PHYLOGEOGRAPHY AND CONSERVATION GENETICS OF THE LESSER WHITE-FRONTED GOOSE (*ANSER ERYTHROPUS*)

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OULU 2001

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Academic Dissertation to be presented with the assent of the Faculty of Science, University of Oulu, for public discussion in Kajaaninsali (Auditorium L6), Linnanmaa, on April 20th, 2001, at 12 noon.

OULUN YLIOPISTO, OULU 2001

Manuscript received 21 March 2001
Manuscript accepted 28 March 2001

Communicated by
Professor Wolfgang Stephan
Professor Robert Zink

ISBN 951-42-5948-3 (URL: http://herkules.oulu.fi/isbn9514259483/)

ALSO AVAILABLE IN PRINTED FORMAT
ISBN 951-42-5947-5
ISSN 0355-3191 (URL: http://herkules.oulu.fi/issn03553191/)

OULU UNIVERSITY PRESS
OULU 2001

Ruokonen, Minna, Phylogeography and conservation genetics of the lesser white-fronted goose (*Anser erythropus*)
Department of Biology, University of Oulu, P.O.Box 3000, FIN-90014 University of Oulu, Finland
2001
Oulu, Finland
(Manuscript received 21 March 2001)

Abstract

Analyses of mitochondrial control region sequences were used to infer phylogeny of *Anser* species, phylogeography of the lesser white-fronted goose, and genetic background of a captive stock.

The genetic distances among the *Anser* species ranged from 0.9 to 5.5% in the complete control region sequences and supported the view of close relatedness of these species. Among the four most closely related species, the bean, pink-footed, white-fronted and lesser white-fronted goose, the branching order is uncertain. The short internal branches and low support for the branching order suggest that the species have diverged recently within short time-intervals. The mtDNA tree obtained is incongruent with the traditional view of the species relationships, but the reasons for this remain to be clarified.

Two diverged mitochondrial lineages were found in the lesser white-fronted goose and a refugial origin was proposed. Basal haplotypes are geographically widespread and indicate a recent common ancestry for populations. The derived haplotypes are confined to singular breeding populations and suggest restrictions to the present female gene flow. A shift in the frequency of the mtDNA lineages approximately coincides with a migratory divide in the Taimyr Peninsula. Low mtDNA diversity and significant difference in the haplotype frequencies observed in Fennoscandian subpopulation suggested that it should be considered as a management unit. The fossil record was examined to gain additional information about the colonisation history of the species, but was found to be of limited use.

The captive lesser white-fronted goose stock used for reintroduction/restocking was shown to be incompatible with the Fennoscandian wild population. Some captive individuals carried the mtDNA of the white-fronted goose suggesting a hybrid origin. Hybridisation has probably occurred during captive propagation, but to clarify further the extent of introgression, nuclear markers should be applied.

The structure and evolution of the control region were studied by comparing complete avian sequences. Saturation was found to occur at pairwise divergences of 10% as shown for third codon positions of the mitochondrial genes previously. In pairwise comparisons of the control region and cytochrome *b* sequences, the rate of divergence varied among the lineages. Two conserved sequence blocks showed considerable sequence conservation when compared to mammalian sequences.

Keywords: Anser, phylogeography, conservation genetics, mitochondrial control region

Acknowledgements

This research was carried out at the Department of Biology, University of Oulu. I am grateful to my supervisor Docent Jaakko Lumme and Professor Markku Orell for their guidance and support during this work. I thank Professor Emeritus Seppo Lakovaara and Professor Pekka Pamilo for providing excellent working facilities. I thank Professor Outi Savolainen for support while I was contemplating whether to go to work abroad or not. I am indebted for encouragement and guidance from Professor Håkan Tegelström, Uppsala University, who also led me through some moments of despair during the project. Thank you for letting me to join your group for a while!

I thank my former and present colleagues Arttu Ahola, Jussi Kuusela, Päivi Kuuva, Tuija Liukkonen-Anttila, Maria Meinilä, Helena Penttilä, Seppo Pöykkö, Anni Tonteri, Leena Uimaniemi, Kerttu Vihula, Keijo Viiri, Marek Zietara and especially Laura Kvist for sharing. I am grateful to my vegetating and flowering colleagues at the lower levels of the food chain, Auli Karhu, Päivi Hurme, Sami Oikarinen and Mona-Anitta Riihimäki, for bringing joy and having refreshing alienated discussions with me. At the same time I apologize if it sometimes happened against your will and occasionally at your expense! Auli, thank's for walking this through with me! I thank Hannele Parkkinen and Miia Makkonen for their technical assistance in parts of this work. Staff of our office, especially Sisko Veijola, is gratefully acknowledged for sorting out all kinds of messy paperwork and bureaucracy. Everybody in the Department deserves my gratitude for making the working days pleasant!!!

I wish to express my gratitude to Anna Carin Andersson, Martin Carlsson, Vadim Fedorov, Anna Goropashnaya, Mats Isaksson, Ciia Olsson & Perttu Seppä and Carl-Gustaf Thulin from the Department of Conservation Biology and Genetics, Uppsala University, who made my stay at Uppsala a pleasant (and a groovy) experience.

I am indebted to Tomas Aarvak, Juha Markkola, Ingar J. Øien and Petteri Tolvanen for their companionship and discussions during the field trips and meetings in Finland, Sweden, Norway, England, Belgium, Bulgaria, Poland, Russia, Japan and China. During these years I have learned a lot about geese, but equally as much about life! I am especially grateful to Tomas for sharing the same attitude towards 'science', which has been a motivation for all our discussions about the lesser white-fronted goose, and his never-failing patience in answering my stupid questions. Cheers, mates!

I also thank my other co-authors and colleagues Lei Gang, Shigeki Iwabuchi, Vladimir V. Morozov and Eugeny E. Syroechkovsky Jr. for their invaluable contribution. I would like to extend my thanks to all the members of LWfG team, WWF Finland, and especially to Aki Arkiomaa, Toni Eskelin, Heikki Holmström & Muri, Risto Karvonen, Jarmo Pääläinen, Kalle Ruokolainen, Sami Timonen and many others. With you guys it's difficult to draw the line between a professional relationship and a friendship, but maybe that's unnecessary?

I am grateful to Professor Wolfgang Stephan and Professor Robert Zink for the valuable comments on my thesis and to Dr. Bohdan Sklepkovych for revising the language. This work was supported by the Academy of Finland, University of Oulu, Thule Institute at the University of Oulu and NorFA.

My daughter Saana has done her best to keep me in contact with the realities of life. Looking at her, I often marvel in nature's way of creating amazing things from modest ingredients. I'm also proud that she carries my mitochondria! I thank Tommi for the long-lasting friendship in all of its forms. Last but not the least I thank my parents Aila and Kari Turkki, my sister Annamari and my brother Juha and their families for the encouragement.

Oulu, January 2001, Minna Ruokonen

What if the world refuses its turn?
What if the stars hesitate?
What if what is isn't true?
Smashing Pumpkins: Appels + Oranjes

Abbreviations

AMOVA	Analysis of molecular variance
bp	Base pair
BP	Before present
cpDNA	Chloroplast DNA
CSB	Conserved sequence block
dNTP	Dinucleotidetriphosphate
H strand	Heavy strand
IS	Isotope stage
kb	Kilo base pair
L strand	Light strand
MHC	Major histocompatibility complex
ML	Maximum likelihood
mtDNA	Mitochondrial DNA
My, Mya	Million years, million years ago
nDNA	Nuclear DNA
numt	Nuclear copy of a mitochondrial sequence
OTU	Operational taxonomical unit
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
tRNA	Transfer ribonucleic acid
ts	Transition
tv	Transversion

List of original papers

This thesis is based on the following publications, which are referred to in the text by their Roman numerals.

I Ruokonen M & Kvist L (2001) Structure and evolution of the avian mitochodrial control region, manuscript.

II Ruokonen M, Kvist L & Lumme J (2000) Close relatedness between mitochondrial DNA from seven *Anser* goose species. Journal of Evolutionary Biology 13: 532-540.

III Ruokonen M, Kvist L, Aarvak T, Gang L, Iwabuchi S, Markkola J, Morozov V, Øien IJ, Syroechkovsky E Jr, Tolvanen P & Lumme J (2001) Phylogeography and population genetic structure of the endangered lesser white-fronted goose (*Anser erythropus*), submitted.

IV Ruokonen M, Kvist L, Tegelström H & Lumme J (2001) Hybrids, captive breeding and restocking of the Fennoscandian lesser white-fronted goose (*Anser erythropus*). Conservation Genetics, in press.

Contents

1 Introduction

Variation in nature is partitioned into different levels of hierarchy: genes, individuals, populations, species and ecosystems, and together constitute the spatially and temporally variable biodiversity of nature. The heritable basis for this diversity is genetic variation, of which a large proportion is neutral or nearly so (Kimura 1968, 1983), and once introduced into a population by mutation, recombination and migration, its fate is determined largely by random genetic drift. Genetic variation affecting the fitness of an individual is the target for natural selection (Fisher 1930, Wright 1931, Haldane 1932).

Organisms live in an environment that is not constant over time. The present patterns of species' diversity are affected by both past evolutionary history and current population processes and their relative significance are often difficult to distinguish based on contemporary observations. For example, genetic homogeneity among the populations of a species can be due to a recent common ancestry or contemporary gene flow. The ecological, life-history and behavioural characteristics of species, such as population size, population subdivision, dispersal and social structure, affect present diversity. Increasingly, environmental changes caused by man, such as habitat fragmentation, are involved.

Among the most influential factors in history affecting the structuring of present populations are the ice ages. During the past 2.5 My, the climatic and environmental fluctuations of the Pleistocene have forced species to adjust the distributional areas according to their adaptive ability. During cold periods, species responded by moving southwards to refugia and during milder periods they migrated northwards or to high altitudes, or expanded their distributional ranges without leaving the refugia (Roy *et al.* 1996, Blondel & Mourire-Chauviré 1998). The homogeneity and low level of diversification in the Northern Hemisphere have been explained as a consequence of repeated mixing of populations during the climatic oscillations preventing differentiation or limited availability of refugia and consequent high extinction rate (Webb & Bartlein 1992, Blondel & Mourer-Chauviré 1998, for empirical evidence see e.g. Klicka & Zink 1997).

1.1 Phylogeny and phylogeography

By using a comparative approach genetic diversity can be organised into a meaningful estimation of the evolutionary relationships among lineages of organisms i.e. a phylogeny. Phylogenetic inference is an attempt to estimate the branching order of taxa and to define monophyletic groups of taxa (topology), and sometimes the process includes also the estimation of the evolutionary rates along the lineages (branch lengths). In practise a phylogenetic tree can be reconstructed by using different statistical methods, most often based on two kinds of criteria: by defining an algorithm which determines the tree (e.g. based on genetic distances: the estimated genetic distances between pairs of OTUs reflecting the degree of relatedness), or an optimality criterion used in selecting the best tree among all possible alternative trees (e.g. maximum parsimony: the number of evolutionary changes to explain the data is minimised) (Hillis *et al.* 1996). A variety of traits and characters, such as morphology, behavioural, physiological or life-history features and, nowadays, molecular characters have been increasingly employed to infer the phylogenetic relationships of taxa (for a review, see Hillis *et al.* 1996). A fundamental difference exists between a species tree, representing the true evolutionary pathways of a group of species, and a gene tree, often constructed based on one gene. The gene tree and the species tree are not necessarily congruent in terms of topology or branch lengths, owing to e.g. polymorphism at the time of divergence, reticulate evolution and sampling errors (Nei 1987).

The term phylogeography was first introduced by Avise *et al.* (1987). The developments in phylogeography mainly rely on the use of (animal) mtDNA: due to the maternal inheritance and nonrecombining nature of the mtDNA the haplotypes can be ordered phylogenetically, whereas most nuclear markers, e.g. microsatellites and allozymes, provide allele frequency-based data. Phylogeography deals with the genealogy of lineages and their geographical location, at the intra- and interspecific levels, and it can be considered as a bridge linking the study of micro- and macroevolutionary processes (Avise 2000). In the analysis and interpretation of lineage distributions, other fields of natural sciences such as population genetics, ecology, ethology, phylogenetics, paleontology and geological history are employed.

