Genomic data reveals strong differentiation and reduced genetic diversity in island golden eagle populations

Title:

Genomic data reveals strong differentiation and reduced genetic diversity in island golden eagle populations

Running Head:

Genomic diversity of golden eagle populations

Authors: Yu Sato[1, 2], Emily Humble[2], Rob Ogden[2]

Affiliations:

1. Wildlife Research Center, Kyoto University, Kyoto 606-8203, Japan
2. Royal (Dick) School of Veterinary Studies and the Roslin Institute, Easter Bush Campus, University of Edinburgh, EH25 9RG, United Kingdom

Abstract

Understanding population structure and the extent and distribution of genetic diversity are recognised as central issues in endangered species research, with broad implications for effective conservation management. Advances in whole genome sequencing (WGS) techniques provide greater resolution of genome-wide genetic diversity and inbreeding. Subspecies of golden eagles (*Aquila chrysaetos*) in Scotland (*A. c. chrysaetos*) and Japan (*A. c. japonica*) are endangered; it is therefore important to understand genetic diversity and inbreeding of these small island populations to increase the chances of conservation success. We investigated this using WGS data from golden eagles in Scotland, continental Europe, Japan, and the USA. Following determination of population genetic structure, analysis of heterozygosity and nucleotide diversity revealed reduced levels of genetic diversity together with runs of homozygosity (ROH), suggesting evidence of inbreeding due to recent shared parental ancestry in the island populations. These results highlight the need to consider genetic reinforcement of small isolated golden eagle populations from neighbouring outbred populations, alongside existing efforts to boost population size through within-island conservation translocations and captive breeding programmes.

Additional keywords: *Aquila chrysaetos* – South of Scotland Golden Eagle Project – reintroduction – population genomics – conservation genetics

Introduction

Conservation genetic studies are important for effective species management. Understanding population structure and connectivity helps conservationists to manage populations at an appropriate scale, and to consider the prioritization of conservation units (Fraser and Bernatchez, 2001; Weeks et al., 2016). Small, isolated populations require particular attention, as they have an elevated risk of losing genetic diversity through both genetic drift and inbreeding, which in turn reduces the fitness of individuals and threatens the survival of populations (Frankham et al., 2009). Individual inbreeding affects population growth and viability of natural populations (Kardos et al., 2016) and has long been recognised as a driver of the extinction vortex (Gilpin and Soulé, 1986). However, it has historically been difficult to measure in the wild. Inbreeding coefficients estimated from microsatellites or small panels of SNPs have insufficient power to resolve inbreeding across the genome (Kardos et al., 2016; Goszczynski et al., 2018). Pedigree reconstruction and subsequent pedigree-based inbreeding coefficients (F_{PED}) have been shown to be more accurate estimators of inbreeding (Pemberton, 2008), but are rarely possible in the wild because of a lack of reliable pedigree information, or appropriate samples required for molecular based pedigree reconstruction.

Recent advances in DNA sequencing and chromosomal assembly allow us to measure genetic variation at millions of single nucleotide polymorphisms (SNPs) at physically mapped locations across the genome. Such genome-wide polymorphic data is providing unparalleled resolution of genetic variation across landscapes (e.g., crested ibis (Feng et al., 2019), manta and devil rays (Hosegood et al., 2020), and tigers (Armstrong et al., 2021)). These previous studies highlight the importance of

62 understanding geographic boundaries and phylogenetic distinctiveness of populations,
63 which underpins the definition of management units for conservation.
64 In addition, chromosomal patterns of genetic polymorphism can also reveal
65 sections of the genome that are identical-by-descent (IBD), meaning that both copies
66 of DNA in a diploid organism are derived from a recent common ancestor. Mating of
67 related individuals that share a single copy of a chromosomal region results in
68 stretches of homologous sequence that are IBD, also known as homozygosity,
69 commonly referred to as *runs of homozygosity* (ROH) (Kardos et al., 2016; Ceballos et
70 al., 2018). Analysis of ROH is therefore a powerful method for quantifying inbreeding
71 in endangered species. Furthermore, the length distribution of ROH can provide
72 insights into past population dynamics and historical demography (Ceballos et al.,
73 2018, Humble et al 2023). Thus, ROH, and the associated inbreeding coefficient,
74 F_{ROH}, are increasingly used in conservation genomic studies to provide new insights
75 into inbreeding, for example, concerning source populations of reintroduced
76 Scandinavian wolves (Kardos et al., 2018), or killer whales, which demonstrated long-
77 term inbreeding resulting from an ancestral bottleneck (Foote et al., 2021). It has been
78 shown that inbreeding estimates based on F_{ROH}, calculated from millions of genome-
79 wide SNPs, are more accurate and less biased than estimates generated from pedigree
80 data (Kardos et al., 2015). In this study, the power of whole genome re-sequencing is
81 applied to investigating population structure and inbreeding (F_{ROH}) in the golden eagle.
82 The golden eagle (*Aquila chrysaetos*) is a circumglobal avian species,
83 categorized by the IUCN Red List (2021) as Least Concern (LC) based on its
84 estimated global population size (~160,000 individuals). Taxonomically, it has been
85 divided into six subspecies (*A. c. chrysaetos*: northern Europe, *A. c. homeyeri*:

central/southern Europe and mediterranean countries, *A. c. daphanea*: central Asia, *A. c. kamtschatica*: north east Asia, *A. c. japonica*: Japan and Korea, and *A. c. canadensys*: North American continent) based on phenotypic characters such as body size, feather colour, and habitat type (Cramp and Simmons, 1980). However, this taxonomy does not consider molecular genetic data which has become available through a series of studies over the past decade. An analysis of mitochondrial (mt) DNA haplotypes revealed that golden eagle populations are divided into two lineages, namely the Mediterranean (M) haplogroup, restricted to a relatively small distribution around the Mediterranean Sea, and the Holarctic (H) haplogroup, comprising all other global populations (Nebel et al., 2015). In Europe, these lineages overlap in the Alps, with the Swiss, Austrian, German, and Italian populations, consisting of a mixture of both M and H haplotypes. Nuclear DNA microsatellite analysis has demonstrated contemporary geneflow between northern European populations and Mediterranean populations, with Baltic countries supporting an apparent hub population for gene flow within Europe (Nebel et al., 2019).

The same study found Scottish golden eagles to be isolated from Continental Europe, a finding supported by SNP data (Sato et al. 2020), which also suggested that island populations of golden eagles in Scotland and Japan have been isolated from continental populations since the Last Glacial Period. Small island populations of golden eagle, such as the Scottish golden eagle (*A. c. chrysaetos*, 500 breeding pairs), which has been considered part of the continental European population, and Japanese golden eagle (*A. c. japonica*, 500 individuals) are considered to be at elevated risk of extinction (Whitfield et al., 2008; Hayhow et al., 2017; Ministry of the Environment, Japan, 2020; Ogden et al., 2020) compared to larger contiguous continental

populations. Current island population sizes are considered to be the result of declines caused by hunting and poisoning in the UK during the early 20th century (Whatson, 1997), and ongoing habitat degradation, leading to reduced prey availability and low breeding success in Japan (Ogden et al., 2020). As a result, captive breeding programmes have been developed in Japan to act as an insurance for potential future reintroductions. It is important to understand genetic diversity not only of the Japanese subspecies, but also Scottish birds in order to assess the current genetic status of island populations and consider genetically effective conservation strategies.

Previous studies of these island golden eagle populations indicated that the genetic diversity (measured as number of alleles and observed heterozygosity) is not critically low (Ogden et al., 2015; Sato et al., 2017; Naito-Liederbach et al., 2021), and that inbreeding (estimated as *Fis*, an indicator of inbreeding due to non-random mating (Wright, 1965)) may also be low enough to avoid inbreeding depression (*Fis* = 0.06 in Scottish and 0.00 in Japanese, according to Naito-Liederbach et al., 2021). However, these studies were conducted with microsatellites, which provide a poor representation of genome-wide processes and, furthermore, the use of *Fis* itself has been shown to lead to biases in estimating inbreeding, especially with small population sizes that typically limit conservation genetic studies (reviewed by Kardos *et al.* 2016).

The aim of this study is to more precisely measure diversity and inbreeding in island populations, relative to continental populations, using whole genome re-sequencing (WGS) data. We analyse European birds from five localities (Scotland, Switzerland, Mediterranean birds from Spain and Italy, and Northern European birds from Norway) alongside birds from Japan and the USA. First, we re-assessed genomic population structure and phylogenetic relationships among European golden eagles

from across their range and compare genome-wide diversity using millions of SNP markers. Next, we estimate inbreeding of Scottish and Japanese birds using ROH analysis and compare findings with other populations from across the globe. These genomic analyses are used to provide novel insights into the demographic history and contemporary population dynamics of golden eagles at greater resolution than before, providing more information to improve effective *in-situ* and *ex-situ* conservation management.

Materials and methods

Samples

DNA samples of 105 golden eagles from 17 European localities were received from five sets of collaborators for potential inclusion in the study. After checking DNA quality and quantity (QC steps), 24 samples were passed to WGS. Four Japanese birds were also sequenced using samples collected from founder birds in captive (originally rescued from the wild) provided by three zoos in Japan (Akita Omoriyama Zoo (n = 2), Morioka Zoological Park (n = 1) and Tama Zoological Park (n = 1)).

