Genetic variation and structure in Norwegian red deer

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Thesis for the degree Philosophiae Doctor

Norwegian School of Veterinary Science

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Oslo July 2008

Series of dissertations submitted to the
Norwegian School of Veterinary Science
No. 46

ISSN 1890-0364
ISBN 978-82-7725-161-5

Cover: Unipub AS
Printed in Norway: AiT e-dit AS, Oslo, 2008.

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Acknowledgements

This work was performed at the Department of Environmental and Health Studies, University College of Telemark (HiT; 2001 – 2005) and at Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Sciences (NVH; 2001 – 2008). It was financed by the Norwegian Ministry of Education and Research. A separate scholarship was granted by Lise og Arnfinn Hejes fond, which was most welcome at the time.

I am grateful to my supervisor's professor Olav Rosef (HiT) and professor Knut Røed (NVH) for the opportunity to carry out this doctor degree, and for their help and supervision during my work and writing. For a great introduction to and help in the laboratory I am in debt to Ing. Liv Midthjell at NVH.

I thank Frode Bergan, Hege-Ann Skaret and Monica Solevaagseide for field work assistance, and the red deer farms Dagsrud Hjorteoppdrett and Norsk Hjortesenter (Svanøy) for providing the family material. For samples of wild deer I am much obliged to the Section for Wildlife Diseases at National Veterinary Institute (NVI), Dr. Jon Arnemo, Harald Holm, M Pearson, Halvor Ovastrøm, Oddegeir Hårstad, Ander Børsstad, and the many participating Norwegian hunters, Per Larrson, Göran Anderson, Dr. Aligmantas Paulauskas at Vytautas Magnus University in Lithuania, Dr. László Szemethy at St.Stephen University and Professor Habil Lászlo Sugar at University of Kaposvar in Hungary, for handling samples Turid Vikøren, Astrid Stovner and Liv Midthjell, and for information about Swedish red deer Björn Molitor.

For helpful assistance and providing computational power I thank Kjetil Olsen and the IT group at NVH. I wish to thank Dr. Øystein Flagstad (NINA) and Professor Atle Mysterud (UiO) for helpful comments and discussions during compilation of the manuscripts, Professor Howard Parker (HiT) and Dr. Håkon Hansen for proof-reading and. Dr. Øivind Øines and Dr. Anders Jørgensen (NVI) for both phylogenetic discussions and proof-reading.

Finally, I would like to thank family and friends for being patient during heavy work loads and through strenuous times, their support was really necessary to be able to fulfil this thesis.

Thank you all.

'*Endurance is one of the most difficult disciplines, but it is to the one who endures that the final victory comes*'

- Buddha (alias Hindu Prince Siddharta, 563-483 B. C.)

Contents

Summary

In all species the abundance and distribution of populations vary through time and space, but recently major changes in the size and range of many populations have been observed. Population contractions and founder events during spatial expansion may involve differentiation of or even loss of genetic variation, which is important for the sustainability of species. This thesis addresses the genetic variation of microsatellite and mitochondrial DNA in a cervid population that is presently spatially expanding but that has been exposed to previous population reductions and translocations from non-indigenous populations.

In Norwegian red deer genetic variation is significantly differentiated compared to in other European populations and a lower level of variation demonstrate loss of genetic variation by previous genetic drift. Separate bottleneck analyses of microsatellite data support loss of gene copies during an old common European bottleneck but the additional Scandinavian losses imply other and more recent bottlenecks. Historic records and genetic estimates suggest bottlenecks to have taken place around postglacial colonisation and from 300 to 100 years ago. All together, strong genetic drift has involved strong genetic differentiation of the European populations.

In an island population of indigenous Norwegian red deer a translocation of genetically distinct continental red deer one century ago has involved full interbreeding and a resultant hybrid population. Genetic measures show that the hybrid population on Otterøya is an intermediate of Norwegian and introduced red deer, which were of a Hungarian – German blood cross. A similar to larger body size of hybrids than indigenous Norwegian deer indicates positive effects that may stem from introduced additive genetic variation, positive gene interactions or hetrozygote overdominance. The mainland Norwegian population has however been little affected since gene flow from Otterøya until now has been very low.

Within the mainland Norwegian red deer population a present genetic structure of five subpopulations coincides very well with the historically recorded distribution from 300 to 100 years ago, lending support to strong genetic drift during this period. Founder events during spatial population expansion the last century have by comparison not involved any significant genetic differentiation. Rather existing genetic structure has been maintained by physical barriers for dispersal, like steep - sided Norwegian fiords and broad mountain ranges, during spatial expansion. Further, dispersal is male-biased and significant isolation by distance may be explained by female philopatry, which is supported by a stronger female than male genetic structure.

Summary in Norwegian (Sammendrag)

Hos alle arter endrer populasjonene størrelse og utbredelse gjennom tid og rom, men nylig har spesielt store endringer blitt observert i mange populasjoner. Sammentrekninger i størrelse og nyetableringer ved romlig ekspansjon av populasjoner kan medføre differensiering eller til og med tap av genetisk variasjon, som er viktig for artenes opprettholdelse. Denne doktorgraden tar for seg genetisk variasjon av mikrosatellitt og mitokondrie DNA i en hjortepopulasjon som for øyeblikket ekspanderer geografisk, men som tidligere har vært utsatt for reduksjoner i størrelse og utsettinger av hjort fra fremmede populasjoner.

I norsk hjort er genetisk variasjon markant differensiert i forhold til i andre europeiske populasjoner, og et lavere nivå variasjon viser at genetisk variasjon har blitt tapt ved tidligere genetisk drift. Forskjellige analyser av mikrosatellitt DNA støtter at gen kopier har blitt tapt i en gammel felles europeisk flaskehals, men ytterligere tap i de skandinaviske populasjonene tyder på andre og mer nylige flaskehalser. Historiske nedtegninger og genetiske estimater antyder flaskehalser både ved postglasial kolonisering og fra 300 til 100 år siden. Til sammen har genetisk drift medført sterk genetisk differensiering av de europeiske populasjonene.

I en øy – populasjon av hjemmehørende norsk hjort har innføringen av genetisk forskjellig kontinental hjort for et århundre siden medført full krysning og en medfølgende hybrid populasjon. Genetiske mål viser at hybrid populasjonen på Otterøya er en mellomting mellom den norske hjorten og den innførte, som var en krysning mellom ungarsk og tysk hjort. En lik til større kroppsstørrelse hos hybridene enn hos norsk hjort tyder på positive effekter av hybridiseringen som kan skyldes tilført additiv genetisk variasjon, positive gen – interaksjoner eller overdominans av heterozygoter. Den norske fastlandspopulasjonen har imidlertid blitt lite påvirket ettersom spredning fra Otterøya inntil nå har vært svært lav.