In comparative phylogeography, the phylogeographic patterns among the species are compared in order to find general patterns of evolutionary history and to reveal the evolutionary processes behind the patterns (Bermingham & Avise 1986, Zink 1996, Hewitt 2000). Comparative phylogeography can identify groups of species that have a common history of vicariance. For example, the post-glacial colonisation routes of mammals, amphibians, arthropods and plants in Europe showed that northern regions were generally colonised from refugia in the Iberian peninsula and the Balkans, whereas Italian lineages were often isolated by the Alpine barrier, although the distributions of the phylogroups were not spatially congruent among all the species (Taberlet *et al.* 1998). In studies of plant phylogeography, the cpDNA has been mostly employed (argan tree: el Mousadik & Petit 1996, oaks: Dumolin-Lapeque *et al.* 1997, Ferris *et al.* 1998). As a regional approach, comparative phylogeography can be used for conservation purposes to localise areas of high biodiversity and thus of high conservation value (Moritz & Faith 1998).

1.2 Conservation biology and genetics

Conservation biology is an applied cross-disciplinary science of maintaining the biological diversity at the levels of genetic diversity, species diversity and ecosystem diversity. The beginnings of conservation biology lie in the 1970's and 80's when the first conference of conservation biology was held and soon after, the first book on the topic was edited and published by Soulé and Wilcox (1980). The detailed priorities, targets and principles of conservation biology are a matter of debate. The problematics are closely related to developments in other fields of biology e.g. how to define a conservation unit (i.e. species concept), the need to create uniform and objective criteria to be applied in decision-making or how to quantify conservation worth to preserve as much as possible with limited economical resources. The scientific foundations of conservation biology, as Bowen (1999) suggests, can be seen to include three complementing fields: 1) systematics: identification of organismal lineages, 2) ecology: protection of the life-support system for the lineages and 3) evolutionary biology: maintenance of conditions that generate new lineages.

During the past few decades the theoretical framework of population and quantitative genetics and empirical data collected with the help of molecular genetic methods have been increasingly used in conservation biology. The background for employing molecular genetics in conservation biology lies in the 1960's when protein and allozyme electrophoresis was first used to reveal the patterns of genetic diversity, levels of polymorphism and heterozygosity, both in species and in populations (see Avise 1994, O'Brien 1994a). Soon, it was noticed that endangered species or populations may show reduced levels of genetic variability (Bonnell & Selander 1974, O'Brien *et al.* 1983, Wildt *et al.* 1987) and the costs of inbreeding were demonstrated by differences in juvenile mortality in offspring of related and unrelated captive ungulates (Ralls *et al.* 1979). This may have led to an overemphasis of the importance of genetic variation and in neglecting the demographic and environmental factors in species' conservation (Lande 1988, Caughley 1994) for which more emphasis has been recently placed, especially in short-term management issues surrounding endangered taxa. The corner stones of conservation genetics are the theory of evolutionary genetics, molecular genetic methods and the ecology of the species.

Molecular genetic techniques may be considered applicable at the levels of ecosystems, species, populations and individuals. The empirical data have management implications, i.e. genetic diversity in populations, patterns of mate choice, parentage and kinship, gene flow, hybridization and phylogenetic relationships among taxa. One of the main tasks in conservation biology is the recognition and identification of possible conservation units at different levels of taxonomic hierarchy. Often, the taxonomical assignment has been based on a typological view of a species, which can potentially fail in preserving the diversity, maintaining the geographical variation and evolutionary potential within a species (Rojas 1992, Soltis & Gitzendanner 1999). Molecular genetic methods provide an objective means to reveal and assist the determination of taxonomic units (Avise 1994, King & Burke 1999). The study of genetic variation provides an insight to current processes, but also to the historical evolutionary processes that have created present biodiversity (Mace *et al.* 1996). Genetic data can also provide information

for management planning by making it possible to evaluate the effect of genetic changes in the survival and persistence of the populations (O'Brien 1994a, Mace *et al.* 1996).

Neutral genetic markers, such as nuclear microsatellites or mtDNA commonly applied in conservation genetics, are assumed to reflect the evolutionary prospects of the species of interest. Some conservation biologists oppose of the use of neutral markers in studies related to conservation issues. Instead, the use genetic markers directly involved in adaptive traits, such as loci related to immune defence, reproduction and physiological functions, has been proposed (Hughes 1991, O'Brien 1994b) and in some studies, the levels of variation in the MHC genes of the endangered species have been analysed (Yuhki & O'Brien 1990, Ellegren *et al.* 1993, Hedrick & Parker 1998). However, the general trend in conservation genetics seems to be that multiple independent markers are more commonly used in conservation related studies (King & Burke 1999).

1.3 Mitochondrial DNA: the tool

1.3.1 Mitochondrial DNA

The animal mitochondrial DNA is a circular molecule of 15-20 kb in length and in vertebrates it contains genes for 22 tRNAs, 2 rRNAs and 13 mRNAs coding for proteins involved in electron transport and oxidative phosphorylation. The only major noncoding area of the mtDNA is the control region, typically 1 kb, involved in the regulation and initiation of mtDNA replication and transcription (see 1.3.2). The use of mtDNA has become increasingly popular in phylogenetic and population genetic studies, first with the developments in methodology for mtDNA isolation and use of restriction enzymes to detect nucleotide differences (Lansman *et al.* 1981), and further with the development of PCR methodology and applicability of 'universal' primers (Kocher *et al.* 1989) for amplification of mtDNA. Much of the interest is related to the fast rate of substitutions in mtDNA. The approximate mutation rate in mtDNA is 10^{-8}/site/year (Brown *et al.* 1979, Ferris *et al.* 1983, De Salle *et al.* 1987) compared to 10^{-9}/site/year in nuclear genes. Most differences between mtDNA sequences are point mutations, with a strong bias for transitions over transversions (Brown *et al.* 1982).

The mtDNA is haploid and uniparentally inherited (with some exceptions, see below) and thus the variability is introduced by mutations alone. Compared to diploid nuclear autosomal genes with biparental transmission, the effective population size of mtDNA is one quarter of that for nuclear autosomal genes (Moore 1995). Therefore, a mtDNA tree is more likely to be congruent with a species tree due to a high probability of coalescence even when speciation events have occurred within short time-periods.

Mitochondrial genes are inherited as one linkage group in the absence of recombination (Hayashi *et al.* 1985, Hoech *et al.* 1991). Recently, the clonal nature of mtDNA has been questioned and the possibility of recombination has been advocated based mainly on linkage disequilibrium in human and chimpanzee mtDNA, excess homoplasy in human control region and the existence of a globally rare transitional mutation found in more than one well-supported mtDNA clade present in one population (Awadalla *et al.* 1999, Eyre-Walker *et al.* 1999, Hagelberg *et al.* 1999). Subsequently, the

methodology used has been criticised (Ingman *et al.* 2000, Kumar *et al.* 2000) and alternative explanations have been preferred, such as the presence of hypervariable nucleotide positions or selection (Wallis 1999). The necessary enzymatic machinery for recombination does exist in mammalian mitochondria (Thyagarajan *et al.* 1996), and mtDNA recombination has so far been shown to occur in plants, fungi, protists (Gray 1989) and phytonematodes (Lunt & Hyman 1997). The prerequisite for recombination to create new variants is that different types of mitochondria are present in the same cell. Although individuals usually carry one type of mtDNA in their cells, heteroplasmy (existence of more than one extranuclear DNA sequence type in an organism) has also been reported. The most common cases are length variants in repetitive areas found within an individual (e.g. Berg *et al.* 1995), but it has been suggested that these could be created *de novo* within a lineage (Lunt *et al.* 1998). Contrary to common belief, the sperm mitochondria have been shown to enter the oocyte in most mammals (Ankel-Simons & Cummins 1996) and paternal leakage or biparental inheritance of mtDNA have been reported (Kondo *et al.* 1990, Gyllensten *et al.* 1991, Zouros *et al.* 1992). However, in mice it has been shown that the paternal mitochondria are usually eliminated although the process of elimination does not work for interspecific crosses (Kaneda *et al.* 1995). Then, if mtDNA recombination takes place, the hybrids of deeply diverged lineages could produce recombinant genomes e.g. in hybrid zones (Wallis 1999), but as such this has yet to be reported.

1.3.2 Mitochondrial control region

The control region is the main regulatory region and the only major non-coding area in animal mtDNA. It contains the heavy-strand origin of replication (Desjardins & Morais 1990) and the promoters for heavy and light strand transcription (L'Abbé *et al.* 1991). The control region is situated in between tRNAglu and tRNAphe in most avian species studied and between tRNAthr and tRNApro in Picidae, suboscine and at least one oscine Passeriformes, Falconiformes and Cuculidae (Mindell *et al*. 1998, Bench & Härlid 2000). In avian species, the length of the control region varies from 1028 bp in *Struthio camelus* (Härlid *et al.* 1997) to 1580 bp in *Cyanoramphus auriceps* (Boon *et al.* 2000), and in mammals approximately from 880 to 1400 bp (Sbisà *et al.* 1997). The variation in length has been attributed to variation in the tandem repeat number (Berg *et al*. 1995) and small insertions/deletions usually in the 5' and 3' ends of the control region.

Despite its functional importance, the control region is suggested to be the most variable part of the mtDNA. In the human control region, the estimates of the rate of substitution were found to range between 2.8 (Cann *et al.* 1984) to 5 times (Aquadro & Greenberg 1983) the rate of the rest of the mtDNA. Most of the studies in which control region sequences have been used have focused on intraspecific patterns of variability and phylogenetic relationships of closely related species, a prominent example being the study of human population history (see Cavalli-Sforza *et al.* 1994, and references therein). A high mutation rate also means that the phylogenetic utility of the control region sequences diminishes in deep divergences due to saturation and ambiguities in homology determination.

Based on the distribution of the variable nucleotide positions and differential nucleotide frequencies in different parts of the control region, it is divided into three domains (Brown *et al.* 1986). Domains I and III are rich in L-strand adenine, whereas the central domain II is low in adenine. Most of the variability, both nucleotide substitutions and deletions/insertions, is concentrated in domains I and III whereas domain II is more conservative. Based on sequence similarity, tens of conserved sequence blocks with putative functional importance have been described (e.g. Southern *et al.* 1988, Lee *et al.* 1995, Sbisà *et al.* 1997, Randi & Lucchini 1998). The general structure of the control region and an overview of the sequence blocks are depicted in Fig. 1.

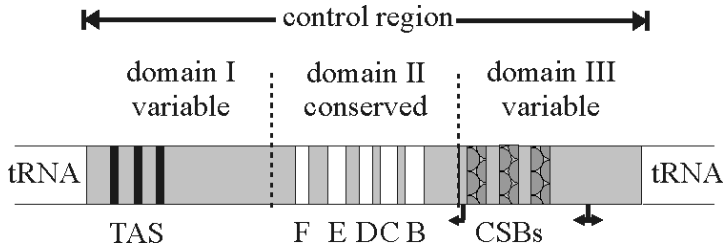

Fig. 1. General structure of the vertebrate mitochondrial control region. The arrows indicate the location of the H-strand replication origin and the bidirectional promoter for L- and H-strand transcription. TAS, termination associated sequence; F through B, conserved sequence boxes in the central domain; CSBs, conserved sequence blocks.

1.4 The geese

The geese, subfamily Anserinae, tribe Anserini, are divided into two genera, 'the grey geese' *Anser* (10 species) and 'the black geese' *Branta* (five species). Earlier, the geese were divided into five genera: *Anser*, *Philacte*, *Chen* (present genus *Anser*), *Branta* and *Nesochen* (present genus *Branta*), and these old taxonomic affiliations are still used by some authors (Miller 1937, Delacour 1954, Bellrose 1980, Quinn *et al.* 1991). Goose species are considered relatively young, a notion that is supported by molecular genetic studies. The divergence time of genera *Anser* and *Branta* has been estimated to 5 My based on fossil data (Shields & Wilson 1987).