WGS data collection

DNA samples of European birds were sequenced on an Illumina HiSeq X platform (Genewiz, Germany). WGS data were successfully obtained from 17 of the 24 individuals and represent five localities in Europe: Switzerland (n = 8), Scotland (5), Spain (2), Norway (1), and Italy (1). WGS of the four Japanese birds was conducted by Macrogen, Japan, on an Illumina NovaSeq 6000. WGS data of two Japanese birds were available from a previous study (Sato et al., 2020), and data for two American

158 birds were also downloaded from NCBI (SRA: SRX363774 (Doyle et al., 2014: a
159 male bird caught in California) or Hi-C genome data originally from a female bird in
160 Texas State Aquarium, downloaded from DNA zoo
161 (https://www.dnazoo.org/assemblies/aquila_chrysaetos), meaning that in total, WGS
162 data from 25 golden eagles from seven localities were included in the study (Table
163 S1).
164
165 *Bioinformatics and SNP calling*
166 The chromosomal level assembled genome bAquChr1.4 (BioProject: PRJEB27699,
167 Sanger Institute) was used as the reference assembly. Sex-chromosomes (ChrZ and
168 ChrW) were removed, and simple repeat elements were masked using RepeatMasker
169 v4.1.2 (Smit et al., 1996). The final genome size of the assembly was 1.1 Gb. Raw
170 WGS data were filtered for adaptor sequences using Platanus_trim v1.0.7 (Kajitani et
171 al., 2014), and mapped to the reference assembly using BWA-MEM v0.7.17 (Li et al.,
172 2009; Li, 2013). GATK v4.2.2.0 was then used to remove PCR duplicates (McKenna
173 et al., 2010). SNPs were called using two different methods: ANGSD and GATK.
174 SNPs called by ANGSD v.0.936 (Korneliussen et al., 2014) were used for calculating
175 population admixture, reconstructing phylogenetic relationships (Neighbour-Joining
176 method) and estimating genome-wide heterozygosity and nucleotide diversity (π).
177 SNPs called by GATK v4.2.2.0 were used in Principal Components Analysis (PCA),
178 reconstructing phylogenetic relationships (Maximum-likelihood method), and
179 assessment of inbreeding by ROH analysis. See Supplementary Methods for detailed
180 SNP calling and filtering procedures.
181

Population genetic structure

Population structure was first assessed through PCA using PLINK 1.9 (Purcell et al., 2007), to investigate the presence of genetic clustering among birds and localities. Intra-specific phylogenetic reconstruction was conducted using maximum likelihood tree reconstruction in RAxML-NG v.1.1 (Kozlov et al., 2019); node support was calculated from 500 bootstrap replicates and the tree was visualised in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). A second tree was generated with the Neighbour-Joining method using ANGSD. In both trees, a white-tailed eagle (*Haliaeetus albicilla*), originally in Japan, was used as the outgroup (NCBI: DRR191146). Population admixture simulations (Rius and Darling, 2014) were conducted in NGSadmix (Skotte et al., 2013) to investigate the most likely number of population genetic clusters (K) and inter-populations (intraspecific) introgression, using three runs at each number of putative clusters (K = 1 to 7). The most suitable result was selected based on likelihood scores of each K.

Comparative analysis of genetic diversity and inbreeding among regions

Genetic diversity was assessed using estimates of genome-wide heterozygosity and nucleotide diversity (π). Both indicators were calculated using the 'real Site-Frequency Spectrum (realSFS)' computed by ANGSD. Our measures of genome-wide heterozygosity were calculated on an individual basis, while π was calculated on a locality/population basis depending on the outputs of population structure. Note that π was estimated from only Japan, Scotland, Switzerland or Central/South Europe (Switzerland + Italy + Spain) which have more than five samples in this study.

Inbreeding in each locality/population was assessed using estimates of ROH. The

analysis was conducted using PLINK following the recommended settings in Meyermans et al. (2020) and a homozygous length threshold > 250 Kb. The inbreeding coefficient of ROH (F_{ROH}) was then calculated from the total length of ROH segments >1 Mb over the total assembly genome size (1.1 Gb) as the proportion of ROH length on the genome (Keller et al., 2011; Thompson, 2013). We also calculated the total number of ROH segments > 1 Mb (NROH) and sum and average length (Mb) of ROH > 1 Mb (SROH and ALROH, respectively). To explore the contribution of different ROH lengths to overall homozygosity, we further calculated F_{ROH} for different ROH length classes. For this, ROH were classified into five length classes at 0.5 Mb increments: 0.25-0.5 Mb, 0.5-1.0 Mb, 1.0-1.5 Mb, 1.5-2.0 Mb, and > 2.0 Mb. The fraction of the genome in ROH for each length class was then calculated. ROH calculations were conducted for each individual golden eagle in this study, with results compared across localities /populations and visualised in R (4.3.0) and RStudio (2023.06.2+561) with ggplot2 contained in tidyverse package (2.0.0) (R Core Team, 2022; RStudio Team, 2022; Wickham et al., 2019).

In addition, pairwise relatedness of every combination of birds in each population was calculated by NgsRelates software (Hanghøj et al., 2019) with the same SNPs data set of our ROH analysis.

Results

Genome sequencing, SNP discovery and filtering

The average read depth across the seventeen European birds successfully sequenced was 13.67x (min: 11.91, max: 19.29) after mapping and removing PCR duplicates (Table S1). The average sequence read depth of the six Japanese birds was 34.08x

(30.62-37.23), and that of two American birds was 24.36x (25.06 and 23.66). A total of 6,611,278 SNP sites were detected across all 25 golden eagles before SNP filtering and these sites were used for the ROH analysis. Following sequential filtering, a minimum panel of 1,742,617 SNP sites was used for the PCA and phylogenetic calculations.

Genomic re-assessment of population structure

The PCA plot divides the 25 golden eagles from seven breeding localities into five genetic clusters (Figure 1A). The first axis (PC1) distinguishes American (n = 2), Japanese (n = 6) and European birds (n = 17), with the European birds segregated into three clusters on the second axis (PC2): Central/South Europe (n = 11, Italy, Spain, and Switzerland), Northern Europe (n = 1, Norway), and Scotland (n = 5).

Population admixture analysis revealed that K = 5 is the best supported model (log-likelihood = 31.42), reinforcing the broad geographic clusters found under PCA (Figure 1B). There is no evidence from the more contemporary nuclear genome analysis of the historic mitochondrial divergence between the Mediterranean and Holarctic lineages (Nebel et al., 2015) where they overlap in Switzerland, with birds displaying different haplotypes forming a single nuclear DNA genetic cluster.

Phylogenetic reconstruction yielded well resolved ML and NJ trees, both of which recovered clear geographic groupings and showed Japanese and USA birds to be sister clades to all European birds, with the latter branching into Norwegian, Scottish, Italian, Spanish and Swiss nodes, in broad agreement with PCA and admixture analyses (Figure 2 and Figure S1).

Comparative analysis of genetic diversity and inbreeding

Golden eagles in Scotland and Japan displayed consistently lower genetic diversity than other European localities and the USA, measured either by individual genome-wide heterozygosity (Figure 3), or population-wide nucleotide diversity (π) (Table S2).

Consistent with the theory that inbred populations have more and longer ROH, Scottish birds and Japanese birds had more ROH segments > 1 Mb (NROH) (on average, 84.8 (s.d. = 11.86) segments in Scotland, 72.5 (9.43) in Japan, but 25–53 in others, Figure 4A and Table S2). However, the Italian bird in this study had the longest average length of ROH (ALROH, 1.98 Mb), with those of Japan and Scotland slightly shorter (1.90 (s.d. = 0.16) and 1.87 (0.07) Mb, respectively) (Table S2, Figure S2 and S3). The correlation of NROH and SROH (Sum of ROH length) reflected the relationship with population size: the small populations of Scotland and Japan showing higher NROH with longer SROH than those of other localities which maintain larger population sizes (Figure 4A). The inbreeding coefficient of ROH (F_{ROH}) revealed that Scottish and Japanese birds display markedly higher levels of inbreeding than continental birds (Figure 4B and Table S2). In addition, although there was a wide variety of ROH lengths within eight Swiss and two US birds, their inbreeding levels were similar to those of other European continental birds.

Our results above suggest that both Scottish birds and Japanese birds experienced inbreeding, but the proportion of ROH segments in each length class differed between these populations (Figure 5A and S4). Scottish birds had more ROH segments in every length class, including shorter lengths (0.25–1.0 Mb), than other continental European populations. On the other hand, Japanese birds had similar proportions of ROH segments in shorter length classes to continental birds, but at longer ROH length classes, were similar to Scottish birds between 1.0–2.0 Mb in

length, and higher than Scottish birds in the longest class (more than 2 Mb). This resulted in Scottish birds having more ROH segments in every length class, while Japanese birds had much longer ROH segments than other populations.

In addition, Japanese birds and Scottish birds showed slightly higher values of pairwise relatedness (0.35-.043 or 0.31-0.40, respectively) while the range of Central/South European was 0.03-0.20 and that of USA was 0.38, although our samples were collected from birds considered to be unrelated, based on sampling locations (Table S3).

Discussion

This study enabled a genome-wide assessment of golden eagles from three continents for the first time, revealing the nuclear genetic relationships between four of the six subspecies (*A. c. chrysaetos*, *A. c. homeyeri*, *A. c. japonica*, and *A. c. canadensys*) and significantly improving our understanding of comparative diversity and inbreeding in the small island populations of the UK and Japan.

Population structure of golden eagles

Within Europe, the results of PCA, phylogenetic analysis and admixture analysis confirm the genetic distinctiveness of Scottish, northern European and central/southern European eagles, with the Swiss birds showing greater affinity to Mediterranean birds. This finding largely corroborates previous microsatellite analyses (Nebel et al., 2019), which suggests that contemporary geneflow is no longer congruent with historic mtDNA lineages. The earlier study indicated connectivity, albeit reduced, between the Swiss alps and Scandinavian / Baltic region and more samples for resequencing from

Germany, Poland and Denmark, as well as the far north of Scandinavia, are now being sought to examine this transition in more detail. These findings contribute to the wider discussion of golden eagle subspecies taxonomy, with some discordance between traditional classifications and contemporary population genetic structure in southern Europe.

The marked separation of Scotland and Japan from continental Eurasian and North American birds in the PCA analysis is likely to be driven by a reduced diversity in the island populations, rather than particularly novel distinct genetic variation. The fact that European, Asian and American birds segregate along the first PC axis, with variation within Europe on the second axis, suggests that more genetic variance is distributed cirumglobally than north-south within Europe, in contrast to previous mtDNA studies (Nebel et al., 2015). This was also clearly suggested from our phylogenetic analyses; the common ancestor of Asian and American birds was separated before the divergence of European birds. It is known that the golden eagle is a truly circumglobal species distributed from west coast to east coast of the Eurasian and north American continents (Watson, 1997). Further sampling throughout this range to assess genomic admixture and re-assess subspecies classifications and distribution is underway.

