IS}), or per-individual (F_H, and F_{ROH}), as the reference population varied. When it was based solely on individuals from their own populations (F_{IS}), no inbreeding or deviation from Hardy–Weinberg equilibrium was observed. However, when inbreeding was estimated based on comparing each individual with the variation within the total sample, a clear difference was observed. Then the levels of inbreeding (F_H) for the island populations were comparatively very large (0.35–0.46), while staying close to zero in the mainland populations, and much lower proportions of heterozygous sites were observed in the islands than in the mainland populations. The two coefficients based on the individual genomic patterns F_H and F_{ROH}, known to reflect well the "true" level of inbreeding (Forutan et al., 2018; Kardos et al., 2015) gave similar outcomes and suggest substantial inbreeding in both island populations. Generally, larger genetic distances between individuals were observed in contemporary samples than in the historical populations. Such changes could result from larger scatter (i.e., drift) due to fewer heterozygous sites in the island populations than in the mainland and longer runs of homozygosity as have been seen for example, in studies of human populations (Price et al., 2006). Similarly, genetic variance in quantitative traits has been found to increase within populations with inbreeding, where genetic factors segregate among different lines in linkage disequilibrium (Wang et al., 1998), contrary to the more general assumption that a population bottleneck should reduce gene diversity and genetic heterozygosity per loci in the population (Crow & Kimura, 1970). All sampled populations show signs of a recent bottleneck. However, the analyses of genetic variation reveal strong signals of ancient bottlenecks and lower diversity in the island populations than in the mainland populations.

Temporal comparisons within localities generally displayed similar genetic signatures, except for the samples from Denmark and, to a lesser extent, Iceland. In Denmark, we find a difference between the historical and contemporary samples in the PCA and the admixture composition. This is not surprising as the population went extinct in Denmark and has since been reestablished (Skelmose & Larsen, 2021), and thus the two temporal populations do not necessarily share a direct recent common ancestor. This is consistent with the pattern seen in a Japanese population of the closely related golden eagle, where following a local collapse an influx of genetic diversity from North America was observed (Sato et al., 2020). The population in Iceland, which went through a strong recent bottleneck (Petersen, 1998; Skarphéðinsson, 2003, 2013), also shows a difference between the contemporary and historical samples as revealed by the PCA, ADMIXTURE, and IBS comparison. Furthermore, less heterozygosity and higher inbreeding are observed in the contemporary Icelandic sample compared to the historical sample. Although this difference may be biased due to the small historical sample size, missing data, and overestimation of heterozygosity due to post-mortem damage, the signal is consistent for the different assessments of diversity and is also supported by the effective population size analysis that shows an ongoing decline. The post-mortem damage was just restricted to 5 bp at the ends of reads and had only a minor potential effect on the heterozygosity. Thus, even though the white-tailed eagle is long-lived, and the recent bottleneck is moderately short (c. 150 years), it has affected the variation within the Icelandic population. Although no clear reduction is otherwise observed in heterozygosity between the temporal samples, as has been observed for several species since the industrial revolution (Leigh et al., 2019), all populations exhibit considerable population reductions for the last centuries and over larger time scales.

Effective population sizes were large during the interglacial Eemian period 110–150 kyr ago and dropped around the onset of the last glacial period. The populations remained relatively stable until the last glacial maximum about 20–25 kyr ago, when three other declines were reflected in the analysis. (1) During the

