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ORIGINAL ARTICLE

Phylogeography of the white-tailed eagle, a generalist with large dispersal capacity

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ABSTRACT

Aim Late Pleistocene glacial changes had a major impact on many boreal and temperate taxa, and this impact can still be detected in the present-day phylogeographic structure of these taxa. However, only minor effects are expected in species with generalist habitat requirements and high dispersal capability. One such species is the white-tailed eagle, *Haliaeetus albicilla*, and we therefore tested for the expected weak population structure at a continental level in this species. This also allowed us to describe phylogeographic patterns, and to deduce Ice Age refugia and patterns of postglacial recolonization of Eurasia.

Location Breeding populations from the easternmost Nearctic (Greenland) and across the Palaearctic (Iceland, continental Europe, central and eastern Asia, and Japan).

Methods Sequencing of a 500 base-pair fragment of the mitochondrial DNA control region in 237 samples from throughout the distribution range.

Results Our analysis revealed pronounced phylogeographic structure. Overall, low genetic variability was observed across the entire range. Haplotypes clustered in two distinct haplogroups with a predominantly eastern or western distribution, and extensive overlap in Europe. These two major lineages diverged during the late Pleistocene. The eastern haplogroup showed a pattern of rapid population expansion and colonization of Eurasia around the end of the Pleistocene. The western haplogroup had lower diversity and was absent from the populations in eastern Asia. These results suggest survival during the last glaciation in two refugia, probably located in central and western Eurasia, followed by postglacial population expansion and admixture. Relatively high genetic diversity was observed in northern regions that were ice-covered during the last glacial maximum. This, and phylogenetic relationships between haplotypes encountered in the north, indicates substantial population expansion at high latitudes. Areas of glacial meltwater runoff and proglacial lakes could have provided suitable habitats for such population growth.

Main conclusions This study shows that glacial climate fluctuations had a substantial impact on white-tailed eagles, both in terms of distribution and demography. These results suggest that even species with large dispersal capabilities and relatively broad habitat requirements were strongly affected by the Pleistocene climatic shifts.

Keywords

Control region, Eurasia, Falconiformes, *Haliaeetus albicilla*, mtDNA, population expansion, postglacial colonization.

INTRODUCTION

The multiple glacial advances and retreats that occurred during the Pleistocene not only altered the landscape topography of the northern hemisphere, but also had a dramatic influence on the abundance and distribution of living forms. Most species underwent dramatic reductions in numbers and range (summarized in Hewitt, 2000), and many lineages went extinct (e.g. Shapiro *et al.*, 2004). For many temperate animal and plant species from Europe, peninsulas on the Mediterranean coast acted as glacial refugia (Taberlet *et al.*, 1998; Hewitt, 2000). Conversely, some Arctic species appear to have reacted in an opposite manner to the Pleistocene climatic shifts, with contraction into northern refugia during warm periods and major population expansions during cold periods (Flagstad & Roed, 2003; Dalén *et al.*, 2005).

Taxa with low mobility and narrow habitat requirements are expected to have been the most strongly affected by the drastic habitat changes accompanying the glacial cycles, while the genetic structure of species with higher dispersal capability that exploit a broader ecological niche may have been less influenced by climatic changes. For example, the grey wolf (*Canis lupus*, Linnaeus, 1758) shows very little phylogeographic structure in mitochondrial DNA (mtDNA) at a continental level (Vilà *et al.*, 1999; but see Sharma *et al.*, 2004).

Another species group with high dispersal potential as well as broad habitat and dietary requirements is that of eagles in the genus *Haliaeetus*. The three largest species in this genus occur in the northern hemisphere, including the white-tailed eagle *H. albicilla* (Linnaeus, 1758), its North American sister species the bald eagle, *H. leucocephalus*, Linnaeus, 1766, and the south-east Asian Steller's sea eagle, *H. pelagicus*, Pallas, 1811 (Wink *et al.*, 1996).

Among these three, the white-tailed eagle has the widest distribution: it occurs from Greenland and Iceland in the west, throughout Europe, northern and central Asia, to the Pacific coast and Japan in the east. Breeding habitats are mostly in coastal and freshwater regions from the Arctic to the subtropics. Prey taken in these regions are fish and waterfowl, but in drier areas medium-sized mammals are common food items (Katzner, 2002). White-tailed eagles also feed on carrion, especially in winter. Nests are built in trees as well as on cliffs or on the ground. Except in some northern populations, studied territorial pairs are mainly sedentary (Glutz von Blotzheim *et al.*, 1971; Helander & Stjernberg, 2003). Younger birds are vagrant: long-distance wandering behaviour has been recorded for juveniles, for example from northern Europe down to Bulgaria (Glutz von Blotzheim *et al.*, 1971). Despite this, ringing data from Europe suggest strong philopatry, with individuals typically settling to breed close to their natal area (Helander, 2003), a pattern in accordance with variation at mitochondrial DNA (mtDNA) and autosomal microsatellite markers in north European populations (Hailer *et al.*, 2006). Nevertheless, over long time-scales white-tailed eagles have been shown to be capable of long-distance dispersal and colonization, as documented by the colonization of Iceland, Greenland, and Hawaii (population today extinct; Fleischer *et al.*, 2000). Moreover, the fossil record indicates that the white-tailed eagle colonized northern latitudes very soon after glacial retreat (Ericson & Tyrberg, 2004), compatible with its being a habitat generalist and indicating high dispersal potential.

Given this flexibility in habitat and food choice, the white-tailed eagle offers a good opportunity to test for the expected weak population structure at a continental level in a generalist species with high dispersal potential. We therefore utilized genetic markers to deduce the effect of the Pleistocene climatic shifts on this species. We chose mtDNA as a marker because it is especially suitable for the detection of phylogeographic structure within animal species (Avise, 2000).

MATERIAL AND METHODS

Study populations, sampling and DNA extraction

A total of 237 white-tailed eagle individuals from 11 breeding populations throughout the range of the species were analysed (sampling locations are indicated in Fig. 1; sample sizes for each population are shown in Tables 1 and 2). For 228 of the individuals we took blood samples of nestlings ($n = 209$) or collected moulted feathers ($n = 19$) from breeding adults in close vicinity to their nests. This ensured that we did not mix local breeders with vagrants or dispersing animals in the analysis, which is important when studying a species in which wintering individuals can be found several hundreds to thousands of kilometres away from their original breeding population (Helander & Stjernberg, 2003). We sampled only one offspring per breeding pair, to avoid inclusion of close relatives, at least with regard to the present generation. Furthermore, in order to survey for additional haplotypes we sampled nine individuals whose natal origin could not be safely assigned to any of the 11 regions: one adult found injured in southern Sweden during the winter season, two nestlings from the Baltic island of Gotland (situated between Sweden and the Baltic States, two well-sampled regions), one feather found in north-eastern Poland during late winter, one adult individual found dead in Kazakhstan during summer, and four presumably unrelated individuals from a zoo in Kazakhstan. These samples were not included in the population-level analyses.

Blood samples were stored in EDTA/SSC buffer and kept frozen until treatment in the laboratory, feather samples were kept at room temperature under dry and dark conditions. DNA from blood was extracted using a standard phenol-chloroform procedure after digestion with proteinase K (Sambrook *et al.*, 1989). For feather quills we used the DNeasy Tissue Kit (Qiagen, Hilden, Germany) and followed the protocol of Horváth *et al.* (2005). DNA from feathers from Kazakhstan was extracted using an ammonium acetate precipitation protocol, as described in Rudnick *et al.* (2005).

