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# Conservation Genetics of the White-Tailed Eagle

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**Abstract**

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The white-tailed eagle is a formerly threatened raptor that is commonly used as a flagship and indicator species in conservation work. This thesis uses molecular genetic methods to study sex determination of nestlings, genetic variability, population structure and phylogeography of the white-tailed eagle.

Fourteen microsatellite markers were developed and tested for the white-tailed eagle.

A method to sex white-tailed eagle nestlings in the field is presented. The method is based on just one tarsus measure, and is suitable for situations where a single person is handling the nestlings alone in a treetop.

Most European white-tailed eagle populations underwent extreme declines during the 20th century. The results presented here show that bottlenecked populations have maintained significant levels of genetic diversity. Gene flow between regions is not a main explanation for this, as indicated by both genetic and ringing data. Instead, the long generation time of white-tailed eagles has acted as an intrinsic buffer against rapid loss of genetic diversity. Additionally, local conservation led to protection of more genetic diversity than if conservation had focused on the large remnant population in Norway.

Mitochondrial DNA of white-tailed eagles is structured in two main clades with a predominantly eastern and western Eurasian distribution. The clades likely correspond to separate Ice Age refugia but do not grant classification as evolutionary significant units given their current extensive overlap across large parts of Eurasia.

Microsatellite variation was studied in populations across Eurasia. Variability was rather constant across the continent, but clearly lower on Iceland and Greenland. This is best explained by founder effects during their colonisation, but only weak bottlenecks during colonisation of and persistence on the continent. Current population differentiation between Europe and eastern Eurasia is not compatible with a zero gene flow model but requires some amount of gene flow over evolutionary time scales.

*Keywords:* *Haliaeetus albicilla*, microsatellites, mtDNA, molecular sexing, population structure, bottleneck, phylogeography, raptors

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*to my parents,  
and my sister and brother*

Colour artwork on front page  
kindly provided by Anna Widén.

## List of papers

The thesis is based on the following papers, hereafter referred to by their roman capitals.

- I.** Hailer F, Gautschi B, Helander B (2005): Development and multiplex PCR amplification of novel microsatellite markers in the white-tailed sea eagle, *Haliaeetus albicilla* (Aves: Falconiformes, Accipitridae). *Molecular Ecology Notes* 5: 938-940.
- II.** Helander B, Hailer F, Vilà C: Morphological and genetic sex identification of white-tailed eagle nestlings. *Manuscript*.
- III.** Hailer F, Helander H, Folkestad AO, Ganusevich SA, 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*, in press.  
(published online: doi:10.1098/rsbl.2006.0453)
- IV.** Hailer F, Helander B, Folkestad AO, Ganusevich SA, Garstad S, Hauff P, Koren C, Masterov VB, Nygård T, Rudnick JA, Saiko S, Skarphedinsson K, Volke V, Wille F, Vilà C: Phylogeography of the white-tailed eagle, a generalist with large dispersal capacity. *Submitted manuscript*.
- V.** Hailer F, Helander B, Olsson M, Folkestad AO, Ganusevich SA, Garstad S, Hauff P, Koren C, Masterov VB, Nygård T, Saiko S, Skarphedinsson K, Volke V, Wille F, Ellegren H, Vilà C: Signatures of coancestry and gene flow between populations of the white-tailed eagle. *Manuscript*.

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# Abbreviations

c.i.	confidence intervals
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DDT	dichloro-diphenyl-trichloroethane
$H_E$	expected heterozygosity
$H_O$	observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IUCN	World Conservation Union
LD	linkage disequilibrium
mtDNA	mitochondrial DNA
$N_A$	mean number of alleles per locus
$N_e$	(genetic) effective population size
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
s.d.	standard deviation



## Introduction: vulnerability of top consumer populations

Numerous top consumers, including avian and mammalian species, are today prime targets of conservation efforts (Soulé & Terborgh 1999). Many of them are under legal protection and closely monitored by conservation biologists. Top predators are endangered by intrinsic and extrinsic factors, and by interactions among those factors. As an example of the latter, life history traits can make predators susceptible to alterations of their habitat by human activities.

### Life history traits (intrinsic factors)

Population sizes of predator species are usually quite small since, from an ecosystem perspective, each step towards the end of the food chain means that a lower biomass can be sustained. This has been suggested theoretically and also been shown empirically (Cohen *et al.* 2003; Jonsson *et al.* 2005). An effect of their smaller population size is that predators are generally more prone to become demographically endangered than more abundant species (Schaffer 1987). In combination with this, life history traits such as low reproductive rate, large home ranges and complex social systems contribute to their vulnerability (Purvis *et al.* 2001; Cardillo *et al.* 2005). The importance of these factors in shaping predator populations can be illustrated by the fact that small islands generally do not harbour large-bodied predators (Frankham *et al.* 2002).

### Extrinsic factors

The conflict between humans and our carnivore “competitors” has a broad and intricate background, rendering their conservation a complex task (*e.g.* Breitenmoser 1998). Competition for common resources (wild game species and livestock) explains part of this conflict, as does human fear of attacks (primarily regarding mammalian predators; Nowell and Jackson 1996). In numerous human societies, predators are associated with evil and subject to intense persecution.

The population history of avian top predators in Scandinavia illustrates this conflict. Major population declines occurred during the last two centuries, followed by recent population recovery, *e.g.* of the peregrine falcon *Falco peregrinus*, golden eagle *Aquila chrysaetos*, eagle owl *Bubo bubo* and white-tailed eagle *Haliaeetus albicilla* (Lindberg *et al.* 1988; Tjernberg 2005; Olsson 1997; Gerdehag and Helander 1988).

Raptors are some of the most threatened birds in Europe and many of them are classified as endangered (Tucker and Heath 1994). However, the factors underlying raptor population declines in many cases reach beyond direct persecution by humans. Among the various human-mediated threats to raptor populations, habitat loss, decline of prey populations, electrocution at power lines and collision with traffic are among the most common causes of death (summarised in Whitfield *et al.* 2004). Another major threat to raptor populations is the accumulation of harmful substances in the environment..

### Accumulation of harmful substances in the food chain

The decline of raptor populations across large parts of Eurasia and North America during the mid-20<sup>th</sup> century was related to their high trophic position in the food chain. Bioaccumulating substances (*i.e.* substances that persist at higher concentrations in the food web than in the surrounding abiotic environment) are found at increasing concentrations as one goes from a lower to a higher trophic level (*e.g.* Atwell *et al.* 1998). Raptors thus generally show elevated levels of environmental pollutants in their bodies.

Many bioaccumulating substances are developments of the chemical industry (Birnbaum and Staskal 2004). One such class of chemicals, polychlorinated biphenyls (PCBs), was introduced in the late 1920s, among other reasons initially for usage as a treatment for closed electronic systems. During the 1940s, dichloro-diphenyl-trichloroethane (DDT) was introduced as an insecticide around large parts of the world, primarily to eliminate mosquito populations conveying malaria. The use of DDT was later increased, with extensive application in agriculture and other insect “control” measures. Already in the early 1950s, accumulation of DDT was detected and some associated risks were known (Laug *et al.* 1951). Somewhat later, PCBs were discovered as global contaminants (Jensen 1966).

The bioaccumulation of organochlorine substances such as DDT and PCB can cause eggshell thinning and depressed reproduction in birds (Riesebrough 1994). These substances were a major cause of the mid-20<sup>th</sup> declines of raptor populations around the world. Many persistent chemicals have been found to accumulate in human fatty tissues, are present in breast milk, and concerns have thus been raised regarding possible detrimental effects on humans (Laug *et al.* 1951).

Lead poisoning is another factor contributing to increased raptor mortality. The source of the lead is often ammunition used by hunters. Scavenging and raptorial birds can ingest lead when preying on shot-dead or crippled prey (in many cases waterfowl). For instance, about 25% of 80 recovered carcasses of white-tailed eagles analysed in Germany showed lead concentrations in their liver tissue at presumed lethal concentrations (Kenntner *et al.* 2003).

## Some reflections on why to protect top predators

There are a number of reasons as to why top consumers deserve special protection. From a purely anthropogenic perspective, they have often served as symbols of endangered habitats and species. For this reason, they are commonly used as **flagship species** in conservation (see table 1 for definition). Carnivores are thus commonly selected by conservation organisations to attract public opinion.

Table 1. *Concepts of using focal species for conservation planning (adapted from Lindell et al. 2000).*

concept	explanation
<b>flagship</b>	“...normally a charismatic large vertebrate, is one that can be used to anchor a conservation campaign because it arouses public interest and sympathy... .” (Simberloff 1998)
<b>indicator</b>	“ an organism whose characteristics (e.g., presence or absence, population density, dispersion, reproductive success) are used as an index of attributes too difficult, inconvenient, or expensive to measure for other species or environmental conditions of interest.” (Landres <i>et al.</i> 1998) Can further be subdivided into health, population and biodiversity indicators (Caro and O’Doherty 1999).
<b>keystone</b>	“...certain species have impacts on others, often far beyond what might be expected from a consideration of their biomass or abundance.” (Simberloff 1998)
<b>umbrella</b>	“...a species that needs such large tracts of habitat that saving it will automatically save many other species.” (Simberloff 1998)

Predators are often considered to be **indicator species** (table 1) representing healthy ecosystems. Although this is not necessarily true for mammalian carnivores (*e.g.* Lindell *et al.* 2000), it is the case for many raptors, due to the impact of environmental pollutants on their populations (see section

above). In Sweden, for example, the white-tailed eagle is used as indicator by the National Environment Monitoring Programme.

From an ecological point of view, predators play a complex role in ecosystems that after their extinction could lead to severe imbalance in the ecosystem (Croll *et al.* 2005; Gittleman *et al.* 2001; Terborgh *et al.* 2001; see also Roemer *et al.* 2002). By exhibiting demographic control of prey populations (and also influencing behaviour and habitat use; reviewed in Miller *et al.* 2001), predators can therefore be considered to be **keystone species** (table 1) in their environment. Since many predators prey on herbivores, their interaction can also affect regeneration patterns of vegetation (*e.g.* in Yellowstone National park where the reintroduction of wolves led to a marked change in riparian vegetation; Ripple *et al.* 2001; Ripple and Beschta 2004). Further, the presence of carnivores leads to increased availability of carrion, thus affecting the guild of carrion feeders. This mechanism has been suggested to act as a buffer for facultative carrion feeders against climatic fluctuations (Wilmers and Getz 2005).