Genetic diversity and inbreeding

The low genetic diversity highlighted by both heterozygosity and nucleotide diversity in Scottish and Japanese golden eagles, compared with other European localities and the USA agrees with previous population genetics studies with smaller scale marker panels (Ogden et al., 2015; Sato et al., 2017; Nebel et al., 2019; Naito-Liederbach et

al., 2021). In addition, a recent assessment of major histocompatibility complex (MHC) diversity revealed lower diversity in Japanese golden eagles compared with non-endangered raptor species (Naito-Liederbach et al., 2021). Overall, this highlights how Scottish and Japanese island populations have reduced genetic diversity at both neutral and functional genomic regions. Inbreeding will increase the number of homologous alleles in an individual's genome, raising the risk of inbreeding depression through the inheritance of deleterious recessive homozygous genotypes (Ceballos et al., 2018). We might therefore infer that populations with elevated levels of inbreeding are at greater risk from inbreeding depression. However, the relationship between levels of heterozygosity/inbreeding and individual fitness remains untested in the golden eagle highlighting the need for further work using empirical data or assessing mutation load.

Bottleneck events and ongoing reductions in population size will increase the number of ROH, with longer ROH segments (hundreds Kb to over 1–2 Mb) resulting from recent shared parental ancestry, because recombination has had little opportunity to break them up. In contrast, shorter ROH are indicative of smaller population sizes in the more distant past (Ceballos et al., 2018). It follows that recent or ongoing population reductions are likely to confer a greater inbreeding effect than small populations that declined many generations ago (Charlesworth and Wills, 2009). Our ROH results clearly suggest that Scottish birds have been subject to close inbreeding relative to other European birds. The Scottish population experienced strong bottleneck events in 19^{th} ~ early 20^{th} century and its population size was reduced to around 100-200 pairs at that time (Watson, 1997). Our results of Scottish birds probably refer to this bottleneck history, especially as they were characterised with

more ROH segments in shorter length classes indicating the effect of meiotic recombination during the population recovery through the 20th century (Figure 5 and S4). The long generation time of the golden eagle (17 years, according to the IUCN) might act as a buffer to the loss of genetic diversity per generation. On the other hand, the population size of Japanese birds has decreased much more recently, since the 1970's (SRGE-J, 2017), and has experienced reduced breeding success. Our result of more ROH segments in longer length classes in Japanese birds probably reflects this pattern.

Unlike previous estimates of inbreeding coefficients from microsatellites (*Fis*) (Sato et al., 2017; Naito-Liederbach et al., 2021), the individual inbreeding inferred from F_{ROH} data in this study, on average 0.144 in Scotland and 0.126 in Japan, also suggests that golden eagles in Scotland and Japan have a higher proportion of homologous regions in their genome than birds in other regions. A review of ROH analysis in 78 mammalian species including both non-endangered and endangered taxa revealed that the average of F_{ROH} of these animals was 0.0745 (± 0.134 SD) (Brüniche-Olsen et al., 2018). Our results show that golden eagles on islands have much higher inbreeding levels, when compared to other species. We conclude that previously applied small microsatellite panels had insufficient power to detect inbreeding in golden eagles.

Swiss and American birds presented a wide range of ROH lengths among individuals, but their inbreeding levels were almost the same as non-endangered populations in Europe. It is known that genomic data of American birds (n = 2) were obtained from both a wild (lower inbreeding level, Doyle et al., 2014) and a captive individual (higher inbreeding level, Texas State Aquarium), therefore it is easy to

understand the variety in American samples. On the other hand, samples of Swiss birds were all wild, collected from across their national range. Here, the difference in ROH lengths probably represents the natural variation in inbreeding levels within the Swiss population, highlighting the need to achieve good sample numbers for comparative ROH analysis wherever possible.

On the other hand, our results of pairwise relatedness indicate that while relatedness varied across populations and was notably high in Japan and Scotland, this is likely to be a function of population level inbreeding, rather than individuals relatedness, and there were no pairs of birds in any population showing significantly increased relatedness compared to their population averages.

Conservation implications for Scottish and Japanese golden eagles

While the overall patterns of population genetic structure observed in this study were largely concordant with previous work, our ROH analysis revealed evidence of a reduced genetic diversity and inbreeding events in both Scottish and Japanese birds, which were not suggested from previous genetic studies using microsatellites. Given the lack of gene flow between continental populations and these endangered island populations today (Nebel et al., 2019; Sato et al., 2020), it is unlikely that genetic diversity will recover through natural gene flow. There is no veterinary or ecological evidence reported to indicate that island eagles are suffering from inbreeding depression; however very low reproductive rates in certain parts of Scotland and Japan remain unexplained.

It may be appropriate to start considering genetic supplementation of Scottish and Japanese golden eagle populations with continental birds. It is still unclear how

functional genetic variation contributes to local adaptation to the various habitats distributed widely in the northern hemisphere. It is also known that local adaptation raises the possibility of outbreeding depression during population reinforcement, which may cause a loss of fitness due to reproduction between birds that are adaptively divergent (Templeton, 1986; Frankham et al., 2011). The use of genomic data to differentiate neutral and non-neutral genetic variation and further investigation of the genetic basis of golden eagle subspecies at a global scale is underway; however it is important to pay close attention to maintaining genetic diversity of both Scottish and Japanese golden eagles through applied conservation genetic management, in order to avoid the opposing risks of inbreeding depression, particularly as both countries are considering national range expansion through localised reintroductions.

In the case of the Scottish golden eagle, efforts have begun to extend the species distribution in the south of Scotland (South of Scotland Golden Eagle Project; https://www.goldeneaglessouthofscotland.co.uk/) where a series of conservation translocations have been conducted to reinforce an outlying population. Further translocations into England and Wales have been proposed. To limit genetic bottlenecking due to local founder effects, it is important that genomic data are made available to inform individual selection and to monitor the changes in genetic diversity over time. Similar population genomic analysis is underway to support the expansion of the UK white-tailed eagle population, first introduced into Scotland and now being serially translocated to England.

In Japan, an intensively managed captive breeding programme is underway as a source for future reinforcement attempts (Ogden et al., 2020), which has included previous rounds of population genetic analysis. Our findings in the current study

contrast somewhat with earlier microsatellite data that suggested genetic diversity was not particularly low when comparing wild Japanese birds with captive Japanese birds and wild Scottish birds (Sato et al., 2017; Naito-Liederbach et al., 2021). On the other hand, comparative microsatellite data from the Mongol-Altai Mountains, Central-Northeast Asia, and Northern Europe indicate that genetic diversity in Japan is lower than other parts of Asia (Nebel et al., 2023); broadly supporting our findings here. The results of our genomic analysis indicate that reduced diversity may already be leading to significant inbreeding in Japan.

The transition towards genomic data in conservation genetic management is technically challenging, but is starting to deliver genuine value by revealing the genetic effects of very recent demographic change. The outcomes of this study are presented as a starting point for conservation genomic analysis in golden eagles, hopefully contributing to their conservation not only in Scotland and Japan, but globally.

Acknowledgements

We are grateful to sample providers of golden eagles in Europe: South Scotland Golden Eagle project; Natural History Museum, Vienna, Austria; Swiss Ornithological Institute; University of Copenhagen, Denmark; and Zoobotánico de Jerez, Cadiz, Spain. In addition, we also thank to zoos in Japan for providing samples of Japanese birds: Akita Omoriyama Zoo, Morioka City Zoological Park (ZOOMO), and Tama Zoological Park.

We obtained great suggestions for this study from members of Japan × UK Bilateral program on the Conservation of Golden Eagles (JSPS grant no:

JPJSBP120219921; Royal Society of Edinburgh SAPHIRE grant no: 2808). We are especially grateful for beneficial discussions with Prof. Miho Inoue-Murayama and Ms. Annegret M. Naito-Liederbach (Kyoto University), and Dr. Taku Maeda (Iwate Prefectural Research Institute for Environmental Sciences and Public). In addition, we thank Frank Zachos and two anonymous reviewers for their comments which helped improve this paper. This work was mainly conducted during the period that YS was at the University of Edinburgh as a JSPS Overseas Research Fellow and financially supported by JSPS Overseas Research Fellowship in 2020–2022.

Data accessibility

WGS re-sequencing data of European golden eagles and Japanese are available with given BioSample Accession of SAMD00454443-SAMD00454467 in Table S2 or BioProject Accession of PRJDB13385. Genome sequencing data of American birds are available from SRA: SRX363774 (Doyle et al., 2014) or DNA zoo (https://www.dnazoo.org/assemblies/aquila_chrysaetos).

References

Armstrong EE, Khan A, Taylor RW, Gouy A, Greenbaum G, Thiéry A, Kang JT, Redondo SA, Prost S, Barsh G, Kaelin C, Phalke S, Chugani A, Gilbert M, Miquelle D, Zachariah A, Borthakur U, Reddy A, Louis E, Ryder OA, Jhala YV, Petrov D, Excoffier L, Hadly E, Ramakrishnan U. 2021. Recent evolutionary history of tigers highlights contrasting roles of genetic drift and selection. *Molecular biology and evolution*, 38(6), 2366-2379. https://doi.org/10.1093/molbev/msab032

Bonfield JK, Marshall J, Danecek P, Li H, Ohan V, Whitwham A, Keane T, Davies RM. 2021. HTSlib: C library for reading/writing high-throughput sequencing data. *Gigascience*, 10(2), giab007. https://doi.org/10.1093/gigascience/giab007

Brüniche-Olsen A, Kellner KF, Anderson CJ, DeWoody JA. 2018. Runs of homozygosity have utility in mammalian conservation and evolutionary studies. *Conservation Genetics*, 19(6), 1295-1307. https://doi.org/10.1007/s10592-018-1099-y

Byers O, Lees C, Wilcken J, Schwitzer C. 2013. The One Plan Approach: The philosophy and implementation of CBSG's approach to integrated species conservation planning. *World association of Zoos and Aquariums Magazine*, 14, 2–5.

Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. *Nature reviews genetics*, 10(11), 783-796. https://doi.org/10.1038/nrg2664

Ceballos FC, Joshi PK, Clark DW, Ramsay M, Wilson JF. 2018. Runs of homozygosity: windows into population history and trait architecture. *Nature Reviews Genetics*, 19(4), 220-234. https://doi.org/10.1038/nrg.2017.109

Cramp S, Simmons KEL. 1980. The Birds of the Western Palearctic. Vol 2. University Press, Oxford.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R,1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156-2158. https://doi.org/10.1093/bioinformatics/btr330

Doyle JM, Katzner TE, Bloom PH, Ji Y, Wijayawardena BK, DeWoody JA. 2014. The genome sequence of a widespread apex predator, the golden eagle (*Aquila*

494 *chrysaetos*). *PloS one*, 9(4), e95599.
495 https://doi.org/10.1371/journal.pone.0095599
496 Feng S, Fang QI, Barnett R, Li C, Han S, Kuhlwilm M, Zhou L, Pan H, Deng Y, Chen
497 G. Gamauf A, Woog F, Prys-Jones R, Marques-Bonet T, Gilbert MTP, Zhang G.
498 2019. The genomic footprints of the fall and recovery of the crested ibis. *Current*
499 *Biology*, 29(2), pp.340-349. https://doi.org/10.1016/j.cub.2018.12.008
500 Flouri T, Izquierdo-Carrasco F, Darriba D, Aberer AJ, Nguyen LT, Minh BQ, von
501 Haeseler A, Stamatakis A. 2014. The Phylogenetic Likelihood Library. *Systematic*
502 *Biology*, 64(2): 356-362. https://doi.org/10.1093/sysbio/syu084
503 Foote AD, Hooper R, Alexander A, Baird RW, Baker CS, Ballance L, Barlow J,
504 Brownlow J, Collins T, Constantine R, Dalla Rosa L, Davison NJ, Durban JW,
505 Esteban R, Fordyce Martin SL, Forney KA, Gerrodette T, Gilbert MTP, Guinet C,
506 Hanson MB, Li S, Martin MD, Robertson KM, Samarra FIP, de Stephanis R,
507 Tavares SB, Tixier P, Totterdell JP, Wade P, Wolf JBW, Fan G, Zhang Y, Morin
508 PA. 2021. Runs of homozygosity in killer whale genomes provide a global record
509 of demographic histories. *Molecular Ecology*, 30(23), 6162-6177.
510 https://doi.org/10.1111/mec.16137
511 Frankham R, Ballou JD, Briscoe DA, McInnes KH. 2009. Introduction to
512 Conservation Genetics. Cambridge Univ Press, Cambridge, UK.
513 Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, Fenster CB.
514 2011. Predicting the probability of outbreeding depression. Conservation Biology,
515 25(3), 465-475. https://doi.org/10.1111/j.1523-1739.2011.01662.x
516 Fraser DJ, Bernatchez L. 2001. Adaptive evolutionary conservation: towards a unified
517 concept for defining conservation units. *Molecular ecology*, 10(12), 2741-2752.

518 https://doi.org/10.1046/j.0962-1083.2001.01411.x
519 Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of species
520 extinction. In Conservation biology: the science of scarcity and diversity. Edited
521 by M.E. Soulé. Sinauer Associates, Sunderland, Mass., USA.
522 Goszczynski D, Molina A, Terán E, Morales-Durand H, Ross P, Cheng H,
523 Giovambattista G, Demyda-Peyrás S. 2018. Runs of homozygosity in a selected
524 cattle population with extremely inbred bulls: Descriptive and functional analyses
525 revealed highly variable patterns. *PLoS One*, 13(7), e0200069.
526 https://doi.org/10.1371/journal.pone.0200069
527 Hanghøj K, Moltke I, Andersen PA, Manica A, Korneliussen TS. 2019. Fast and
528 accurate relatedness estimation from high-throughput sequencing data in the
529 presence of inbreeding. *Gigascience*, 8(5), giz034.
530 https://doi.org/10.1093/gigascience/giz034
531 Hayhow DB, Benn S, Stevenson A, Stirling-Aird PK, Eaton MA. 2017. Status of
532 golden eagle *Aquila chrysaetos* in Britain in 2015. *Bird Study*, 64(3), 281-294.
533 https://doi.org/10.1080/00063657.2017.1366972
534 Hosegood J, Humble E, Ogden R, De Bruyn M, Creer S, Stevens GM, Abudaya M,
535 Bassos-Hull K, Bonfil R, Fernando D, Foote AD, Hipperson H, Jabado RW,
536 Kaden J, Moazzam M, Peel LR, Pollett S, Ponzo A, Poortvliet M, Salah J, Senn
537 H, Stewart JD, Wintner S, Carvalho G. 2020. Phylogenomics and species
538 delimitation for effective conservation of manta and devil rays. *Molecular*
539 *Ecology*, 29(24), 4783-4796. https://doi.org/10.1111/mec.15683
540 Humble E, Stoffel MA, Dicks K, Ball AD, Gooley RM, Chuven J, Pusey R, Remeithi
541 MA, Koepfli K, Pukazhenthi B, Senn H, Ogden R. 2023. Conservation

management strategy impacts inbreeding and mutation load in scimitar-horned oryx. *Proceedings of the National Academy of Sciences*, 120(18), e2210756120. https://doi.org/10.1073/pnas.2210756120

Kajitani R, Toshimoto K, Nogushi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, Kohara Y, Fujiyama A, hayashi T, Itoh T. 2014. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. *Genome research*, 24(8), 1384-1395. https://doi.org/10.1101/gr.170720.113

Kardos M, Åkesson M, Fountain T, Flagstad Ø, Liberg O, Olason P, Sand H, Wabakken P. Wikenros C, Ellegren H. 2018. Genomic consequences of intensive inbreeding in an isolated wolf population. *Nature ecology & evolution*, 2(1), 124-131. https://doi.org/10.1038/s41559-017-0375-4

Kardos M, Luikart G, Allendorf FW. 2015. Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. *Heredity*, 115(1), 63-72. https://doi.org/10.1038/hdy.2015.17

Kardos M, Taylor HR, Ellegren H, Luikart G, Allendorf FW. 2016. Genomics advances the study of inbreeding depression in the wild. *Evolutionary applications*, 9(10), 1205-1218. https://doi.org/10.1111/eva.12414

Keller MC, Visscher PM, Goddard ME. 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics*, 189(1), 237-249. https://doi.org/10.1534/genetics.111.130922

Korneliussen TS, Albrechtsen A, Nielsen R. 2014. ANGSD: analysis of next generation sequencing data. *BMC bioinformatics*, 15(1), 1-13. https://doi.org/10.1186/s12859-014-0356-4

566 Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: A fast,
567 scalable, and user-friendly tool for maximum likelihood phylogenetic inference.
568 *Bioinformatics*, 35 (21), 4453-4455 https://doi.org/10.1093/bioinformatics/btz305
569 Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with
570 BWA-MEM. arXiv preprint arXiv:1303.3997
571 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler
572 transform. *Bioinformatics*, 25(14), 1754-1760.
573 https://doi.org/10.1093/bioinformatics/btp324
574 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella
575 K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis
576 Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing
577 data. *Genome research*, 20(9), 1297-1303. https://doi.org/10.1101/gr.107524.110
578 Meyermans R, Gorssen W, Buys N, Janssens S. 2020. How to study runs of
579 homozygosity using PLINK? A guide for analyzing medium density SNP data in
580 livestock and pet species. *BMC genomics*, 21(1), 1-14.
581 https://doi.org/10.1186/s12864-020-6463-x
582 Naito-Liederbach AM, Sato Y, Nakajima N, Maeda T, Inoue T, Yamazaki T, Ogden R,
583 Inoue-Murayama, M. 2021. Genetic diversity of the endangered Japanese golden
584 eagle at neutral and functional loci. *Ecological Research*, 36(5), 815-829.
585 https://doi.org/10.1111/1440-1703.12246
586 Nebel C, Gamauf A, Haring E, Segelbacher G, Villers A, Zachos FE. 2015.
587 Mitochondrial DNA analysis reveals Holarctic homogeneity and a distinct
588 Mediterranean lineage in the Golden eagle (Aquila chrysaetos). *Biological*
589 *Journal of the Linnean Society*, 116(2), 328-340.

https://doi.org/10.1111/bij.12583

Nebel C, Gamauf A, Haring E, Segelbacher G, Väli Ű, Zachos FE. 2019. New insights into population structure of the European golden eagle (*Aquila chrysaetos*) revealed by microsatellite analysis. *Biological Journal of the Linnean Society*, 128(3), 611-631. https://doi.org/10.1093/biolinnean/blz130

Nebel C, Haring E, Murgatroyd M, Sumasgutner SC, Gombobaatar S, Sumasgutner P, Zachos FE. 2023. Genetic Analysis of Golden Eagles (*Aquila chrysaetos*) from the Mongol-Altai: A Hotspot of Diversity and Implications for Global Phylogeography. *Journal of Raptor Research*, 57 (3), 359–374. https://doi.org/10.3356/JRR-22-57

Ogden R, Heap E, McEwing R, Tingay R, Whitfield DP. 2015. Population structure and dispersal patterns in Scottish golden eagles *Aquila chrysaetos* revealed by molecular genetic analysis of territorial birds. *Ibis* 157:837–848. https://doi.org/10.1111/ibi.12282

Ogden R, Fukuda T, Funo T, Komatsu M, Maeda T, Meredith A, Miura M, Natsukawa H, Onuma M, Osafune Y, Saito K, Sato Y, Thompson D, Inoue-Murayama M. 2020. Japanese golden eagle conservation science: current status and future needs. *Japanese Journal of Zoo and Wildlife Medicine*, 25(1), 9–28. https://doi.org/10.5686/jjzwm.25.9

Ortiz EM. 2019. vcf2phylip v2. 0: convert a VCF matrix into several matrix formats for phylogenetic analysis. URL https://doi org/105281/zenodo, 2540861.