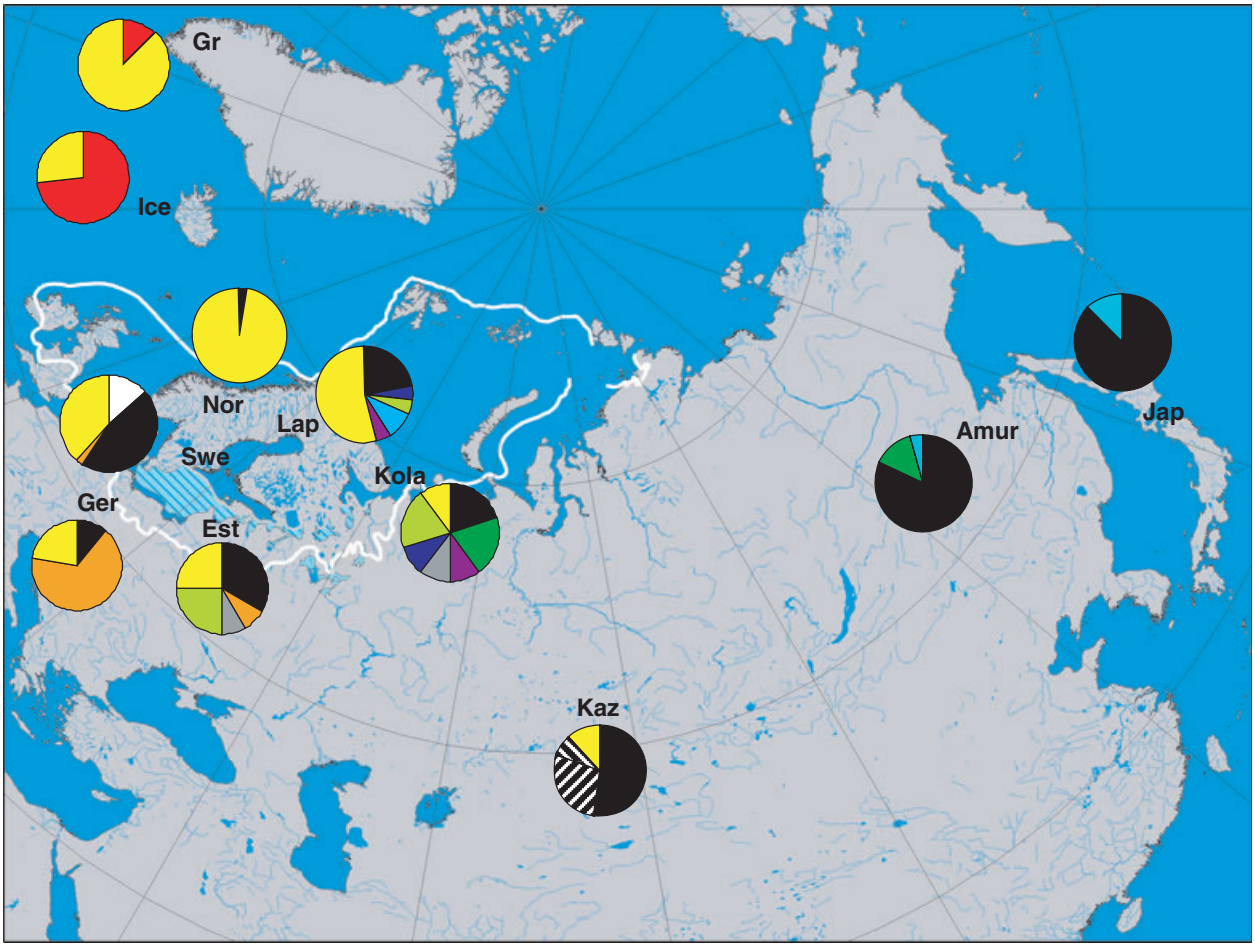

Figure 1 Locations and haplotype frequencies of studied white-tailed eagle populations. Haplotype colours correspond to those in Fig. 2, and the positions of population names indicate approximate sampling locations. Locality codes are explained in Table 1. For northern Europe, limits of the ice sheets during the last glacial maximum (LGM) reconstructed by Svendsen *et al.* (2004) have been superimposed on the map (white line; ice margins in other regions are *not* shown). Inside the glacial limit, younger (about 14,000 yr BP) ice-dammed lakes in the Baltic Sea depression and around Lake Onega are shown in light blue (following Mangerud *et al.* 2004).

Polymerase chain reaction (PCR) amplification and DNA sequencing

In order to find a suitable marker for the present study we initially amplified and sequenced 400 base pairs (bp) of the mtDNA *cytochrome b* (*Cyt-B*) gene in a total of 32 individuals (21 from Sweden, four from Greenland, four from Germany and three from eastern Russia). This recovered four variable sites defining four haplotypes (data not shown). Given this restricted amount of sequence variation, we subsequently focused on the non-coding control region instead.

The complete mtDNA control region (> 1500 bp, including two heteroplasmic tandem repeats of 11 bp each that hampered exact length estimation) was amplified in a few individuals from different geographic origins as described in Hailer *et al.* (2006). Next, a 544-bp region spanning domains I and II that contained most of the control-region variability was targetted using the primers *Hal-HVR1F* (5′-CCCCCCCTATG-TATTATTGT-3′) and *Hal-HVR1R* (5′-TCTCAGTGAAGAGC GAGAGA-3′), both located within the control region. PCR reactions were carried out in 10-μL volumes containing approximately 15 ng of genomic DNA, 0.3 μM of each primer, 0.2 mM of each dNTP, 0.25 units of HotStarTaq DNA polymerase (Qiagen) in 1× HotStarTaq (Qiagen) reaction buffer containing Tris-Cl, KCl, $(NH_4)_2SO_4$ and 1.5 mM $MgCl_2$. PCR was performed in a PTC-225 instrument (MJ Research, Watertown, USA) using the following thermal profile: 15 min at 95°C prior to 36 cycles of 30 s at 56°C, 30 s at 72°C and 30 s at 95°C; followed by a final 1-min step at 56°C and an extension step of 10 min at 72°C. PCR products were cleaned using the ExoSAP enzyme kit (Amersham Biosciences, Uppsala, Sweden), and DNA sequencing of both strands was performed using the original PCR primers and the DYEnamic ET Terminator kit (Amersham Biosciences). Sequencing reactions were cleaned using AutoSeq plates (Amersham Biosciences) and run on a MegaBACE 1000 (Amersham Biosciences) capillary instrument according to the manufacturer's recommendations. Electropherograms were assembled, checked manually, and aligned using SEQUENCHER 4.1.4 (Gene

Table 1 Variable sites and absolute frequencies of the mtDNA control-region haplotypes in the 11 study populations.

	Site no.												Population											
Haplotype	005	008	009	041	092	108	172	177	192	201	494	497	Gr	Ice	Nor	Ger	Swe	Lap	Est	Kola	Kaz	Amur	Jap	Count
A01	T	T	C	T	A	T	C	A	G	C	C	G	7	7	32	4	17	12	3	1	3			86
A02	.	.	.	A	.	.	.	.	.	.	.	.				12	1		1					14
A03	.	.	.	.	.	.	.	.	.	.	.	T	1	19										20
B01	.	C	T	.	.	C	.	.	A	.	T	A			1	2	20	5	4	2	13	18	7	72
B02	.	C	T	.	.	.	.	.	A	.	T	A						1		2		3		6
B03	.	C	T	.	.	C	.	.	A	.	T	.							1	1				2
B04	.	C	T	.	.	C	.	.	A	T	T	A						2				1	1	4
B05	.	.	T	.	.	C	.	.	A	T	T	A						1		1				2
B06	.	C	T	.	G	C	.	.	A	.	T	A						1		1	(1)*			2
B07	.	C	T	.	.	C	.	G	A	.	T	A							3	2				5
B08	.	C	T	.	.	C	T	.	A	.	T	A									7			7
B09	.	C	T	.	.	C	.	.	.	.	T	A									2			2
C01	C	.	T	.	.	.	.	.	A	.	.	.					6							6
Total													8	26	33	18	44	22	12	10	25	22	8	228

Gr: Greenland, Ice: Iceland, Nor: Norway, Ger: Germany, Swe: Swedish coast, Lap: Swedish Lapland, Est: Estonia, Kola: Kola Peninsula, north-west Russia, Kaz: Kazakhstan, Amur: Amur river, eastern Russia, Jap: Japan.

*Data from an individual found dead in Kazakhstan (not certified to be a local breeder) are included in brackets, but not included in the count.

Table 2 Estimates of within-population variability of partial control-region sequences of white-tailed eagle mtDNA. Sample size (n), number of unique haplotypes (N_H), haplotype diversity (H), nucleotide diversity (π), and the frequency of group *A* haplotypes are reported.