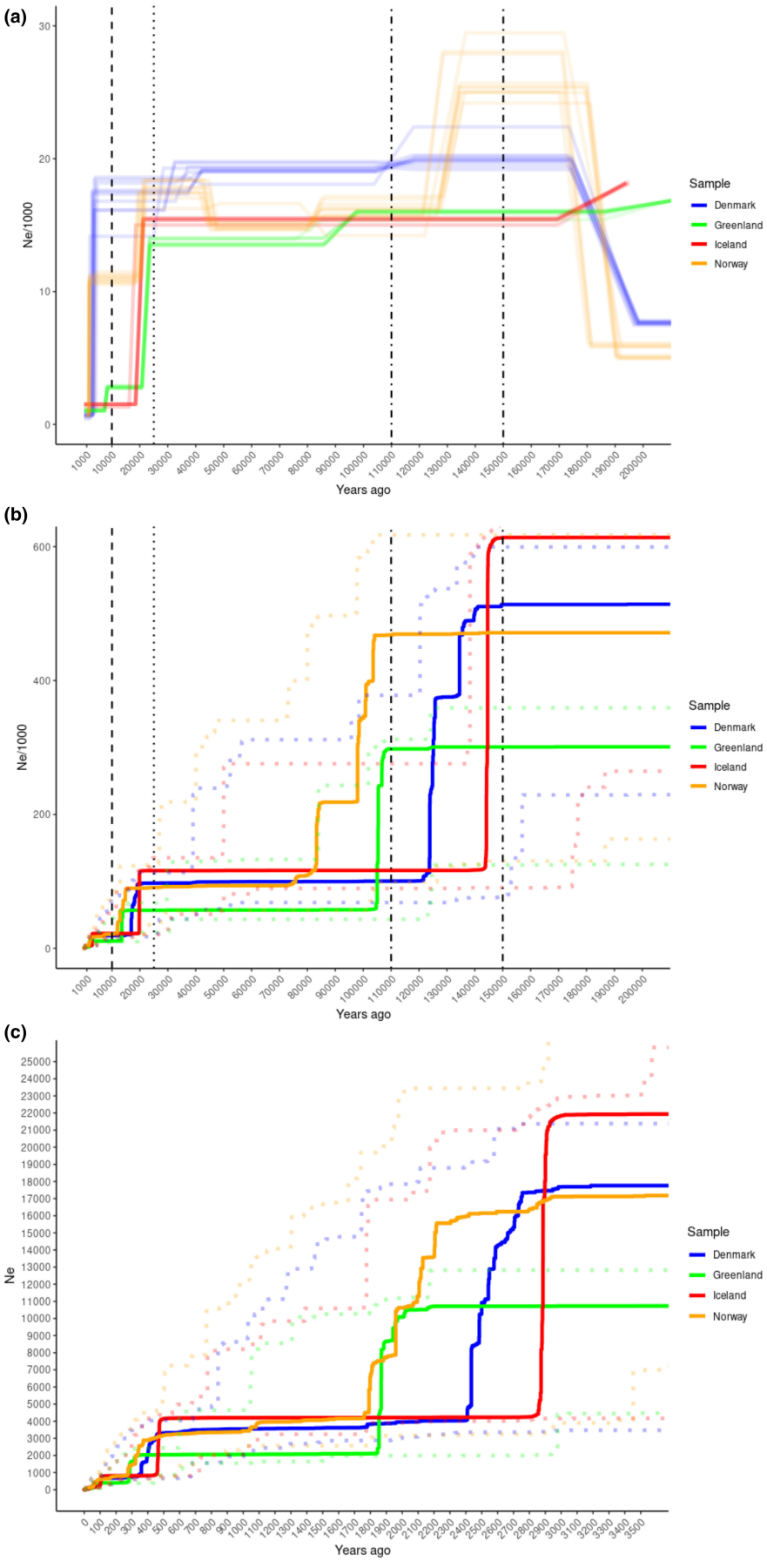

FIGURE 6 Effective population size estimates over time, including only contemporary samples from Iceland ($N = 25$), Greenland ($N = 12$), Norway ($N = 12$), and Denmark ($N = 11$). Generation time is fixed at 8 years, and mutation rate at 3.2×10^{-9} per site per year. Note that the scale of the y-axes differ between each plot. (a) Results from the SMC++ "estimate" analysis. Each population analysis was replicated 10 times, and the result of each replicate is represented as a single transparent line. (b) Results from the stairway plot analysis. Solid lines represent the median N_e value for each population, while dotted lines represent the upper (97.5%) and lower (2.5%) estimates for each population. Both (a) and (b) show a temporal range on the x-axis between 1000 and 200,000 years ago. Broken lines represent key paleogeographic events: a dashed line at 10,000 years ago shows the end of the last glacial period when Iceland and Greenland became feasible habitats; a dotted line at 25,000 years ago shows roughly the last glacial maximum; and finally two dash-dotted lines between 110,000 and 150,000 years ago show the range of the last interglacial warming period (Eemian). (c) A zoomed in view of the stairway plot analysis showing the recent fluctuations in N_e over the last 3500 years.