Pemberton JM. 2008. Wild pedigrees: the way forward. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), 613-621. https://doi.org/10.1098/rspb.2007.1531

614 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar
615 P, de Bakker PIW, Daly MJ, Sham PC. 2007. PLINK: a tool set for whole-
616 genome association and population-based linkage analyses. *The American*
617 *journal of human genetics*, 81(3), 559-575. https://doi.org/10.1086/519795
618 R Core Team. 2022. R: A language and environment for statistical computing. R
619 Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-
620 project.org/.
621 Rius M, Darling JA. 2014. How important is intraspecific genetic admixture to the
622 success of colonising populations? *Trends in ecology & evolution*, 29(4), 233-
623 242. https://doi.org/10.1016/j.tree.2014.02.003
624 RStudio Team. 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston,
625 MA URL http://www.rstudio.com/.
626 Sato Y, Ogden R, Komatsu M, Maeda T, Inoue-Murayama M. 2017. Integration of
627 wild and captive genetic management approaches to support conservation of the
628 endangered Japanese golden eagle. *Biological Conservation*, 213, 175-184.
629 https://doi.org/10.1016/j.biocon.2017.07.008
630 Sato Y, Ogden R, Kishida T, Nakajima N, Maeda T, Inoue-Murayama, M. 2020.
631 Population history of the golden eagle inferred from whole-genome sequencing
632 of three of its subspecies. *Biological Journal of the Linnean Society*, 130(4), 826-
633 838. https://doi.org/10.1093/biolinnean/blaa068
634 Skotte L, Korneliussen TS, Albrechtsen A. 2013. Estimating individual admixture
635 proportions from next generation sequencing data. *Genetics*, 195(3), 693–702.
636 https://doi.org/10.1534/genetics.113.154138
637 Smit A, Hubley R, Green P. 1996. REPEATMASKER Open-3.0

https://www.repeatmask er.org.

Society for Research of Golden Eagle Japan (SRGE-J). 2017. Population and breeding success of the golden eagle *Aquila chrysaetos* in Japan, 1981 to 2015. *Aquila chrysaetos* 26*,* 1-16 (in Japanese with English abstract).

Templeton AR. 1986. Coadaptation and outbreeding depression. Pages 105–166 in M. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts.

Thompson EA. 2013. Identity by descent: variation in meiosis, across genomes, and in populations. *Genetics*, 194(2), 301-326. https://doi.org/10.1534/genetics.112.148825

Van Den Bussche RA, Judkins ME, Montague MJ, Warren WC. 2017. A resource of genome-wide single nucleotide polymorphisms (Snps) for the conservation and management of golden eagles. *Journal of Raptor Research*, 51(3), 368-377. https://doi.org/10.3356/JRR-16-47.1

Wang S, Haynes C, Barany F, Ott J. 2009. Genome-wide autozygosity mapping in human populations. *Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society*, 33(2), 172-180. https://doi.org/10.1002/gepi.20344

Watson J. 1997. The golden eagle. A & C Black. London, UK.

Weeks AR, Stoklosa J, Hoffmann AA. 2016. Conservation of genetic uniqueness of populations may increase extinction likelihood of endangered species: the case of Australian mammals. *Frontiers in Zoology*, 13(1), 1-9. https://doi.org/10.1186/s12983-016-0163-z

Whitfield DP, Fielding AH, McLeod DRA, Haworth PF. 2008. A conservation

framework for golden eagles: implications for their conservation and management in Scotland. Scottish Natural Heritage Commissioned Report No.193 (ROAME No. F05AC306).

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. 2019. Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), 1686. http://doi:10.21105/joss.01686.

Wright S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution, 19(3), 395-420. https://doi.org/10.2307/2406450

Figures

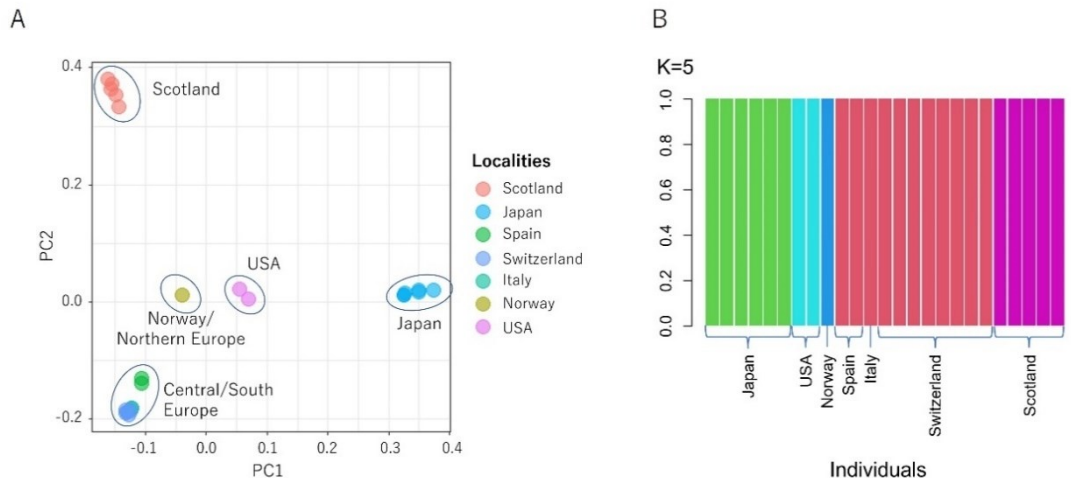


Figure 1. Population reassessment reveals seven breeding localities of golden eagles into five populations. A: The PCA plots based on the genome-wide SNPs of seven localities of golden eagles in Europe, Japan, and USA. Each point represents an individual. Genomic SNPs re-grouped seven sampling localities with four phenotypic subspecies into five genetic clusters. B: Population admixture result of K =5 (the best supported model). Each vertical bar represents an individual. This reinforces the broad geographic clusters found under PCA.

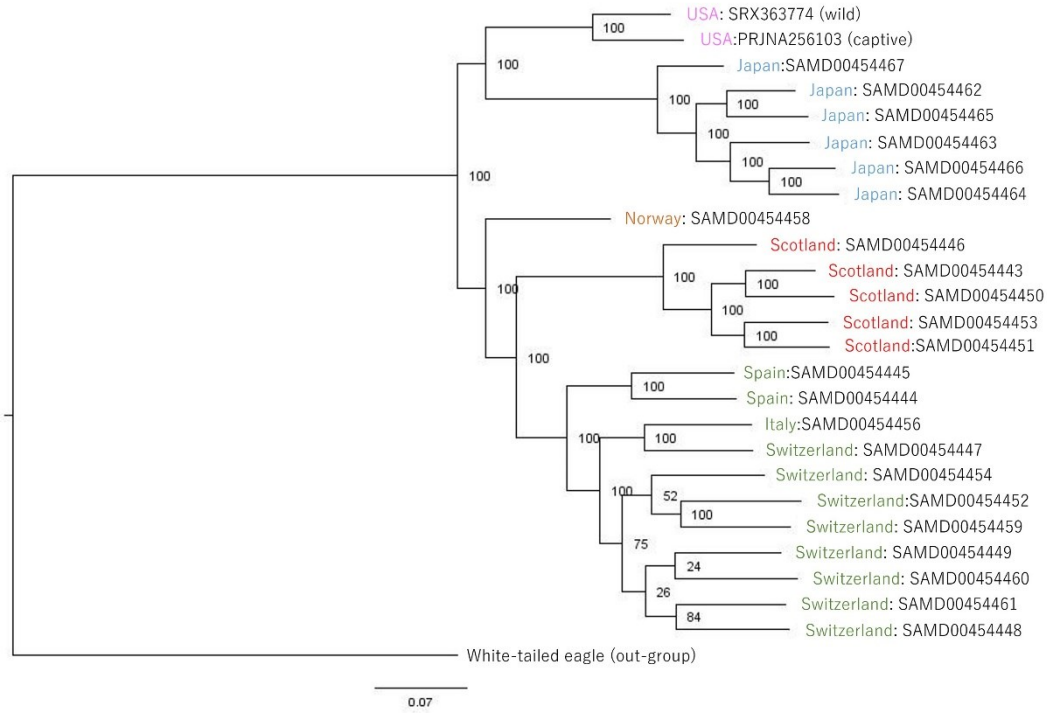
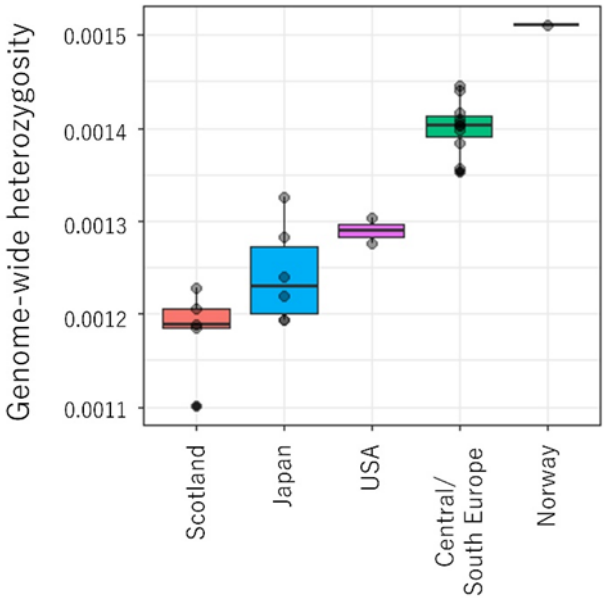


Figure 2. The Maximum-Likelihood (ML) tree of golden eagles calculated from only variant sites after SNPs filtering steps. The bootstrapping of 500 times was conducted and its value was shown for each node. Japanese and USA birds were distinguished from European birds. European birds were potentially separated into three populations: Scotland, Northern Europe (Norway), Central/Southern Europe including Italy, Spain, and Switzerland. This phylogenetic result also reinforced the broad geographic clusters found under PCA.