Innen den norske fastlandspopulasjonen er en nåværende genetisk struktur av fem sub – populasjoner svært godt overensstemmende med den historisk nedtegnede utbredelsen fra 300 til 100 år siden, og dette støtter at den genetiske driften var sterk i denne perioden. Nyetableringer ved geografisk ekspansjon det siste århundret har til sammenligning ikke medført noen signifikant genetisk differensiering. Snarere har eksisterende genetisk struktur blitt opprettholdt av fysiske barrierer for spreding, som bratt – sida fjorder og store fjellkjeder, i løpet av den geografiske ekspansjonen. Spredning er også høyere blant hanner og signifikant isolasjon i forhold til geografisk avstand kan forklares av hjemmekjærhet hos hunnene, noe som støttes av en sterkere genetisk struktur mellom hunner enn hanner.

List of papers

I. Haanes H, Rosef O, Veiberg V, Røed KH (2005). Microsatellites with variation and heredity applicable to parentage and population studies of Norwegian red deer (*Cervus elaphus atlanticus*). *Animal Genetics* **36**, 454-455.

II. Haanes H, Røed KH, Perez-Espona, S, Rosef, O. Low genetic variation support bottlenecks in Scandinavian red deer. Manuscript.

III. Haanes H, Røed KH, Mysterud, A, Langvatn, R., Rosef O. Consequences on genetic diversity and population performance of introducing continental red deer into the northern distribution range. Submitted to Molecular Ecology.

IV. Haanes H, Røed KH, Flagstad Ø, Rosef O. Genetic structure in an expanding cervid population after population reduction. Submitted to Conservation genetics.

V. Haanaes H, Røed KH, Rosef O. Sex-biased dispersal in an expanding population. Manuscript.

Introduction

General background

The last centuries human exploitation of natural resources and land areas have affected biodiversity both locally and globally (McNeely 1992; Parsons 1992; Begon *et al.* 1996) and there is a need to preserve bio – resources at sustainable levels through management of species, habitats and ecosystems (Lande 1988; Soulé & Mills 1992). According to prevailing environment and available habitats the demography and distribution of species varies through time and space (Begon *et al.*, 1996), greatly affecting levels of genetic variation and population structure (Hartl & Clark, 1997; Hedrick, 2000). However, since pre-industrial times large significant changes of the climate and an altered use of land by humans have resulted in major changes in the abundance and geographical range of many species (IPCC 2001; IPCC 2007). The changes are alarming, considering potential effects on genetic variation, adaptation and evolution of species.

During the Quaternary ice ages extensive climatic oscillations and rapid changes in ice sheet distribution produced large successive shifts in the demography and geographical range of many species. In many newly colonised areas isolation after leading edge expansions involved loss of genetic variation, increased homozygosity and development of new adaptations, and in some cases speciation (Hewitt 2000; Hewitt 2001). More recently, the natural distributions of many species have also been altered by human-mediated translocations (Allendorf *et al.* 2001). It is important to increase our knowledge about how the genetic variation of species is affected by range shifts and human-mediated translocations so we can better conserve existing species and manage them optimally at different scales. Knowledge of genetic structure is for example important for estimation of the effective size of populations and for identification of management units when making conservation plans (Wang & Caballero 1999; Nunney 2000).

One group of appreciated game species with great economic importance that has been heavily affected by human activities, are the cervids. Most cervid populations in North America and Europe have been heavily exploited the last centuries and many were nearly extirpated, but recently most have increased in size and distribution (Gill 1990; Jedrzejewska *et al.* 1997). Cervid species have commonly been translocated to avoid local extinction or for reintroduction purposes, often affecting their genetic variation (Hartl 1991; DeYoung *et al.*

2003; Randi 2005). Investigations of cervids are therefore of economical value and may offer a possibility to investigate the genetic effects of population fluctuations and translocations.

The red deer, *Cervus elaphus* Linneaus, 1758, is a typical cervid species, which has experienced many population fluctuations and human mediated translocations (Gill 1990; Kuehn *et al.* 2003; Randi 2005), often obscuring natural distributions of genetic variation (Hartl *et al.* 1995; Hartl *et al.* 2003). In the Norwegian population (*Cervus elaphus atlanticus*) archaeological finds indicate isolation since postglacial colonisation (Ahlèn 1965) and only one translocation is historically recorded. This was to an island isolated in the outer range of red deer distribution (Collett 1909; Ingebrigtsen 1924). Like most other cervid populations, the Norwegian red deer population was severely reduced in size and distribution in the 1800th and 1900th century (Collett 1909; Ingebrigtsen 1924), but since then the population has substantially grown and expanded spatially (Gill 1990; Forchhammer *et al.* 1998; Langvatn 1998). It thus offers a good study population for the genetic effects of population contraction and expansion, as well as for the consequences of human mediated translocations.

Factors affecting genetic variation within populations

In most species an abundant geographic variation is found in morphology and genetic composition (Wright 1978; Slatkin 1987). Evolution is the process of change in the genetic makeup of populations (Graur & Li 2000), and through studies of how genetic variation is generated and maintained inference can be made about evolution (Nei 1987; Nei 2000). Existing variation within populations may change from natural selection, migration or through random processes affecting levels of genetic variation and population structure (Endler 1992; Hartl & Clark 1997; Hedrick 2000). Mutations are the main source of new genetic variation but genetic variants “new” to a population may also be introduced through immigration. Adaptations may develop through natural selection acting on inheritable phenotypic variation (Darwin 1859) and populations change genetically when fitness improves in relation to local environment (Fisher 1930; Endler 1992). Genetic variation in a population may therefore reflect local or offer future adaptations. In isolated populations of limited size genetic variation also change by random genetic drift (Fisher 1930; Wright 1931; Wright 1969) and when gene flow is limited among populations or subpopulations they may become genetically differentiated and structured (Hartl & Clark 1997; Hedrick 2000).

Random genetic drift and effective population size

In any population of finite size the sampling of gametes for each next generation is likely to involve a change of gene frequencies. When a limited number of gametes are passed on to the next generation, these are unlikely to reflect parental gene frequencies with total accuracy. The fewer gametes that are sampled the less likely these are to represent the gene frequencies of the parental gene pool. If the next generation is small and only a few gametes are sampled, the random change of gene frequencies may be large from generation to generation. This "sampling error" is called random genetic drift and becomes more pronounced the smaller populations are in size (Fisher 1930; Wright 1931; Wright 1969). In very small populations genetic drift can be very strong and change gene frequencies drastically and rapidly (Chakraborty & Nei 1977) and gene copies may become fixed or lost (Nei & et al. 1975). In large populations the effect of genetic drift is small per generation but with population isolation changes may accumulate over long periods of time (Hartl & Clark 1997).

In actual populations it is seldom that all individuals participate in reproduction, as some may be passing through juvenile, adolescence and senescence stages. Further, in most mammals mating of adult males with several females (polygony) involves variation in individual reproductive success (Clutton-Brock 1989). Individuals that reproduce to a higher degree will contribute correspondingly more to the genetic pool of the next generation, and some adults may not contribute at all. The numbers of reproducing males and females may be unequal and members of one sex may contribute more. Calculation of random genetic drift is based on an ideal population without such deviations in individual contribution to the gene pool of the next generation. The population size used to calculate genetic drift must therefore be corrected for any fluctuations in population size, age structure, skewed sex ratio or skewness in reproduction success. Such a corrected population size is called the effective size of a population (N_e), and it equals a theoretic population size with random genetic drift of the same magnitude as observed in the actual population (Hartl & Clark 1997). In other words, the effective population size with regard to maintenance of genetic variation will be smaller than the observed population size (Nunney 1993; Hedrick 2000). With non-random mating among subdivisions within a population, genetic structure also becomes an important parameter for the estimation of effective population size, which is necessary to consider when making management and conservation plans (Wang & Caballero 1999; Nunney 2000).