Population	n	N_H	H (±SE)	π (±SE)	Frequency of haplogroup *A*
Greenland	8	2	0.250 ± 0.180	0.00050 ± 0.00071	1.00
Iceland	26	2	0.409 ± 0.083	0.00082 ± 0.00087	1.00
Norway	33	2	0.061 ± 0.056	0.00073 ± 0.00080	0.97
Germany	18	3	0.523 ± 0.112	0.00345 ± 0.00236	0.89
Lapland	22	6	0.667 ± 0.092	0.00686 ± 0.00406	0.55
Sweden	44	4	0.640 ± 0.038	0.00661 ± 0.00385	0.41
Estonia	12	5	0.818 ± 0.070	0.00706 ± 0.00435	0.33
Kola peninsula	10	7	0.933 ± 0.062	0.00507 ± 0.00336	0.10
Kazakhstan	25	4	0.657 ± 0.071	0.00371 ± 0.00245	0.12
Amur	22	3	0.325 ± 0.117	0.00068 ± 0.00078	0
Japan	8	2	0.250 ± 0.180	0.00050 ± 0.00071	0
Overall	228	13	0.746	0.00680 ± 0.00012	0.53

Codes, Ann Arbor, MI, USA). After removal of primer sequences and some additional bases close to the primers, this yielded a 500-bp fragment for analysis.

Several lines of evidence indicate that we did not sequence a nuclear copy of the mitochondrial control region (a *numt*). First, we found only three individuals with double peaks (i.e. potential heterozygote positions, confirmed by resequencing of new PCR product) among all analysed electropherograms. All three instances were from blood samples. Given the overall high haplotype diversity ($H = 0.746$), and assuming Hardy–Weinberg equilibrium, we would expect 177 (0.746×237) heterozygotes among the 237 analysed individuals if our sequences were from nuclear autosomal inserts. Thus, heteroplasmy is a much more likely explanation for the observed double peaks. Second, we obtained identical sequences from the same individual using a variety of PCR primers. Third, all haplotypes obtained from feather samples were also encountered in blood samples, compatible with an identical genomic origin. Hardened feather quills have earlier been suggested to be particularly good sources of mitochondrial DNA sequences (Sorenson & Quinn, 1998). Fourth, the observation of a transition–transversion ratio around 7 (see Table 1 and Fig. 2) is typical of mitochondrial rather than nuclear DNA (Nei & Kumar, 2000). Fifth, identical sequences were obtained on amplifying fragment sizes between 416 and 1990 bp. Since nuclear insertions of mtDNA tend to be of rather restricted length (Sorenson & Quinn, 1998), this also suggests that the analysed fragment is mitochondrial.

Data analyses

DNASP 4.10 (Rozas *et al.*, 2003) was used to determine Tajima's *D* (Tajima, 1989), calculated based on the total number of mutations in the alignment. To measure within-population

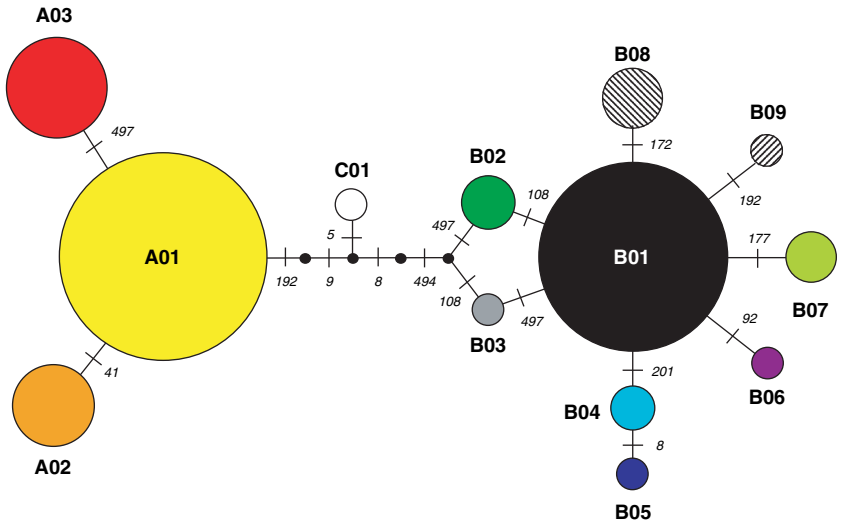

Figure 2 Unrooted statistical parsimony network of Eurasian white-tailed eagle mtDNA control-region haplotypes. Circle area is proportional to haplotype frequency. Dashes indicate inferred mutational steps, and numbers refer to the corresponding sites in the alignment. Small black circles denote inferred intermediate haplotypes. Haplotype colours correspond to those in Fig. 1.

variability, ARLEQUIN 3.0 (Excoffier *et al.*, 2005) and DNASP were used to calculate haplotype diversity (H) and nucleotide diversity (π, based on uncorrected genetic distances p). To verify that our comparisons of population genetic variability (π and H) were not affected by sample size, we employed a bootstrap resampling procedure using a macro in Microsoft EXCEL: eight or ten sequences (corresponding to the sample sizes from Greenland, Japan and the Kola peninsula) were sampled with replacement from each of the more extensively sampled populations 1000 times, and π and H were calculated for each resampling. From that we calculated the mean across resamplings and the 95% confidence intervals (CI) (percentile method) of π and H.

A statistical parsimony network (Templeton *et al.*, 1992) of the nucleotide sequences was constructed using the program TCS 1.21 (Clement *et al.*, 2000) with the default setting of 95% parsimony connection limit. Compared with bifurcating trees, networks are better suited for the typically shallow intra-species phylogenies in which divergence is low and ancestral haplotypes may still exist in the population (Posada & Crandall, 2001).

We used the software MODELTEST 3.7 (Posada & Crandall, 1998) to identify the model of sequence evolution that best fits the data. The suggested model was more complex than any of the models available in the software used for subsequent calculations. However, Nei & Kumar (2000) show that, for sequence divergences as small as those observed in this study, complex models of sequence evolution do not greatly modify distance estimates. To estimate the divergence time between major phylogenetic clades, we applied the Tamura & Nei (1993) distance correction when calculating the 'net average distances between groups' using MEGA 3 (Kumar *et al.*, 2004). Standard errors were estimated with 1,000 bootstrap replicates across sites. The obtained divergence value, which is corrected for within-clade diversity to mimic ancestral polymorphism (Nei, 1987), was then divided by the divergence rate (two times the mutation rate μ). To our knowledge, independently calibrated estimates of the mtDNA control-region divergence rate have not been published for raptors. We therefore used the divergence rate for the combined hypervariable regions I and II of 14.8% per site per million years estimated by Wenink *et al.* (1996) for *Calidris alpina*. We also considered a range of other plausible rates (5–20%, see Brito, 2005) since the rate is known to vary between species (Garcia-Moreno, 2004) and between different parts of the mtDNA control region (Ingman & Gyllensten, 2001). Comparisons of sequence divergence among white-tailed eagles sequenced for both *Cyt-B* and the control region indicated that the control region evolved at least 3–4 times faster (data not shown). Therefore, we did not consider a 2% rate estimate in our analyses.

To investigate phylogeographic structure in the data set, we partitioned the amount of genetic variation into components within and between regions by performing an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) as implemented in ARLEQUIN. For this analysis we used Tamura–Nei-corrected distances between sequences. Significance of the covariance components was assessed using a permutation procedure, thus avoiding dependence on normality of the data (Excoffier *et al.*, 1992). We also calculated K_{XY}, the divergence between groups of sequences as measured by the uncorrected average number of nucleotide substitutions per site between populations (Nei, 1987), using DNASP. The obtained pairwise distances between populations were used for a neighbour-joining (NJ) analysis in MEGA 3. The resulting tree is influenced by many population genetic factors, including mutation, drift and migration. Therefore, it depicts present-day overall similarity of populations, but does not necessarily reflect ancestry relationships.

The presence of limited gene flow can be indicated by a pattern of isolation by distance across regions. We determined the geographical coordinates of the approximate distribution midpoints for each of the sampled populations. The GEOD program (US Geological Service) was used to calculate distances assuming a spherical Earth surface. Following Rousset (1997), we then plotted $\Phi_{ST}/(1 - \Phi_{ST})$ against the log of the geographic distances using the IBDWS software (Jensen *et al.*, 2005). The same program was used to investigate statistical significance using Mantel tests with 10,000 randomizations.