Lastly, predators can serve as **umbrella species** (see table 1; Shrader-Frechette and McCoy 1993): predators generally possess large home ranges, thus their effective conservation encompasses extensive areas and habitats. Further, their terminal position in the food chain implies that predator conservation also may require conservation of numerous other species in their environment.

It should however be mentioned that the use of such 'focal' species for popularisation and delineation of conservation strategies has been criticised. Especially, the usefulness of the concept of umbrella species may be highly debatable (*e.g.*, Andelman and Fagan 2000; Lindell *et al.* 2000). Further, detailed predictions based on indicator species on the demography of other species may in fact be hard to make.

The use of indicator species within an ecotoxicological framework is much more accepted (Caro *et al.* 2005), and many countries employ monitoring programs, whereby species are being surveyed to check for early warning signs of environmental change. Further, the discussion on the role of predators as keystone species has been revitalised in recent years by empirical findings (*e.g.* Ripple *et al.* 2001; Croll *et al.* 2005; Wilmers and Getz 2005), emphasising their importance in ecosystems..

## The study species

The white-tailed eagle *Haliaeetus albicilla* (Linnaeus, 1758) (also called ‘white-tailed sea eagle’ or ‘grey sea eagle’) is the largest eagle in Europe. The eagle is used as a major flagship species for conservation work in many European and Asian countries. In addition to being a conspicuous and charismatic species, it is also used as a biosentinel (*sensu* International Joint Commission 1984), *i.e.* an indicator species for surveillance of the environmental quality of freshwater and marine ecosystems. This is possible due to the high exposure and sensitivity of the species to environmental pollutants. As a consequence of persistent chemicals (especially DDT and PCB) accumulating in the food chain, it experienced dramatic population declines throughout Europe during the 20<sup>th</sup> century. However, the species is presently recovering in numbers and is recolonising parts of its former distribution.

## Legal status

The white-tailed eagle is classified as ‘Least Concern’ (LC) on the IUCN Red List 2005<sup>1</sup> (BirdLife International 2005) and is listed in Appendix I of CITES (trade with specimens is only permitted in exceptional circumstances). It is also listed on Appendices I and II of the Convention on Migratory Species (CMS or Bonn Convention; *i.e.* classified as an endangered migratory species), Appendix II of the Berne Convention on the Conservation of European Wildlife and Natural Habitats (strictly protected species) and on Annex I of the EC Birds Directive (species to be the subject of special conservation measures concerning their habitat in order to ensure their survival and reproduction in their area of distribution).

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<sup>1</sup> The IUCN red list conservation status of the white-tailed eagle was recently changed from ‘threatened’ to ‘near threatened’, and last year to ‘least concern’. This was to reflect the recovery of the species in many areas of Europe during recent decades (see below for details on recent population history).

## Taxonomy, distribution and life history traits

The white-tailed eagle belongs to the order Falconiformes and the family Accipitridae. The genus *Haliaeetus* encompasses a total of eight extant species distributed across most continents. The closest living relative is the bald eagle *Haliaeetus leucocephalus*, which replaces the white-tailed eagle in North America. The two species diverged during the Pleistocene, possibly about one million years ago (Wink *et al.* 1996). Hence, they resemble each other in many morphological and ecological characteristics.

The white-tailed eagle is classified into two subspecies: *Haliaeetus albicilla groenlandicus* (endemic to Greenland) and *H. albicilla albicilla* (the remaining distribution range). Body proportions differ between the subspecies, with non-overlapping size of the breast bone and pelvis, but considerable overlap in wing length (Salomonsen 1979).

The white-tailed eagle has a wide distribution across large parts of the Palaearctic. It occurs throughout much of Europe and Asia, from tundra regions in the north to the Mediterranean in Europe and central Asian steppe in the south. The westernmost part of its range breeds on south-western Greenland (Nearctic), and the easternmost populations are found in Japan and north-eastern Siberia. The total world population has been estimated at 6,800-7,400 breeding pairs, the majority located in Europe (Helander and Stjernberg 2002). The population of the *groenlandicus* subspecies is estimated at 150-170 pairs (Helander and Stjernberg 2002).

The white-tailed eagle occurs in a range of different habitats, from arctic steppe via boreal and nemoral forests to the subtropical region. While it is generally more frequent in coastal and freshwater biomes (Glutz von Blotzheim 1971; Helander and Stjernberg 2003), its plasticity also allows it to breed in steppe regions far from major water occurrence (Katzner 2002). Nesting places are usually in trees, but can also be on cliffs or, rarely, directly on the ground. Nesting trees are generally old, usually well above average tree ages in managed forests. A reason for this is that nests can become very heavy, with reported weight of several hundred kilos (Gerdehag and Helander 1988 give a range of 200 to 1200 kilograms).

The species is rather opportunistic and adaptive regarding its food choice, and also partly a kleptoparasite (stealing food items from other species). Where available, birds and fish comprise more than 90% of the summer diet (*e.g.* Helander 1983, Wille and Kampp 1983). In dryer habitats, the white-tailed eagle can shift its diet to small mammals, reptiles and other non-aquatic prey (Katzner 2002). Carrion is also an important food item in some regions, especially during winter, which links high survival chances of the eagles to the presence of other large predators in the landscape.

Sexual maturity is normally reached at the age of five years. During the juvenile phase, the species is mostly vagrant or migratory and can cover large geographic areas (Glutz von Blotzheim 1971; Helander and Stjernberg

2003). Adult individuals are mostly sedentary, although populations in northern regions, which often do not yield enough food during winter, are migratory. Pairs remain faithful to their territories, and continue to breed in the same area year after year. Even succeeding generations of pairs tend to re-use old territories - some have been occupied by white-tailed eagles for more than a century (Gerdehag and Helander 1988). Mated pairs are stable, partners normally breed with each other until one partner dies. The generation time of white-tailed eagles is long for being a bird. Individuals have been reported to live up to 42 years in captivity (Glutz von Blotzheim 1971). Survival in the wild is probably lower, but birds up to 36 years old have been found (Struwe-Juhl 2003). Studies from southern Sweden and Germany indicate a mean life span of breeders of 17 years (Struwe-Juhl 2003; Helander 2003b). However, these results may have been affected by being obtained in growing and geographically expanding populations - with potentially lower impact of intraspecific competition.

Wing span can reach 2.5 meters and adult birds usually weigh between four and seven kilograms. As in most other raptors, sexual dimorphism is present but not very pronounced in the white-tailed eagle: females are generally bigger than males (average weight around 25%, and wing length around 9% bigger; Gerdehag and Helander 1988).

## Recent population history: decline and subsequent recovery

Historically, the white-tailed eagle was distributed across most parts of Europe, including coastal and inland regions. It was less abundant in regions far away from the coast or larger wetlands (floodplains and lakes), but many historical records describe the species as rather common and abundant even in landscapes with relatively limited surface water (Glutz von Blotzheim 1971; Helander and Stjernberg 2003).

Starting from a wide distribution and large population sizes, populations in Europe have experienced two major demographic bottlenecks during the last two centuries. Alike many other raptors, white-tailed eagles were long regarded as competitors and therefore their nests and eggs were destroyed, poisoned baits were laid out for them, and many were shot (Bijleveld 1974, Helander and Stjernberg 2003). Although breeding data from historical times are scarce, the decline probably became most pronounced during the 1800s when the use of shotguns had become widespread. The last white-tailed eagle on the British Isles was shot in 1916 (Love 1983), and extinction also occurred in a number of other countries including Denmark, France and Italy (reviewed in Dennis 2003). Even in countries with remaining breeding pairs,

local populations sizes were generally low, probably less than 50 pairs in many European countries (for Sweden see Berg 1924; for Germany see review in Hauff 1998). Around the 1920s, many European countries enforced legal protection of the white-tailed eagle (1924 in Sweden). This, and a gradual acceptance of eagles by humans, enabled population recovery during the following decades.

Although (illegal) persecution by humans still accounted for inflated mortality rates, a new threat to white-tailed eagles became obvious during the early 1960s. Apparently undisturbed territorial pairs were found to produce only few, if any, offspring. Consistent breeding failures over several seasons were reported from several European countries, especially around the Baltic Sea.

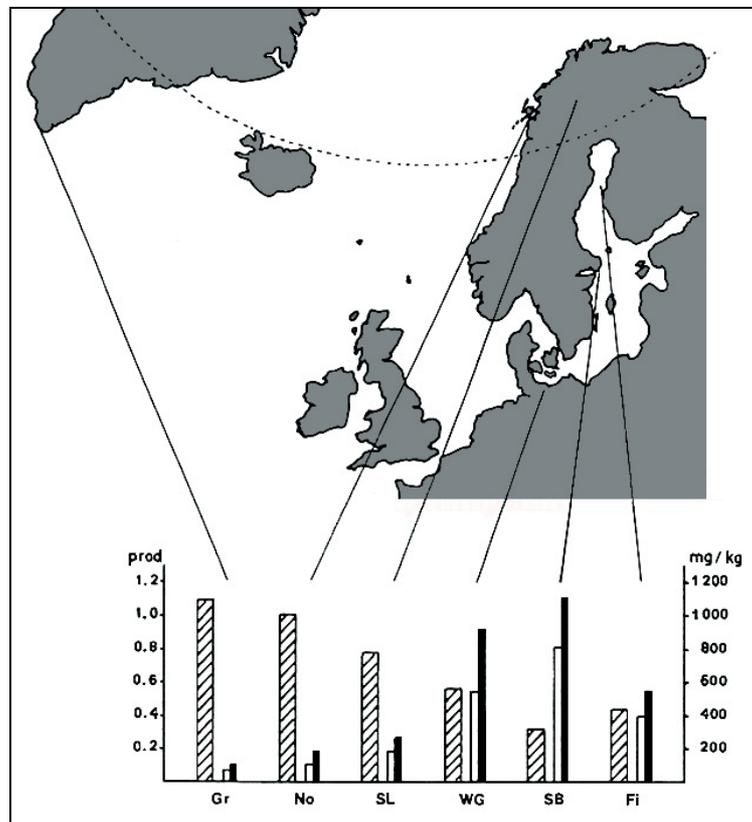


Figure 1. Productivity (average number of young produced per year - striped bars) and mean contaminant levels (DDE - white bars, PCB - black bars) in six white-tailed eagle populations. Gr - Greenland, No - Norway, SL - Swedish Lapland, WG - Western Germany, SB - Swedish Baltic coast, FI - Finland. Figure modified from Helander *et al.* (1982).