Genetic effects of population contraction and expansion

Population reductions involving loss of genetic variation are termed population bottlenecks (Lawrence 2000). When a population decreases in size, the magnitude of random genetic drift within it increases and the probability that an allele is lost by genetic drift is inversely related to its frequency. Rare alleles are therefore the first to be lost during a bottleneck while common alleles with higher gene frequencies take longer to drift to zero or fixed frequencies. Common alleles constitute the genotypes of most heterozygous individuals, and allelic diversity may be relatively quickly reduced during a bottleneck while heterozygosity is reduced at a slower pace (Nei & et al. 1975; Maruyama & Fuerst 1985). Bottlenecks of relatively short duration or little magnitude may therefore involve loss of alleles without any significant reduction of heterozygosity, which is only reduced during severe and long lasting bottlenecks (Allendorf 1986; Amos & Harwood 1998; Amos & Balmford 2001).

Rare alleles may include particular gene copies that can become important for the long-term survival of a population, by potentially offering the genetic variation required for adaptive responses to future environmental or biotic changes. Loss of alleles from genetic drift in small or fragmented populations may therefore involve reduced future adaptability and increased risk of extinction (Allendorf 1986; Soulé & Mills 1992; Nunney 2000). If a bottleneck is severe or long lasting, common alleles may also be lost, and the number of individuals with heterozygous gene copies decrease. If so, population fitness may be reduced by both increased expression of deleterious homozygotes, as well as by a reduction of advantageous overdominant heterozygotes with higher fitness than homozygotes (Amos & Balmford 2001; Keller & Waller 2002). In very small populations selection may be counteracted by genetic drift and even current adaptive genetic variation may be lost (Hedrick 2000). It has been stated that few empirical studies of wild populations have provided convincing evidence of significant loss of genetic variation from genetic drift (Amos & Harwood 1998; Amos & Balmford 2001), encouraging additional studies of the genetic effects of population contractions, especially when severe, and a reduction in heterozygosity is anticipated.

Less is known about the genetic effects of range expansions. Theoretical models and simulation studies show that depending on migration within the population, founding events during spatial expansion may involve genetic differentiation and loss of genetic variation from newly established areas (Austerlitz *et al.* 1997; Excoffier 2004), especially with long-distance dispersal and isolation (Nichols & Hewitt 1994; Ibrahim *et al.* 1996). Such losses of

genetic variation and increased homozygosity were for example the result of many leading edge expansions after the Quaternary ice ages (Hewitt 2000; Hewitt 2001). Similar losses may be anticipated from the present major range shifts of many species associated with recent climatic changes, which are particularly pronounced at higher latitudes (IPCC 2001; IPCC 2007), and investigations of suitable study species may offer valuable information on the potential effects of future range shifts.

When there is a limited number of founders these are unlikely to represent the gene pool of the whole source population, and foundation is likely to involve a change in genetic makeup (Hartl & Clark 1997; Hedrick 2000). Generally, subdivision of a population involves genetic structure (Nei 1977; Slatkin 1987; Nunney 1999), and considering the rapid genetic differentiation during strong genetic drift (Chakraborty & Nei 1977), differentiation from founder events during population expansion may be significant. However, when population growth is substantial, spatial expansion may be accompanied by high or increased migration rates. Migration may have a homogenising effect on the genetic structure of a fragmented population (Hartl & Clark 1997) and high or increased migration may oppose genetic differentiation from founding events during spatial expansion, disintegrating genetic population structure. To be able to better predict the consequences of present and future range shifts on the genetic variation and structure of species, it is therefore important to investigate and understand the processes operating within populations during spatial expansion.

Possible consequences of translocations among populations

Another possible consequence of both spatial population expansions and human-mediated translocations is an increased risk of hybridisation between previously geographically separated and genetically different taxa. For example, during and after the Quaternary ice ages range shifts often involved hybridisation in convergence zones among previously diversified taxa, developing new adaptations or even speciation events (Hewitt 1996; Hewitt 2000; Hewitt 2001). When genetically divergent populations with different gene pools merge, offspring may inherit gene copies from both source populations, and the “isolate break” effect increase the offspring gene pool diversity (Hartl & Clark 1997). Increased genetic variation may have positive consequences for population viability through heterosis effects or reduced inbreeding depression (Frankham 1995; Coulson *et al.* 1998), depending on the genetic divergence of the hybridising taxa (Allendorf *et al.* 2001; Freeland 2005). In small

populations where deleterious alleles have become fixed, introduction of new individuals may involve genetic restoration and avoidance of extinction (Hedrick 2001). Intentional translocations of species within their range for conservation and management reasons have therefore often yielded positive results (Griffith *et al.* 1989; Fischer & Lindenmayer 2000), even though intra-specific hybridisation is rare in some species (Hansen 2002; Lorenzen & Siegismund 2004; Storfer *et al.* 2004).

However, when hybridising taxa are genetically divergent, local adaptations may be lost when non-indigenous gene variants replace native ones (Rhymer & Simberloff 1996) or break up co-adapted gene complexes (Barton 2001; Burke & Arnold 2001). Similarly, gene flow between populations in different environments may prevent development of local adaptations and may lower the short-term fitness of native populations (Storfer 1999). Presently, the hybridisation rates world-wide from human-mediated habitat modifications and animal translocations are increasing, causing extinction of native species, subspecies and locally adapted populations (Allendorf *et al.* 2001). Hybridisation between translocated non-indigenous and native taxa should therefore be closely monitored and the impact of range expansions and human-mediated translocations investigated.

Figure 1. Red deer stag. Photo: Reproduced with kind permission from Frode Bergan

Red deer as study species

The red deer is one of our large and most familiar mammalian wildlife species (Fig.1). It is a member of the deer family (Cervidae) and like in many other ungulates the sexes are segregated by body size dimorphism, habitat use and activity patterns (Clutton-Brock *et al.* 1987; Conradt *et al.* 1999; Conradt & Roper 2000). Each season stags mature sexually before the seasonal rut (Lincoln 1971b; Lincoln 1971a), developing secondary sexual traits like antlers and an increasing body size, which both are regarded as popular traits by trophy hunters. The red deer has been important for hunters since prehistoric times, and is depicted by rock carvings many places in Europe (Fig.2). It has a present wild distribution from Western Europe to central Asia (Whitehead 1972; Clutton-Brock *et al.* 1982b; Whitehead 1993) and constitutes a highly desired and priced game for hunting. It is farmed across the world for its valuable velvet antlers and venison meat (Haigh & Hudson 1993).