To investigate the demographic history of white-tailed eagle populations, we tested for signals of sudden population expansion using the mismatch distribution approach fully implemented in ARLEQUIN. The mismatch distribution is generally multimodal in samples from populations that are in demographic equilibrium, and unimodal when drawn from a population that has undergone a recent demographic expansion (Slatkin & Hudson, 1991; Rogers & Harpending, 1992). The demographic parameters Θ_0, Θ_1 and τ [$\Theta = 2\ u\ N_{e,f}$, where $N_{e,f}$ is the female effective population size before (Θ_0) and after (Θ_1) a single instantaneous expansion, and u is the haplotype mutation rate; $\tau = 2\ u\ t$, where t is the time since population expansion] were estimated with a generalized non-linear least-squares approach. Deviation from the assumed model was tested by parametric bootstrapping (Schneider & Excoffier, 1999) using a coalescent algorithm modified from Hudson (1990). The 95% confidence intervals of the demographic parameters were estimated with 5000 replicates of the same bootstrapping procedure.

We used ARLEQUIN and DNASP to calculate Fu's (1997) test statistic F_S and Fu & Li's (1993) D^* and F^* for each haplogroup and for the whole data set combined. These tests show different degrees of sensitivity to deviation from neutrality caused by demography or selection. Population growth (or genetic hitchhiking, which can produce a similar signal) can be detected from the patterns of significance of these tests: given a population expansion, F_S is expected to deviate significantly from the null expectation, while D^* and F^* are less sensitive to population growth and usually do not show significant deviation. Non-significant values of D^* and F^* justify exclusion of background selection (Ramos-Onsins & Rozas, 2002). In addition, we used the maximum likelihood coalescent-based approach implemented in LAMARC 2.0.2 (Kuhner *et al.*, 1998) to estimate g, a parameter of population growth.

Following Roman & Palumbi (2003) and the formula for Θ given above, we used π (the expected value of Θ; Nei & Kumar, 2000) to determine the current genetic effective population size of females. For this calculation, we used the estimate of μ by Wenink *et al.* (1996) and multiplied it by the generation time of white-tailed eagles (around 14.5 years; author calculations based on data from Struwe-Juhl, 2003).

RESULTS

In the 500-bp sequences analysed in the 237 white-tailed eagle samples we discovered 12 variable sites that defined 13 distinct mtDNA haplotypes (Table 1). The sequences have been deposited in the EMBL database (accession numbers AM156933 to AM156945). Tajima's D for the whole data set was 1.389 ($P > 0.10$), compatible with neutral evolution of the DNA sequences. Haplotype relationships could be deduced with the exception of one loop, as shown in the statistical parsimony network (Fig. 2). No insertions or deletions were observed. Two of the 16 inferred substitutions were transversions, and there were at least three sites with multiple mutations.

All sequences except haplotype *C01* clustered into one of two haplogroups, referred to as *A* and *B* (Fig. 2). Haplotypes *A01* and *B01* occupied the central position in each of the two haplogroups and were the most frequent haplotypes in the total data set, altogether occurring in almost 70% of the studied individuals. Within most populations, one of these two was the predominant haplotype (Fig. 1 and Table 1). Among the haplotypes found at lower frequencies, three (*B02*, *B04*, *B06*) were distributed over large geographic areas, occurring in both the Baltic Sea region and central or eastern Asia. Haplogroups *A* and *B* were found to be admixed over large geographic areas (Fig. 1, Table 1). The two haplogroups showed an east–west cline in their respective frequencies (Table 2). Sequences from haplogroup *B* had a 100% occurrence in the two easternmost populations (Amur and Japan), low to intermediate frequencies in Kazakhstan and around the Baltic Sea, and were largely absent from the populations adjacent to the Atlantic Ocean (Greenland, Iceland and Norway). Conversely, haplogroup *A* went from 100% frequency in Greenland and Iceland, through varying frequencies around the Baltic, to being rare (12%) in Kazakhstan and absent from the two easternmost populations.

Within-population variability

The number of haplotypes per population ranged from two in Greenland, Iceland, Norway and Japan to seven on the Kola peninsula (Table 2). Genetic variability (haplotype and nucleotide diversity) was low on the extremes of the distribution range (Greenland and Japan), and was highest in the Baltic region. Overall, haplotype and nucleotide diversities showed similar patterns. Nucleotide diversity was lowest in Greenland, Iceland, Norway, Amur and Japan, and highest in Estonia, Sweden and Lapland. Nucleotide diversity was strongly affected by the degree of admixture between clades *A* and *B*, as illustrated by the large haplotype but relatively lower nucleotide diversity in Kola. Resampling 1000 times of eight individuals per population (sample size from Greenland and Japan) revealed that the low nucleotide diversity observed in Greenland and Japan was outside the 95% CI of all European populations except Germany and Norway. Resampling 1000 times of ten individuals (sample size from the Kola peninsula) from the other eight populations with larger sample sizes (Table 2) revealed that the high haplotype diversity observed in Kola was outside the 95% CI for all other populations and, therefore, not likely to be an artefact of restricted sample size.

The net average distance between groups *A* and *B* was 0.0098 ± 0.0039 (±SD). Assuming a divergence rate of 14.8% per site per million years (Wenink *et al.*, 1996), the divergence of the two clades was estimated to have occurred 66,200 ± 26,400 years ago. Divergence rates between 5 and 20% yielded average estimates between 49,000 and 196,000 years ago. The assumption that substitution rates may be higher on shorter than on longer time-scales (see Ho & Larson, 2006) would support a more recent date for the divergence. The origin of lineages *A* and *B* was thus confidently placed in the late Pleistocene.

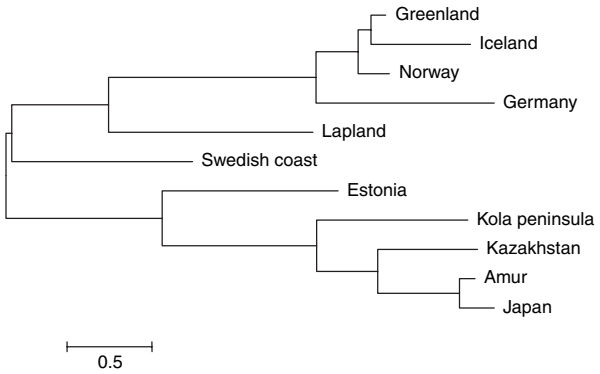

Figure 3 Neighbour-joining tree of populations of the Eurasian white-tailed eagle, based on pairwise K_{XY} distances between mtDNA control-region haplotypes.

Population structure

Overall, white-tailed eagle populations exhibited clear differences in their genetic composition. Φ_{ST} across all populations was 0.512 ($P < 0.001$). Pairwise Φ_{ST} values between populations were significantly larger than zero in most cases (Table 3).

The neighbour-joining tree (Fig. 3) based on the mean number of pairwise nucleotide differences between populations (K_{XY}) roughly reflected the geographic locations of the populations. Atlantic populations clustered closely together, as did the Asian populations. The central and north European populations were located between these clusters. However, genetic distances between European populations were in some cases very small and in other cases substantial, indicating complex differentiation patterns on a smaller spatial scale. For example, the sample from Germany appeared genetically similar to the Atlantic ones, and the Kola population seemed similar to Asian populations.

AMOVA results (Table 4) confirmed the presence of phylogeographic structure in our data. Grouping white-tailed eagle populations according to large-scale geographic regions (Atlantic islands, Europe, Asia; grouping 5) yielded a high and significant Φ_{CT} value, indicating strong geographic differentiation. In contrast, separating Greenland from the remaining populations (grouping 2), which would reflect current taxonomical segregation into two recognized subspecies, *H. albicilla groenlandicus* and *H. a. albicilla*, did not explain much of the variance and was non-significant. Groupings 3 and 4 indicated that Greenland and Iceland were less differentiated from the remaining populations than Japan and Amur were from all others. The best grouping we explored was number 7. This, however, was an *a posteriori* grouping based on the results of the neighbour-joining population tree (Fig. 3).

A plot of genetic differentiation in relation to geographic distance (Fig. 4) indicated a weak but significant correlation ($Z = 1726.40$, $r = 0.424$, one-sided $P < 0.001$). This correlation was largely the result of strong differentiation between the geographically most distant populations, namely Greenland and Iceland compared with Amur and Japan. The exclusion of Greenland, Iceland, Amur and Japan (but still including Kazakhstan) yielded a non-significant correlation ($Z = 142.41$, $r = 0.248$, one-sided $P > 0.15$).