The geese are largely holarctic in their distribution and all species are highly migratory, with the exception of the endemic Hawaiian goose (*Branta sandvicensis*). The breeding areas are typically in the tundra and taiga, and for most species in the arctic or in high altitudes, as the bar-headed goose (*Anser indicus*). The only goose species having a strong association with the sea is the brent goose (*Branta bernicla*) which breeds mainly on small islands and winters in coastal areas.

Geese breed for the first time at the age of 2-3 years, and pair bonds are considered to be monogamous and long-term (Cramp & Simmons 1977). As in general for arctic species the incubation and fledging times are short due to a short favourable breeding season. Goose species breeding in high latitudes, e.g. the lesser white-fronted goose (*Anser erythropus*) and the brent goose, do not lay replacement clutches. Mainly due to

unfavourable weather conditions and high numbers of native or introduced predators (mainly the arctic fox *Alopex lagopus* and, nowadays to a greater extent in Fennoscandia, the red fox *Vulpes vulpes*) there is a large variance in the yearly number of offspring produced.

Most non-breeding geese perform moult migrations at locations generally north of breeding areas. The evolution of moult migrations has been explained by the possibility of gaining access to easily digestible high-protein food resources provided by the early growth of grasses and sedges starting later in more northern areas to compensate for energy-loss during feather replacement and to avoid food competition with family groups (Salomonsen 1968, Owen & Ogilvie 1979). These moult flights can be extreme and sometimes involve crossing an open sea, as in bean geese (*Anser fabalis*) which moult largely on the Siberian archipelago (Ogilvie 1978, Alerstam 1990) or in the pink-footed goose which migrate from Iceland to Greenland (Christensen 1967). Breeding individuals remain with their mates and families and moult near the breeding areas where the moult of the parents is synchronised with the fledgling of their offspring (Cramp & Simmons 1977). The juveniles stay with parents at least through the first autumn and winter, but in some species juveniles may remain through to the following breeding season (Fox *et al.* 1995).

The culturally transmitted migratory flyways of the geese lead to the wintering areas through traditional stopover sites. Earlier, it was assumed that the breeding groups of the geese remained largely isolated from other breeding groups also during the nonbreeding season (Cramp & Simmons 1977). This was recently contradicted by ring-recovery data on the European white-fronted goose (*Anser albifrons albifrons*) indicating that the birds breeding in Taimyr Peninsula migrated as a wide front and were distributed over several wintering sites (Mooij 1996). However, it has been shown in the Pacific white-fronted goose (*Anser albifrons frontalis*) that while the spatially segregated breeding groups use the same staging areas, the groups still are temporally segregated (Ely & Takekawa 1996).

Geese generally overwinter in lowlands, farmlands and marshes. Recently, many goose species have shifted from natural habitats to using agricultural land for feeding in Western Europe in winter. As during the breeding season, weather plays an important role during winter. If the weather is harsh the geese move southwards to winter. Goose species are gregarious during the non-breeding season and families usually remain together throughout the winter (Cramp & Simmons 1977).

The spring migration routes of geese are not necessarily the same as in autumn. To maximise the chances for successful reproduction during the breeding season, the geese should arrive at the breeding grounds in good condition and with optimal timing to be able to use the short favourable season effectively. Therefore, good-quality wintering and staging areas along the migratory route are of utmost importance in augmenting body reserves prior to the onset of nesting (Ebbinge *et al.* 1982, Johnson & Herter 1990).

1.4.1 Life-histories and genetic consequences

The genetic structuring of geese populations are shaped by life-history and behavioural characteristics. The amount of inter-population movement defining the amount of gene

flow and ultimately population structuring is an interplay of factors such as the degree of natal and breeding philopatry, winter philopatry, timing of pair-formation, pair-bond stability, assortative mating and gregarious behaviour.

The hypothesis proposed to explain the evolution of philopatric behaviour is related to the selective advantage of being familiar with an area and maintaining social bonds with conspecifics leading to a higher reproductive success as compared to dispersing individuals (ecological mechanisms) and mating with partners with which they share a specific level of genetic relatedness to gain optimal outbreeding and at the same time optimal inbreeding (genetic mechanisms) (Greenwood 1980, Weatherhead & Forbes 1994). Compared to most other bird species, geese show female-biased natal and breeding fidelity (Greenwood 1980). In the Canada goose (*Branta canadensis*) up to 79% of females returned to their natal site to breed, whereas 63% of the males were observed to breed outside their hatching site (Lessells 1985). None of the individuals exhibited breeding dispersal in subsequent years. However, the study was conducted with introduced mainly non-migratory Canada geese in Britain, and therefore the results may not apply to migratory geese. In breeding populations of the lesser snow goose (*Anser caerulescens caerulescens*) banding results clearly showed that female geese frequently returned to the natal colony to breed, whereas males seldom did (Cooke *et al*. 1975). Because a large proportion of the 2nd calendar year males were still seen in their natal colony during the breeding season but considerably fewer 3rd calendar year or older males were observed, the disappearance of the males may well be related to pair-formation and consequent emigration to the female's natal colony. However, despite of female philopatry suggested by ringing data, a low level of genetic structuring in mtDNA among the breeding populations of the snow goose has been shown (Avise *et al.* 1992, Quinn 1992).

Winter philopatry, on the other hand, is considered male-biased in geese. The return rates (proportion of banded individuals returning to a wintering area in subsequent years) estimated for goose species have ranged from 50% in the brent goose to 85% in the white-fronted goose (Robertson & Cooke 1999 and references therein). However, as mentioned earlier, geese can use more than one area during one winter depending on the weather conditions and there are few data on the maintenance of flock integrity throughout the winter.

Timing of pair formation in migratory geese is essential in determining the amount of gene flow between breeding locations. Some studies have shown that pairing in mid-winter is not a rule in geese, as often has been assumed for waterfowl in general. In the lesser snow goose and the brent goose pair formation occurs in winter or spring, whereas in the emperor (*Anser canagicus*), white-fronted and the Canada goose pair formation occurs during spring migration or in summer (reviewed in Ely & Scribner 1994). If the pairs are formed on the wintering grounds where mixing of individuals breeding in different localities takes place and if a non-assortative pairing is assumed, a chance to pair with an individual from a non-natal group exists. If pair-formation occurs during the spring migration or on the breeding grounds when the individuals are already at least partially segregated, the magnitude of gene flow between breeding groups depends mainly on the degree of natal philopatry. On the other hand, if the species exhibit winter philopatry and flock integrity is maintained, the amount of gene flow can be restricted even if pair-formation takes place on the wintering grounds. Additionally, at least in the

snow goose an assortative mating between the two colour-phases is known to take place (Cooke *et al.* 1972, Cooke & McNally 1975).

The life-history characteristics described above have different consequences for the amount of genetic differentiation observed in biparentally versus uniparentally inherited genetic markers. In avian species the female is the heterogametic sex (ZW) so that both the W sex chromosome and mtDNA are maternally inherited. The autosomal chromosomes and the Z chromosome are inherited through both parents and paternally inherited markers, such as mammalian Y chromosome, do not exist. In Fig. 2 the effect of these characters is depicted in terms of genetic structuring.

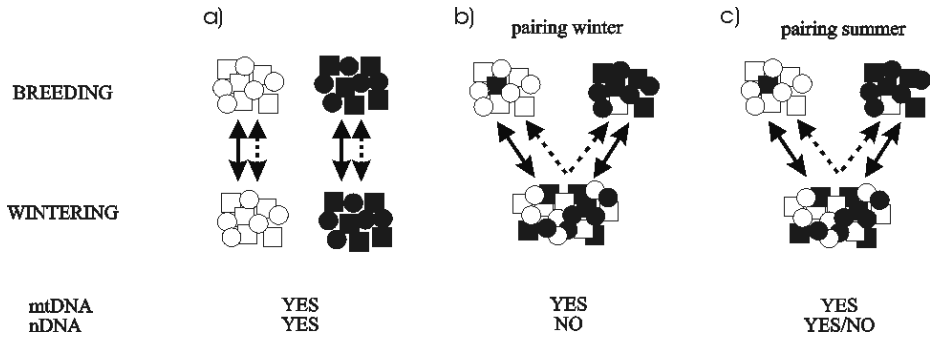

Fig. 2. The effect of some life-history characteristics to the genetic structuring of the goose populations in maternally inherited mitochondrial DNA and W chromosome, and in biparentally inherited nuclear autosomal genes. In all the cases, the assumption is that the females show natal philopatry and the males wintering philopatry. In c) the degree of differentiation in nuclear genes depends on the degree of male natal philopatry. If assortative mating is involved, genetic structuring in both maternally and biparentally inherited markers may take place in a) trough c). Circle, a female; square, a male; solid line, female movements; dotted line, male movements.

1.4.2 Geese and conservation cases

During the past century, almost all goose species have faced threats leading to declining population trends. A common denominator for most cases showing a declining population trend is excessive hunting or a combination of hunting and other factors, such as habitat destruction and agricultural conflicts mainly at stopover sites and wintering grounds, and disturbance and predation especially on the breeding grounds. As with any migratory species, geese are constrained by factors at breeding, staging and wintering areas, all of which may have a different set of adaptive requirements. Also, some goose species show very narrow and specialised habitat requirements. For example, the population decline of the brent goose (*Branta bernicla bernicla*) in the 1930s was at least partially related to reduced abundance of the main food plant eelgrass *Zostera marina* in the wintering areas (Atkinson-Willes & Matthews 1960). Generally, geese are considered to have a good ability to adapt and have responded by e.g. changes in culturally

transmitted migratory behaviour, shifts in distributional areas, use of agricultural land for feeding and the avoidance of predation by specialised nesting places.

One of the most famous examples of a conservation programme in geese is the endemic Hawaiian goose, which underwent a severe population bottleneck at the beginning of the last century due to habitat loss, introduced predators and over-hunting. By 1950 only 30 individuals of the estimated original 25 000 birds were left, and a captive propagation program was established (Kear & Berger 1980). By 1992 more than 2000 captive birds were released, but the population numbers are still today dependent on the number of birds released (Black *et al.* 1991). Because the captive stocks were founded by few individuals, high relatedness and consequent low levels of genetic variation were potential factors limiting population growth (Rave *et al*. 1994). However, low levels of genetic variation do not necessarily imply that the limited population growth is caused (solely) by inbreeding effects. Most likely, a combination of genetic (e.g. adaptation to captivity, inbreeding) and environmental factors (e.g. lack of grassland reserves, heavy predation) were responsible (Black 1994).

The threatened red-breasted goose (*Branta ruficollis*) breeds on the Russian arctic tundra at Taimyr, Gydan and Yamal Peninsula (Hunter *et al.* 1999). Because the breeding and wintering ranges of the species are very limited, it is especially sensitive to overexploitation by hunting and alterations in habitat quality. In the 1950s the population numbered 50 000-60 000 birds, but because of deterioration of previous wintering grounds in the Caspian region, the population declined to approximately 25 000 during the 1970-80s (Hunter *et al.* 1999). After the switch to the wintering quarters on the Black Sea coast and protective legislation, the present maximum population estimate is 75 000 individuals (Hunter *et al.* 1999). The expansion in the red-breasted goose numbers during the last decades also exemplifies the potential of goose species for expansive population growth.