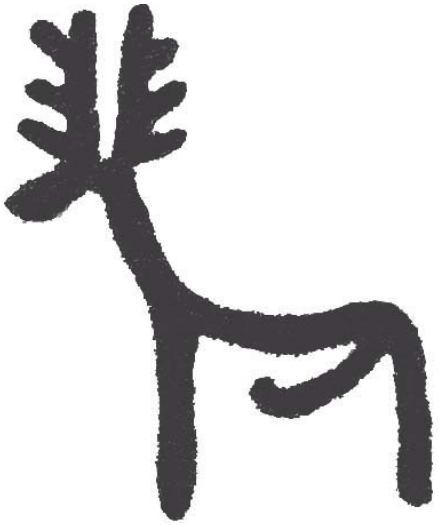

Figure 2. Rock carving of red deer stag (redrawn from picture; www.vitlyckemuseum.se).

Many red deer populations became extremely reduced in size and distribution the last centuries (Strandgaard & Simonsen 1993; Kuehn *et al.* 2003; Kuehn *et al.* 2004) but most have recently increased in density and expanded geographically (Gill 1990; Ward 2005). Many factors have been suggested to affect red deer densities and population fluctuations, like high predation and hunting pressures (Collett 1877; Collett 1909; Jedrzejewska *et al.* 1997), climatic variation (Forchhammer *et al.* 1998; Mysterud *et al.* 2001b) and recent alterations of the use of agricultural land (Ahlèn 1965; Mysterud *et al.* 2002). In Europe the red deer has therefore been thoroughly managed (Groves & Grubb 1987). Translocations have been common among populations to avoid local extinction (Strandgaard & Simonsen 1993; Hartl *et al.* 1995; Zachos *et al.* 2003) or to transfer desirable traits for trophy hunters (Hartl *et al.* 2003). Keeping game in enclosures has played an important role in restocking European populations, serving as reservoirs for different populations and subspecies (Hartl *et al.* 2003). The red deer therefore offers a good study species for assessment of genetic variation and structure after range expansion and translocations between different populations.

Like most mammalian species, the red deer has a polygynous mating system and only some dominant harem holding stags reproduce each season (Gibson & Guinness 1980b; Clutton-Brock *et al.* 1988; Pemberton *et al.* 1992). Females are social, and as in most mammals, female philopatry involves a higher likelihood of male natal dispersal (Greenwood 1980; Clutton-Brock *et al.* 1982b; Clutton-Brock 1989). Generally when dispersal is male-biased and females remain close to their birthplace or maternal relatives, relatedness increase within social groups. In addition, polygony involves increased relatedness among offspring within each social group when females mate with the same male. In social species, philopatry is therefore in concert with polygony, expected to involve genetic differentiation among social groups (Chesser 1991b; Chesser 1991a). Small-scale genetic structure has thus been observed in many ungulates (Mathews & Porter 1993; Petit *et al.* 1997; Coltman *et al.* 2003) and to the extreme in red deer, but decreasingly so with increasing population density as levels of philopatry and polygony decrease (Nussey *et al.* 2005). A possible consequence of sex-biased dispersal is sex-related differences in genetic structure, but such consequences on the subpopulation and meta-population levels have been poorly documented (Prugnolle & de Meeus 2002). The red deer seems to be representative of many mammalian species and may be used as a model species for such studies.

Distribution and genetic differentiation

Presently, the *Cervus* genus extends circum-globally, constituting a species complex of geographically separated subspecies. The American Wapiti (*Cervus canadiensis* Erxleben, 1777) has long been considered con-specific with the red deer (Clutton-Brock *et al.* 1982b; Groves & Grubb 1987; Haigh & Hudson 1993), but differences in morphological and behavioural traits and phylogenetic analyses strongly suggest that they are separate species (Randi *et al.* 2001; Polziehn & Strobeck 2002; Ludt *et al.* 2004). Genetic analyses of maternal mitochondrial DNA (mtDNA) indicate that the red deer is divided into four to six subspecies in Asia, the Middle East, Africa, the Balkan and Western Europe (Ludt *et al.* 2004).

In Europe, red deer populations have previously been divided into several separate subspecies from differences in morphology and genetic variation (Lønnberg 1906; Gyllensten *et al.* 1983; Whitehead 1993). However, the applied morphological traits like measures of antlers and scull may be affected by nutrition (Geist 1998), and many have argued for one common European subspecies (Groves & Grubb 1987; Polziehn & Strobeck 2002; Ludt *et al.* 2004).

Others have argued for subspecies status of indigenous populations like the Carpathian and Corsican red deer because of their genetic distinctiveness (Zachos *et al.* 2003; Feulner *et al.* 2004; Lorenzini *et al.* 2005). Nonetheless, these and other studies show genetic differentiation on many different scales within the European red deer in allozymes (Hartl *et al.* 1990; Strandgaard & Simonsen 1993; Herzog & Gehle 2001), mtDNA (Hartl *et al.* 2005) and microsatellite markers (Zachos *et al.* 2003; Feulner *et al.* 2004; Kuehn *et al.* 2004). Among these, some show differentiation in relation to geography or isolation by distance (Hartl *et al.* 1990; Herzog & Gehle 2001; Kuehn *et al.* 2004) and others show effects of anthropogenic influences like habitat fragmentation (Kuehn *et al.* 2003; Hartl *et al.* 2005), selective hunting and translocations between different populations (Hartl *et al.* 1991; Hartl *et al.* 2003). Mechanisms for genetic differentiation should therefore be addressed, the distinctiveness of different populations established, and the extent and impact of translocations assessed to identify indigenous European red deer populations worthy of conservation.

Red deer first appeared in Pleistocene Europe during the Cromerian interglacial approximately half a million years ago and fossil records suggest a subsequently changing distribution (Flerov 1952; Kurtèn 1968; Lister 1984; Lister 1993). During the last glacial maximum from 21 - 17 000 years before present (BP) the main red deer distribution was across southern Europe (Sommer & Nadachowski 2006) and present mtDNA lineages indicate at least three different glacial refuges (Hartl *et al.* 1995). The ice sheet covered most of northern Europe and retreated through several oscillations until around 8500 BP (Andersen & Borns 1994). The land formerly covered by ice was re-colonised by terrestrial fauna, and after invasion via land bridges from mainland Europe the red deer appeared around 9500 BP in Britain (Lister 1984) and southern Sweden, Scania (Aaris-Sørensen 1992; Jonsson 1995; Liljegren & Ekström 1996). Scania was to the south connected to mainland Europe until the sea level rose (8600 BP) and to the north separated from the rest of Scandinavia by the Närke strait until 9000 BP (Andersen & Borns 1994). After the Närke strait closed red deer colonised the rest of Scandinavia (Jonsson 1995; Hufthammer 2006). Archaeological finds indicate a wide Swedish prehistoric (9000-3800 BP) distribution of red deer (Ahlèn 1965). In Norway, the vast majority of finds have been made on the west coast, (Lønnberg 1906; Ahlèn 1965), dating from 6700 BP to 2000 BP (Hufthammer 2006; Rosvold 2006), except for two south-eastern finds dating 7690 (Mikkelsen & Høeg 1976) and 8000 BP (Hufthammer 2006).