This drop in productivity was to a large degree caused by accumulation of harmful chemicals in the environment, with detrimental effects demonstrated for DDE (a bioaccumulating metabolite of DDT) and PCB (Helander *et al.* 2002). Helander *et al.* (1982) showed that white-tailed eagle populations from the Baltic area had considerably higher levels of contaminants and lower levels of productivity than populations from the Norwegian Atlantic coast and Greenland (see Fig. 1).

Lowered ability to reproduce led to a second demographic bottleneck. A widespread decline was observed not only around the Baltic, but also in most other parts of the European distribution range, leaving only a few tens of breeding pairs in most remnant populations. For instance, 45-50 pairs remained at the Swedish Baltic coast, and many of these pairs never produced any fledgling young (Helander 2003a). A notable exception in Europe was the Norwegian population, which harboured at least some 800 breeding pairs at the time (Helander *et al.* 2003).

As a result, conservation actions were initiated in many European countries. The ban of some harmful chemicals led to a successive decrease of *e.g.* DDT and PCB concentrations in the environment and in white-tailed eagles (Helander *et al.* 2002). Remaining nesting sites were protected and guarded to save them from impacts of forestry and disturbance. Further, supplementary winter feeding was carried out in many places. This was a means of supplying the eagles with uncontaminated food, and probably played a major role in increasing the survival of (especially first calendar year) birds (Helander 1985a).

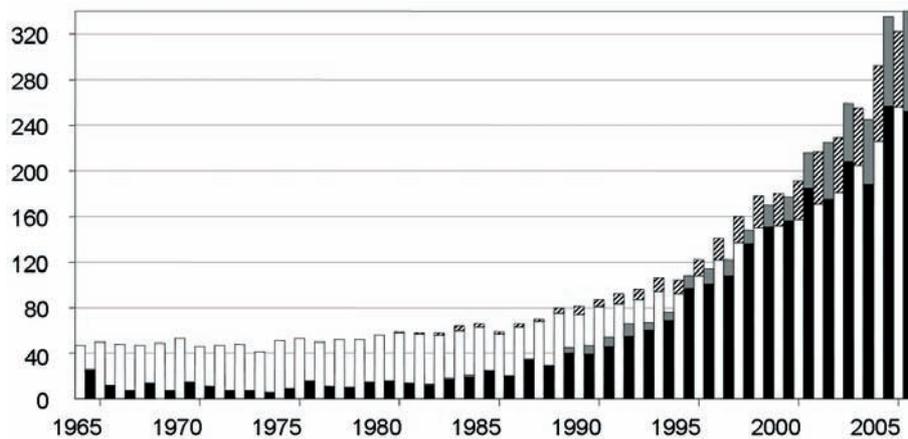


Figure 2. Demographic recovery of the white-tailed eagle in Sweden (excluding the demographically isolated population in Lapland) between 1965 and 2005. White and striped bars show number of breeding pairs at the Baltic coast and inland freshwater lakes, respectively. Black and grey bars show numbers of fledged young in the same areas. (Data assembled and kindly provided by Björn Helander.)

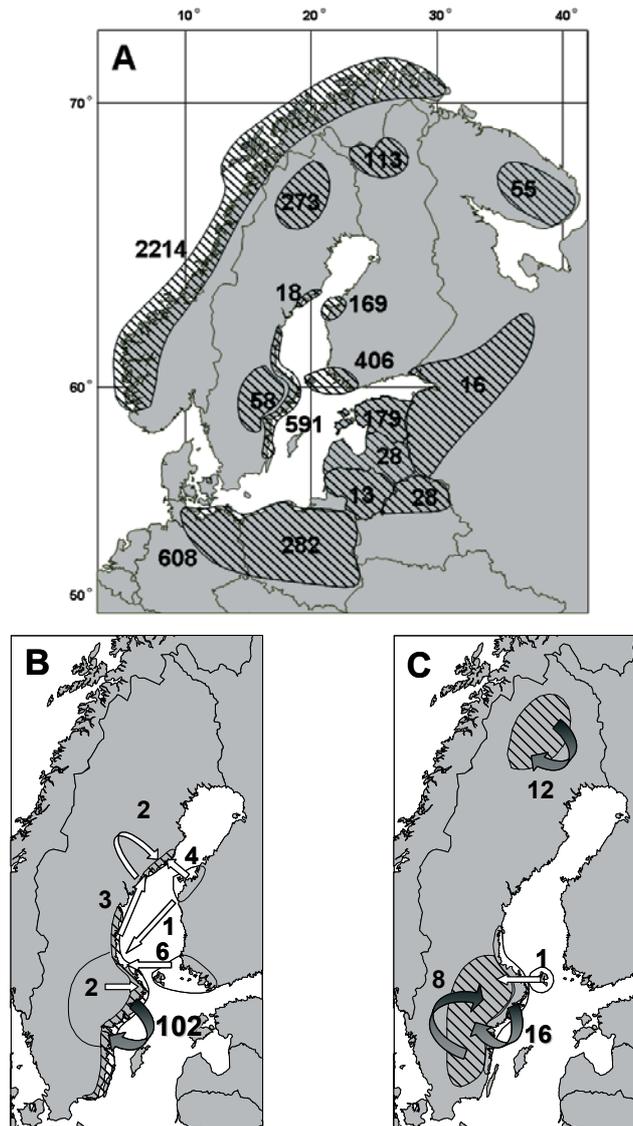
Altogether, these actions led not only to a halt of the decline, but since 1980 also to a strong population growth across Europe (see Fig. 2 for the situation in Sweden; note also the large increase in productivity since the early 1980s). Local population growth has now resulted in recolonisation of several regions where white-tailed eagle previously had gone extinct (Fig. 3). Further, reintroduction of white-tailed eagles from Norway has established a breeding population in Scotland (33 pairs in 2005; Anonymous 2006).



*Figure 3.* Distribution of the white-tailed eagle in north-central Europe. Black shading indicates areas where the species persisted during the mid-20<sup>th</sup> century population crash. Grey shading indicates areas recolonised in the phase of population recovery since the early 1980s.

A colour-ringing program was initiated in 1976 to monitor the remaining white-tailed eagle populations. This program initially focused on northern and central Europe but has come to include also other populations in recent years. The results have so far indicated strong philopatry of the species, with adults generally settling to breed very close to their place of birth. Effective dispersal distances (*i.e.* from birth to nesting place) in Sweden have been

estimated at 90 kilometres for males ( $n=35$ ;  $s.d.=89$ ) and 114 kilometres for females ( $n=37$ ;  $s.d.=55$ ) (Helander 2003b; see Fig. 4). This homing tendency is further supported by the presence of locally restricted partial albinism in some eagles found in Swedish Lapland (Ekman and Helander 1994), and by ring recoveries from other countries (see contributions in Helander *et al.* 2003).



*Figure 4 (A) Ringing areas and numbers of colour-ringed white-tailed eagle nestlings 1976-1995 (i.e. potential breeders in 2000) in north-central Europe. (B) Origin of identified colour-ringed breeders ( $n=120$ ) by the year 2000 on the Swedish Baltic coast, and (C) at freshwater sites in southern and central Sweden, and in Swedish Lapland. Note that none of the large number of nestlings colour-ringed in Norway was found to breed abroad. Figure modified from Helander (2003b).*

## Contribution of population genetics to the conservation of species

As indicated in the previous sections, extensive field-based research has been conducted on white-tailed eagles. However, genetic studies can provide novel information that is not possible to obtain through field research and which can be essential for the development of sensible conservation strategies. The genes present in an individual yield information about processes acting on a wide range of time scales, from processes currently ongoing in the population (*e.g.* correlations between an individual's genetic variability and fitness), to historical processes dating back to the ice ages (traces of climate-induced range contraction and re-expansion) or even several million years ago (phylogeny, speciation).

The application of molecular genetic techniques to conservation resulted during the last decade in the origination of a new scientific discipline: conservation genetics. According to Frankham *et al.* (2002), “*conservation genetics is the application of genetics to preserve species as dynamic entities capable of coping with environmental change. It encompasses genetic management of small populations, resolution of taxonomic uncertainties, defining management units within species and the use of molecular genetic analyses in forensics and understanding species' biology.*” The study of genetic variability in individuals and populations, the partitioning of this diversity among populations and the use of molecular tools to understand a species' natural history are some of the cornerstones of conservation genetics.

### Reduction of genetic diversity in small populations

Many species worldwide experience extensive loss of suitable habitat. In this process, numerous populations have gone extinct, while others have become fragmented, strongly reduced in size, and demographically and genetically isolated. The risk of stochastic demographic and environmental fluctuations to such small populations has been acknowledged for a long time (see *e.g.* Goodman). Population declines are also associated with loss of genetic diversity. In a stable population of a diploid organism, random genetic drift is

expected to reduce the genetic diversity (measured as heterozygosity) by  $1/(2N_e)$  per generation, where  $N$  is the effective population size (Hartl and Clark 1997). This implies that the effect of genetic drift on loss of genetic diversity will be strong at small population sizes. Reduction in size of a population (a bottleneck), or founding of a new population by few individuals (founder effect) are thus expected to increase genetic drift and lead to loss of a large proportion of (mostly rare) alleles (Nei *et al.* 1975; Luikart *et al.* 1998), which has been demonstrated empirically (*e.g.*, Hoelzel *et al.* 2002). Consistent with this theoretical expectation, a meta-analysis by Garner *et al.* (2005) showed that “demographically challenged” species (*i.e.* such that have undergone a reduction in population size or range or whose populations are small and isolated) show lower degree of heterozygosity than populations of unaffected species.

## Genetic variability and population viability

Genetic variability is important for long-term survival of populations by allowing them to adapt to environmental changes (Frankham 2005). Further, both theoretical and empirical results suggest that loss of genetic diversity increases susceptibility to demographic, environmental and stochastic variation and therefore augments the probability of extinction (Mills and Smouse 1994; Lacy 1997; Frankham *et al.* 2002). In addition, when populations are reduced in size, the probability of mating between close relatives increases, which can lead to the emergence of deleterious effects (inbreeding depression; Crnokrak and Roff 1999). For instance, Saccheri *et al.* (1998) showed that populations of Glanville fritillary butterflies (*Melitaea cinxia*) with lower genetic diversity experienced a higher risk of extinction. Similar effects have been demonstrated for plants (Newman & Pilson 1997), and meta-analyses indicate that endangered species have lower genetic diversity than non-endangered ones (Frankham *et al.* 2002).