The bean goose is a widespread species with a breeding distribution covering the Eurasian tundra and taiga zones (Cramp & Simmons 1977). The species shows a declining trend, and since the beginning of the 20th century parts of its former breeding areas in the eastern Palearctic and taiga zone have been abandoned as a consequence of increased human activities (Rogacheva 1992). The world population has declined from 1.5 million individuals in the 1960s to approximately 700 000 individuals in the 1990s (Mooij & Zöckler 1999, and references therein). A decrease in numbers has also been observed in the wintering areas in Europe (Cramp & Simmons 1977, van den Bergh 1999). In Finland, the status of taiga bean goose (*Anser fabalis fabalis*) is considered 'near threatened' in the national 2000 Red List for the first time, but the species is not listed in the 2000 IUCN Red List of Threatened species. The need for future conservation is obvious but at the same time hampered by an unclear taxonomy of the species and a lack of information regarding the connection between breeding, staging and wintering areas. Most of the census counts are carried out during winter in Europe and Asia, and without clear knowledge about migration routes and breeding grounds, the specific threats facing each group cannot be fully understood and proper conservation measures implemented. The taxonomy of the bean goose complex is unclear both at the species and subspecies level. Based on morphology and distributional ranges, most authors recognise two species, the pink-footed goose *Anser brachyrhynchus* and the bean goose with two subspecies breeding in the tundra and two or three subspecies in the taiga zone (Delacour

1954, Cramp & Simmons 1977, Mooij & Zöckler 1999). Recently, the Dutch committee for avian systematics (CSNA) has further divided the bean goose into two species, the taiga bean goose and the tundra bean goose, based on differences e.g. in plumage, vocalization, behaviour and feeding phenology (Sangster *et al.* 1999), although this does not represent a general consensus of opinion.

A recent challenge to the arctic breeding species is related to the impact of climate change to breeding areas. According to a model used by Zöckler & Lysenko (2000), a modest rise in the global temperature of 1.7°C by the end of the 21st century would affect 76% of the current breeding range of the tundra breeding bean geese (*Anser fabalis rossicus* and *A. f. serrirostris*) and 67% of the red-breasted goose breeding area via changes in vegetation. The magnitude of the decrease in population numbers depends largely on the ability of goose species to adapt to the direct effects (e.g. affecting breeding success) and indirect changes (e.g. increased population densities, new species-interactions).

1.4.3 The lesser white-fronted goose

The lesser white-fronted goose is the most threatened of the Palearctic goose species and globally one of the most threatened bird species (Tucker and Heath 1994). The decline of the world population began in the first half of the 20th century and the current estimate is 25 000 individuals (Tolvanen *et al.* 1999). The formal conservation status of the species according to IUCN Red List of Threatened Animals in 2000 was defined as 'vulnerable' by criterion A1 (observed, estimated, inferred or suspected reduction of at least 20% over the last 10 years or three generations). According to BirdLife International the lesser white-fronted goose is classified as a species of European conservation concern SPEC category 1 'globally threatened' with a status of 'vulnerable'. In the EU Birds Directive the species is listed in Annex I (a subject of special conservation measures concerning their habitat to ensure their survival and reproduction in their area of distribution). In the Finnish, Swedish and Norwegian Red Data Lists the species is considered endangered as in most other relevant countries. The lesser white-fronted goose has no formal protection in Kazakhstan, whereas in China the protection status of this species is unclear due to confusion regarding its taxonomical status.

The breeding area of the lesser white-fronted goose extends from Fennoscandian Lapland to northeastern Siberia (Lorentsen *et al.* 1999). The former continuous breeding range has been fragmented into a few geographically distinct breeding areas. Four breeding concentrations are known: the Fennoscandian Lapland, Ural-Yamal, Taimyr and Indigirka (Lorentsen *et al.* 1999, Syroechkovsky 2000). In addition to Indigirka, there are probably other presently unknown breeding localities in the eastern distributional area. A migratory divide exists in Taimyr (Rogacheva 1992, Syroechkovsky 1996). Lesser white-fronted geese breeding in Taimyr and westwards over-winter in Greece and the border areas to Turkey and presently poorly known areas in the Black and Caspian Sea regions, whereas birds breeding eastwards from Taimyr over-winter mainly in central China (Lorentsen *et al.* 1999).

Reasons for a population decline in the lesser white-fronted goose are still somewhat obscure (Madsen 1996, Lorentsen *et al.* 1999). In the breeding areas in Fennoscandia, disturbance, habitat loss and predation by the increasing population of the red fox are likely to contribute to the decline. The breeding success in Fennoscandia is high and comparable to that in other known breeding areas (Aarvak *et al.* 1997) despite the small size of the population. This suggests that the main problems may lie outside the breeding areas. The most important factors causing the negative population trend are deterioration of the habitats and over-hunting in the staging and wintering areas. Adult mortality has been shown to have a more significant effect on the population trend compared to juvenile mortality (Lampila 2000). Therefore e.g. spring hunting in Russia and illegal poisoning of lesser white-fronted geese in winter and early spring in China are especially harmful for population recovery.

At the international level, conservation work directed at the lesser white-fronted goose is coordinated by the Lesser White-fronted Goose Task Force of Wetlands International and yearly updated Urgent Action Plan following the guidelines set in the International Action Plan for the lesser white-fronted goose (Madsen 1996). The main groups carrying out the research and conservation work are WWF Finland Lesser White-fronted Goose project, Lesser White-fronted Goose project of the Norwegian Ornithological Society, Goose and Swan Study Group of Eastern Europe and North Asia (RGG) and Japanese Association for Wild Geese Protection (JAWGP). Additionally, dozens of biologists, ornithologists and volunteers from European countries, Kazakhstan and China have co-operated and contributed to the conservation work.

One of the main aspects in conservation is to gain knowledge regarding a species' ecology. Census estimates (reviewed in Lorentsen *et al.* 1999), estimates of breeding success (Aarvak & Øien 2000) and mortality (Aarvak *et al.* 1997, Markkola *et al*. 2000), behavioural studies, such as time-budget and interactions with other species (T. Aarvak & IJ Øien, unpublished, J. Markkola *et al.*, unpublished), and research on feeding ecology (Aarvak *et al.* 1996, Niemelä 1998) have been mainly carried out in the western distributional area. Further, making firm conclusions is often difficult because studies are limited due to the restricted number of individuals present and, as with any migratory species, the use of many localities throughout the year. Therefore knowledge regarding e.g. breeding and wintering philopatry vs. dispersal and timing of pair-formation in the lesser white-fronted goose is limited.

The main problems facing lesser white-fronted goose conservation are related to the lack of knowledge regarding the breeding, staging and wintering areas, difficulties in accessing remote areas and the low number of (individually marked) individuals for observations. The use of satellite-telemetry for tracking individual movements has been of paramount importance in revealing the migratory flyways of lesser white-fronted geese breeding in Fennoscandia, Yamal and Taimyr (Fig. 3) (Karvonen & Markkola 1998, Lorentsen *et al.* 1998, Markkola & Arkiomaa 1998, Øien *et al.* 1999). Some previously unknown important staging areas, such as the Kanin Peninsula in northwestern Russia and Kustanay in northwestern Kazakhstan, were found with this method and conservation actions have been implemented accordingly (Prokosch 1997, Bragina 2000). Further information on the connections of breeding and non-breeding areas in western Europe have been clarified by identifying geese based on individual differences in belly patches and a few colour ring resightings (Øien *et al.* 1996, Aarvak *et al.* 1999, Aarvak *et al.*

2000). Still, it remains unknown to where lesser white-fronted geese continue from Kazakhstan for the winter.

Despite the main emphasis being on the protection and research of the wild populations, restocking or reintroduction of the lesser white-fronted goose has been carried out in Finland and Sweden. In Finland, captive-reared lesser white-fronted geese were released in the Finnish Lapland during the years 1989-1997 to increase the wild Fennoscandian population (Markkola *et al.* 1999) presently estimated at 30-50 breeding pairs (Lorentsen *et al.* 1999). Another objective was to gain information on the migratory routes, staging and wintering areas through resightings and ring-recoveries of the released birds carrying neck collars and leg-rings. The results indicated that the mortality of the released birds was very high, no breeding attempts were confirmed and the resightings suggested that the individuals joined flocks of bean goose rather than their conspecifics (Markkola *et al.* 1999). The Swedish wild sub-population was considered close to extinction and a reintroduction project was initiated in 1981 (von Essen 1999, Lorentsen *et al.* 1999). To diminish hunting pressure, the wintering area of the reintroduced lesser white-fronted geese has been changed to Western Europe by using barnacle geese (*Branta leucopsis*) as foster parents. Up to 1999, 348 individuals with captive origins have been released in Sweden, and approximately 50 individuals have been seen in the release area during the breeding season (von Essen 1999, von Essen *et al.* 2000). The number of successful breeding attempts exceeds twenty, but the population is neither self-sustaining nor expanding. The natural origins of the captive populations used for restocking and reintroductions are largely unknown. As a consequence of transferring individuals between different stocks, a common ‘practice’ in captive breeding to avoid inbreeding, the composition of the stocks in Finland, Sweden, Britain and central Europe is more or less the same (von Essen 1996).

2 Outlines of the study

Ecological knowledge regarding the lesser white-fronted goose has grown during the period 1996-1999 when part of the conservation work was funded by the LIFE Fund of the European Union. The primary aim of the present work has been to add a genetic perspective to the conservation plan. Genetic data offer insights not otherwise obvious.

At the species level the phylogenetic relationships of seven *Anser* goose species, including the lesser white-fronted goose, were studied (II). This provided a phylogenetic framework for understanding genetic diversity.

At the population level, the genetic structuring of the lesser white-fronted goose was studied (III). When this part of the project was planned, virtually nothing was known about the migratory flyways and staging/wintering areas of the lesser white-fronted goose, with the exception of some old ringing recoveries and observations (reviewed in Lorentsen *et al.* 1999). This information is still fragmentary, especially regarding wintering areas of the western population. The genetic study made it possible to estimate the amount of female gene flow among the breeding localities and the colonization history of the species. Additionally, this work contributes to basic knowledge of the phylogeography of Palearctic species.

For practical conservation purposes, the genetic composition of a captive lesser white-fronted goose stock was studied (IV) in light of the results from a wild population. According to the guidelines of the Re-introduction Specialist Group of the IUCN's Species Survival Commission, reintroduction and restocking should be carried out using individuals of the same type as the original wild population. This, together with the ambiguous history of the captive population and the results from population genetic structure of the wild population (III), was the motivation for studying the genetic background of the captive lesser white-fronted goose stock.

During this project, both the characteristics and limitations in the use of mtDNA as a genetic marker became obvious. Although the discussion regarding clonal inheritance and possible recombination of mtDNA continue to be debated, other characteristics of mtDNA need to be (re)considered. These include e.g. the applicability of the molecular clock and the variation in the mutation rate among different parts of mtDNA. In addition to sequences analysed in our group, further support was obtained by using sequences from GenBank. Therefore, in I mtDNA control region and cytochrome *b* sequences from altogether 68 avian species fetched from the GenBank were used to characterise mtDNA as a genetic marker.

3 Materials and methods

Brief outlines of the materials and methods will be provided here, more detailed descriptions can be found in the original papers.

3.1 The species and populations

For paper I, all complete, or nearly so, avian mtDNA control region sequences were fetched from the GenBank (Table 1 in I). Altogether, control region sequences from 68 species comprising 25 genera, 13 families and eight orders of avian fauna were used. For the species used for pairwise comparisons, also cytochrome *b* sequences were fetched, when available.

Altogether seven *Anser* goose species (Tribe Anserini) were included in paper II. The lesser snow goose (*Anser caerulescens caerulescens*) and the Ross' goose (*A. rossii*) are Nearctic species, and the white-fronted goose (*A. albifrons*) is almost circumpolar, whereas the lesser white-fronted goose *(A. erythropus*), the greylag goose (*A. anser*), the bean goose (*A. fabalis*) and the pink-footed goose (*A. brachyrhynchus*) are Palearctic species (Fig. 3). One to three individuals from each species were included in the study. Three of the species, the Ross', the lesser white-fronted and the pink-footed geese, are considered monotypic. For the snow goose, the nominate race (*A. caerulescens caerulescens*) and for the bean goose, the tundra bean goose (*A. fabalis rossicus*) were studied. Two of the total 4-5 races of the white-fronted goose (*A. albifrons albifrons*, *A. albifrons flavirostris*) and both races of the greylag goose (*A. anser anser, A. anser rubrirostris*) were included. Two of the species, the snow goose (numt sequence) and the Ross' goose (control region and numt sequence) and one of the races, the eastern greylag goose (control region and numt sequence) were represented by captive individuals.