The study population

The Norwegian population (*Cervus elaphus atlanticus*) has been considered a separate subspecies of red deer (Lønnberg 1906; Whitehead 1972; Dolan 1988). Allozyme studies show it is most genetically similar to Scottish red deer, but that it has a much lower level of genetic variation (Gyllensten *et al.* 1983). Compared to Scottish, the Norwegian red deer have smaller but coarser bodies, with male slaughter weights from 110 to 150 kg in autumn (Collett 1909). Norwegian red deer are also much smaller than the Swedish, and have distinct scull morphologies in the nasal and foramen bones (Lønnberg 1906; Collett 1912). A low general level of genetic variation has been verified by microsatellite markers and implies that the Norwegian population has been isolated for a long time (Røed 1998). It could also indicate that genetic variation has been lost during founder events or previous population reduction. Written records describe an abundance of red deer in southern Norway before the 1700th century (Claussøn Friis 1599) but in the 1800th century it was common only along the western coast (Pontoppidan 1753; Melchior 1834). From the middle of the 1800th century the population declined drastically and it was confined to a few locations along the west coast most of the 1900th century (Collett 1909; Ingebrigtsen 1924). Since the beginning of the 2000th century it has expanded and now counts more than 100 000 individuals across southern Norway (Langvatn 1988; Forchhammer *et al.* 1998; Langvatn 1998). The Norwegian population should therefore be very suitable for studies of population contraction and spatial expansion. Any loss of genetic variation after post-glacial colonisation may be assessed from comparisons with other European reference populations, like the Swedish, Scottish, Lithuanian and Hungarian red deer populations.

In addition, one Norwegian stock offers a possibility to study the genetic impacts of human-mediated translocations. Around year 1900, the local stock on Otterøya (64.5°N 11.2°E) was reduced to only 12 - 14 individuals (Collett 1898; Collett 1912). To avoid local extinction, 17 individuals of a blood cross between the Hungarian (*C. e. hippalphus*) and German (*C. e. germanicus*) populations were introduced from a German zoo (Die-Woche 1902; Collett 1909; Finsberg 1934). These populations are geographically well separated from the Norwegian population and have from differences in morphology been described as separate subspecies (Whitehead 1972; Dolan 1988). Characteristic traits of these populations, like large body size and large backwards inclined antlers, were observed for decades after the introduction on Otterøya (Finsberg 1934), indicating hybridisation between translocated and native red deer. Presently however, the red deer on Otterøya have a relatively similar

morphology as the mainland population (Langvatn 1988). The stock thus offers an opportunity to examine interbreeding between populations from different environments with different morphologies and possibly different local adaptations, and may offer insight to the effects on fitness and population performance.

Genetic markers

Genetic variation within and among taxa is commonly addressed through protein variation or genetic markers. Individuals from each taxon are characterised through markers of different types of DNA, often reflecting the base-sequence or size of a specific DNA fragment. High quantities of each marker fragment are amplified through the polymerase chain reaction (PCR) and the sequence or size determined through electrophoresis. The combination of gene copies from different specific DNA fragments possessed by each individual constitutes its genotype. Different types of DNA have different modes and rates of mutation, and different markers therefore have different areas of application. Highly variable markers are generally used to differentiate closely related organisms, whereas relationships among more distantly related taxa are resolved with less variable markers that mutate slower (Freeland 2005).

In the circular genome of animal mitochondria (mtDNA), bases are substituted by an average mutation rate of 10^{-8}, which is five to ten times faster than in nuclear genes (Brown *et al.* 1979; Brown *et al.* 1982). The maternal mode of inheritance enable tracing of maternal lineages in time and space, and mtDNA markers have proved to be powerful for studies of phylogeny, evolution and population structure (Moritz *et al.* 1987). The non-coding control region (D-loop) evolves faster than the mitochondrial genes and its high level of polymorphy is a powerful tool for intra-specific studies (Saunders & Edwards 2000; Wan *et al.* 2004). However, the uni-parental mode of heredity reflects only the matrilineal story and involves an effective population size only one quarter as large as for nuclear genes, and inferences made on population history and structure are therefore likely to be biased (Zhang & Hewitt 2003).

Microsatellites are repetitive sequences of short nuclear DNA motifs (< 6 bp) with 10^{-6}-10^{-2} mutations per locus per meiosis event (Dallas 1992; Weber & Wong 1993; Zhang & Hewitt 2003) and a high degree of size polymorphism (Litt & Luty 1989; Tautz 1989; Weber & May 1989). They are generally found in regions of nuclear DNA that does not code for proteins and are considered neutrally selective (Li *et al.* 2002). Mechanisms of mutation are believed

to be unequal crossover during recombination (Smith 1976) and especially slipped-strand mis-pairing during replication (Levinson & Gutman 1987). Classically, mutation in selectively neutral loci has been described by the infinite allele model (IAM), which assumes that all new alleles are unique (Kimura & Crow 1964). In microsatellites, the majority of mutations involve addition or deletion of one repeat, and infrequently two or more (Weber & Wong 1993; Di Rienzo *et al.* 1994). Mutation is therefore better described by the stepwise mutation model (SMM; Ohta & Kimura 1973; Kimura & Ohta 1978) or as a combination of single and multiple steps by the two-phase mutation model (TPM; Di Rienzo *et al.* 1994). Microsatellites are abundant across the eukaryote genome and the high genetic variability has made them a marker of choice for population genetic studies (Bruford & Wayne 1993; Bowcock *et al.* 1994; Jarne & Lagoda 1996). The co-dominant mode of inheritance involves that genotypes can be identified as homozygous or heterozygous (Wan *et al.* 2004). They are suitable for high-resolution studies of closely related populations and within-population studies of recent demographic history, genetic structure and relatedness (Goldstein *et al.* 1999; Zhang & Hewitt 2003).

Comparison of results from different laboratories may however not be straightforward because of microsatellite size calling errors depending on machines and running conditions (Pasqualotto *et al.* 2007). Genotyping of microsatellites often involve scoring errors associated with stutter bands, large-allele dropout, as well as from null alleles (DeWoody *et al.* 2006). Homozygous genotypes may be mistyped as heterozygous because of large stutter bands or nonspecific amplified products (Hoffman & Amos 2005), and heterozygous genotypes may be missed because of reduced amplification. Another potential problem is posted by microsatellite alleles with the same size but different mutational origins. Such homoplasy, or use of different mutation models, nevertheless do not pose any significant problems for population genetics of closely related populations, since genetic divergence among these is essentially due to random genetic drift (Estoup *et al.* 2002).