The exact mechanisms behind this effect of genetic diversity on individual fitness are in many cases unclear. The detrimental effect of single alleles on individual fitness is well documented (Charlesworth & Charlesworth 1999). An overall correlation between heterozygosity and fitness has been suggested (Coulson *et al.* 1998; Reed and Frankham 2003; Markert *et al.* 2004). However, a number of empirical studies have reported the lack of such an association (*e.g.* Savolainen & Hedrick 1995). This could be due to the fact that the correlation between fitness and genetic diversity is dependent on population and environmental characteristics (Lesbarrères *et al.* 2005), or reflect local rather than genome-wide effects of genetic diversity (Hedrick *et al.* 2001; Hansson and Westerberg 2002). If local effects are the main explanation for the published significant heterozygosity-fitness correlations, then intrinsic benefits of local heterozygosity could be rare.

There has been a long debate over the relative importance of genetic threats to survival compared to demographic threats ignited by Lande (1988). He suggested that “*demography is usually of more immediate importance than population genetics in determining the minimum viable sizes of wild populations*”. There is today a wide consensus that both factors can play a major role (Hedrick and Kalinowski 2000; Frankham *et al.* 2002; Lukas and Keller 2002). Importantly, a meta-analysis by Spielman *et al.* (2004) indicated that “*most species are not driven to extinction before genetic factors impact them*”.

In summary, overwhelming evidence points to the importance of genetic diversity for the persistence of populations and species. The protection of genetic diversity has thus become a priority for the World Conservation Union (IUCN).

## Phylogeography, population structure and other information

The possibility of establishing phylogenetic relationships between alleles and studying their geographic distribution has allowed the development of a new scientific discipline during the last two decades: phylogeography. This term was introduced for the first time by Avise *et al.* (1987). Phylogeography is “*a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species*” (Avise 2000). Phylogeographic approaches have since their advent proven useful to understand the origin of populations and how species have responded to climatic changes during the Pleistocene (Taberlet *et al.* 1998; Hewitt 2000).

One aspect of phylogeography that is of particular relevance for conservation is the possibility to detect distinct phylogenetic lineages within-species. For instance, cryptic genetic variation may exist within species (or at higher taxonomic levels) that potentially requires taxonomic subdivision and independent conservation (*e.g.* Barrat *et al.* 1997; Omland *et al.* 2000).

Distribution of alleles in different populations, even if they can not be related phylogenetically, can also provide insights into patterns of gene flow. The distribution of genetic diversity within and between populations has led to the possibility of indirectly estimating gene flow between populations at equilibrium situations (Wright 1965). More recently, several methods have been developed to estimate genetic exchange between non-equilibrium populations which are more likely to portray current instead of historic gene flow (Paetkau *et al.* 1995; Davies *et al.* 1999; Pritchard *et al.* 2000; Falush *et al.* 2003; Manel *et al.* 2005).

Detailed knowledge about the ecology and behaviour of species is necessary for the development of efficient conservation strategies. During the last two decades, molecular genetic approaches have increasingly been used to improve the knowledge about natural systems. For instance, many species show a sex bias in dispersal behaviour, which can be identified with genetic means (*e.g.* Hammond *et al.* 2006). Molecular genetic techniques can also be used to study various other factors, *e.g.*, paternity (Pena & Chakraborty 1994), adaptation (Albertson *et al.* 1999), reproductive strategies and social behaviour (Hughes 1998; Freeman-Gallant *et al.* 2003), sex allocation (Ellegren *et al.* 1996), sperm competition (Karr & Pitnick 1999), and for the design of management units (Moritz *et al.* 1994; Crandall *et al.* 2000). Molecular approaches have thus taken a central part in numerous research programs in ecology, behaviour and conservation.

## Goals of the thesis

1. Development of genetic markers for the study of variability within and structure among populations of the white-tailed eagle
2. Evaluation and refinement of morphology-based methods to identify the sex of white-tailed eagle nestlings in the field
3. Study the genetic consequences of population declines during the 20<sup>th</sup> century in Europe
4. Reconstruct phylogeography and past demography, as well as the post-glacial colonisation of Eurasia
5. Analyse the structure and genetic diversity of populations across the species distribution range
6. Assessment of the role of gene flow in shaping the patterns of genetic diversity in this highly philopatric species

## Present investigations

### Paper I: Development and multiplex PCR amplification of novel microsatellite markers in the white-tailed eagle *Haliaeetus albicilla*

Since a few years after their discovery (Weber and May 1989; Litt and Luty 1989), microsatellites have been a marker of choice for numerous studies of within-population variation, relatedness, fine scale gene flow patterns and large scale population structure (Goldstein and Schlötterer 1999, Schlötterer 2004). Microsatellites are thus widely applicable, generally yield high resolution power and the results have a high reproducibility within and between laboratories (Karp *et al.* 1997). In comparison to many other genetic markers (especially allozymes, RAPDs and AFLPs), this is generally also true for samples exhibiting rather low DNA concentration and/or quality.

However, a relative drawback of microsatellites as population genetic markers is that a given locus is generally only useful in a rather limited taxonomic group, *i.e.* is restricted to close relatives of the species from which the marker was originally developed. At the starting point of this project, therefore, microsatellite markers were developed for the white-tailed eagle.

#### Results and discussion

A size-selected and microsatellite-enriched library was constructed by ligation of white-tailed eagle DNA with TSPAD-linkers (Tenzer *et al.* 1999). Next, an enrichment procedure was performed by magnetic bead selection using biotin-labelled (CA)<sub>13</sub>, (CA)<sub>20</sub>, (CAAA)<sub>9</sub> and (AGG)<sub>10</sub> oligonucleotide repeats as described in Gautschi *et al.* (2000). Out of 960 colonies hybridised, 142 gave a positive signal indicating successful enrichment and integration of a microsatellite-like motif into the corresponding bacteria. We sequenced the inserts of 109 of these clones and designed primers for 21 different microsatellite loci. Fourteen markers gave reproducible and interpretable results, and appeared polymorphic on an initial testing panel of four to 15 individuals. Next, a multiplexing protocol for those 14 markers was developed. The markers were genotyped in a sample of 40 individuals from the white-tailed eagle population in southern and central Sweden.

The markers yielded between two and eight alleles per locus, with average observed and expected heterozygosity values of 0.463 and 0.468 respec-

tively. A significant heterozygote deficit ( $p < 0.05$ ) was found for one marker (*Hal 10*), possibly due to the presence of null allele(s).

These results show that the developed markers are polymorphic, and should be useful for investigations of genetic variability and population structure in white-tailed eagles. The optimised multiplexing procedure enables time- and cost-efficient amplification of the markers in only four PCRs.

## Paper II: Morphological and genetic sex determination of white-tailed eagle nestlings

Sex identification of individuals can yield a better understanding of the ecology and behaviour of bird species (Ellegren and Sheldon 1997) and can also be important for their management and conservation (Morris and Doak 2002). Although the sex of adults in many raptor species can be assessed based on plumage or sexual size dimorphism, the sex of nestlings or subadults is generally hard to discern.

We here ascertain the value of different morphological measurements taken from white-tailed eagle nestlings to correctly identify their sex. White-tailed eagle nestlings are often ringed in treetops by one person alone, and under a mandate to reduce the time and intensity of disturbance at the nest as much as possible. We therefore focus our investigation on few measurements, and such that are possible to take under large restrictions on handling.

### Material and methods

We analysed 211 white-tailed eagles nestlings from Sweden, comprising two demographically and ecologically separate groups in (i) Lapland and (ii) coastal regions and freshwater lakes in southern and central Sweden. Nestlings were measured in their nests during ringing. Four measurements were recorded: tarsal thickness (two diameters at the thinnest section: *tars1* and *tars2*), length of the folded wing from the carpal joint to the tip of the longest primary (*wing*), and nestling *weight* (corrected for estimated crop content as in Helander 1981). A blood sample was taken from each individual to allow sex determination using molecular techniques. For the latter, we used the method by Fridolfsson and Ellegren (1999).

Since earlier studies have indicated that nestling survival is lower and body growth rates are lower in Lapland than in the southern population (Helander 1985b), we analysed samples from those regions separately.

### Results and discussion

The molecular method revealed the sex of 208 individuals, only three individuals could not be classified due to repeatedly unsuccessful PCR amplifi-

cation. Except for wing length, which is known to be tightly correlated with nestling age (Helander 1981), all morphological measurements were larger in females than in males ( $p < 0.001$  in all cases) for the samples from southern and central Sweden, indicating clear sexual size dimorphism.

Single measurements allowed the correct sex identification of a large proportion of nestlings in southern and central Sweden (table 2). The best measure for sex identification in both sexes was *tars1*, which allowed for correct classification of 95% and 98% of females and males respectively from southern and central Sweden. None of the composed measurements created to standardize for nestling age (and thus size) performed better.

Table 2. Morphometric characteristics of female ( $n=98$ ) and male ( $n=84$ ) white-tailed eagle nestlings from southern and central Sweden.

measure	females		males		sexual dimorphism <sup>□</sup>	% of females/males classified correctly <sup>#</sup>
	average	S.D.	average	S.D.		
1. <i>tars1</i>	14.8	0.6	12.8	0.5	$p < 0.001$	95 / 98
2. <i>tars2</i>	16.92	0.8	15.6	0.7	$p < 0.001$	81 / 92
3. <i>weight</i>	4.48	0.66	3.76	0.50	$p < 0.001$	77 / 74
4. <i>wing</i>	326.6	72.1	323.5	66.8	$p = 0.759$	48 / 49
5. [ <i>tars1</i> * <i>tars2</i> ]	249.86	20.31	196.78	15.12	$p < 0.001$	91 / 96
6. [ <i>tars1</i> / <i>tars2</i> ]	0.87	0.03	0.84	0.03	$p < 0.001$	68 / 65
7. <i>all</i> (1-6)	-	-	-	-	-	95 / 98
8. [( <i>tars1</i> * <i>tars2</i> )/ <i>wing</i> ]	0.80	0.17	0.63	0.13	$p < 0.001$	63 / 80

<sup>□</sup> two-tailed Student *t* tests

<sup>#</sup> based on a discriminant function analysis

Overall, nestlings in Lapland during the studied growth period were smaller than those in the more southern population. Therefore, the criteria developed for sexing ‘southern’ samples incorrectly classified many Lapland individuals. In another attempt to correct for nestling growth and thus correlations between variables, we calculated a linear regression for all measurements in females from the ‘southern’ population, using *wing* as independent variable. Stepwise discriminant analysis was then performed on the residuals of male and female measurements against this regression. This improved classification of Lapland females (92% correctly identified), but to the price of low success rates in males (46%).