The individuals we had left unassigned to any breeding populations generally carried haplotypes found in individuals

Table 3 Genetic differentiation between white-tailed eagle populations. Pairwise Φ_{ST} values based on Tamura–Nei distances are shown below the diagonal. Corresponding significance as assessed by 2024 permutations is indicated by a plus ($P < 0.05$) or minus (nonsignificant) above the diagonal. More conservatively, to account for multiple testing, values in bold indicate significance at the 0.05 level after sequential Bonferroni correction.

	Greenland	Iceland	Norway	Germany	Lapland	Sweden	Estonia	Kola	Kazakhstan	Amur	Japan
Greenland		−	−	+	+	+	+	+	+	+	+
Iceland	0.473		+	+	+	+	+	+	+	+	+
Norway	−0.028	**0.566**		+	+	+	+	+	+	+	+
Germany	0.236	**0.495**	**0.346**		+	+	+	+	+	+	+
Lapland	0.258	**0.423**	**0.380**	0.269		−	−	+	+	+	+
Sweden	0.310	**0.428**	**0.390**	**0.305**	−0.010		−	+	+	+	+
Estonia	0.510	**0.661**	**0.666**	**0.452**	0.042	0.021		−	+	+	+
Kola	**0.724**	**0.811**	**0.824**	**0.637**	0.195	0.154	−0.008		−	+	−
Kazakhstan	**0.746**	**0.800**	**0.809**	**0.683**	**0.298**	**0.237**	0.108	0.024		+	−
Amur	**0.947**	**0.937**	**0.939**	**0.837**	**0.470**	**0.365**	0.307	0.105	0.101		−
Japan	**0.960**	**0.940**	**0.943**	**0.798**	0.387	0.320	0.204	0.048	0.052	−0.006	

Table 4 Analysis of molecular variance (AMOVA) describing the partitioning of mitochondrial DNA haplotype variation across a range of conceivable population (pop) groupings.

Population grouping	Among groups (Φ_{CT})	Among populations within groups	Within populations
1. All in one group	–	0.512	0.488
2. [Gr] [all remaining pops]	$0.038^{n.s.}$	0.491	0.471
3. [Gr/Ice] [all remaining pops]	$0.211^{n.s.}$	0.367	0.422
4. [Amur/Jap] [all remaining pops]	0.373*	0.267	0.359
5. [Gr/Ice] [Eur[1]] [Kaz/Amur/Jap]	0.401**	0.185	0.414
6. [Gr/Ice] [Eur[1]/Kaz] [Amur/Jap]	0.308*	0.272	0.421
7. [Gr/Ice/Nor/Ger] [Swe/Lap/Est] [Kola/Kaz/Amur/Jap]	0.523**	0.053	0.424

[1]Eur denotes the northern and central European populations [Nor, Swe, Lap, Kola, Est and Ger], each treated separately within the group.
$^{n.s.}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, as assessed by 10,100 permutations.

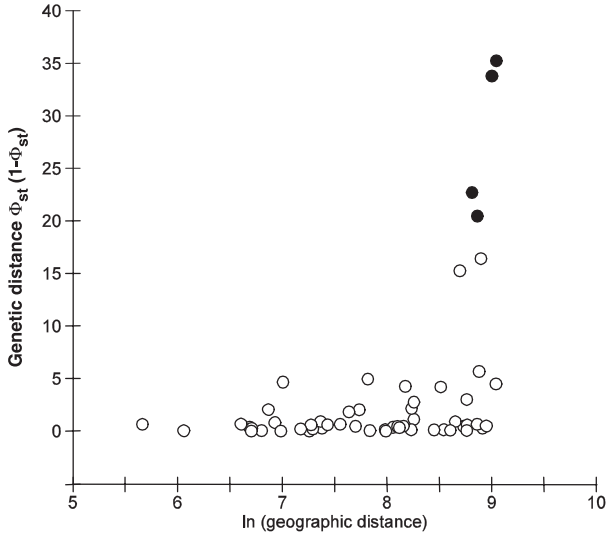

Figure 4 Isolation-by-distance plot of Eurasian white-tailed eagle populations, with genetic distance plotted against the log of geographic distances. Filled circles correspond to comparisons between the most geographically distant populations (Greenland or Iceland vs. Amur or Japan).

from neighbouring populations. Interestingly, however, the adult found dead in Kazakhstan carried haplotype *B06*, otherwise present in Lapland and the Kola peninsula, but not previously detected in the Kazakhstan sample.

Demography

Phylogenetic analysis (Fig. 2) revealed the presence of two distinct clades (*A* and *B*) and thus suggested past evolution of white-tailed eagles in two groups. We therefore investigated population history not on the basis of the current geographically defined populations, but instead on the basis of the past subdivision and evolutionary divergence of the major phylogenetic lineages (as in Flagstad & Roed, 2003; Godoy *et al.*, 2004). Haplogroup *B* exhibited a frequent central haplotype together with several closely related derived haplotypes at lower frequencies. Such a star-shaped pattern is a common characteristic of lineages following a demographic expansion (Slatkin & Hudson, 1991). Haplogroup *A* also shows a predominant central haplotype. However, only two other derived haplotypes were encountered within this clade.

Fu's (1997) F_S statistic was significant for clade *B* (-5.54, $P < 0.001$), but not for clade *A* (0.80, $P > 0.10$), while Fu & Li's (1993) F^* and D^* were non-significant ($P > 0.05$) for both *B* ($F^* = 0.38$, $D^* = 1.26$) and *A* ($F^* = 0.71$, $D^* = 0.001$). Results consistent with this were obtained with the program LAMARC, which yielded a growth parameter of $g = 2830$ (95% CI: 669 to 9062) for clade *B*, but a value for clade *A* ($g = 1172$) whose 95% CI included negative values (95% CI: -12 to 8793), thus not excluding population decline or stasis for *A*. In summary, these tests indicate a population expansion in haplogroup *B*, but give inconclusive results for haplogroup *A*.

The mismatch distribution analyses yielded a bimodal pattern for the total data set (results not shown). For haplogroup *A*, the least-squares procedure in ARLEQUIN did not converge when fitting the model to the observed data. For lineage *B*, deviation from the sudden expansion model was not significant ($\Theta_0 = 0.000$, $\Theta_1 = 483.1$, $P > 0.05$). The peak of the corresponding mismatch distribution was at $\tau = 0.689$ (95% CI: 0.335 to 0.951). Using the formula $t = \tau/(l\ 2\ \mu)$, where l and μ represent the sequence length and the substitution rate per site and million years, and using the divergence rate calibration by Wenink *et al.* (1996) of 14.8% per bp and million years, we estimated that the expansion of haplogroup *B* took place 9311 yr BP (95% CI: 4527 to 12,851). Using divergence rates of between 5% and 20% (see Brito, 2005) yielded average values for the time since expansion of between 6890 and 27,560 yr BP – thus around the Pleistocene–Holocene transition across a wide range of parameters.

Values of nucleotide diversity (π) for the eastern (*B*) and western (*A*) clade were 0.00120 and 0.00098, corresponding to a genetic effective population size of females ($N_{e,f}$) of around 560 and 460 females, respectively.

DISCUSSION

Species-level diversity

Our study revealed relatively few haplotypes and a shallow within-species divergence between the white-tailed eagle haplogroups. Over a broad range of conceivable mutation rates, the divergence of the major lineages present in the species dates back 50,000 to 200,000 years, pointing to an intra-species lineage splitting during the late Pleistocene. This pattern has been observed in several other Northern Hemisphere, especially boreal, species of birds (Lovette, 2005). Pleistocene climatic shifts are a likely explanation for both the splitting into an eastern and western group and the recent diversification of each haplogroup.

Another related large raptor with a wide geographic distribution is the bearded vulture (*Gypaetus barbatus*, Linnaeus, 1758). The white-tailed eagle harbours less overall nucleotide diversity than the bearded vulture (about 0.7%, as compared with 2.9% in the bearded vulture; Godoy *et al.*, 2004). The bearded vulture thus appears to have retained higher effective population sizes than the white-tailed eagle has. This may indicate a higher sensitivity of white-tailed eagles to climatic fluctuations, and/or be related to the wider distribution range of the bearded vulture, which extends to southern Africa. Furthermore, the amount of mtDNA diversity in the white-tailed eagle is similar to that described for control-region sequences of another raptor, the red kite (*Milvus milvus*, Linnaeus, 1758; Roques & Negro, 2005), despite the distribution of the latter being largely restricted to Europe.