last glacial maximum (Clark et al., 2009; Lisiecki & Raymo, 2005) 10–20 kyr ago, the Iceland and Greenland population sizes decreased substantially, during which time the metapopulation may have become isolated in two or more refugia, as has previously been suggested (Hailer et al., 2007; Hansen et al., 2022; Langguth et al., 2013), possibly before the colonization of the islands. This signature of diversification within multiple possible refugia can be contrasted with the study by Pujolar et al. (2017) on pink-footed geese (*Anser brachyrhynchus*), which despite a similar dispersal capacity as the white-tailed eagle shows a signature of only a single surviving refugia population diversifying following the last glaciation period.

This drop is not reflected for the Norwegian and Danish populations in the SMC++ output, whereas the main drop in the mainland populations only occurred during the last 1–2 kyr, although the stairway plot results estimate a Norwegian and Danish population collapse around roughly the same time as the island populations. Thus the split of the island ancestral population could have occurred before the colonization of the islands at the end of the glaciation period 10–15 kyr ago, as they may have been derived from an extinct lineage, for example, from the British isles or western Europe, which had started to diverge from the mainland population during a period of multiple glacial refugia. (2) A second drop apparent in the stairway plot results happened for all populations between ~2–4 kyr ago, which could indicate the establishment of the different populations identified in the PCA, ADMIXTURE and IBS analysis after the end of the last glacial period within the sampled localities (Clark et al., 2009; Clark & Mix, 2002). Finally, (3) the most recent large-scale drop in population size on the mainland around a thousand years ago coincides with a period of human expansion in northern Europe (Kremer, 1993), including settlement in Iceland around the year 871 (Batt et al., 2015). White-tailed eagle bones have been found in human settlements and may thus have been hunted by humans (Price et al., 2018). Finally, other than these three major declines in population size, we also see a drop in effective population size for all populations during the last centuries, which could well be an anthropogenic effect following the industrial revolution, including human persecution in the 19th century and organic toxic pollutants known to have had a detrimental effect on reproductive success in the eagles during the 20th century (Bijleveld, 1974; Helander et al., 1982, 2002; Love & Ball, 1979; Walker et al., 2009).

Nadachowska-Brzyska et al. (2015) analysed the demographic changes based on a single individual from Greenland with PSMC (Li & Durbin, 2009) and obtained the same pattern of population decline following the Eemian interglacial period, albeit yielding much higher effective population size estimates than our SMC++ analysis. While SMC++ and stairway plot generally yielded similar effective population fluctuation patterns, effective population size estimates varied considerably, with stairway plot estimating population sizes 30–60× higher than SMC++. Furthermore, we do not see as large a difference in N_e between the island and mainland populations as one might expect based on the census sizes, although the former have clearly fewer heterozygotic sites and thus higher inbreeding per individual. This could be explained by the island populations having a higher estimated recombination rate than the mainland populations which boosts the N_e estimates. In a study by Sellinger et al. (2021), a ratio of rho/theta = 10 gave less reliable estimates of the coalescence times than lower ratios. Here, we saw ratios between 60–100, and as stated by Sellinger et al. (2021), such a high ratio impedes the detection of any recombination events which may occur before the introduction of a new mutation. Whether this high recombination rate stems from the genetic map is unclear, but separate SMC++ analyses for each of the chromosomes gave similar recombination rate estimates.