Other traits than those we measured may prove to be more efficient for sex determination across populations. Further evaluation of the best discriminatory variables could provide more reliable results in cases where

nestlings can be handled longer and other measurements can be taken (Bortolotti 1984; Masterov 2000; Shephard *et al.* 2004). In field conditions where this is not attainable, the present study shows that a simple measurement like tarsus thickness, easily recorded when ringing, can be used to provide correct sex identifications in a majority of individuals – in this case 95-98% of nestlings. However, the criteria used to identify the sexes need to be adjusted to the study population.

### Paper III: Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline

Historically, white-tailed eagles were generally abundant and widespread across large parts of Europe (Glutz von Blotzheim 1971; Helander and Stjernberg 2003). Starting in the mid-1900s, however, a dramatic population decline occurred. Reproduction of white-tailed eagles was strongly reduced as a consequence of the accumulation of harmful persistent chemicals such as DDT and PCB in the environment (Helander *et al.* 2002). Eagles from countries around the heavily polluted Baltic Sea were especially strongly affected, while pairs nesting along the Norwegian Atlantic coast were much less exposed and hence did not decline significantly in numbers. In fact, the Norwegian population harboured at least 800 breeding pairs at that time and accounted for more than 70% of the total northern and central European population (Helander *et al.* 2003). Other remaining sub-populations were considerably smaller, in most countries only some tens of pairs, and included many pairs not able to reproduce.

After the ban in the 1970s of DDT, PCB and other harmful chemicals, white-tailed eagle populations started to recover, a still on-going process (see Figures 2 and 3). Notably, though, the recovery appeared to have been based mainly on local population growth rather than immigration from other populations, since ringing data indicated strong philopatry (Helander 2003b, Köppen 2003; Fig. 4).

In the absence of gene flow from other regions, fragmentation and population declines result in loss of genetic diversity by random genetic drift. Reduced genetic variability has been shown to negatively impact individual fitness, population viability (Saccheri *et al.* 1998), and a species' evolutionary potential for future adaptation (Frankham 2005). This led us to investigate whether extant white-tailed eagle populations in Europe might be depleted of genetic diversity as a result of population declines.

### **Material and methods**

Samples of 218 presumably unrelated nestlings or breeding adults were taken from six populations in north-central Europe: Norway, southern and central Sweden, Germany, Estonia, Swedish Lapland and the Kola peninsula (north-western Russia). Genetic diversity in 500 base pairs (bp) of mitochondrial DNA (mtDNA) control region sequences and at 26 autosomal microsatellite loci was investigated.

### **Results and discussion**

After correction for differences in sample size, genetic variability at microsatellite markers was rather homogeneous across populations. Importantly, genetic diversity in the non-bottlenecked Norwegian was not higher than that in the populations which recently had undergone population size reductions. Similarly, no significant shifts in allele frequencies normally associated with strong demographic bottlenecks were found ( $p > 0.05$ ; Cornuet and Luikart 1996). As for the microsatellite results, mtDNA data did not reveal higher diversity in Norway than in other populations. In fact, many mtDNA haplotypes found across Europe were not encountered in Norway, refuting the notion that currently recovering European populations would have derived to a large extent from the coastal Norwegian population.

As indicated by ringing data, gene flow between regions could not be the main explanation for the locally preserved genetic diversity. Our results were consistent with limited genetic exchange, since more than 80% of the individuals assigned to their natal population and because all pairwise population comparisons revealed significantly differentiated gene pools ( $p < 0.01$ ). Simulations of the expected amount of genetic variability (Kuo & Janzen 2004) lost in the past bottleneck showed that the long generation time of the eagles (mean life span of breeders: 17 years; Struwe-Juhl 2003; Helander 2003b) acts as an intrinsic buffer against rapid loss of genetic diversity.

A contributing factor for the sustained diversity was probably also the variety of local conservation measures. Altogether, these actions enabled remaining pairs to raise fledgling young and led to increased recruitment of new breeders. Local conservation together with the long generation time of eagles thus enabled the preservation of much genetic diversity. This finding gives hope for the preservation of genetic variability in other endangered long-lived species and stresses the importance of their local remnant populations.

## Paper IV: Phylogeography of the white-tailed eagle, a generalist with large dispersal capacity

Knowledge of large-scale genetic population structure is important for the delineation of conservation strategies by allowing the definition of evolutionary significant units and management units (for different approaches see Moritz 1994 and Crandall *et al.* 2000). The Pleistocene glaciations had a major impact on Northern hemisphere landscapes, species distributions, and their genetic make-up. While many temperate and boreal taxa survived the Ice Ages in distinct refugia (Hewitt 2000), generalist species capable of long-distance dispersal may not have been affected in the same way. One species for which this has been shown is the grey wolf *Canis lupus*, which only displays weak phylogeographic structure at mtDNA across most of its range (Vilà *et al.* 2003).

The white-tailed eagle is rather flexible regarding both its diet and choice of nesting sites (Helander & Stjernberg 2003; Katzner 2002). Furthermore, it appears in the fossil record as an early coloniser of Scandinavia (Ericson and Tyrberg 2004), and long distance movements are documented from especially juveniles. On the other hand, strong philopatric behaviour has been revealed in Europe (see paper III), which altogether yields an unclear picture regarding the impact of long distance gene flow in this species.

We therefore tested for phylogeographic structure by analysing mtDNA from populations across its distribution range.

### Material and methods

A 500 bp fragment of the mtDNA control region was sequenced in 237 individuals from 11 populations spanning the entire distribution range of the white-tailed eagle (Greenland, Iceland, Norway, southern and central Sweden, Germany, Estonia, Swedish Lapland, Kola peninsula (north-western Russia), Kazakhstan, Amur region (eastern Russia) and Japan. The data was analysed with regard to genetic variability, phylogenetic relationships between haplotypes, population differentiation and structure, and statistics for demographic inference.

Table 3. Estimates of within-population variability for white-tailed eagle mtDNA control region sequences. Sample size ( $n$ ), number of unique haplotypes ( $N_H$ ), haplotype diversity ( $H$ ), nucleotide diversity ( $\pi$ ) and the frequency of group A haplotypes are shown.

Population	$n$	$N_H$	$H$ (+/- S.E.)	$\pi$ (+/- S.E.)	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

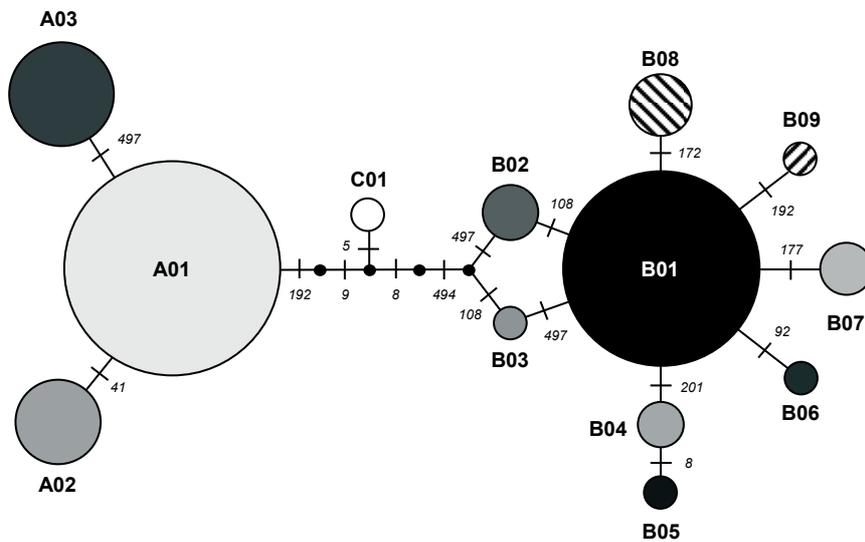


Figure 5. Statistical parsimony network of mtDNA control region haplotypes in the white-tailed eagle. For haplotype names and their occurrence in the populations see table 1 in paper IV. Circle size is proportional to haplotype frequencies. Dashes and associated numbers refer to inferred mutational steps and their position in the alignment. Small black circles denote inferred intermediate haplotypes.

## Results

Across all individuals, a total of 13 haplotypes defined by 12 variable sites were discovered. Their phylogenetic relationship is shown in Fig. 5. Haplotype and nucleotide diversity (Table 3) was lowest in populations on the extremes of the distribution range (Greenland and Japan) than in most of the more central populations (Kola peninsula, Estonia, Lapland, Sweden) and Kazakhstan. Haplotype diversity on the Kola peninsula was significantly higher than in all other populations except Estonia.

Population structure was pronounced, with  $\Phi_{ST}$  across all populations amounting to 0.512 ( $p < 0.001$ ). AMOVA results and a neighbour-joining tree of populations indicated strong geographic structuring of genetic variation, which reflected differentiation between large-scale geographic regions (Atlantic islands, Europe and Asia).

Haplotypes clustered into two main clades (Fig. 5) with a predominantly western and eastern distribution, respectively, and with considerable admixture in many European populations (see table 3). As confirmed by summary statistics, mismatch analysis and a coalescent-based maximum likelihood approach, the 'eastern' clade showed a pattern compatible with a recent sudden expansion model. Based on the mismatch analysis and a divergence rate estimate for the avian control region domains I and II by Wenink *et al.* (1996) of 14.8%, expansion of that eastern clade was estimated to have occurred between 4,500 and 12,900 years before present (YBP), *i.e.* most likely in the last stages or just after the last glacial maximum.

The subspecies *Haliaeetus albicilla groenlandicus* from Greenland appeared as closely related to eagles from Iceland and Norway, and did not show any unique characters for the analysed control region fragment. For instance, separation of samples into two groups according the subspecies classification yielded a non-significant  $\Phi_{CT}$  value.