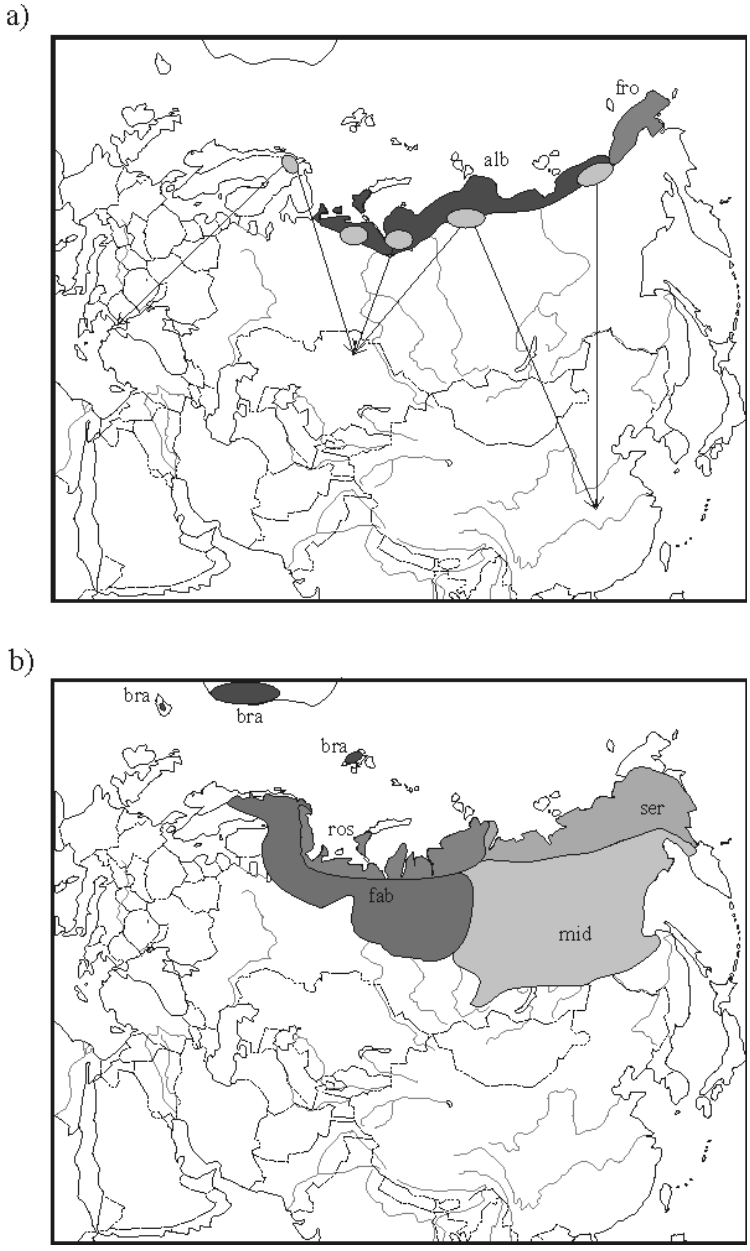

Fig. 3. Breeding distributions of a) the lesser white-fronted goose (circles), the white-fronted goose (alb = *Anser albifrons albifrons*, fro = *A. albifrons frontalis*), b) the pink-footed goose (bra = *Anser brachyrhynchus*), the bean goose (fab = *A. fabalis fabalis*, mid = *A. fabalis middendorffii*, ros = *A. fabalis serrirostris*, mid = *A. fabalis middendorffii*). The connections of the known lesser white-fronted goose breeding areas to the wintering areas in Greece and China and to a staging area in Kazakhstan are indicated by arrows.

In paper III, the population genetic structure of the lesser white-fronted goose was studied. All of the four known breeding areas of the lesser white-fronted goose in the western and central distributional areas were sampled for the study (Fennoscandia, Bolshezemelskaya Tundra, Yamal and Taimyr). Only one of the eastern breeding areas, Indigirka in Yakutia, is presently known. Because it was not possible to obtain samples of lesser white-fronted geese from the eastern breeding areas for the study, their wintering area in East Donting, China, was sampled. Additionally, material was collected from two non-breeding areas, Kazahkstan and Bulgaria. The material used consisted of blood, muscle and feathers collected during the years 1988-1999. To clarify the levels of genetic diversity of the lesser white-fronted goose prior to the population decline, feathers from museum skins from the years 1889-1945 were obtained in Finland and Norway. Only five out of the 20 feathers obtained were successfully analysed (listed in Table 1 in III).

There are tens of wildfowl farms raising captive stocks of the lesser white-fronted goose in Europe. The species has long been a favourite in captivity and the first record of an attempt to propagate the species in confinement is from the London Zoo in the 1850's (Delacour 1954). The natural origins of the captive stocks are unknown, but as a consequence of frequent exchange of individuals between the stocks, the genetic composition of the stocks is probably very similar. From one of the captive populations of the lesser white-fronted goose in Hailuoto, Finland, 15 out of 28 individuals present in the stock were sampled (IV).

3.2 Molecular methods

Total DNA was isolated from blood, muscle, liver or kidney with a standard phenol-chloroform extraction (Sambrook 1989) or from feathers with a Chelex-based method (Walsh *et al.* 1991).

The complete mitochondrial control region and the flanking areas (in total 1227 bp) were amplified and sequenced to study the phylogenetic relationships of the *Anser* species (II). To study the population genetic structure and phylogeography of the lesser white-fronted goose (III) and the genetic composition of the captive lesser white-fronted goose population (IV), the hypervariable 5' fragment of the control region was sequenced. In study II, this 221 bp fragment was shown to be the most variable part of the control region among geese, and it contains approximately half of the variable nucleotide positions in the complete control region. PCR was used to amplify the mitochondrial fragments with specific primers (primer sequences presented in the original papers, see also 3.3. for discussion regarding the specificity of primers).

PCR amplification products were purified from 1% agarose gel (Glenn & Glenn 1994) to remove excess primers and dNTPs. All sequencing was done using double-stranded PCR products directly as templates to detect possible heteroplasmy and to diminish the problems stemming from errors made by DNA polymerase during the PCR. At first, the sequencing was done manually (Bernatchez *et al.* 1992). Later on, sequencing was performed with the ABI 377 DNA Sequencer according to manufacturer's instructions.

3.3 Numts, nuclear copies of mitochondrial sequences

In recent years, a wealth of nuclear mitochondrial-like sequences (numts) in both vertebrates and invertebrates have been reported in the literature. Although found in many organisms, numts seem to be especially common in some avian taxa (*Anser*; Quinn 1992; *Aythyini*; Sorenson & Fleischer 1996). Numts present a challenge for phylogenetic and population genetic studies due to unwitting inclusion of paralogous nuclear sequences instead of mitochondrial sequences (Zhang & Hewitt 1996; Sorenson & Quinn 1998). Nuclear insertions are under different mutation constraints compared to the mitochondrial counterparts and therefore the nucleotide substitutions in nuclear copies occur more randomly. In non-coding areas, such as mitochondrial control region, the identification of a nuclear copy is more difficult due to a lack of codon structure compared to a coding area.

The first attempts to amplify the mitochondrial control region from *Anser* geese (II) resulted in amplification of a nuclear copy. Subsequently, mtDNA enriched isolates were used (Jones *et al.* 1988) as templates, an approximately 10 kb mitochondrial fragment spanning from cytochrome oxidase I to tRNAphe was amplified and comparison of sequences obtained by using different tissues (with differing ratio of nuclear DNA to mtDNA) as templates as well as comparison of sequences obtained with different primer pairs were used to assure the mitochondrial origin of the sequences. The mtDNA specific primers were designed by comparing the sequences of the nuclear copy and the mitochondrial DNA.

3.4 Sequence analysis

The sequences were aligned using programs PILEUP and LINEUP in Genetic Computer Group Program Package (available in CSC) and ClustalW (Thompson *et al.* 1994), when needed. All alignments were checked and edited manually. The pairwise genetic distances were estimated using Kimura's two-parameter method (Kimura 1980), HKY85 (Hasekawa *et al.* 1985), F81 (Felsenstein 1981), TAMNEI (Tamura & Nei 1993), TVM (Rodriquez *et al.* 1990) with or without gamma distribution parameter. In I, Modeltest program (Posada & Crandall 1998) was used to choose the model of sequence evolution that best fits the data. Neighbor-joining method in MEGA (Saitou & Nei 1987, Kumar *et al.* 1993), maximum parsimony method in PAUP3.1 (Swofford 1993), maximum likelihood method in PAUP*4.0b6 (Swofford 1998) and maximum likelihood method in fastDNAml (Olsen *et al.* 1994) were used for inferring the phylogenetic relationships of the taxa or mtDNA haplotypes.

At the intraspecific level, haplotype ($\hat{h}$) and nucleotide (π) diversity were calculated as in Nei (1987, eqs. 8.5 and 10.5, respectively). The amount of genetic differentiation among the localities was estimated by F_{ST} (based on haplotype frequencies) and ϕ_{ST} (incorporating sequence variation) using AMOVA and their significance was tested with a randomisation procedure in AMOVA (Excoffier *et al.* 1992). The amount of female gene flow was estimated based on population pairwise F_{ST} values using the equation $N_f m = 1/2(1/F_{ST}-1)$ (Nei 1987, eq. 13.25).

4 Results and discussion

4.1 Evolution of the avian mitochondrial control region

In I, the sequence characteristics and applicability of one of the most commonly employed regions of mitochondrial DNA, the noncoding control region, were examined. The conserved sequence blocks in the control region have been determined using alignments of relatively closely related taxa (e.g. Brown *et al.* 1986, Taylor *et al.* 1993, Randi & Lucchini 1998), which may over-emphasise the degree of sequence conservation. Athough there is no criteria to determine a sequence block, a background assumption is that the degree of conservation potentially indicates functional significance in the regulation of replication or transcription of mtDNA. In that case, sequence conservation should be universal and extend across e.g. vertebrate classes, unless for example nuclear-mitochondrial co-evolutionary processes are involved (e.g. Sbisà *et al.* 1997). For this reason, in I the comparison of conserved sequence boxes determined for avian sequences were extended to mammalian, fish and amphibian sequences. Although e.g. Southern *et al.* (1988) localised nine conserved sequence blocks in mammalian control region sequences, only two of them with a considerable amount of sequence conservation were found when avian and mammalian sequences were compared. The D-box, for which no specific function has been suggested, in the domain II, consists of 25 nucleotides of which 10 are conserved in the avian-mammalian sequence alignment (Fig. 2b in I). CSB-1 is situated in the 5' end of the domain III and is believed to be involved in the regulation of transcription and replication of mtDNA (Ghivizzani *et al.* 1994). When avian and mammalian CSB-1 sequences were aligned (Fig. 2d in I), an insertion of 14-21 bp was found in *Struthio* and *Rhea* (Palaeognathae) and in Passeriformes (Neognathae), but not in other avian sequences or in mammals. This interruption, and the fact that the conserved nucleotides among mammals and avian species are concentrated to the 3' end of the block, suggest that CSB-1 is shorter than usually considered (e.g. Baker and Marshall 1997). With these new boundaries for the CSB-1, nine out of the 22 nucleotide positions were conserved among mammalian and avian sequences.

On the other hand, some studies have analysed relatively distantly related species-pairs to characterise the patterns of variability, up to the point when saturation is present and the sequence alignment becomes ambiguous due to difficulties in determining

homologous nucleotide positions (e.g. Baker & Marshall 1997). Therefore, the alignments of the complete control region sequences were always done within each genus. Both closely and distantly related species and genera were included. Within genera, the genetic distances varied from 0 to 37.88 % among the species-pairs, whereas the mean genetic distances of the 12 avian genera studied ranged from 0.54% in *Camarhynchus* (Passeriformes, Fringillidae) to 26.24% in *Phylloscopus* (Passeriformes, Sylviidae). The saturation, as detected with ts/tv ratio, became pronounced at approximately 10% divergence in the control region. Similarly, the saturation has been reported to occur at 10-13% divergences in the third codon positions of the mitochondrial coding genes (Hackett 1996, Griffiths 1997, Johnson & Sorenson 1998).