In population genetic studies of red deer a variety of microsatellites have been applied (Slate *et al.* 2000b; Kuehn *et al.* 2004) but in the Norwegian population many of these show little or no genetic variation (Røed 1998; Røed & Midthjell 1998). Additional microsatellites are thus needed for population genetic studies. Mendelian inheritance is required in most population genetics models and the commonness of non-Mendelian inheritance like gene duplication, sex linkage and null alleles in microsatellites demonstrates the need to conduct species-specific

inheritance studies of these loci (Ardren *et al.* 1999; de Meeus *et al.* 2004). Null alleles are alleles that fail to amplify to detectable levels in PCR because of mutations in the primer region leading to poor hybridisation, competition among different alleles in the PCR or PCR failure because of low or poor quality template DNA (Callen *et al.* 1993; Jones *et al.* 1998; Dakin & Avise 2004). In red deer null alleles have been identified in three of 16 microsatellite loci (Pemberton *et al.* 1995), and inheritance studies should be performed before applying other microsatellites. Undetected non-Mendelian heredity like null alleles may otherwise involve offspring-parent mismatches in parentage studies or problems like apparent heterozygote deficiencies and deviations from Hardy-Weinberg expectations in population genetics (Callen *et al.* 1993; Jones *et al.* 1998; Ardren *et al.* 1999).

Population genetic analyses

In microsatellites, missing alleles within in the repeat size ranges of loci may indicate a recent bottleneck. Once repeats are lost from a population only new mutations (or immigration) can fill the repeat size range, and for a period the number of mutational steps will be lower than expected. Variation lost during a bottleneck can therefore be expressed as the ratio between the observed and expected number of repeats within the size range of microsatellite loci, the M-ratio (Garza & Williamson 2001). With strict stepwise mutation, this size range grows or decreases with one repeat at a time and holes in the size ladder indicate that alleles have been lost. Two-phase mutation may involve addition or deletion of more than one repeat at a time and may generate holes in the ladder until filled by new mutations. Multiple step mutations are observed with 5 -15 % frequency in microsatellites (Di Rienzo *et al.* 1994) and may thus introduce a slight bias on the interpretation of such holes as losses during bottlenecks.

The probability of a recent bottleneck can also be assessed by comparing the number of observed alleles in a population with the number of alleles expected from the observed heterozygosity, using the software BOTTLENECK (Cornuet & Luikart 1996). Since alleles of low frequency are the first to be lost during bottlenecks, heterozygosity is not necessarily affected since few individuals are carriers of these alleles. Bottlenecks of short duration or low severity therefore involves that allelic diversity is reduced to a larger extent than heterozygosity, and are followed by a transient period of allele deficiency compared to what is expected from the observed heterozygosity (Nei & et al. 1975; Maruyama & Fuerst 1985;

Cornuet & Luikart 1996). By comparison, heterozygosity is reduced during very severe and long lasting bottlenecks (Allendorf 1986; Amos & Harwood 1998; Amos & Balmford 2001).

Parameters of the demographic history of a population may also be assessed from tracking microsatellite genealogies backwards in time on basis of their coalescent (Beaumont 1999; Storz & Beaumont 2002). Estimates may be made from the posterior distributions of Marcow Chain Monte Carlo (MCMC) simulations of an algorithm (MSVAR) operating on specified priors using Bayes' theorem to draw inference from the data set according to the expected coalescent of genealogies. This allows estimation of ancestral and present population sizes, and based on the number of generations, the time frame since onset of any change in size.

When a population is subdivided, the degree of genetic differentiation between subpopulations may be estimated from Wright's F-statistics (Wright 1951; Wright 1965). When mating is not random within a population, differentiation of gene frequencies will involve a lower number of observed heterozygous genotypes in the population as a whole than expected from Hardy-Weinberg equilibrium. The genetic structure may thus be expressed by the fixation index, F_{st}, which is a measure of inbreeding within subpopulation relative to total population (Hartl & Clark 1997).

Population subdivision may also be assessed through MCMC – based Bayesian assignment analyses like the STRUCTURE algorithm (Pritchard *et al.* 2000). Without prior information about sampling localities the algorithm clusters individuals on basis of their genotypes to maximize the Hardy-Weinberg signal, estimating the probability of assignment of each individual to each of a number of specified clusters. From geographic clusters of genetically similar individuals' genetic structure may be identified. By applying different numbers of clusters to the algorithm the most optimal Hardy - Weinberg signal can be identified and the main division of genetic variation in a data set may be assessed. The algorithm can also be used to estimate dispersal (Berry 2004). The clusters suggested by the algorithm may be compared with where individuals were sampled and dispersers identified as individuals assigned to geographic clusters that do not correspond with their sampling locality. Alternatively, prior information about individual sampling localities may be incorporated in the model, which then by default suggests which individuals that are dispersers (Pritchard *et al.* 2000).

Aims of the study

1) Assess genetic variation and drift in a previously reduced cervid population
   a. Establish a genetic tool (microsatellite battery) suitable for assessment of genetic variation in the Norwegian red deer population (Paper I)
   b. Compare the level of genetic variation in Norwegian red deer with other European populations and investigate for population bottlenecks (Paper II)

2) Investigate the impact on genetic diversity and population performance of translocating non-indigenous individuals into a native cervid population (Paper III)
   a. Determine interbreeding and admixture between translocated red deer of Hungarian / German origin and a native Norwegian island population
   b. Assess any impact on population performance in the potential hybrid stock
   c. Estimate gene flow between the potential hybrid stock and mainland to assess the genetic impact on the indigenous Norwegian population

3) Address dispersal and genetic structure within an expanding cervid population
   a. Investigate structure of genetic variation to assess genetic drift and limitations to gene flow in the spatially expanding Norwegian red deer population (Paper IV)
   b. Assess sex-biased dispersal in the expanding Norwegian population (paper V)

Results – a brief account of the papers

Genetic variation in a previously reduced cervid population

Establishment of a genetic tool to assess genetic variation in Norwegian red deer

Twenty-five microsatellite loci developed for deer, reindeer, goat, sheep and cattle that were known to be polymorphic in other red deer populations were first screened in the Norwegian population. Overall the level of genetic variation was low, with an observed heterozygosity of 0.11 to 0.72 and from two to nine alleles per locus (paper I). In some loci the observed and expected heterozygosity was lower than reported from other European red deer populations (Table 1). Exclusive male homozygosity was not observed in any loci and sex linkage ruled out.

Table 1. Genetic variation measured by microsatellite allele number (A) and either observed heterozygosity (H(0)) or expected heterozygosity (H(E)) in red deer (*Cervus elaphus*) from Norway (n=94), Scotland (n=364, ** = null allele (Slate *et al.* 2000b)$_1$, and Germany (n=30 (Poetsch *et al.* 2001)$_2$).

	Norway			Scotland$_1$		Germany$_2$	
	A	**H (O)**	**H (E)**	**A**	**H (E)**	**A**	**H (0)**
McM58	6	0.72	0.78				
FCB304	6	0.72	0.76	9	0.79		
BM4208	5	0.70	0.73	11	0.86		
BM888	9	0.70	0.74	11	0.84		
FCB193	6	0.70	0.70	11	0.79		
NVHRT48	5	0.62	0.68			8	0.67
OarCP26	3	0.59	0.64	9	0.69		
NVHRT73	5	0.56	0.57			7	0.70
RT7	4	0.55	0.65			5	0.63
RT1	3	0.53	0.54	8	0.84	5	0.60
RT5	3	0.46	0.49	8	0.77		
RT6	3	0.44	0.52	10**	0.72	6	0.80
CSSM066	4	0.43	0.47	8**	0.83		
BM757	2	0.33	0.40	7	0.61		
BM203	3	0.27	0.25	7	0.69		
ETH225	4	0.23	0.23	6	0.65		
NVHRT21	4	0.16	0.43			5	0.57
NVHRT16	2	0.11	0.20			4	0.37

Heredity was investigated for 21 of the 25 screened loci (Paper I). A linkage map for red deer was published (Slate *et al.* 2002) during our study, showing linkage among two pairs of the investigated loci. Mendelian heredity was therefore only investigated for one locus of each linkage pair. Two other loci were also excluded, one because it seldom yielded PCR products and the other from its low applicability in parentage assignment with two alleles.