## Discussion

This study revealed rather limited mtDNA variability at the species level for the white-tailed eagle. Divergence of the two main intraspecific lineages was estimated to have occurred during the last 200,000 years, possibly as a consequence of climatic fluctuations. The current eastern and western distribution of the two clades indicates survival during the Ice Ages in two refugia, followed by incomplete postglacial lineage mixing. This is further corroborated by the finding of strong overall genetic differentiation as well as significant differentiation in most pairwise population comparisons. Hence, our results do not lend support to the notion that white-tailed eagle form a largely panmictic group across the Eurasian continent.

Our data indicate that one of the glacial refugia was located in Europe, and the other was more centrally placed in Eurasia. Based on climatic reconstructions (Svendsen *et al.* 2004), habitat requirements of the white-tailed

eagle and on comparisons with other species we suggest that those refugia may correspond to the European Atlantic coast (possibly including the Mediterranean) and the Aralo-Caspian and Black Sea basin, a pattern which would coincide with that found in many ecologically similar species (see *e.g.* Liebers *et al.* 2004). Such congruence in patterns among species with similar ecological requirements supports the importance of ecological factors in shaping their patterns of mtDNA variability.

The finding of high genetic diversity in northern populations (especially Estonia and Kola peninsula) may indicate rapid postglacial colonisation of and major population expansion in northern latitudes. This may be related to the occurrence of vast suitable habitats around proglacial lakes in northern Europe during the late Pleistocene (Mangerud *et al.* 2004).

The *groenlandicus* subspecies was confirmed to be rather young, its mtDNA variation being compatible with postglacial founding around 5,000 years ago (Salomonsen 1979) and subsequent demographic separation from Western European populations. Such small and isolated populations deserve special attention and high conservation priority.

## Paper V: Signatures of coancestry and gene flow between populations of the white-tailed eagle

A central concept in population genetics is that gene flow between populations can be estimated using genetic markers. Standard approaches like measures of population differentiation by  $F_{ST}$  rely on equilibrium assumptions and may therefore not be applicable to situations where equilibrium has not yet been attained (Neigel 2002; Whitlock and McCauley 1999). However, assignment tests and Bayesian model-based clustering methods are believed to better cope with such situations.

The white-tailed eagle is a long-lived species that has colonised its current distribution range after the last Ice Age. Population differentiation at neutral markers is therefore a quite slow process in this species (see paper III), which could affect gene flow estimates. Specifically, this slow effect of genetic drift will lead to an increased signal of coancestry. We here describe population genetic structure across the species' distribution range.

### Methods

Twenty-six microsatellite markers were analysed in 384 samples from ten populations across the distribution range of the species. Surveyed populations fall into three geographic regions: Atlantic islands (Greenland, Iceland), Europe (Norway, Sweden, Germany, Estonia, Lapland and Kola peninsula) and eastern Asia (Amur river region, eastern Russia, and Japan).

Statistical analyses included calculation of genetic differentiation ( $F_{ST}$ ) values, factorial correspondence analysis (FCA), analysis of molecular variance (AMOVA), construction of a neighbour-joining tree of populations, assignment tests, a Bayesian model-based clustering method implemented in STRUCTURE 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003) and testing for isolation by distance. We also used a coalescent-based Markov chain Monte Carlo (MCMC) method implemented in 2MOD (Ciofi *et al.* 1999) to test whether a pure genetic drift or a drift and immigration balance model would better explain our data. Lastly, the expected differentiation between white-tailed eagle populations over time in the complete absence of gene flow was assessed using simulations in BOTTLESIM (Kuo and Janzen 2004).

## Results

Genetic variation was lowest on Greenland and Iceland, but quite homogeneous across the remaining populations (table 4). Overall differentiation across populations was moderate ( $F_{ST}=0.10$ ) and significantly different from zero (95% c.i.: 0.087 - 0.113). Further, all pairwise populations comparisons showed  $F_{ST}$  values significantly larger than zero ( $p<0.05$ ). As indicated by an FCA plot, AMOVA analyses and a NJ tree of populations, population structure was pronounced with regard to large-scale geographic origin of the samples (Atlantic islands, Europe or Asia).

Table 4. Genetic diversity at 26 microsatellite markers in white-tailed eagle populations.  $n$ , number of samples;  $H_O$  and  $H_E$ , observed and expected heterozygosity;  $N_A$ , mean number of alleles per locus.

Population	$n$	$H_O$	$H_E$	$N_A$
Greenland (Gr)	9	0.286	0.277	1.9
Iceland (Ice)	31	0.182	0.202	1.8
Norway (Nor)	44	0.480	0.485	3.9
Sweden (Swe)	172	0.500	0.510	4.9
Lapland (Lap)	26	0.488	0.504	4.2
Germany (Ger)	18	0.415	0.434	3.4
Estonia (Est)	13	0.503	0.495	3.5
Kola peninsula (Kola)	20	0.518	0.516	4.2
Amur	38	0.506	0.508	4.3
Japan (Jap)	13	0.437	0.444	3.0

As indicated by the AMOVA, grouping the samples according to taxonomic (subspecies) classification, *i.e.* Greenland versus all remaining populations, did not explain a significant portion of the total variation ( $p>0.05$ ).

In the assignment test about 85% of the individuals assigned to their (known) natal population. However, assignment to a population other than the known sampling locality could not necessarily be interpreted as a sign of

gene flow. Consistent with this, another assignment test employing the Monte-Carlo probability computation by Paetkau *et al.* (2004) revealed that for most individuals, at least two different populations were compatible with being possible sources (exclusion probability  $p < 0.95$ ).

Results obtained in STRUCTURE did not reveal any clear-cut previously unexpected population structure. Some of the cluster co-occurred in the same geographic region, indicating that they did not correspond to groups reproducing in (geographic or reproductive) isolation from each other. Results obtained from 2MOD indicated that the gene flow model fitted the data best in all three geographic regions. Further, simulations of white-tailed eagle populations in BOTTLESIM indicated that, despite their long generation time, evolution over longer time periods in the absence of gene flow would lead to a much stronger population structure than that found in this study: comparing European and Asian populations, isolation during the last 10,000 years would without any gene flow lead to much higher  $F_{ST}$  values than actually observed ( $F_{ST} = 0.1$ ). Alternatively, very large long-term effective population sizes would be required (at least several thousand individuals per group; Fig. 6).

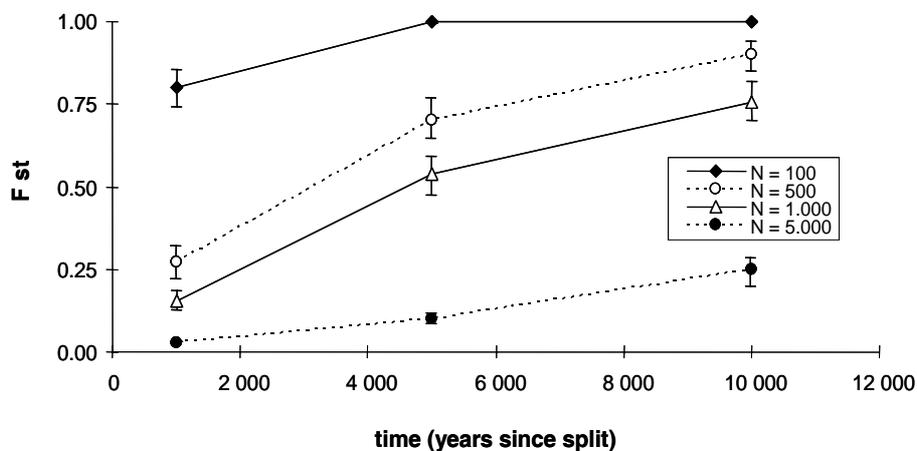


Figure 6. Genetic differentiation over time in a simulation of white-tailed eagle populations diverging from each other in the complete absence of gene flow. Error bars show 95% confidence intervals.

Consistent with this, we found a significant pattern of isolation by distance across all populations ( $r = 0.4312$ ;  $p < 0.05$ ). This relationship became more clear ( $r = 0.8365$ ;  $p < 0.0002$ ) when the highly divergent populations from Greenland and Iceland were excluded from the analysis. Solely for

Europe, this relationship was not significant ( $r = 0.4722$ ;  $p > 0.05$ ). Altogether, this indicated a large-scale pattern of limited dispersal and local drift across the Eurasian continent.

### Discussion

This study corroborates the notion of a buffering effect of long generation time during reductions in population size (paper III), by showing similar amounts of genetic variability in populations spanning large parts of the Eurasian distribution. On Iceland and Greenland, however, heterozygosity was approximately 50% lower than in the other populations, likely a consequence of strong founder effects during their colonisation. Such founder effects may otherwise in fact have been weak during the postglacial recolonisation of Eurasia. Following Austerlitz (2000) we suggest that the observed high within-population diversity and low among-population differentiation in white-tailed eagles can partly be explained by that slow initial population growth rate following colonisation of a new habitat opens the door for the arrival of additional immigrants, thus reducing the founder effect.

However, our results indicate that large-scale patterns of diversity in this species are not compatible with a model of differentiation without any gene flow: despite the long generation time, long-term population sizes in the order of several thousand individuals per region would be required to explain the low differentiation between Europe and Asia. This scenario appears unlikely given the current world population size of white-tailed eagles of around 7,000 breeding pairs (Helander & Stjernberg 2003) and the lack of evidence for very much larger populations in the past.

Given this reasoning and the observed pattern of isolation by distance we conclude that our data is best explained by a pattern of at least some gene flow over evolutionary time scales. Regarding the strong philopatry of white-tailed eagles in Europe indicated by ringing data, we believe this may be explained by two things: either there was more gene flow in the past and/or there is on-going gene flow at a level not detectable from ringing recoveries. Moreover, the observed philopatric behaviour of white-tailed eagles in Europe during recent decades may result from density-dependent migration: lower dispersal rates (or distances) could be typical of populations occurring at lower densities (Swenson *et al.* 1998; Travis *et al.* 1999; Forero *et al.* 2002; Matthysen 2005).

Our data lends only weak support for the classification of local or regional populations as unique genetic groups. However, our results together with the philopatric behaviour observed from ringing data indicates that management should tentatively treat populations in different regions as separate management units. In this light, the current population size of the likely isolated population on Greenland (around 150-170 breeding pairs; Helander & Stjernberg 2003) may be too low to support a long-term viable population.

## Possible future threats for white-tailed eagle populations

Not many decades ago, the future of especially European populations of the white-tailed eagle looked dim. During the last 10-25 years, however, many populations worldwide have shown an on-going marked recovery in numbers, and also extended their distribution range. Consequently, the white-tailed eagle is no longer listed as 'threatened' by the IUCN, but has lately been down-listed to category LC (Least Concern).