Commonly, only a fraction of the control region is sequenced in population studies and, to improve resolution, the target is the most variable region. However, the distribution of the variable sites within the control region varies among the genera (Fig. 1 in I). In most of the genera, variation was concentrated in domain I (*Alectoris*, *Anser*, *Cepphus*, *Cyanoramphus*, *Geospiza*, *Grus*), whereas in some genera, domain III was the most variable (*Camarhynchus*, *Cyanocorax*, *Parus*). It has been suggested earlier that the distribution of the variation may depend on the level of divergence (Baker & Marshall 1997), but no strong support for this was found. The divergences of the genera were overlapping being 0.56-10.89% for the genera showing most of variation in domain I and 0.54-17.30% for genera in which domain III was the most variable of the domains. In other genera the variation was distributed almost evenly to the whole control region. Two of the genera in this group were among the most diverged ones showing ts/tv ratios of 1.63 for *Phylloscopus* and 1.33 for *Emberiza* suggesting that saturation affects the patterns observed. In the genus *Polioptila*, both 5' and 3' variable species-pairs exist. Within the species, the pattern of variability among the domains was generally congruent with the pattern observed within genera, as has been suggested earlier (Lee *et al.* 1995).

Based on earlier studies, it has become a general assumption that the mitochondrial control region is the most variable region of the mtDNA (e.g. Walberg & Clayton 1981, Aquadro & Greenberg 1983, Cann *et al.* 1984, Chang & Clayton 1985, Saccone *et al.* 1993) and evolves at a ten-fold rate compared to the mtDNA cytochrome *b* gene (Greenberg *et al.* 1983). Furthermore, because the control region and the cytochrome *b* gene are parts of the same molecule, a (more or less linear) correlation in the levels of divergence of the two regions could be assumed. To test this, the divergence in control region sequences versus cytochrome *b* divergence was examined from pairwise comparisons of the species. Surprisingly, the ratio of the control region distance vs. cytochrome *b* distance ranged from 0.13 to 21.65 (Fig. 4 in I) and the ratio did not depend on the level of divergence. In some genera the ratio was always more than one (e.g. 5.14-21.65 in *Cyanoramphus*), whereas in others it was always less than one (e.g. 0.46-0.94 in *Alectoris*) suggesting a genus-specific trend. Recently, the mutation rate of the control region was also reported to vary between human lineages, whereas the coding regions were shown to evolve in an approximately constant rate (Ingman *et al*. 2000).

The results of the present study have practical implications both for planning sequence studies and for interpreting results. Taken together, the control region is not necessarily the first choice in searching for the most sensitive marker of the mtDNA, but also the levels of divergence among the taxa and among the domains of the control region may not be comparable. Therefore, the molecular clock should be calibrated separately for e.g.

each genus by using external evidence. Up to now, the molecular clock estimate of 20.8%/million years between the sequences for domain I (snow goose; Quinn 1992), 14.8% for domains I and II (dunlin; Wenink *et al.* 1996), the 'conservative' 2% originally estimated for the whole mtDNA (Brown *et al.* 1982), or one of their derivatives have been commonly applied in avian studies to date divergence events.

4.2 MtDNA phylogeny of the *Anser* geese

Geese (Tribe Anserini) are divided into two genera, *Anser* (11 species) and *Branta* (5 species), and they are considered evolutionarily young. Based on differences in morphology, mainly in osteological characters, it has not been possible to confirm even the monophyletic status of the goose genera (Livezey 1986). Based on the divergence in mtDNA and paleontological evidence, the divergence time for the two genera is suggested to be 5 My (Shields & Wilson 1987). Previously, allozyme studies have shown that species within *Anser* are extremely closely related: no fixed allele differences have been found among species and the minor variation detected is at the individual level (Baker & Hanson 1966, Patton & Avise 1986, Kuznetsov 1995). The phylogenetic relationships of seven *Anser* species were studied in paper II. Considering their extremely close relationships, a sensitive marker was needed to clarify the phylogenetic relationships of the species and thus the complete mitochondrial control region sequence (approximately 1230 bp) was chosen for the study. In the absence of suitable outgroup species (even *Branta* sequences were too variable to be aligned unambiguously), a nuclear mitochondrial-like sequence common for all the species studied was used as an outgroup in the mtDNA tree.

Among the *Anser* species, genetic distances ranged from 0.9 to 5.5% in the complete mitochondrial control region sequences. The sequence divergence among *Anser* is at the lower end of the scale reported for other avian genera (Fig. 4). The genetic distances among the mtDNA of three species, the lesser white-fronted, the bean and the pink-footed goose, are of equal magnitude (range 0.9-1.1%) and the divergence of the mtDNA clades found within both the lesser white-fronted and the white-fronted goose are similar (0.8 and 1.1%, respectively). This suggests that the geese have undergone a period of rapid cladogenesis leading to speciation events and/or divergence of mtDNA within the species.

Traditionally, morphological similarity has been considered a basis for determining phylogenetic relationships among goose species (e.g. Delacour 1954). The lesser white-fronted and the white-fronted goose have been considered close relatives based mainly on a few synapomorphic characters, such as the white frontal patch and dark belly patches, and one allozyme locus among the otherwise inconclusive loci among *Anser* species supports this view (Baker & Hanson 1966). The pink-footed goose was earlier considered a subspecies of the bean goose (e.g. Delacour 1954). The mtDNA tree, however, does not support this view: the lesser white-fronted goose mtDNA lineages group rather with the bean and the pink-footed goose, although the bootstrap support for this is moderate (maximum parsimony 81%, maximum likelihood 67%, Fig. 2 in II). The internal branches are short and no structuring among the species within this group is evident. Whether this incongruence is due to convergence in morphology, historical hybridization and introgression or ancestral polymorphism and lineage sorting in mtDNA remains to be clarified.

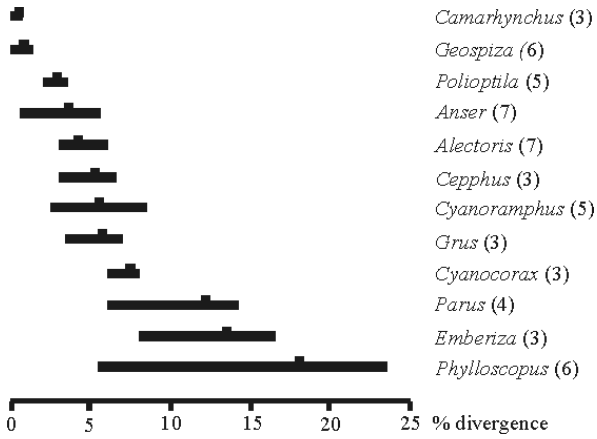

Fig. 4. Levels of mtDNA divergence among the complete avian control region sequences. The mean distance value and the range in pairwise comparisons within the genera are indicated. The number of species is shown in parenthesis, for a list of the species see Table 1 in I.

4.3 Ice ages and *Anser* fossil data

Additional information regarding the divergence of *Anser* species and the distribution of species before the present breeding areas were colonised during the Holocene was obtained from the fossil record. After the relatively warm climatic conditions of the Pliocene, many species went extinct or declined in numbers due to cooling climate in the Pleistocene (ca. 2.5-0.01 Mya). During the glacials, the ice sheet generally covered most of Fennoscandia and the British Isles reaching 52° N during the Weichselian glacial maximum. The climate fluctuated between cold glacials and warm interglacials, but also within these periods (cool stadials and mild interstadials). In The Netherlands for example mean summer temperatures varied from +2 to +20°C during the Pleistocene (Andersen & Borns 1997). In the eastern Palearctic, the extent of glaciers was more restricted and scattered, but known in lesser detail compared to Europe (Alekseev 1997, Siegert *et al.* 1999, Svendsen *et al.* 1999). The existence of large glaciers in northern Russia (e.g. in Taimyr Peninsula) and the Tibetan Plateau have been a matter of debate, but presently most scientists agree that they did not exist during the Pleistocene. The growth and melting of glaciers caused changes in the sea level and in general, sea level was lower than today. Therefore, islands and even continents were connected by land bridges, such as Beringia.

During the ice ages, species shifted their distributional ranges according to the prevailing climate and vegetation, but some species responded to periods of milder climate by expanding northwards without leaving the refugia (Blondel & Mourer-Chauviré 1998) or by moving into high altitudes (Danell *et al.* 1999). Also, the present patterns of migratory behaviour in northern species have been created during the ice ages and shaped after the glacial retreat (Alerstam 1990). Even if some of the fossils of *Anser* have been dated, the localities in which the fossils have been found may represent either

breeding, staging or wintering areas for the species. Information on the reconstructed vegetation and species composition (e.g. sedentary species with a present narrow optimal habitat) may be used to determine which period of the annual cycle is represented, but the species assemblages themselves are not necessarily similar to the present ones (Webb & Bartlein 1992).

As discussed also by Tyrberg (1998), the fossil record is biased by numerous factors. Avian fossils are rather common, the distribution of the records is uneven and undoubtedly an artefact related to the differences in activity of investigation and in accessing this information. In Tyrberg's (1998) catalogue more than one thousand localities with fossils are noted for Europe whereas only approximately one hundred locations are included for Russia and China together. Such bias poses a problem when using fossil data to determine and calibrate divergence times of Palearctic avian species. The first appearance in the fossil record does not necessarily correlate with the divergence time of the species, but instead it can indicate a shift in the species distributional area postdating the speciation event.

The dating of fossils often poses difficulties and uncertainties, but fortunately many deposits have been radiocarbon dated or can be associated with human cultural periods through other coexisting artefacts. As an example, the Natufian, the dominant Epipaleolithic culture that existed east of the Mediterranean Sea, dates within 12 000-10 000 BP, i.e. the last millennia of the late Pleistocene.

Some conservative conclusions about the historical phylogeography of *Anser* during the last ice ages were made based on fossil findings. During the early and the middle Pleistocene, the first records of the white-fronted, the lesser white-fronted, the bean and the pink-footed goose appear in Europe and date to approximately 1.3 Mya for the white-fronted goose, 0.5 Mya for the lesser white-fronted and the bean goose, and 0.4 Mya for the pink-footed goose (Tyrberg 1998). As discussed above, these dates can be considered as minimum estimates for the divergence of the species. The mean divergence estimate based on mtDNA (II) for the lesser white-fronted, the bean and the pink-footed goose is 1.02%, and the estimated divergence of the white-fronted goose from the previously mentioned species is 1.86%. This would give a molecular clock estimate of 1.4-2.5% between the two lineages in million years for the whole control region sequence of *Anser*. However, if the appearance in the fossil record does not correlate with speciation, the species determination is incorrect or if hybridisation is involved, the molecular clock estimates may be incorrect.

During the late Pleistocene (130 000-10 000 BP), a total of 91 fossils of the lesser white-fronted, the white-fronted, the bean and the pink-footed goose in 57 localities have been reported (Tyrberg 1998). All fossils for which the species determination was uncertain (either two probable species suggested or 'affinity' to a species was suggested) were excluded. Out of these, 22 fossils were radiocarbon dated and an additional 37 fossils were associated with relatively short stratigraphic zones or cultural periods which allowed for placing the fossil into one of the isotope stages and sometimes to a more defined period within an isotope stage. The most abundant fossil record of *Anser* geese is found during the last two isotope stages, IS3 (60 000-24 000 BP) and IS2 (24 000-10 000 BP) of the late Pleistocene and, IS1 of the Holocene (10 000 BP-present) and therefore these stages were selected for a more detailed inspection. This period is also the most

interesting for trying to understand the population histories before the colonization of the present breeding areas of *Anser* geese.