Phylogeographic structure and range contraction during cold periods

MtDNA control-region sequences of the white-tailed eagle clustered into two distinct major haplogroups with an east–west cline through Eurasia (Fig. 1, Table 2). Such a pattern has been observed in many other Eurasian and North American taxa (Hewitt, 2000; Ruokonen *et al.*, 2004; Lovette, 2005) and probably reflects range contraction into two allopatric refugia, followed by postglacial re-expansion. The finding of haplotype *C01* in the data set may result from retained ancestral polymorphism, or indicate the presence of a third refugium.

For species like the white-tailed eagle it is not clear if a glacial refugium can be envisioned in a similar way as the traditional refugia of temperate forest species. White-tailed eagles have a large dispersal capability and vagrant individuals can cover vast areas. Populations live at rather low densities, and it is possible that large areas were required to sustain viable populations during glacial maxima. A glacial refugium for the white-tailed eagle may therefore have spanned large areas and varied spatio-temporally along with climatic and other environmental changes.

The restricted number of region-specific haplotypes prevents us from establishing precise locations for the refugia. However, our data suggest that the white-tailed eagle survived the last glaciation period in at least two Eurasian regions, neither of which was likely to have been on the Pacific coast. The populations in eastern Russia and Japan show low haplotype and nucleotide diversity and share all their haplotypes with the European populations. This pattern of genetic diversity is characteristic of postglacially founded populations. The only extant breeding population of Steller's sea eagles (*Haliaeetus pelagicus*) is located on the Pacific coast of Asia. In areas of sympatry with the white-tailed eagle, competitive advantage with regard to both nesting sites and prey has been documented for Steller's sea eagle (Masterov, 1992). Whether or not competitive exclusion by Steller's sea eagles had an impact on the survival and distribution of the white-tailed eagle during glacial times is not known.

The western refugium could have been located in western Europe, possibly along the Atlantic coast. A glacial refugium somewhere in that region has been postulated for many species, for example coastal birds (seagulls and eider ducks; Tiedemann *et al.*, 2004; Liebers *et al.*, 2004), several fishes (e.g. Volckaert *et al.*, 2002) and a seaweed (Provan *et al.*, 2005). Compatible with this, the white-tailed eagle has been reported as the most abundant diurnal raptor in the Pleistocene fossil record of the Iberian peninsula (Sánchez-Marco, 2004; Antonio Sánchez-Marco, personal communication). A glacial refugium in Iberia has been postulated for the Spanish imperial eagle (*Aquila adalberti*, C. L. Brehm, 1861 Ferrer & Negro, 2004).

The location of the eastern refugium could be the region around the Aralo-Caspian and Black Sea basin. Water levels of the Caspian and Black Sea during the last glacial maximum (LGM) were considerably higher than they are today (Grosswald & Hughes, 2002), implying a larger surface and thus longer coastline. The region has a high degree of endemism (Dumont, 1998) and has been proposed as glacial refugium for many other species linked to aquatic habitats, for example fishes (Bernatchez, 2001; Kotlik *et al.*, 2004), crustaceans (Audzijonyte *et al.*, 2005) and seagulls (Liebers *et al.*, 2004).

The roles of the Danube river system and the Mediterranean coast as possible glacial refugia for the white-tailed eagle remain unclear. These alternatives are difficult to assess because many historic populations have gone extinct in Spain, Italy and France, or have recently undergone dramatic declines in Greece, Albania, Serbia, Croatia and Romania (Helander & Stjernberg, 2003).

The survival of white-tailed eagles in these refugia is very similar to the scenario proposed by Liebers *et al.* (2004) for the herring gull complex, a taxonomic group resembling the white-tailed eagle in many ecological features regarding their predominant habitat choice and foraging requirements. Also for the herring gull complex, mtDNA indicates Ice Age survival in a Western European and one central Eurasian refugium (Liebers *et al.*, 2004). Such congruence of phylogeographic patterns among different species with similar requirements supports the importance of ecological factors in shaping current mtDNA variation.

Demography: population expansion out of the refugia

The fossil record indicates that the white-tailed eagle was an early postglacial colonizer of northern regions. Bone remains (9000 ^{14}C yr BP) have been recovered from southern Sweden (Ericson & Tyrberg, 2004) and from near Stavanger, Norway (7000–8000 yr BP; J Mangerud, University of Bergen, Norway, personal communication). In accordance with this, results from the mismatch distribution analysis for the eastern clade (*B*) indicated a sudden population expansion from the eastern refugium at or after the latest stages of the Pleistocene (< 30,000 yr BP; average estimate with rate calibration by Wenink *et al.*, 1996: 9311 yr BP). This demographic growth may have been triggered by glacier retreat and climate warming following the LGM (20–15,000 yr BP; Svendsen *et al.*, 2004). After the LGM, climate warming led to an increased availability of coastal landscapes. Extensive new suitable habitats appeared where meltwater from retreating glaciers accumulated, and an abundance of ice-dammed lakes, rivers and other water systems existed from the Caspian Sea towards the Baltic region (Mangerud *et al.*, 2004; see also Fig. 1).

The demography of the western white-tailed eagle clade *A* is difficult to deduce from our data. While it is possible that this group did not expand as markedly as the eastern group *B*, or that it expanded later, a third option is also conceivable. During the last 150 years, white-tailed eagles have disappeared from vast parts of their historic distribution range in southern and western Europe (Helander & Stjernberg, 2003). Especially if those regions harboured refugial populations, loss of diversity during recent centuries may have influenced our results (see Leonard *et al.*, 2005). We may thus underestimate the original clade *A* diversity and miss signals of a postglacial population expansion.

Our results indicate that Iceland and Greenland were colonized by white-tailed eagles from northern or western Europe, possibly via the Faroe islands. Two haplotypes were encountered in Iceland and Greenland, namely *A01* and *A03* (Table 1). *A01* is the presumed western refugial haplotype, while *A03* is a derived form currently restricted to Iceland and Greenland and which probably arose during postglacial range expansion. A similar colonization history has been documented for many other animal taxa now present in Greenland and/or Iceland (Sadler, 1999; Tiedemann *et al.*, 2004; Muñoz-Fuentes *et al.*, 2006), i.e. a predominantly Palaearctic rather than Nearctic origin.

Demographic responses to climate change have previously been proposed to vary in timing and intensity at different latitudes (Hewitt, 2000; Lessa *et al.*, 2003). In the white-bellied sea eagle (*Haliaeetus leucogaster*, Gmelin, 1788), which is mainly distributed in tropical and subtropical regions (India through Southeast Asia to Australia), a major population expansion was dated to have occurred around 160,000 yr BP (Shepard *et al.*, 2005; also analysing the mtDNA control region). The authors related this expansion to a period of lowered sea levels, enabling colonization of new habitats.

Current population-level diversity

For several European populations of the white-tailed eagle, levels of mtDNA diversity are relatively high, reflecting the admixture of sequences from divergent clades. However, the finding of high mtDNA variation in northern populations that inhabited regions covered by glaciers during the LGM is noteworthy. Confirmed by all diversity measures, the highest variability was found in the populations surrounding the Baltic Sea (Table 2), and not in regions that were unglaciated during the LGM. Several factors can explain this pattern of high genetic diversity in northern populations. First, an eradication of diversity in the south might have occurred. However, the species has a long generation time and has been shown to be relatively resilient against loss of genetic diversity, at least within the time perspective of a few decades (Hailer *et al.*, 2006). Second, the population expansion could have occurred mainly in the north. Most haplotypes are separated from either *A01* or *B01* by just one or two mutational steps. Our dating of the population expansion of clade *B* indicates that the age of these derived haplotypes postdates the LGM and thus points towards an origin in the postglacially expanding populations. Following glacier retreat, the vast water masses arising in proglacial regions of northern Europe (see above) might have constituted highly productive habitats enabling early colonization and pronounced population expansion. In summary, we propose a scenario of Ice Age survival in southerly regions, followed by rapid postglacial colonization of northern habitats, major population expansion, and generation of a large proportion of the current mtDNA gene pool of the species.