The results of this thesis indicate that white-tailed eagles in north-central Europe appear to have endured the past phase of dramatic declines without dramatic loss of genetic variability in remnant populations (but note the extinction of southern European populations). On a short to intermediate time scale, threats to population viability associated with inbreeding depression appear to be rather low. It is noteworthy that conservation actions likely were a major contributor to the preservation of genetic diversity in white-tailed eagles around the Baltic Sea. Without local conservation actions, the last remaining pairs could have gone extinct in many regions, as happened in most areas in southern Europe.

However, some threats persist and new ones have been identified. I will here briefly describe some threats that may affect white-tailed eagle populations in the near future, but for a detailed discussion see Helander & Stjernberg (2003).

### Persecution and disturbance

The current impact of direct persecution is not known for many regions of the distribution range. In Germany, five out of 58 found-dead and X-rayed individuals were revealed to have been shot at (non-lethally) during their lifetime (Krone *et al.* 2002). Persecution may be a significant threat in some regions, *e.g.* in southern Europe and parts of Asia (Helander & Stjernberg 2003). Poisoning of white-tailed eagles from baits has been reported from several European and Asian countries. In some populations, disturbance and persecution at nesting sites occurs. Increased recreational activity in many regions can disturb the eagles, especially during the breeding period.

## Environmental pollutants

Each year, hundreds of new chemicals are being introduced to the market (Birnbaum and Staskal 2004). Within short time, many of those are widely in use, despite associated risks for the environment. There is evidence for overall increasing contaminant levels in different top predator species worldwide, but the possible ecosystem impacts of this are not clear yet (Birnbaum and Staskal 2004). The Stockholm convention (United Nations Environment Programme 2004) may prove to be an important step towards a more careful use of potentially harmful chemicals (Porta and Zumeta 2002) than we have had in earlier decades.

Ingestion of lead from prey items as well as foraging on poisoned baits aimed at other species is reported as a death cause in white-tailed eagles from several regions.

## Habitat changes

Availability of suitable nesting sites is crucial for the white-tailed eagle. Logging of nesting trees by forestry, as well as disturbance of forestry work close to actively used nests can threaten the success of breeding pairs. Old trees are especially important as nesting sites, and their disappearance resulting from forestry reduces the availability of suitable nesting locations. In general, the ongoing loss of natural, relatively unmodified habitats (Jenkins *et al.* 2003), may imply a risk for future population declines.

Although the species is largely a food generalist, some populations depend on a rather narrow prey base and are therefore sensitive to population declines of their prey. This is especially true for northern populations which have been demonstrated to be limited in productivity by food resources (Heller 1985b).

## Accidental killing by collision and electrocution

An significant mortality factor today is collision with traffic (trains and cars). Collision with wind turbines has also been reported (Krone & Scharnweber 2003), and electrocution at power lines is a common cause of death.

## Diseases and stochastic events

To what degree various stochastic events have the potential for having a major influence on white-tailed eagle populations is not clear. The current outbreak of bird flu has affected other raptors (goshawk and common buzzard), but so far not the white-tailed eagle. However, given the scavenging behaviour of white-tailed eagles and large overlap of its wintering areas with that of waterfowl, the risk of cross-infection could be high.

# Svensk sammanfattning

## Bakgrund

I denna avhandling undersöks olika aspekter av havsörnens (*Haliaeetus albicilla*) genetiska variation samt populationsstruktur med molekylärgenetiska metoder. Havsörnen är Europas största örn och används inom naturvården som flaggskepps- och indikatorart i stora delar av sitt utbredningsområde. Arten är ofta associerad med kusttrakter eller större sjöar och floder. I dessa områden utgör fisk och sjöfågel en huvuddel av havsörnens föda. Trots detta är havsörnen ganska flexibel i sitt habitatval, t ex förekommer den även som häckfågel i centralasiatiska stäppområden, där den främst livnär sig på marklevande däggdjur, ödlor och dylikt.

För 200 år sedan häckade havsörnen utspritt över hela Sverige. Detta ursprungliga bestånd har försiktigt uppskattats till som minst 500 par. På liknande vis var havsörnen förr ganska vanlig och utbredd över många delar av Europa där den inte finns kvar idag (exempelvis de brittiska öarna, Frankrike och Italien). Havsörnens tillbakagång började under 1800-talet och förorsakades av förföljelse. Havsörnen sköts, förgiftades, dess bon förstördes och/eller plundrades. Total fridlysning i många länder kring 1920-talet (i Sverige 1924) ledde till att bestånden kunde återhämta sig något.

Under 1960-talet uppdagades dock ett nytt hot för havsörnen. Som toppkonsument är den speciellt känslig för miljögifter som anrikas i näringskedjan. Vi vet idag att särskilt DDT och PCB bidrog till kraftigt nedsatt reproduktionsförmåga och sjunkande beståndssiffror för havsörnen. Efter förbudet av dessa kemikalier dröjde det omkring tio år innan deras halter i miljön hade avtagit märkbart och havsörnens reproduktion förbättrades. Under tiden vakade naturvården i många länder kring östersjön över de kvarvarande havsörnsbestånden. Botråd skyddades från avverkning, boplatser bevakades för att förhindra störningar under häckningstiden och ett omfattande program för vinterutfodring startades. Det sistnämnda hade som syfte att erbjuda havsörnar giftfri föda, vilket ledde till en kraftig ökning av ungararnas överlevnad.

Efter ca. 1980 började Europas havsörnstammar att åter öka i antal. Idag har havsörnen återhämtat sig märkbart med avseende på antalet häckande par och den har även återtagit vissa regioner där den dog ut under 1800 och 1900-talet. Det totala svenska beståndet omfattar numera (2005) minst 400 revirhållande par.

De norska havsörnarna drabbades inte i samma utsträckning av miljögifter som de i resten av Europa. Detta berodde på att de huvudsakligen sökte sin föda i Atlanten, där gifterna fanns i mycket lägre koncentration än exempelvis i östersjön. Medan havsörnsbestånden under mitten av 1900-talet alltså var mycket låga i de flesta europeiska länderna, överlevde ett relativt stort bestånd på minst 800 par vid den norska Atlantkusten. Detta motsvarade ca. 70% av det totala beståndet i hela Norden samt norra Central-europa (se fig. 3 för avgränsning av området).

År 1976 initierades ett internationellt samstämt färgringmärkningsprogram för havsörnen. Inom detta hade t.o.m. år 2000 totalt mer än 8000 boungar märkts med färgringar. Uppföljningen av ringmärkta örnar gav en bild av stark 'hemortstrohet' (filopatri) hos havsörnen – nästan samtliga avlästa örnar återvände inför häckningen (könsmognad inträffar vanligtvis vid ca. 5 års ålder) 'hem' till trakten där de föddes. Framför allt har inte en enda av de mer än 3100 ringmärkta norska havsörnarna hittats som häckfågel utanför Norge.

## Hur populationsgenetik kan bidra för att bevara hotade arter

Genetisk variation är viktig för arters överlevnad, både i ett kortare perspektiv (eftersom låg variation kan medföra sänkt överlevnads- och/eller reproduktionsförmåga) och i ett långsiktigt perspektiv (för att kunna anpassa sig till kommande miljöförändringar). Genetisk variation försvinner främst i små populationer (demografiska flaskhalsar) där det låga antalet individer medför att många genetiska varianter inte bidrar med avkommor till nästa generationen (s k genetisk drift). Forskning under de senaste åren har visat att låg genetisk variation kan vara en avgörande faktor för att populationer dör ut (inavelsdepression).

Eftersom Europas havsörnspopulationer reducerades kraftigt i antal under de senaste decennierna, och eftersom ringmärkningsresultaten tyder på att genetiskt utbyte mellan olika populationer verkar ha varit mycket ovanligt, skulle lokala havsörnsbestånd kunna ha förlorat avsevärda mängder av genetisk variation. Detta kunde i så fall medföra ett framtida hot, även om populationerna på sistone har växt till sig rent antalsmässigt.

Med genetiska metoder kan variationsgrader i populationer mätas och jämföras, för att belysa potentiella risker associerade med förlust av genetisk variation.

Genetiska metoder gör det dessutom möjligt att beskriva huruvida populationer av samma art skiljer sig signifikant mellan olika regioner. Hos många arter har lokala populationer visats ha så egenartad genuppsättning att man bör undvika att blanda dem med andra populationer. Stora genetiska skillna-

der mellan populationer kan exempelvis uppstå efter långtida anpassning till olika förhållanden i avsaknad av frekvent utbyte. En mekanism som ledde till att populationer av europeiska arter utvecklade betydande skillnader var istiderna. Under istiderna förekom många eurasiatiska arter endast i begränsade områden vid Medelhavet där de isolerades från varandra på olika halvöar (t ex på den iberiska halvön och på Balkan).

Utöver det nämnda tillåter genetiska metoder också att ta reda på mer om en arts ekologi. I fallet fåglar kan exempelvis könet av boungar identifieras, vilket är viktig kunskap för olika studier om ekologi och beteende, samt för övervakning av hotade bestånd eller djurparkspopulationer.

## Artikel I: Utveckling av havsörnspecifika mikrosatellitmarkörer

I ett första skede utvecklades s.k. mikrosatellitmarkörer för havsörn. Dessa mikrosatelliter är ett vanligt använt verktyg inom populationsgenetik. Totalt utvecklades initialt 21 sådana markörer och deras användbarhet testades på 40 individer från populationen vid den svenska östersjökusten samt sjösystemen i södra och mellersta Sverige.

Fjorton av de nyutvecklade markörerna gav entydiga och reproducerbara resultat samt visade sig vara 'polymorfa' dvs variabla. Ett vanligt sätt att ange mikrosatelliters variationsgrad är antalet alleler och heterozygoti. På de 40 undersökta individerna visade de 14 markörerna mellan två och åtta alleler per markör samt en observerad heterozygotigrad på 0,463. Preliminära analyser visade alltså att markörerna var lämpliga för att studera genetisk variation inom och mellan olika havsörnspopulationer.

## Artikel II: Morfologisk och genetisk könsbestämning av boungar

Fåglar har som bekant inga yttre könsorgan som skulle tillåta könsklassificering av individer. Många rovfåglar visar dock storleksskillnad mellan könen, vilket även är fallet hos havsörn. Med genetiska metoder kan en individs kön bestämmas baserat på att hanar och honor bär på olika uppsättningar av könskromosomer. Hanar har två likadana kromosomer (ZZ) medan honor har två olika (ZW).