In general, the fossil locations from IS1 through IS3 (Fig. 5) are places where *Anser* species are presently observed at least occasionally during the non-breeding season. However, because species identification based on a few bones is uncertain (Barnes *et al.* 2000) species determination could also be biased by their present distribution. The pink-footed goose is found only in Western Europe with the exception of one fossil location found in Italy during Dryas III, the last cold period before the onset of Holocene, which was harsh especially in the Western Europe. Comparatively, lesser white-fronted goose fossils are not found in Western Europe, which is in agreement with their present distribution in Europe during the non-breeding season. The white-fronted and the bean goose are the most abundantly represented *Anser* species in the fossil record and also the most widely distributed.

It is likely that some of the goose fossil localities in northern Europe during milder periods and in southern Europe down to the Mediterranean during colder periods of IS2 and 3 were breeding areas. These were also distributional areas for other arctic species like mammoth, wooly rhinoceros, muskox and reindeer as well as other arctic avian species such as the extinct great auk (*Pinguinus impennis*) and the snowy owl (*Nyctea scandiaca*) (Mourer-Chauviré 1993). During the last glacial maximum (18 000-20 000 BP), goose fossils are found in Southern France and northern-central Italy as well as in Egypt (Fig. 5). Based on vegetation and climatic reconstructions (Peyron *et al.* 1998), areas north of the Mediterranean were covered by a non-analogue ice age vegetation, a cool steppic vegetation with some evidence of tundra scrubs in France and forest stands in the Mediterranean regions and as far north as Hungary (Willis *et al.* 2000). The temperatures of the coldest month were approximately -20ºC indicating that these were probable breeding areas, if anything. The areas in northern Africa south from the Mediterranean were covered by temperate desert type of vegetation suggesting that the fossil locality in Egypt could have been a wintering locality. The fossil record does not support Ploeger's (1968) suggestion that the nominate race of the bean goose would have been breeding in central Spain and the Greenland white-fronted goose (*A. albifrons flavirostris*) as well as the pink-footed goose would have been breeding in the North Sea area during the last glacial maximum.

The fossil record for the geese is remarkably sparse (four species, 57 localities) compared to that of some other northern species, such as the ptarmigan (*Lagopus mutus*, ca. 240 localities) and the snowy owl (*Nyctea scandiaca*, ca. 110 localities). Considering the scarcity of *Anser* in the fossil record it seems possible that either the main breeding areas of the *Anser* species were outside Europe during the Pleistocene or that the habitats used by the geese may not have been favourable for the preservation of bones. Because sea level was much lower than today, some of places could be presently covered by the sea.

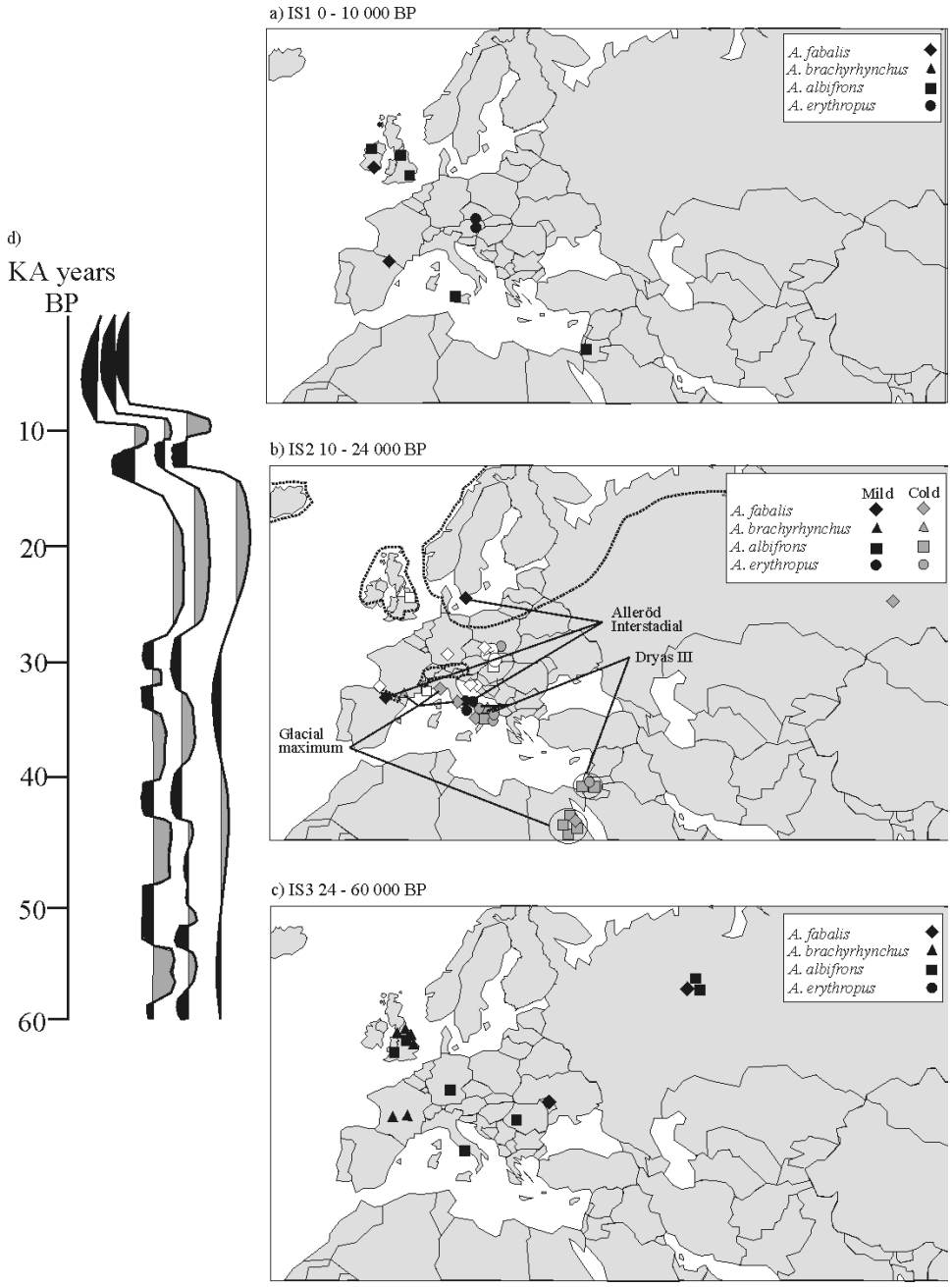

Fig. 5. Localities for fossils of four *Anser* goose species during a) the Holocene, b) Isotope stage 2, and c) Isotope stage 3. In d) climatic fluctuations during the last 60 000 years are shown for northern France (left), Holland-northern Germany (centre) and Fennoscandia (right) (modified from Andersen & Borns 1997). Black, warm or relatively warm climate; grey, cold or relatively cold climate.

4.4 Intraspecific diversity of the lesser white-fronted goose

The intraspecific diversity of the lesser white-fronted goose was studied by using the hypervariable fragment of the mitochondrial control region (III). This 221 bp region was shown to contain approximately half of the variable sites in the complete control region sequences of the goose species (nucleotides 189-410, Fig. 1 in II). The lesser white-fronted goose sequences defined two mitochondrial lineages (W and E) with a mean divergence of 2.0%. In principle, mtDNA lineages could have been maintained in a single panmictic population with a very large historical population size, but it is probable that all the northern species have encountered population bottlenecks during the ice ages. Therefore, the most commonly advocated explanation in intraspecific mtDNA divergences is a vicariant origin, i.e. the lineages have diverged in separate refugia.

Fig. 6 presents the intraspecific mtDNA trees among all goose species studied to date (II, III, IV, Quinn 1992, Pierson *et al.* 2000, Scribner *et al.* unpublished). For all studies, approximately the same fragment of control region has been sequenced and therefore divergences are roughly comparable, under the assumption that the rates of divergence are the same across species. In all the studies more than one major mtDNA lineage has been found. Among major lineages of the lesser white-fronted and the white-fronted goose, the divergence is shallow when compared to the Canada and snow goose, but approximates the divergence of 2.3% of two mtDNA lineages found within the snow goose clade II (Quinn 1992). However, the grouping of the snow goose haplotypes in the neighbor-joining tree in Fig. 6b is not identical to the parsimony tree presented in the original paper (Fig. 5 in Quinn 1992, based on one synapomorphic nucleotide position).

The pattern for the lesser white-fronted goose mtDNA divergence and the geographic localisation of haplotypes corresponds mainly to phylogeographic category V as defined by Avise *et al.* (1987). The basal haplotypes W1 and E1 are the most common of the haplotypes (W1 38%, E1 32%) and geographically widespread (Fig. 1 in III), whereas the rare haplotypes (W2-8, E2-6) are private, i.e. found in a singular population each. This kind of pattern suggests low or modest contemporary gene flow between populations that have a recent evolutionary connection (Avise 2000). The rare haplotypes were separated by one or two substitutions from the main haplotypes indicating a recent bottlenecking of the populations.

Genetic structuring among the localities was significant (F_{ST}=0.136, P=0.000 and ϕ_{ST}=0.234, P=0.000). The estimates of gene flow based on pairwise population F_{ST} values ranged from 0.6 to 13.8 females per generation suggesting high levels of gene flow among some of the populations. F-statistics do not distinguish between contemporary and historical gene flow (discussed in e.g. Templeton *et al.* 1995), but the latter was favoured on three grounds. First, although no divergence time was estimated based on a molecular clock, the divergence of the major mtDNA lineages predates the colonisation of the present breeding areas within the last 10 000 years and therefore it seems probable that there have been opportunities for mixing of the mtDNA ineages during the late Pleistocene. Second, the frequencies of the W and E lineages differ between the western and eastern distributional areas (western area: 0.70 W and 0.30 E, eastern area: 0.28 W and 0.72 E).