706

707 Figure 3. Genome-wide heterozygosity of each bird in each population by boxplot.

708 The Central/South Europe includes birds from Switzerland, Italy and Spain. The

709 Northern Europe includes a bird of Norway. Boxplots show median, and interquartile

710 ranges of genome-wide heterozygosity, and each point represents an individual.

711

712

713

714

715

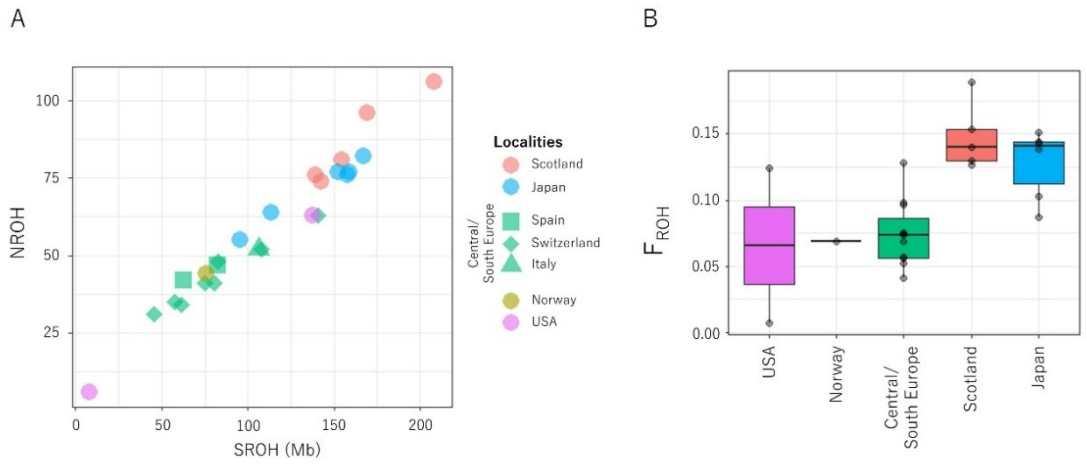


Figure 4. The results of ROH analysis for revealing inbreeding. A: the number of ROH segments (NROH) and its total length (sum) (SROH (Mb)) of golden eagles. Each dot shows each individual. Green dots are birds from Central/South Europe, with different shapes of dots referring Spain, Switzerland, or Italy. B: The inbreeding coefficient of ROH (F_{ROH}) which was derived from the proportion of ROH on the length of genome assembly (1.1 Gb of golden eagle). Boxplots show median, and interquartile ranges of F_{ROH} in each population, and each dot shows each individual. Scottish and Japanese birds have more and longer ROH, and F_{ROH} is also higher than other localities.

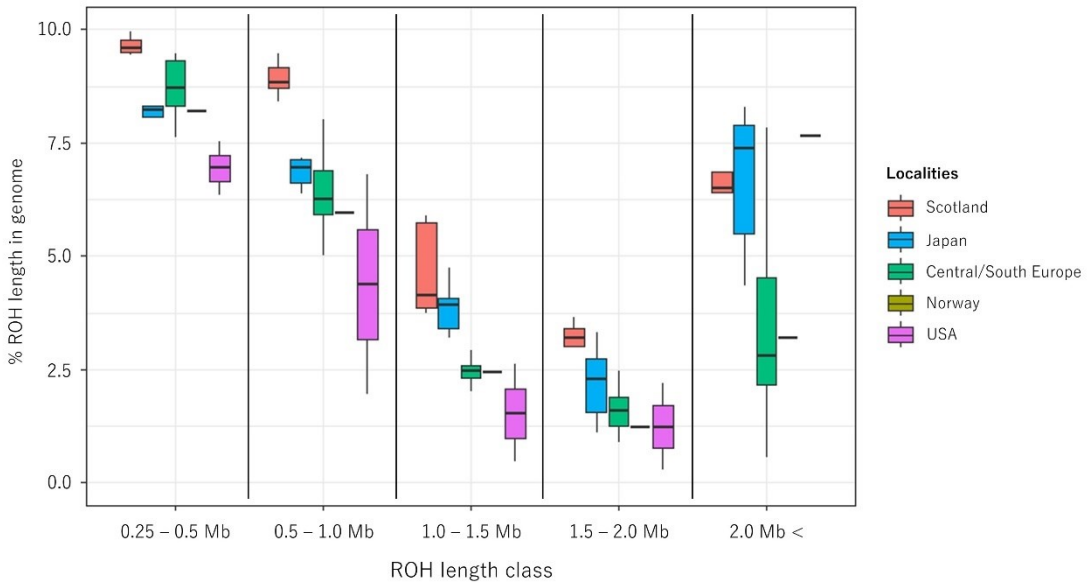


Figure 5. Distribution of ROH segments in five length classes and comparing among five populations. The ratio (%) of ROH length was calculated by sum of ROH segments/genome assembly (1.1 Gb) in each class. Scottish birds have more ROH than others in every length class. On the other hand, Japanese birds also have more and longer ROH segments especially in longer length classes of more than 1.0 Mb, however the genomic ratio of shorter segments (0.25–0.5 Mb and 0.5–1.0 Mb) is similar as other continental European birds.

Appendix

Supplementary Methods

Samples

DNA extraction

In total 105 samples of European golden eagles in 17 localities, 93 samples were extracted DNA, and 12 samples were tissues (ten from Scotland and two from Spain, liver or blood). DNA was extracted from each of these tissues using a DNeasy Blood & Tissue kit (Qiagen, Germany) following the manufacturer's protocol, eluting the DNA in TE buffer. All DNA samples were stored at -20 ℃ in Roslin institute, UK, until quality checking ahead of genome re-sequencing. Furthermore, samples of four Japanese founder birds were also blood samples. DNA was also extracted with the same procedure from whole blood samples and stored at -20 ℃ in Wildlife Research Centre, Kyoto university, Japan.

DNA quality checking (QC) and WGS data collection

DNA quantity and quality was assessed using Qubit and gel electrophoresis (1% agarose gel, 20 min) in Roslin. Based on this assessment, 35 samples from nine localities (Denmark, Estonia, Italy, Latvia, Norway, Scotland, Spain, Sweden, and Swiss) out of 105 samples were passed and sent to Genewiz for sequencing. Twenty-four samples out of 35 subsequently passed QC steps of Genewiz and WGS was conducted on an Illumina HiSeq X platform targeting 10-15 X coverage depth of the golden eagle genome (around 1.4 Gb).

It is known that golden eagles in the world maintain two types of lineages in mtDNA

control region; M (Mediterranean) and H (Holarctic). The M type is maintained only in Mediterranean countries, and H type is maintained in all other regions. However, it is also known that Swiss birds have both M and H lineages, exceptionally (Nebel et al., 2015). In this analysis, our Swiss samples included both haplotypes: five samples were M and three were H. On the other hand, all Italian (n = 1) and Spanish (n = 2) birds displayed an M lineage haplotype. All other birds from European, Japanese and American were from the H lineage.

Bioinformatics and SNP calling, population genetic structure, and comparative analysis of genetic diversity and inbreeding

Actual bioinformatic commands are in the supplementary command text file.

- Mapping and removing PCR duplication

Raw sequence reads for each of 26 birds were trimmed to remove adaptor sequences and low-quality reads using Platanus_trim v1.0.7 (Kajitani et al., 2014). Trimmed reads were mapped to the reference assembly using BWA-MEM v0.7.17 (Li, 2013), and outputted as BAM format using SAMtools v1.9 (Li et al., 2009). During this step, unmapped reads were discarded with -F4 option of SAMtools. After mapping, each output BAM files were added the information of read group containing sample ID and WGS platform using GATK -AddOrReplaceReadGroups function. Removing PCR duplications step was also conducted with GATK -MarkDuplicates function.

- SNP calling (GATK)

A vcf file was created for each sample using GATK -HaplotypeCaller. Then variants of each bird were filtered based on its sequencing depth using VCftools min-meanDP

(mean_coverage*1/3) and max_meanDP (mean_coverage*2). All 25 vcf files were merged into one vcf file and genotyped using GATK -CombineGVCFs and –GenotypeGVCFs. SNPs and INDELs were divided separately, and hard-filtering was applied in each other (please see bellow commands for the criteria) with -VariantFiltration. Using output files from filtration, Base Quality Score Recalibration (BQSR) was applied following GATK's best practices workflow (Poplin et al., 2017) BQSR to BAM file of each bird with -BaseRecalibrator and -ApplyBQSR options. Finally, SNPs were called from calibrated BAM files with repeating process from haplotype-calling to hard-filtering. For PCA and ML tree analysis, called SNPs were filtered with some statistical processes (known as soft-filtering) by VCFtools: the ratio of missing data (0% allowed), Hardy-Weinberg equilibrium exact test ($P < 0.001$), minor allele frequency (10%).

- Analysis of PCA, ML tree, and ROH

PCA was simply computed with -pca function in PLINK with filtered SNPs. On the other hand, ML tree also used the same dataset of PCA, but it was required to convert vcf to phylip format using vcf2phylip.py (https://github.com/edgardomortiz/vcf2phylip). The best suitable model for computing the ML tree was explored using ModelTest-NG (Darriba et al., 2020; Flouri et al., 2014), and TVM+G4 model was selected as the best for our dataset. The phylogenetic analysis of ML tree was conducted by RAxML-NG with 500 times bootstrapping, and the tree itself was visualised on FigTree.

Inbreeding assessment by ROH was conducted using -homozyg function of PLINK with a vcf file from GATK without any statistical filtering. This analysis

also required to convert non-filtered vcf to plink-specific file formats (.ped and .map), and VCFtools was used for converting. The ROH detection was conducted using the -homozyg function in PLINK 1.9 following recommended settings by previous report (Meyermans et al., 2020). On the other hand, the selection of a suitable sliding window size (scanning window size; the number of SNPs contained in the window) must be explored (Meyermans et al., 2020). We tested (size = 5, 10, 15, 20, 25, and 50) and selected 10 SNPs (-homozyg-window-snp 10) for maximizing the number of analysed individuals and detected ROH segments for this study.