On a regional scale, despite clear general phylogeographic structuring of mtDNA diversity (Fig. 3, Table 4), some observed patterns deviate from the large-scale picture. Comparing the neighbouring populations from Norway and Kola peninsula, very different patterns of genetic diversity are observed. While the Norwegian population is largely fixed for haplotype *A01*, a variety of different haplotypes were found on Kola (Table 1). This difference between neighbouring populations is indicative of low gene flow and stresses the importance of local population history for present-day genetic variability. Because the Norwegian population has been the largest in Europe during the last century, this pattern is not the result of any recent declines (Hailer *et al.*, 2006).

Conservation implications

White-tailed eagle mtDNA suggests the absence of populations that should be defined as evolutionary significant units (ESUs; see Moritz, 1994). The two major lineages (*A* and *B*) have a broad zone of admixture and are thus largely sympatric today, at least in Europe. However, the present distribution range and recent history of some populations indicate that they should be regarded as largely isolated reproductive units.

For Greenland, some authors have proposed a separate subspecies (*H. albicilla groenlandicus*; see Glutz von Blotzheim *et al.*, 1971). The fossil record indicates that the white-tailed

eagle colonized Greenland during the Holocene. Salomonsen (1979) suggested that this might have been during the hypsithermal interval, between 6000 and 4000 yr BP. Given the absence of well-differentiated haplotypes from Greenland, and the likely postglacial origin of haplotype *A03*, our data are compatible with this recent colonization. However, given the lack of reciprocal monophyly, our data do not lend strong support to the subspecies distinction. MtDNA structuring and subspecies distinctions commonly do not coincide for avian taxa (Zink, 2004), suggesting that ecological and/or morphological distinction may be attained more quickly than mtDNA lineage sorting (Bulgin *et al.*, 2003). As emphasized by Crandall *et al.* (2000), ESUs should also be defined on the basis of ecological characteristics. A series of morphological measurements (e.g. bill length, wing length and egg size) are known to vary along a cline from north-west to south-east throughout Eurasia, which led Glutz von Blotzheim *et al.* (1971) to refute the status of white-tailed eagles from Greenland as a separate subspecies (*H. a. groenlandicus*). However, as Salomonsen (1979) pointed out, skeletal measurements of *H. a. groenlandicus* and *H. a. albicilla* do not overlap, possibly supporting the subspecies distinction. The low mtDNA diversity in Greenland and Iceland, and the unique occurrence of haplotype *A03* strongly suggest that these populations have long been isolated from other white-tailed eagle populations, and they may today also be isolated from each other. Such small and isolated populations deserve special attention and high conservation priority.

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REFERENCES

Audzijonyte, A., Damgaard, J., Varvio, S.L., Vainio, J.K. & Vainola, R. (2005) Phylogeny of Mysis (Crustacea, Mysida): history of continental invasions inferred from molecular and morphological data. *Cladistics*, **21,** 575–596.

Avise, J.C. (2000) *Phylogeography. The history and formation of species*. Harvard University Press, Cambridge, MA.

Bernatchez, L. (2001) The evolutionary history of brown trout (*Salmo trutta*) inferred from phylogeographic, nested clade and mismatch distribution analyses of mitochondrial DNA variation. *Evolution*, **55,** 351–379.

Brito, P.H. (2005) The influence of Pleistocene glacial refugia on tawny owl genetic diversity and phylogeography in western Europe. *Molecular Ecology*, **14,** 3077–3094.

Bulgin, N.L., Gibbs, H.L., Vickery, P. & Baker, A.J. (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionarily distinct populations in the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*). *Molecular Ecology*, **12,** 831–844.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9,** 1657–1660.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. & Wayne, R.K. (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15,** 290–295.

Dalén, L., Fuglei, E., Hersteinsson, P., Kapel, C.M.O., Roth, J.D. & Samelius, G. (2005) Population history and genetic structure of a circumpolar species: the arctic fox. *Biological Journal of the Linnean Society*, **84,** 79–89.

Dumont, H.J. (1998) The Caspian Lake: History, biota, structure, and function. *Limnology and Oceanography*, **43,** 44–52.

Ericson, P.G.P. & Tyrberg, T. (2004) *The early history of the Swedish avifauna. A review of the subfossil record and early written sources. Kungl. Vitterhets Historie och Antikvitetsakademins Handlingar. Antikvariska Serien 45*. Almqvist & Wiksell International, Stockholm.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131,** 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin version 3.0, An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1,** 47–50.

Ferrer, M. & Negro, J.J. (2004) The near-extinction of two large European predators: super-specialists pay a price. *Conservation Biology*, **18,** 344–349.

Flagstad, Ø. & Roed, K.H. (2003) Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **57,** 658–670.

Fleischer, R.C., Olson, S.L., James, H.F. & Cooper, A.C. (2000) Identification of the extinct Hawaiian Eagle (*Haliaeetus*) by mtDNA sequence analysis. *The Auk*, **117,** 1051–1056.

Fu, Y.-X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147,** 915–925.

Fu, Y.-X. & Li, W.-H. (1993) Statistical tests of neutrality of mutations. *Genetics*, **133,** 693–709.

Garcia-Moreno, J. (2004) Is there a universal mtDNA clock for birds? *Journal of Avian Biology*, **35,** 465–468.

Glutz von Blotzheim, U.N., Bauer, K.M. & Bezzel, E. (1971) *Handbuch der Vögel Mitteleuropas. Band 4: Falconiformes*. Akademische Verlagsgesellschaft, Frankfurt am Main.

Godoy, J.A., Negro, J.J., Hiraldo, F. & Donázar, J.A. (2004) Phylogeography, genetic structure and diversity in the endangered bearded vulture (*Gypaetus barbatus*, L.) as revealed by mitochondrial DNA. *Molecular Ecology*, **13,** 371–390.

Grosswald, M.G. & Hughes, T.J. (2002) The Russian component of an Arctic Ice Sheet during the LGM. *Quaternary Science Reviews*, **21,** 121–146.

Hailer, F., Helander, H., Folkestad, A.O., Ganusevich, S.A., Garstad, S., Hauff, P., Koren, C., Nygård, T., Volke, V., Vilà, C. & Ellegren, H. (2006) Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters*, **2,** 316–319.

Helander, B. (2003) The international colour-ringing programme – adult survival, homing, and the expansion of the White-tailed Sea Eagle in Sweden. *Sea Eagle 2000. Proceedings from an international conference* (ed. by B. Helander, M. Marquiss and W. Bowerman), pp. 145–154. Swedish Society for Nature Conservation/SNF & Åtta. 45 Tryckeri AB, Stockholm.

Helander, B. & Stjernberg, T. (2003) *Action plan for the conservation of white-tailed eagle (Haliaeetus albicilla)*. Recommendation 92/2002, adopted by the Standing Committee of the Bern Convention in Dec., 2002. BirdLife International, Strasbourg, France.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405,** 907–913.

Ho, S.Y.W. & Larson, G. (2006) Molecular clocks: when times are a-changin'. *Trends in Genetics*, **22,** 77–83.

Horváth, M., Martínez-Cruz, B., Negro, J.J., Kalmár, L. & Godoy, J.A. (2005) An overlooked DNA source for non-invasive genetic analysis in birds. *Journal of Avian Biology*, **36,** 84–88.

Hudson, R.R. (1990) Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology*, **7,** 1–44.

Ingman, M. & Gyllensten, U. (2001) Analysis of the complete human mtDNA genome: methodology and inferences for human evolution. *Journal of Heredity*, **92,** 454–461.

Jensen, J.L., Bohonak, A.J. & Kelley, S.T. (2005) Isolation by distance, web service. *BMC Genetics*, **6,** 13 (http://phage.sdsu.edu/~jensen/).

Katzner, T.E. (2002) *Ecology and behavior of four coexisting eagle species at Naurzum Zapovednik, Kazakhstan*. PhD thesis, Arizona State University, Tempe, AZ.

Kotlik, P., Bogutskaya, N.G. & Ekmekci, F.G. (2004) Circum Black Sea phylogeography of *Barbus* freshwater fishes: divergence in the Pontic glacial refugium. *Molecular Ecology*, **13,** 87–95.

Kuhner, M.K., Yamato, J. & Felsenstein, J. (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, **149,** 429–434.

Kumar, S., Tamura, K. & Nei, M. (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, **5,** 150–163.

Leonard, J.A., Vilà, C. & Wayne, R.K. (2005) Legacy lost: genetic variability and population size of extirpated US gray wolves (*Canis lupus*). *Molecular Ecology*, **14,** 9–17.

Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100,** 10331–10334.

Liebers, D., de Knijff, P. & Helbig, A.J. (2004) The herring gull complex is not a ring species. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **271,** 893–901.

Lovette, I.J. (2005) Glacial cycles and the tempo of avian speciation. *Trends in Ecology & Evolution*, **20,** 57–59.

Mangerud, J., Jakobsson, M., Alexanderson, H., Astakhov, V., Clarke, G.K.C., Henriksen, M., Hjort, C., Krinner, G., Lunkka, J.-P., Moeller, P., Murray, A., Nikolskaya, O., Saarnisto, M. & Svendsen, J.I. (2004) Ice-dammed lakes and rerouting of the drainage of northern Eurasia during the last glaciation. *Quaternary Science Reviews*, **23,** 1313–1332.

Masterov, V.B. (1992) *Ecological energetic and interspecies relationships of sea eagles H. albicilla and H. pelagicus in the Lower Amur Region and Sakhalin*. PhD thesis, Moscow State University (in Russian).

Moritz, C. (1994) Defining "evolutionarily significant units" for conservation. *Trends in Ecology & Evolution*, **9,** 373–375.

Muñoz-Fuentes, V., Green, A.J., Sorenson, M.D., Negro, J.J. & Vilà, C. (2006) The ruddy duck in Europe, natural colonization or human introduction? *Molecular Ecology*, **15,** 1441–1453.

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press, New York.

Nei, M. & Kumar, S. (2000) *Molecular evolution and phylogenetics*. Oxford University Press, New York.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14,** 817–818.

Posada, D. & Crandall, K.A. (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, **16,** 37–45.

Provan, J., Wattier, R.A. & Maggs, C.A. (2005) Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel. *Molecular Ecology*, **14,** 793–803.

Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19,** 2092–2100.

Rogers, A.R. & Harpending, H. (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9,** 552–569.

Roman, J. & Palumbi, S.R. (2003) Whales before whaling in the North Atlantic. *Science*, **301,** 508–510.

Roques, S. & Negro, J.J. (2005) MtDNA diversity and population history of a dwindling raptorial bird, the red kite (*Milvus milvus*). *Biological Conservation*, **126,** 41–50.

Rousset, F. (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145,** 1219–1228.

Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19,** 2496–2497.

Rudnick, J.A., Katzner, T.E., Bragin, E.A., Rhodes, A.E., Jr & DeWoody, J.A. (2005) Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Molecular Ecology*, **14,** 2959–2967.

Ruokonen, M., Kvist, L., Aarvak, T., Markkola, J., Morozov, V.V., Øien, I.J., Syroechkovsky, E.E., Jr, Tolvanen, P. & Lumme, J. (2004) Population genetic structure and conservation of the lesser white-fronted goose (*Anser erythropus*). *Conservation Genetics*, **5,** 501–512.

Sadler, J.P. (1999) Biodiversity on oceanic islands: a palaeoecological assessment. *Journal of Biogeography*, **26,** 75–87.

Salomonsen, F. (1979) Ornithological and ecological studies in SW Greenland. *Meddelelser om Groenland, 204(6)*. Nyt Nordisk Forlag Arnold Busck, Copenhagen.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sánchez-Marco, A. (2004) Avian zoogeographical patterns during the Quaternary in the Mediterranean region and paleoclimatic interpretation. *Ardeola*, **51,** 91–132.

Schneider, S. & Excoffier, L. (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. *Genetics*, **152,** 1079–1089.

Shapiro, B., Drummond, A.J., Rambaut, A., Wilson, M.C., Matheus, P.E., Sher, A.V., Pybus, O.G., Gilbert, M.T., Barnes, I., Binladen, J., Willerslev, E., Hansen, A.J., Baryshnikov, G.F., Burns, J.A., Davydov, S., Driver, J.C., Froese, D.G., Harington, C.R., Keddie, G., Kosintsev, P., Kunz, M.L., Martin, L.D., Stephenson, R.O., Storer, J., Tedford, R., Zimov, S. & Cooper, A. (2004) Rise and fall of the Beringian steppe bison. *Science*, **306,** 1561–1565.

Sharma, D.K., Maldonado, J.E., Jhala, Y.V. & Fleischer, R.C. (2004) Ancient wolf lineages in India. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **271** (S3)**,** S1–S4.

Shepard, J.M., Hughes, J.M., Catterall, C.P. & Olsen, P.D. (2005) Conservation status of the White-Bellied Sea-Eagle *Haliaeetus leucogaster* in Australia determined using mtDNA control region sequence data. *Conservation Genetics*, **6,** 413–429.

Slatkin, M. & Hudson, R.R. (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129,** 555–562.

Sorenson, M.D. & Quinn, T.W. (1998) Numts: a challenge for avian systematics and population biology. *The Auk*, **115,** 214–221.

Struwe-Juhl, B. (2003) Age-structure and productivity of a German White-tailed Sea Eagle population. *Sea Eagle 2000. Proceedings from an international conference* (ed. by B. Helander, M. Marquiss and W. Bowerman), pp. 181–190. Swedish Society for Nature Conservation/SNF & Åtta. 45 Tryckeri AB, Stockholm.

Svendsen, J.I., Alexanderson, H., Astakhov, V.I., Demidov, I., Dowdeswell, J.A., Henriksen, M., Hjort, C., Houmark-Nielsen, M., Hubberten, H.W., Ingólfson, O., Jakobsson, M., Kjær, K., Larsen, E., Lokrantz, H., Lunkka, J.P., Lysså, A., Mangerud, J., Maslenikova, O., Matioushkov, A., Murray, A., Möller, P., Niessen, F., Saarnisto, M., Siegert, C., Stein, R., Siegert, M.J. & Spielhagen, R. (2004) Late Quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews*, **23,** 1229–1271.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7,** 453–464.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123,** 585–595.

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **9,** 512–526.

Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132,** 619–633.

Tiedemann, R., Paulus, K.B., Scheer, M., von Kistowski, K.G., Skirnisson, K., Bloch, D. & Dam, M. (2004) Mitochondrial DNA and microsatellite variation in the eider duck (*Somateria mollissima*) indicate stepwise postglacial colonization of Europe and limited current long-distance dispersal. *Molecular Ecology*, **13,** 1481–1494.

Vilà, C., Amorim, I.R., Leonard, J.A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F., Crandall, K.A., Ellegren, H. & Wayne, R.K. (1999) Mitochondrial DNA phylogeography and population history of the gray wolf *Canis lupus*. *Molecular Ecology*, **8,** 2089–2103.

Volckaert, F.A.M., Hänfling, B., Hellemans, B. & Carvalho, G.R. (2002) Timing of the population dynamics of bullhead *Cottus gobio* (Teleostei: Cottidae) during the Pleistocene. *Journal of Evolutionary Biology*, **15,** 930–944.

Wenink, P.W., Baker, A.J., Rösner, H.-U. & Tilanus, M.G.J. (1996) Global mitochondrial DNA phylogeography of Holarctic breeding Dunlins (*Calidris alpina*). *Evolution*, **50,** 318–330.

Wink, M., Heidrich, P. & Fentzloff, C. (1996) A mtDNA phylogeny of sea eagles (genus Haliaeetus) based on nucleotide sequences of the cytochrome b-gene. *Biochemical Systematics and Ecology*, **24,** 783–791.

Zink, R.M. (2004) The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **271,** 561–564.

BIOSKETCH

Frank Hailer did his PhD work in the research group of **Carles Vilà** and **Hans Ellegren**, studying conservation genetics of the white-tailed eagle.
Jamie Rudnick uses genetic analyses on non-invasively collected feathers to investigate eagle biology in central Asia.
Björn Helander is senior scientist responsible for the monitoring of white-tailed sea eagle reproduction and population trends within the National Environmental Monitoring Programme under the Swedish Environment Protection Agency. He is also leader of Project Sea Eagle run by the Swedish Society for Nature Conservation/SNF.
Alv O. Folkestad, Sergey Ganusevich, Steinar Garstad, Peter Hauff, Christian Koren, Vladimir Masterov, Torgeir Nygård, Saiko Shiraki, Kristinn Skarphedinsson, Veljo Volke and **Frank Wille** work with various aspects of raptor biology and contributed samples to this study.

Editor: Brett Riddle