A family material of 32 calf-mother relationships was established from observations of parturition and / or repeated suckling behaviour. Among 32 maternities heredity was investigated for 672 pairs of electromorphs in 21 loci and 27 apparent mismatches in Mendelian heredity between mother and offspring were recorded (Paper I). Proof-reading revealed that 16 mismatches were due to incorrect genotyping and an error rate of ~1% was estimated. The remaining 11 mismatches between mother and calf sustained after both repeated PCR and re-isolation of DNA, but were not randomly distributed across loci. They occurred in two loci and involved only homozygous individuals. Two mismatches were identified in the locus NVHRT21, and nine of the mismatches in the locus McM104, involving five hinds and their progeny. Among these were two hinds with triple maternities and one grandmother-mother-daughter relationship, establishing inheritance of the expected null alleles across two generations. Totally 19 loci had a verified Mendelian heredity and 14 were applied in genetic analyses of Norwegian red deer (papers II - V).

Genetic variation in Norwegian compared to European red deer indicates bottlenecks

In a comparative study (Fig. 3), 14 of the microsatellites with Mendelian heredity unambiguously showed lower levels of genetic variation in the Norwegian and Swedish populations than in Scottish, Lithuanian and Hungarian red deer (paper II). Heterozygosity and allelic diversity were as much as 25 % and 41 % lower in the Swedish and 21 % and 40 % lower in the Norwegian population. Compared to the 195 microsatellite repeats observed across the European populations, only 63 and 67 alleles were observed in the Norwegian and Swedish populations, respectively. Further, in contrast to observe complete “ladders” of dinucleotide repeats, totally 50 mutational steps were missing as alleles from the size ranges of the 14 microsatellite loci in all the investigated populations. Population bottlenecks were indicated by three separate analyses of the microsatellite variation.

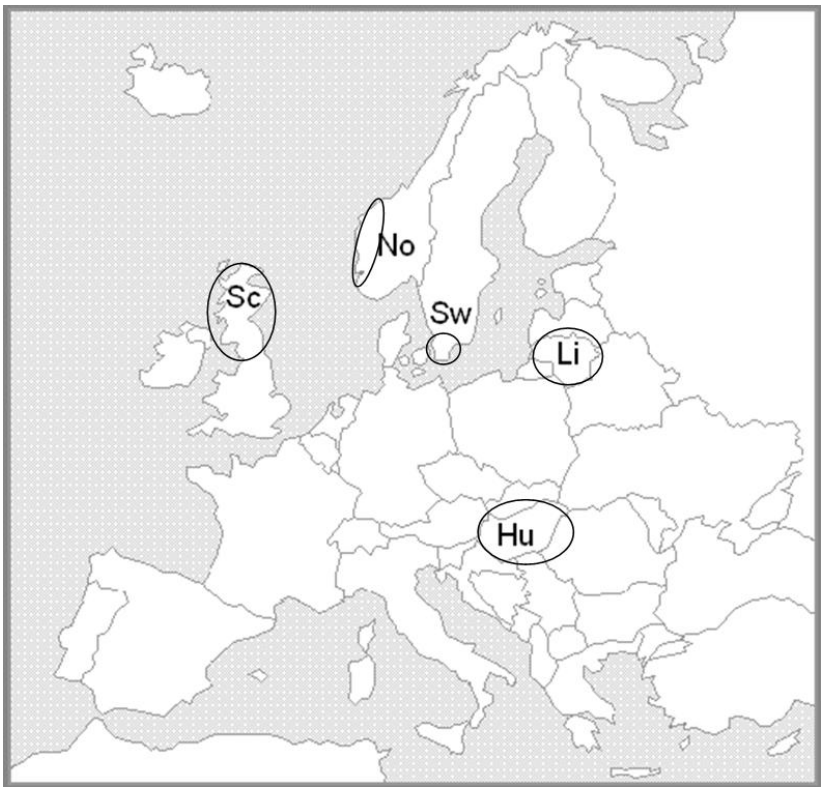

Figure 3. Sampling areas in the Norwegian (No), Swedish (Sw), Scottish (Sc), Lithuanian (Li) and Hungarian (Hu) red deer populations.

First, Low M-ratios' (Garza & Williamson 2001) in all the populations except the Hungarian expressed that the numbers of observed alleles were lower than expected if all mutational steps within the size range of microsatellite loci had been present (Paper II). The additional alleles missing from within the loci size ranges of the Scandinavian populations involved especially low M-ratios. Further, mutational repeats outside the Scandinavian size ranges that were observed in the other European populations would if they had been included in the calculation have involved even lower M-ratios in the Scandinavian populations.

Second, in the Norwegian population, the BOTTLENECK software (Cornuet & Luikart 1996) showed a significant allele deficiency across microsatellite loci compared to what was

expected from their observed heterozygosity (Paper II). This indicates that genetic variation in the form of alleles has been lost to a higher degree than heterozygosity. The outcomes were significant when assuming a two-phase model of mutation (TPM) involving 80 % and 90 % of the stepwise mutation model (SMM) but not when assuming a strict SMM model. Since multiple step mutations are observed with 5 -15 % frequencies in microsatellites (Di Rienzo *et al.* 1994), the results of the TPM models seems most reasonable.

Third, a hierarchical Bayesian algorithm, the MSVAR1.3 software (Storz & Beaumont 2002), supported previous population decline in all the populations through consistent estimates of a large ancestral and a much smaller present population size (paper II). Different runs assuming either high or low ancient population size, or short or long time since onset of decline, gave consistent parameter estimates in each of the populations. Convergence was established from equality of the variation within runs compared to the variation among runs. Among the populations the posterior parameter estimates were similar for ancient population size, mutation rate and time since onset of decline, but varied for present population size.

Finally, in a sequence of the mtDNA control region only one single unique haplotype was observed in the Swedish red deer population, whereas five were observed in each of the Norwegian and Lithuanian populations, and seven and eleven haplotypes were observed in the Hungarian and Scottish populations, respectively (Paper II).