Consistent with previous studies for this species (Nadachowska-Brzyska et al., 2015), and the confamilial golden eagle (Sato et al., 2020), the overall pattern of the effective population sizes follows roughly the main known climatic and anthropogenic influences; however, the confidence intervals are large for the historical estimates. The estimates for the current effective population sizes obtained with the stairway analyses are extremely low for all populations and below the effective population size of 50, the threshold value which has been suggested for populations to avoid inbreeding depression in the short term (Franklin, 1980; Soulé, 1980). Similar estimates have been observed for other species, for example, the Madagascar fish-eagle (*Haliaeetus vociferoides*), for which the ratio of effective population size to population census size (N_e/N_c) is about 10%, which follows the general rule despite variation among taxa (Frankham, 1995). Considering the upper confidence interval for our estimates of N_e and surveys of adult birds, we see a corresponding ratio for Norway and Iceland around 2.5%–5%. The small current-day population sizes could lead to several unfavourable scenarios for the populations; they could not be able to effectively purge deleterious mutations, and beneficial mutations have a higher risk of being lost due to drift (Nielsen & Slatkin, 2013). Further, the small population size may make them less able to adapt per the "500 rule", which has been suggested as the sufficient minimum to retain evolutionary potential (Franklin, 1980; Soulé, 1980); for example, in the case of climate, habitat, or prey/predator change. Our analysis further revealed a reduction in heterozygosity, an increasing inbreeding, and an upsurge in drift during the 20th century for the small Icelandic population, suggesting its existence may be at risk and it may suffer from inbreeding depression (Hartl & Clark, 2007; Nielsen & Slatkin, 2013). Although the Icelandic population has been recovering for the last 40 years, where the number of breeding pairs per year has increased from 20 pairs to about 80, the reproductive rate is low, with only 0.5 chicks per pair per year (Evans et al., 2009; Skarphéðinsson, 2003, 2013) or about one-third of the rate in Sweden (Helander et al., 2013). Although the heterozygosity loss in Iceland is small, a loss of just 5%–10% over 100 years has been suggested to cause a risk of population extinction (Allendorf & Ryman, 2002), though there are examples of species going through ancient bottlenecks but persisting (O'Brien et al., 2017). All these results suggest that an increased conservation effort can become necessary in all the analysed populations, but especially in the small, isolated Icelandic population. Further work linking the genetic variation to variation in fitness-related traits could show whether the

eagle populations in Iceland and Greenland do suffer from inbreeding depression and whether admixture of genetic variants from mainland populations should be considered.

AUTHOR CONTRIBUTIONS

Charles Christian Riis Hansen, Agnar S. Helgason, M. Thomas P. Gilbert and Snæbjörn Pálsson conceived the idea; Charles Christian Riis Hansen, Jacob Agerbo Rasmussen, Jesus Adrian Chimal Ballesteros, Mikkel-Holger S. Sinding, Michael D. Martin, and Snæbjörn Pálsson performed the experiments; Áki Jarl Láruson, Charles Christian Riis Hansen, and Snæbjörn Pálsson analysed the data; Áki Jarl Láruson, Charles Christian Riis Hansen, and Snæbjörn Pálsson wrote the manuscript; Jacob Agerbo Rasmussen, Jesus Adrian Chimal Ballesteros, Mikkel-Holger S. Sinding, Gunnar T. Hallgrimsson, Robert A. Stefansson, Menja von Schmalensee, Kristinn Haukur Skarphédinsson, Aili Lage Labansen, Madis Leivits, Christian Sonne, Rune Dietz, Kim Skelmose, David Boertmann and Igor Eulaers, Michael D. Martin, Agnar S. Helgason, M. Thomas P. Gilbert commented on the manuscript; Charles Christian Riis Hansen, Gunnar T. Hallgrimsson, Robert A. Stefansson, Menja von Schmalensee, Kristinn Haukur Skarphédinsson, Aili Lage Labansen, Madis Leivits, Christian Sonne, Rune Dietz, Kim Skelmose, David Boertmann, Igor Eulaers, Michael D. Martin, Agnar S. Helgason, M. Thomas P. Gilbert and Snæbjörn Pálsson contributed substantial materials, resources; Michael D. Martin, Agnar S. Helgason, M. Thomas P. Gilbert and Snæbjörn Pálsson were responsible for funding.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

Data have been submitted to DRYAD and should be accessible at https://doi.org/10.5061/dryad.fqz612jt8

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