- SNP calling (ANGSD) and analysis of genetic diversity, Admixture, and NJ tree

ANGSD does not produce the vcf file, therefore SNP calling and calculation of each analysis were contained in each command line with suitable filtering and calculating function. The analysis of ANGSD required bqsr.bam files created in a step of above, and the list of the bam files was created as bqsr.bam.list. The genome-wide heterozygosity was calculated in each individual using realSFS function, while nucleotide diversity (pi) was calculated in each population with sliding-window approach. Therefore, input bam file list was created for each population in pi calculation. The calculation provided pairwise theta (tP) and the number of sites (nSites) in the region for that tP; subsequently pi was calculated by pi = tP/nSites. Admixture calculations was conducted by NGSadmix with beagle format output from ANGSD. Three times repeatruns at each number of putative clusters (K = 1 to 7), and the most suitable result was selected based on likelihood scores. The NJ tree with a white-tailed eagle (out group) was also conducted with IBS function of ANGSD.

Supplementary reference

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T. 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution*, 37(1), 291-294. https://doi.org/10.1093/molbev/msz189

Poplin R, Ruano-Rubio V, DePristo MA, Fennell TJ, Carneiro MO, Van der Auwera GA, Kling DE, Gauthier LD, Levy-Moonshine A, Roazen D, Shakir K, Thibault J, Chandran S, Whelan C. Lek M, Gabriel S, Daly MJ, Nealw B, MacArthur DG, Banks E. 2018. Scaling accurate genetic variant discovery to tens of thousands of samples. *BioRxiv*, 201178. https://doi.org/10.1101/201178

867 Table S1. The list of samples used in this analysis and sample providers. WGS data are available using given numbers in this list on any
868 online genome data bases such as NCBI.

	ID	Country	mtDNA lineage	mean depth	Data availability	Sample provider
1	060516-01	Scotland	H	13.00	SAMD00454443	South Scotland Golden Eagle project
2	B29	Scotland	H	12.30	SAMD00454446	South Scotland Golden Eagle project
3	C17	Scotland	H	12.56	SAMD00454450	South Scotland Golden Eagle project
4	C19	Scotland	H	15.74	SAMD00454451	South Scotland Golden Eagle project
5	GE083-19	Scotland	H	11.91	SAMD00454453	South Scotland Golden Eagle project
6	BE9	Switzerland	M	12.17	SAMD00454447	Swiss Ornithological Institute
7	BE10	Switzerland	H	11.98	SAMD00454448	Swiss Ornithological Institute
8	BE13	Switzerland	M	16.47	SAMD00454449	Swiss Ornithological Institute
9	FR2	Switzerland	H	13.37	SAMD00454452	Swiss Ornithological Institute
10	GR43	Switzerland	M	14.71	SAMD00454454	Swiss Ornithological Institute
11	SG6	Switzerland	H	12.58	SAMD00454459	Swiss Ornithological Institute
12	SZ1	Switzerland	M	13.37	SAMD00454460	Swiss Ornithological Institute

13	TI4	Switzerland	M	12.62	SAMD00454461	Swiss Ornithological Institute
14	AGR3	Spain	M	12.78	SAMD00454444	Zoobotánico de Jerez, Cadiz, Spain
15	AGR4	Spain	M	14.26	SAMD00454445	Zoobotánico de Jerez, Cadiz, Spain
16	I-130	Italy	M	19.29	SAMD00454456	Natural History Museum, Vienna, Austria
17	N-62	Norway	H	13.27	SAMD00454458	University of Copenhagen, Denmark
18	12774*	Japan	H	35.79	SAMD00454462	Akita Omoriyama Zoo
19	12775*	Japan	H	34.26	SAMD00454463	Akita Omoriyama Zoo
20	14522	Japan	H	30.62	SAMD00454464	Tama Zoological Park
21	29330	Japan	H	37.23	SAMD00454465	Morioka Zoological Park ZOOMO
22	31232	Japan	H	30.96	SAMD00454466	Akita Omoriyama Zoo
23	31233	Japan	H	35.60	SAMD00454467	Akita Omoriyama Zoo
24	USGE1*	USA	N/A	25.06	SRX363774	NCBI (wild born)
25	USGE2	USA	N/A	23.66	DNA zoo**	DNA zoo (Texas State Aquarium, captive)

869 The mtDNA linages were determined by haplotypes of control region (D-loop, see Nebel et al., 2015). It was not able to obtain the
870 information about haplotypes of American birds, but they should be belonged to Holarctic (H) group. Samples with * were also used in

previous analysis of Sato et al., 2020. **: genome data was downloaded from DNA zoo on Jan/31st/2021 (https://www.dnazoo.org/assemblies/aquila_chrysaetos)

873 Table S2. Genetic diversity of genome-wide heterozygosity and nucleotide diversity (π), and indicators of ROH analysis of golden
874 eagles in Europe, Japan, and USA. Note that π was estimated from only Japan, Scotland, Switzerland or Mediterranean (Switzerland +
875 Italy + Spain) which have more than five samples in this study. All ROH indicators are average value (+ SD) of each population/locality.

Localities	n	mean Het	π	NROH	SROH	ALROH	F_{ROH}
Scotland	5	1.18×10^{-3}	1.27×10^{-3}	84.8 (11.86)	158.35 (23.82)	1.87 (0.066)	0.144
Japan	6	1.24×10^{-3}	1.29×10^{-3}	72.5 (9.43)	138.85 (26.32)	1.90 (0.156)	0.126
US	2	1.29×10^{-3}	-	71.54 (63.42)	71.54 (63.42)	1.75 (0.395)	0.065
Norway	1	1.51×10^{-3}	-	44	75.7	1.72	0.069
Mediterranean	(11)	1.40×10^{-3}	1.54×10^{-3}	44.0 (9.07)	81.12 (26.03)	1.81 (0.236)	0.074
Switzerland	8	1.40×10^{-3}	1.51×10^{-3}	42.8 (9.91)	80.50 (28.40)	1.84 (0.240)	0.073
Spain	2	1.40×10^{-3}	-	44.5 (3.50)	71.63 (10.92)	1.60 (0.119)	0.065
Italy	1	1.45×10^{-3}	-	53	105.09	1.98	0.095

876 n: sample size, mean Het: average of genome-wide heterozygosity in that locality, π: genome-wide nucleotide diversity (of populations

with n ≧ 5), NROH: total number of ROH segments, SROH: sum of the length of ROH segments (Mb), ALROH: average length of ROH segments (Mb), F_{ROH}: indicator of inbreeding which was calculated from the proportion of ROH length on the assembly genome size (1.1Gb of golden eagles).

892 Table S3. Pairwise relatedness of every combination of birds in each population. All other combinations among populations were 0.00,
893 except a couple of combinations of Japanese-American birds (0.01-0.02). It was suggested that there was the geneflow during the Last
894 Glacial Period between Japanese and American (Sato et al., 2020).

ID	Country	12774	12775	14522	29330	31232	31233
12774	Japan	NA					
12775	Japan	0.41	NA				
14522	Japan	0.39	0.39	NA			
29330	Japan	0.39	0.40	0.37	NA		
31232	Japan	0.37	0.41	0.43	0.37	NA	
31233	Japan	0.37	0.37	0.38	0.36	0.35	NA

895

ID	Country	060516-01	B29	C17	C19	GE083-19
060516-01	Scotland	NA				
B29	Scotland	0.33	NA			
C17	Scotland	0.40	0.33	NA		

C19	Scotland	0.36	0.31	0.35	NA	
GE083-19	Scotland	0.38	0.32	0.37	0.36	NA

896

ID	Country	BE10	BE13	BE9	FR2	GR43	SG6	SZ1	TI4	AGR3	AGR4	I-130
BE10	Switzerland	NA										
BE13	Switzerland	0.14	NA									
BE9	Switzerland	0.14	0.09	NA								
FR2	Switzerland	0.10	0.10	0.11	NA							
GR43	Switzerland	0.11	0.13	0.13	0.12	NA						
SG6	Switzerland	0.09	0.13	0.10	0.14	0.14	NA					
SZ1	Switzerland	0.11	0.12	0.11	0.11	0.12	0.14	NA				
TI4	Switzerland	0.14	0.12	0.11	0.10	0.12	0.13	0.12	NA			
AGR3	Spain	0.06	0.04	0.05	0.05	0.04	0.08	0.04	0.05	NA		
AGR4	Spain	0.07	0.04	0.04	0.05	0.05	0.07	0.03	0.05	0.20	NA	
I-130	Italy	0.10	0.07	0.13	0.11	0.08	0.10	0.10	0.08	0.08	0.07	NA