I denna studie jämfördes olika morfologiska karaktärer hos totalt 211 boungar av havsörn med könet som bestämts genom molekylärgenetisk analys av blodprover från samma ungar. Vi begränsade undersökningen till karaktärer som kan tas snabbt och okomplicerat, samt av en enskild person vid

ringmärkningsmetoden, detta för att minimera störningen av häckfågeln vid bokontrollerna.

Vi identifierade ett tarsmått som tillåter korrekt identifiering av könet på totalt 96% av individerna från häckningsbeståndet vid östersjökusten samt insjöar i södra och mellersta Sverige (härefter kallat det 'södra' svenska beståndet). Detta mått tillämpade vi i ett nästa steg på boungar från Lappland. Samma gränsvärde som är användbart i det 'södra' beståndet gav korrekt könsbestämning av 92% av honorna i Lappland, men bara 15% av hanarna. Många boungar i Lappland växer upp under näringsbrist och dödligheten är högre för boungar i Lappland än i det 'södra' beståndet. Detta visar även effekter i boungharnas tillväxtmönster, varför en lokalt anpassad morfologisk metod behöver tillämpas i detta bestånd. Samma tarsmått som används i det 'södra' beståndet kan visa sig vara användbart även i Lappland, men gränsvärdet för skiljelinjen mellan könen måste definieras på ett större material än vi inkluderade i denna studie ( $n=26$ ).

Sammanfattningsvis identifierades i denna studie en enkelt användbar metod för könsbestämning av havsörningar som är snabb att utföra och därmed minimerar störningen av häckningen vid bokontrollen. Metoden verkar behöva anpassas lokalt till det undersökta beståndet och att en kalibrering av mätningen behöver genomföras för att garantera jämförbarhet mellan mätningar.

### Artikel III: Lokalt bevarad genetisk variation i nyligen reducerade havsörnspopulationer

Givet den ovan beskrivna populationshistorien av havsörnen i Norden och Centraleuropa undersökte vi i flera lokala havsörnsbestånd om de visar mindre genetisk variation än det historiskt hela tiden stora norska beståndet. Som nämnt medför låg variation en förhöjd utdöenderisk både på kort och lång sikt.

Totalt undersöktes 218 individer från populationerna i Norge, södra och mellersta Sverige, svenska Lappland, Kolahalvön (Ryssland), Estland och Tyskland. Analyser utfördes både på 26 olika mikrosatelliter (de 14 utvecklade i artikel I, därtill 12 andra som ursprungligen hade utvecklats på andra rovfåglar men även fungerade på havsörn), samt mitokondriellt DNA (mtDNA).

Analyserna visade att samtliga bestånd hade ungefär likadana nivåer av variation på mikrosatelliterna. Framför allt visade det norska beståndet inga tecken på förhöjd genetisk variation, varken för mikrosatelliter eller mtDNA. Bestånden var svagt men signifikant skilda från varandra, vilket talar mot stor betydelse av genetiskt utbyte mellan regioner och överensstämmelse med ringmärkningsresultaten.

Det främst lokala bevarandet av genetisk variation förklarar vi med en kombination av två faktorer: havsörnens långa generationstid (medellivslängd av häckfåglar är ca. 17 år) samt lokala naturvårdsinsatser. Havsörnens långa generationstid minimerade antalet generationer som flaskhalsen varade. Eftersom genetisk variation förloras när en individ inte ger vidare sina gener till nästa generation, kan lång generationstid agera som en inre buffert mot snabb förlust av genetisk variation. En vidare förutsättning för bevarandet av genetisk variation var i detta fall att lokalt återstående havsörnar skyddades så att de kunde få överlevande ungar (boplatsbevakning, skydd av boträd samt vinterutfodring som markant höjde ungöverlevnaden).

Dessutom visar våra resultat att totalt mycket mer genetisk variation bevarades genom det lokala perspektivet än om endast den norska populationen (den största och mest stabila när nedgångarna var som värst under 1960-1980) hade prioriterats.

Förutom det glädjande resultatet angående havsörnen stödjer denna studie det höga värdet av små, lokala återstående populationer av långlivade arter för bevarandet av genetisk variation.

## Artikel IV: Fylogeografisk struktur hos havsörn, en generalist med kapacitet för genflöde över stora avstånd

Kunskap om en arts fylogeografiska struktur (storskaliga geografiska mönster i genetisk variation) är viktig för prioritering och avvägning av olika skyddsåtgärder. En viktig miljöfaktor som formade sådan struktur var istiderna. Det kalla klimatet trängde undan många arter i sydliga refugier. Isoleringen i olika sådana refugier kan ha lett till differentiering inom arter vad gäller viktiga egenskaper. Effekten av istiderna förväntas vara starkast på arter med mycket specifika habitatkrav och begränsad spridningsförmåga. Det är oklart hur effekten bör ha varit på havsörn, som å ena sidan är ganska flexibel i sitt habitat- och födoval samt har potential till spridning över långa distanser, men som å andra sidan har visat uttalad hemortstrohet i Europa (se artikel III).

Vi analyserade 500 baspar mtDNA i 237 individer från elva populationer inom havsörnens utbredningsområde mellan Grönland i väst och Japan i öst. Totalt påträffades ganska begränsade mängder av genetisk variation, 13 haplotyper, kompatibelt med en kraftig reduktion i populationsstorlek under istiderna. Analys av haplotypernas inbördes släktskap visade på förekomsten av två olika evolutionära släktlinjer inom havsörnen. Dessa släktlinjer visar idag en öst-väst struktur i sin förekomst, men överlappar extensivt inom europeiska populationer. Förmodligen motsvarar dessa släktlinjer två refugier där havsörnen överlevde de senaste istiderna i Eurasien. Olika faktorer tyder på att dessa områden var (i) i västra Europa, kanske vid Atlankusten

och (ii) centralt beläget i Eurasien, förmodligen innefattande låglandet kring svarta och kaspiska havet samt Aralsjön.

Havsörnens underart på Grönland, *Haliaeetus albicilla groenlandicus*, visade sig vara mycket närbesläktad med havsörnar i västra Europa, och dess vanligaste haplotyp var även vanlig på fastlandet. Detta resultat överensstämmer med benfynd och klimatologiska överväganden och antyder en kolonisering av Grönland efter den senaste istiden, för några tusen år sedan. De unika morfologiska karaktärerna inom grönländska havsörnar verkar därmed ha utvecklats på tämligen kort tid.

## Artikel V: Spår av gemensam härstamning och genflöde mellan olika havsörnspopulationer

Havsörnen koloniserade sitt nuvarande Eurasiatiska utbredningsområde för omkring 10.000 år sedan (se artikel III). Givet havsörnens långa generationstid har denna tidsperiod kanske inte varit länge nog för vissa traditionella populationsgenetiska analyser som antar jämvikt mellan drift och genflöde. Det är känt att likheter mellan populationer i sådana fall lätt kan feltolkas som nutida genflöde. I själva verket kan det återspegla brist på differentiering sedan grundandet av populationerna. I denna studie undersöks genetisk variation inom prover från stora delar av havsörnens utbredningsområde. För detta används mikrosatelliter som genetisk markör. Genetisk variabilitet inom havsörnspopulationer samt genetisk struktur mellan olika populationer undersöks.

Resultat av traditionella analysmetoder jämfördes med resultat från mer moderna populationsgenetiska analysmetoder, vilka ej antar ovan nämnda drift-migrationsjämvikt.

Den genomsnittliga genetiska differentieringen över hela havsörnens utbredningsområde var endast måttlig. Samtliga populationer på fastlandet och i Japan hyste liknande nivåer av genetisk variation. Detta överensstämmer med slutsatserna dragna i artikel III, d.v.s. effekten av en lång generationstid på variationsnivåer i olika populationer. Det antyder därmed att denna effekt kan vara betydelsefull över stora geografiska avstånd och långa tidsintervall.

Populationerna på Island och Grönland har däremot mycket lägre genetisk variation, ca. 50% av heterozygotigraden i de andra populationerna. Denna låga variation återspeglar sannoligt en kraftig genetisk flaskhals vid kolonisationen av dessa Atlantiska öar. För närvarande verkar populationerna vara helt isolerade från de på fastlandet. Detta innebär att deras aktuella populationsstorlekar kan vara för små för att på längre sikt säkerställa deras överlevnad.

Den låga graden av differentiering över hela Europa och Asien beror delvis på vissa aspekter av havsörnens livscykel. På liknande sätt som det har

föreslagits för populationer av skogsträd (Austerlitz *et al.* 2000) har havsörnen under koloniseringen av Eurasiens fastland efter istiderna inte genomgått några kraftiga flaskhalsar. Antalet immigrater som grundat nya populationer kan ha varit högt. Detta indikeras av den relativt långa juvenila fasen i havsörnens livscykel (5 år) och den endast måttligt höga reproduktionstakten. Innan avkomor av lokala par är könsmogna har många fler immigranter tid att kolonisera regionen.

Med hjälp av simuleringar kunde vi dock visa att populationsstrukturen hos havsörn idag inte överensstämmer med en modell helt utan genflöde. Redan efter 3000 år skulle den genetiska differentieringen vara betydligt starkare än vad som observeras. Detta bekräftas även av att vi mellan populationer ser en differentieringsgrad som tilltar med geografiskt avstånd. Ett sådant mönster tyder på visst genflöde mellan populationer, åtminstone när man ser över längre tidsperioder.

Hur går denna bild av genflöde att förena med europeiska ringmärkningsdata som antyder stark hemortstrohet? Skillnaden kan bero på två faktorer: högre grad av genflöde förr än nu, eller pågående sällsynt genflöde som ej upptäckts med ringmärkta fåglar. Om genflöde var mer utbrett tidigare kan det ha påverkats av densitetsberoende krafter i populationerna. Kanske var genflödet mellan regioner mer vanligt i de historiska havsörnspopulationerna. Dessa präglades av mycket högre individtäthet än vad som var fallet under de senaste decennierna. Om detta antagande stämmer kan man förvänta sig att genflödet mellan regioner snart tilltar. Detta eftersom många europeiska havsörnspopulationer idag börjar närma sig mättnadsnivå och konkurrensen om häckningsplatser och partner ökar.

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