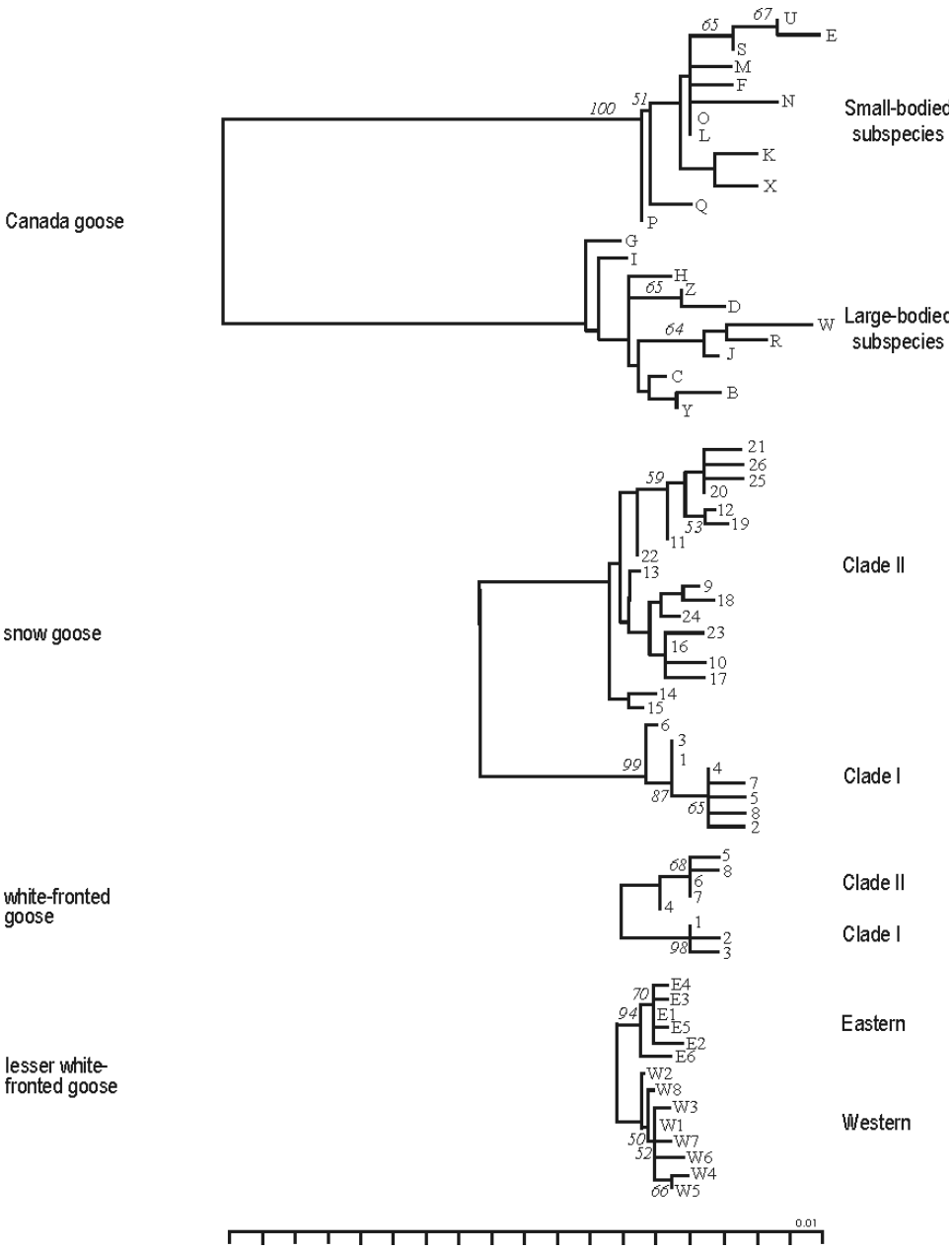

Fig. 6. Comparison of mtDNA divergences within the goose species. The trees are constructed with the neighbor-joining method using the most variable part of the control region (Canada goose 143 bp, accession numbers AF175474-AF175497, Scribner *et al.*, unpublished; snow goose 178bp, Quinn 1992; white-fronted goose 221 bp, IV; lesser white-fronted goose 221 bp, III).

Although the eastern part of the species distributional area is sparsely sampled, it is probable that the change in the frequencies coincides approximately with the migratory divide in Taimyr, but that part of the l breeding population that used to migrate eastwards is presently extinct (Syroechkovsky 1996). The wintering areas of the western subpopulation in eastern Europe and Black Sea-Caspian Sea region and the eastern subpopulation in China are also separate. Together these two facts suggest that dispersal and gene flow between the two subpopulations does not presently take place. However, the pair-formation in the lesser white-fronted goose occurs in spring-summer (Aarvak & Øien, unpublished data) and therefore, Taimyr could have been a dispersal corridor for the western and eastern subpopulations previously. Third, the existence of private haplotypes in all the populations studied suggests that female gene flow is restricted. For reasons mentioned above, the existence of the two mtDNA lineages in the lesser white-fronted goose could be best regarded as a polymorphism within the species because the lineages are no longer strictly ordered geographically. Comparatively, private alleles may have diverged *in situ*.

The Fennoscandian subpopulation is the most endangered of the breeding populations and it also shows the lowest levels of mtDNA variability with a haplotype diversity $\hat{h}$ of 0.342 and a nucleotide diversity π of 0.0065 (n= 16) compared to average values of $\hat{h}$ 0.669 and π 0.0118 (n=37) for other breeding populations. Such low variability is attributed to colonisation history rather than small population size. Five out of 16 individuals studied from Fennoscandia were collected from museums and represent the population when it still numbered thousands of individuals, less than a century ago. Museum samples suggest that the present low level of mtDNA diversity is not a consequence of reduction in variability in a small population: all pre-bottleck individuals are of the same haplotype that is the most common one in Fennoscandia presently. Other studies have shown that marginal or northern populations are often less variable than the central or southern populations due to founder events during colonisation (e.g. Cwynar & MacDonald 1987, Hewitt 1996, Merilä *et al.* 1996). The estimates of gene flow between Fennoscandia and other breeding localities were also the lowest observed in the study, ranging from 0.6 to 2.2 females per generation. It is known that approximately half of the Fennoscandian lesser white-fronted geese use the same staging areas during the autumn as the individuals breeding in Yamal and Taimyr (Karvonen & Markkola 1998, Lorentsen *et al.* 1999, Øien *et al.* 1999). Because the final wintering areas are not known and the spring migration routes are not necessarily the same as in autumn, it is difficult to distinguish between possibilities for birds with different breeding origins to mix, and the possible effect of female philopatry in maintaining population structuring. However, it is known that at least some of the Fennoscandian individuals are spatially segregated during most of the spring migration, and, additionally a bimodal distribution exists in arrival times (Aarvak *et al.* 2000).

4.5 Genetic background of captive lesser white-fronted goose stock

One of the three captive lesser white-fronted goose stocks used for reintroduction/ restocking in Finland and Sweden was sampled to study the genetic background of the

captive lesser white-fronted geese (IV). The founders of the Finnish captive lesser white-fronted goose stock came from a Swedish farm, although some individuals were subsequently transported directly from Central Europe (Markkola *et al.* 1999). The founders of the Swedish stock originated from wildfowl farms in Central Europe (von Essen 1996) and there has been a regular exchange of individuals between different farms. Therefore, the composition of all the present stocks is similar. Of the 15 captive individuals analysed, three were of the same mitochondrial haplotype (W1) as 81% of the wild Fennoscandian breeders and eight captive individuals were of the eastern main haplotype (E1) (see also 4.4). However, the remaining four captive individuals were shown to carry a haplotype, which is 0.45% (1 nucleotide substitution) different from a haplotype found in another goose species, the white-fronted goose. Two possible explanations exist for the occurrence of the white-fronted goose type mtDNA in the captive stock of lesser white-fronted goose. First, white-fronted goose type mtDNA could be present also in the wild lesser white-fronted goose either due to a recent divergence of the species or as a consequence of hybridization under natural conditions. However, as this was not detected in the natural populations (II, III) it can be concluded that hybridization is not very common (at least between female white-fronted and male lesser white-fronted geese) or, if it occurs, it does not lead to introgression of the mtDNA (i.e. restricted to first-generation hybrids in natural conditions). Therefore, a more plausible explanation is that hybridization has occurred during the history of lesser white-fronted goose captive breeding.

The existence of the mtDNA of the white-fronted goose in the captive stock of the lesser white-fronted goose indicates that hybridization has taken place, but does not reveal the degree of introgression. In the absence of pedigree records and other documentation it is impossible to estimate the composition of their nuclear genomes. Because females are the heterogametic sex (ZW) in avian species, it can be reasoned that all captive females carrying the mtDNA of the white-fronted goose also have the W chromosome of the white-fronted goose. On the other hand, any individual in the stock may carry heterospecific nuclear alleles (irrespective of the mtDNA type), because mtDNA and nuclear alleles have different transmission pathways. To assess further the proportion of heterospecific alleles in the stock, the stock should be also analysed with species-specific nuclear markers.

According to the guidelines of the Re-introduction Specialist Group of the IUCN's Species Survival Commission (Kleiman *et al.* 1994, IUCN 1995), the stock used for reintroduction should be similar to the original wild stock to enhance the possibilities of survival and, on the other hand, to prevent the introduction of alien genes to wild populations and possible outbreeding depression. Because the Swedish reintroduction area in Svaipa, Swedish Lapland, is situated within a dispersal range from the breeding areas of the wild lesser white-fronted goose in Norwegian and Finnish Lapland, it would be wise to apply a precautionary principle to avoid possible harmful effects to the remaining wild breeding population. Some individuals released in Sweden have been observed in Finland and Norway, and the possibility of mixing of the wild and released birds with a captive origin is a realistic threat.

In addition to the probable genetic incompatibility of the captive stock, other unresolved obstacles exist for the use of reintroduction or restocking as a successful conservation measure. One of the preconditions for a species reintroduction is that the

original causes of extinction or population decline have been removed (Kleiman *et al.* 1994, IUCN 1995). Despite attempts to protect key-areas and irrespective of the protected status of the lesser white-fronted goose in most countries, the species continues to be hunted. In the Swedish reintroduction project the hunting pressure has been diminished by changing the migration routes to safer areas by using semi-captive barnacle geese as foster parents. Unfortunately, the use of heterospecific foster-parents has led to imperfect imprinting and mixed identity of the goslings, which in turn has led in attempts to pair and hybridise with other goose species (von Essen 1999). During the Finnish restocking project the most prominent problem was that the released individuals joined flocks of bean geese (in the absence of learned migration routes) and were not imprinted to the release area. As a consequence, the resightings were scattered to various wintering localities (within and outside the natural range) and very few individuals returned to the release area (Markkola *et al.* 1999). With regards to the protection of wild populations, the problems in the reintroduction/restocking attempts much centre on the migratory programme of the species. Similarly, it has been shown in other species introductions that the migratory behaviour is the only life-history trait that correlates negatively with introduction success (Veltman *et al.* 1996). The experiences within the lesser white-fronted goose project suggest that even if an appropriate captive stock exists for reintroduction/restocking, the means for carrying out such conservation efforts are presently unavailable.

5 Concluding remarks

The characterisation of the mtDNA control region in avian species (I) showed that some of the common presumptions about the evolution of the control region are not universally applicable. Some of these points have already been suggested or concluded for singular genera. Most importantly, the variation in the relative rates of divergence among the control region and the cytochrome *b* gene suggest that the molecular clock estimates obtained for one pair of taxa may not have broad applicability, that a cross-calibration of the molecular clock of these two regions may be incorrect and that the control region is not always the most rapidly evolving region of mtDNA.

The phylogenetic relationships of seven *Anser* goose species were studied by using complete control region sequences of approximately 1230 bp in length (II). Genetic distances among species were small and also when compared to other avian genera. The topology of the phylogenetic tree was well supported for the basal taxa, but among the four most closely related species, the white-fronted, the lesser white-fronted, the bean and the pink-footed goose, the branching order was less clear. To clarify the phylogeny further, and to determine to what extent the morphological traits correspond with the phylogenetic relationships, a study using nuclear molecular markers would be useful. However, the variation found in allozymes has not been phylogenetically informative and because the rate of evolution in most of the nuclear genes is slower compared to mtDNA, it would not be easy to find a suitable marker for the study.

In the study of the lesser white-fronted goose (III) it was shown that the populations are genetically structured as detected with statistically significant differences in mtDNA haplotype frequencies among the populations. However, because the two main haplotypes were found in a majority of the individuals and the private haplotypes were rare (found from one to four individuals each) it may be possible that the rare haplotypes could be found outside the populations they now exist in if more samples had been studied and if contemporary gene flow takes place. On the other hand, if a larger fragment of the control region had been sequenced, it may have been possible to obtain more phylogenetic resolution and haplotypes. For example one of the individuals carrying haplotype W1 was observed to have a small deletion outside the fragment sequenced from other individuals. These results have practical implications from a species conservation perspective. The Fennoscandian population showed the lowest levels of variation among the breeding

populations and low amount of female gene flow estimated from population pairwise F_{ST} values. This suggests that the Fennoscandian population could be considered a management unit as defined by Moritz (1994). This places more emphasis on the conservation of the Fennoscandian individuals. Because the main problems in species conservation are outside the breeding areas, more effort should be focused to important staging areas, such as Kazakhstan, and to presently unknown wintering areas in the Black and Caspian Sea regions.

In the captive stock of the lesser white-fronted goose some individuals were shown to carry the mtDNA of the white-fronted goose (IV). This only indicates that a hybridisation event has taken place, but because the history of the stock is unknown it is not possible to draw more specific conclusions. However, the results from the wild populations of the lesser white-fronted goose suggest that hybridisation between species is not common or does not lead to introgression in the wild, and therefore the possibility of hybridisation during the captive propagation was favoured. To further evaluate the severity of the problem, the amount of introgression in the nuclear genes of the captive individuals should be determined. In addition to the possible incompatibility of the captive stock for reintroduction or restocking purposes, other unresolved problems exist. For example, and not in the least, the need to increase the size or genetic diversity of the wild population has not been indicated and there are no signs of inbreeding depression in the wild population.

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