ID	Country	usge1	usge2
usge1	USA	NA	
usge2	USA	0.38	NA

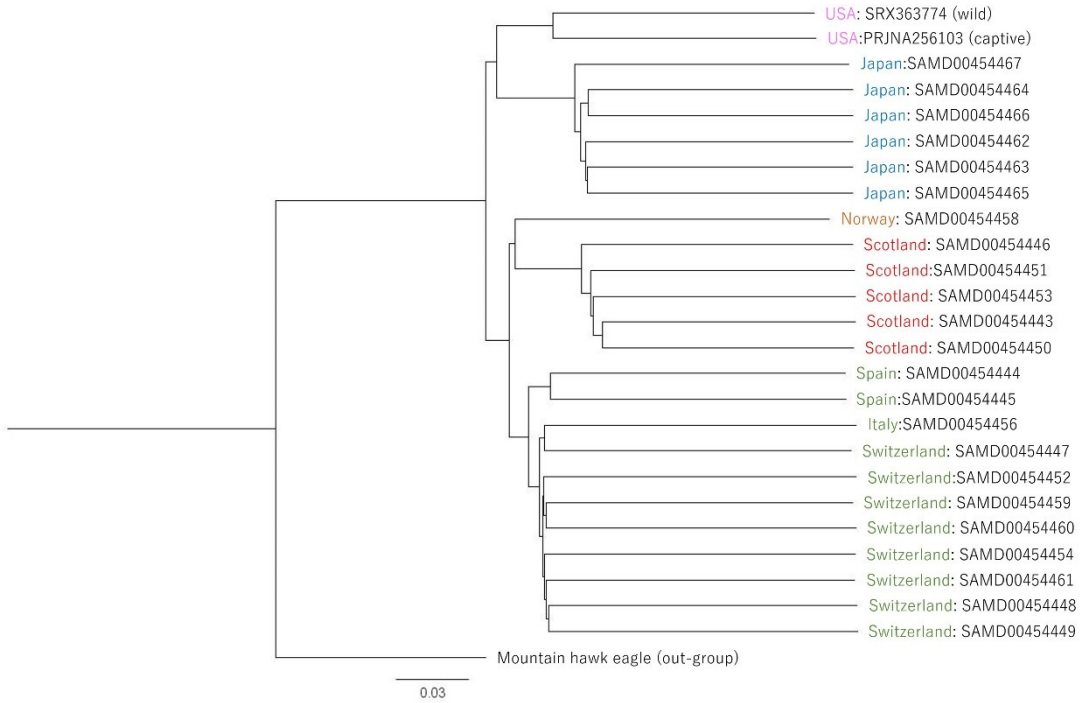
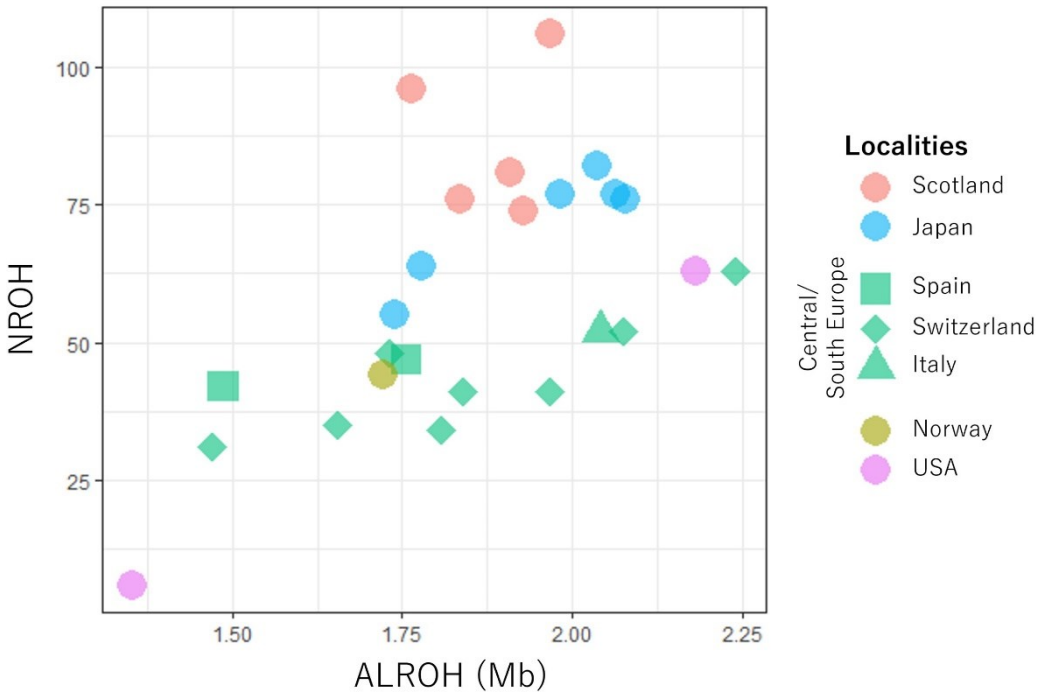


Figure S1. The Neighbour-Joining (NJ) tree of golden eagles with a white-tailed eagle as an out-group based on the WGS data calculated by ANGSD. Countries were coloured based on the result of our PCA. Japanese and USA birds were distinguished from European birds. European birds were separated into three populations: Scotland, Northern Europe, Central/South Europe. This phylogenetic result also reinforced the broad geographic clusters found under PCA.



913

914 Figure S2. The average length of ROH segments of each individual (ALROH, Mb)

915 and the number of ROH segments (NROH). Green dots are birds from Central/South

916 Europe, with different shapes of dots referring Spain, Switzerland, or Italy. Scottish

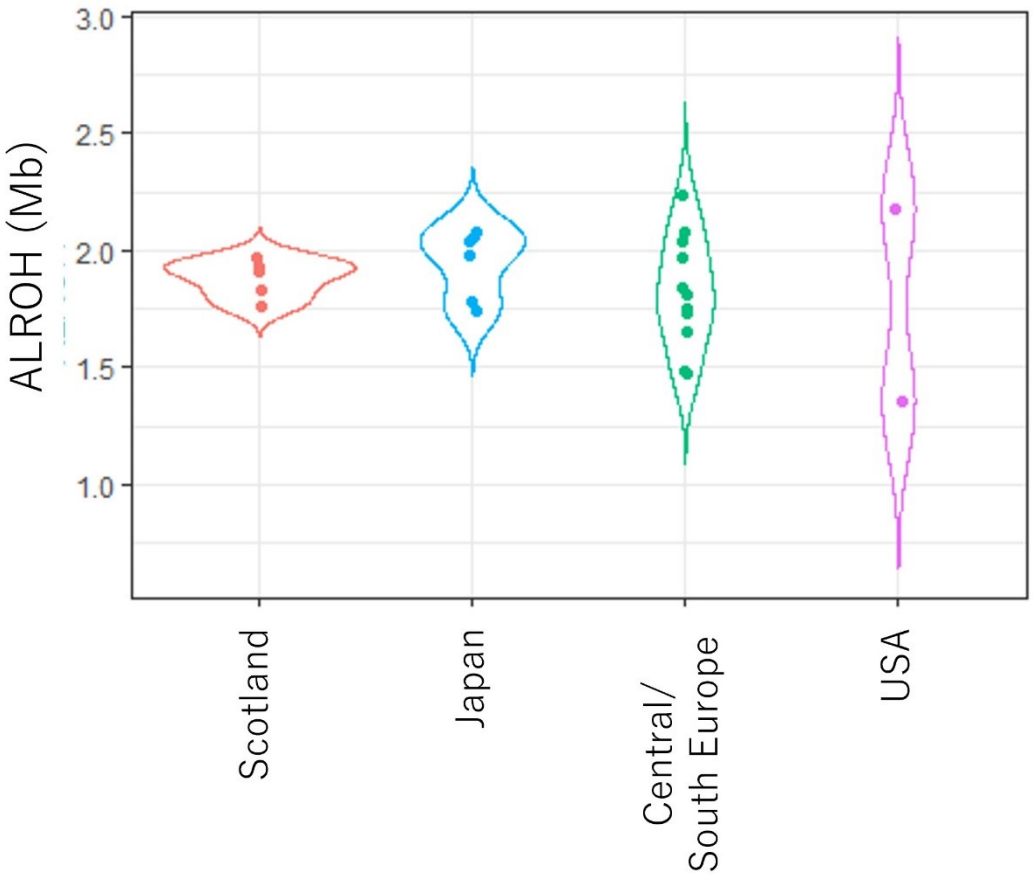
917 and Japanese have more NROH with tight variety of ALROH, although Swiss birds

918 have wide variety of that.

919

920

921



922

923 Figure S3. Violin plots of the average length of ROH segments (ALROH, Mb) of
924 populations with the sample size more than two. Central/South European birds have
925 wide variety of ALROH. Scottish and Japanese have longer ALROH, and Japanese
926 birds form two clusters based on its length, longer one from four birds
927 (SAMD00454463, SAMD004544664, SAMD00454465, and SAMD00454466) and
928 shorter one from two birds (SAMD00454462 and SAMD00454467).

929

930

931

932

933

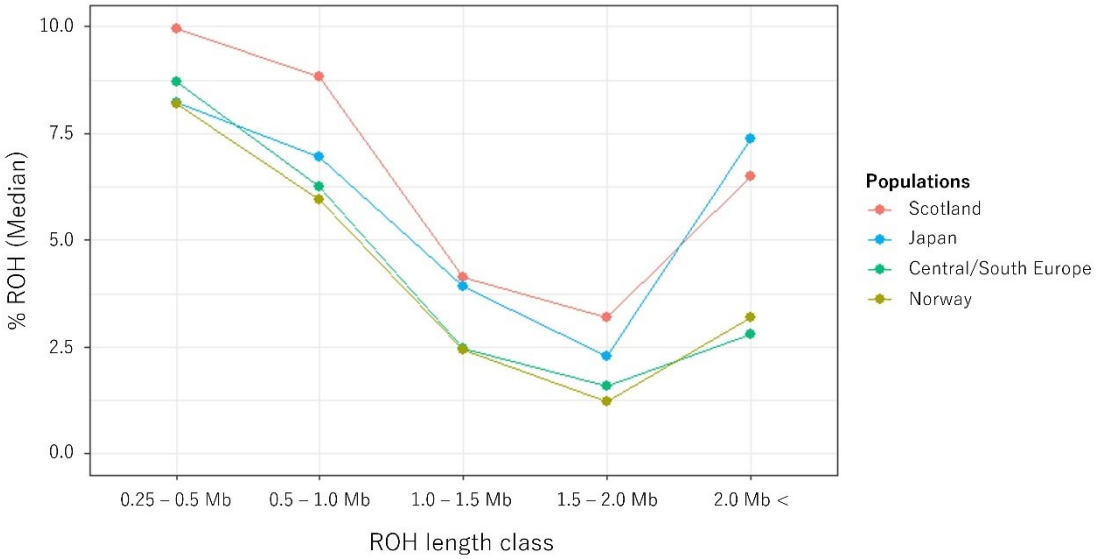


Figure S4. Distribution of ROH segments in five length classes, but only median values of each population were picked up from Figure 5 and shown in here. The result of USA birds was not shown in this figure for simplifying the comparison, because two samples from USA were mixture of wild and captive (probably highly inbred although we were not able to find the information) birds.