The impact of translocating cervids among populations

Interbreeding and admixture between translocated and indigenous red deer

A study of the mtDNA control region and the 14 microsatellite loci demonstrated that the translocated red deer of Hungarian / German origin have interbred and admixed with the native Norwegian island stock on Otterøya (Paper III). The two investigated parental populations in Hungary and Norway are genetically differentiated, as expressed by long genetic distances and F_{st} values of 0.18 for the microsatellites and 0.83 for the mtDNA sequences (Paper II & III). An intermediate level of microsatellite variation, measured by allele richness and heterozygosity in the potential hybrid population on Otterøya compared to in the Norwegian and Hungarian populations, indicates that indigenous and translocated red deer have interbred. The intermediate position was supported by similar genetic distances to

both the Norwegian and the Hungarian population, suggesting it is a hybrid population. Introgression from the translocated red deer was further demonstrated by the occurrence of numerous microsatellite alleles in the Otterøya stock that were otherwise only found in the Hungarian population. In addition, introgression was indicated by one mtDNA haplotype observed on Otterøya that was not found in the mainland Norwegian population.

Further analyses of mtDNA and microsatellite variation indicated more or less even admixture from the Norwegian and Hungarian populations into the Otterøya hybrid stock, and full interbreeding between native and introduced red deer (Paper III). Since the translocated and native red deer were in approximately equal numbers at the time of translocation, the degree of interbreeding was assessed directly from estimates of proportions of admixture from the parental populations. First, the two haplotypes of Norwegian and non-indigenous origin observed on Otterøya were evenly distributed. Further, three different methods using microsatellite variation demonstrated variable estimates of admixture. A moment estimator of admixture based on allele frequencies, m_C (Chakraborty *et al.* 1992), gave equal proportions of admixture from the Norwegian and Hungarian populations (0.55 and 0.45). An estimator based on the coalescence of microsatellite alleles, m_Y (Bertorelle & Excoffier 1998), resulted in a higher admixed Norwegian proportion (0.7) than Hungarian proportion (0.3). Finally, an analysis (LEA) including the effect of genetic drift after the admixture event (Chikhi *et al.* 2001), indicated that an even higher proportion of the Norwegian population (0.7-1.0) and a lower Hungarian proportion (0.0-0.3) was most probable.

Impact on the population performance of a hybrid stock

Assessment of harvest data provided by hunters showed that the population performance of the hybrid stock on Otterøya was similar or even higher than in indigenous Norwegian red deer stocks (Paper III). Average body mass was used as an approximate measure of population performance and generalised linear and additive models controlling for the effects of sex, age, date of harvest and red deer density were used to investigate for spatial differences between the hybrid stock and other stocks in adjacent areas. The same municipalities as where the genetic data were gathered were used for the comparison, and included both inland and coastal areas as well as the island Hitra where red deer density is even higher than on Otterøya. The models showed that the hybrids on Otterøya were larger than red deer from coastal localities and the island Hitra but of similar size as some inland

localities in South Trønderlag. The hybrids were of comparable body size as the red deer in a coastal and an inland locality in North Trønderlag, but this comparison involved few data points scattered in time and between different age classes. Our results therefore demonstrate that population performance is not reduced in the hybrid stock on Otterøya, but rather similar or higher than in indigenous Norwegian localities.

Gene flow between a hybrid stock and the indigenous Norwegian population

The study suggests that recent gene flow from Otterøya and thus introgression from the hybrid stock into the indigenous Norwegian population was very low prior to 2002 (Paper III). Dispersal between Otterøya and adjacent areas was estimated from the microsatellite data through the Bayesian algorithm STRUCTURE (Pritchard *et al.* 2000) and prior information about sampling *was not used* to avoid bias on clustering. The method clustered the absolute majority of individuals within clusters that coincided well with the sampled areas, strongly separating Hungary, Otterøya and the adjacent areas, as expected from the strong observed genetic structure. All the individuals sampled in the Norwegian mainland population were assigned to the "Norwegian" cluster ($0.9 > p > 0.6$) and only three were also partially assigned to the "Hungarian" ($p = 0.15$ & 0.11) or "Otterøya" ($p = 0.28$) clusters (Paper III). These were carriers of alleles seldom or never found in the indigenous Norwegian population and probably have ancestors originating from Otterøya. On Otterøya two dispersers were identified from their assignment to the "Norwegian" cluster ($p = 0.99$ & 0.61) and partial assignment of four others indicated ancestry to the mainland population.

Gene flow and structure in a spatially expanding cervid population

Genetic drift and limitations to gene flow in the Norwegian red deer population

To assess the effects of spatial expansion on genetic variation and structure, the Norwegian red deer population was sampled across its present distribution and genotyped in the 14 microsatellite loci (Paper IV). The sampled localities included both locations along the west coast relict from the period of decline (300 – 100 BP) and locations in the middle and eastern parts of South Norway where red deer have re-established the last century. Totally 100 localities in 25 Norwegian municipalities were sampled, but to attain sample sizes high enough to achieve statistical significance some localities were joined within or among

neighbouring municipalities. Genetic variation and structure was therefore investigated among totally 15 localities.

Significant genetic structure among several of the 15 localities suggested that gene flow is limited within the Norwegian red deer population (Paper IV). F_{st} values between pairs of localities indicated from moderate (0.05 - 0.15) to strong (0.15 - 0.25) genetic structure (Wright 1978; Hartl & Clark 1997), but some localities were not differentiated. A separate test showed that genetic drift because of isolation by distance between the localities was significant and that limited gene flow with geographical distance explains some of the genetic differentiation. We also wanted to investigate whether any genetic structure could be explained by previous genetic drift and the BOTTLENECK software showed significant allele deficiency in 14 of the 15 localities, suggesting loss of alleles from strong genetic drift during some recent bottleneck. However, the level of genetic variation measured by heterozygosity and allele richness was quite similar among the localities, both relict and newly established, and founder events during spatial expansion were ruled out.

A Neighbour Joining tree of genetic distances based on microsatellite loci showed which localities that were connected through high gene flow and thus indicated the origin of many newly established localities (Paper IV). Short genetic distances between a relict locality on the south-west coast and localities to the south and then north-eastwards along the coast indicate dispersal along the coastline during spatial expansion. Similarly, short distances from the relict localities on the north-east coast with a locality towards southeast showed another main route of dispersal on the north side of the massive mountain ranges of central Norway. Further, long genetic distances between the localities along the west coast compared to their geographical distances indicate limited gene flow, especially across the Sognefjorden (Fig. 4).

To investigate the main pattern of genetic structure and gene flow in the Norwegian population, the STRUCTURE algorithm (Pritchard *et al.* 2000) was applied. It revealed that the most probable number of clusters in the microsatellite data set was five (Paper IV), dividing the 15 sampled localities into five separate geographic regions (Fig. 4). The individuals comprising each cluster were mostly sampled in neighbouring localities but in three clusters the assigned individuals were also from localities sampled in other regions. Clusters 1, 2 and 4 consisted of individuals sampled both in relict west coast localities and newly established localities, suggesting the geographical origin of the newly established

localities and routes of dispersal. The first four clusters coincide very well with four of the relict areas of red deer distribution described for the period from three to one hundred years ago (Ingebrigtsen 1924), supporting the strong genetic drift during this population decline suggested by the BOTTLENECK analyses. The fifth cluster comprised of two localities in central Norway, which probably originate from a relict area we did not sample on the Bergen peninsula (Fig 4).

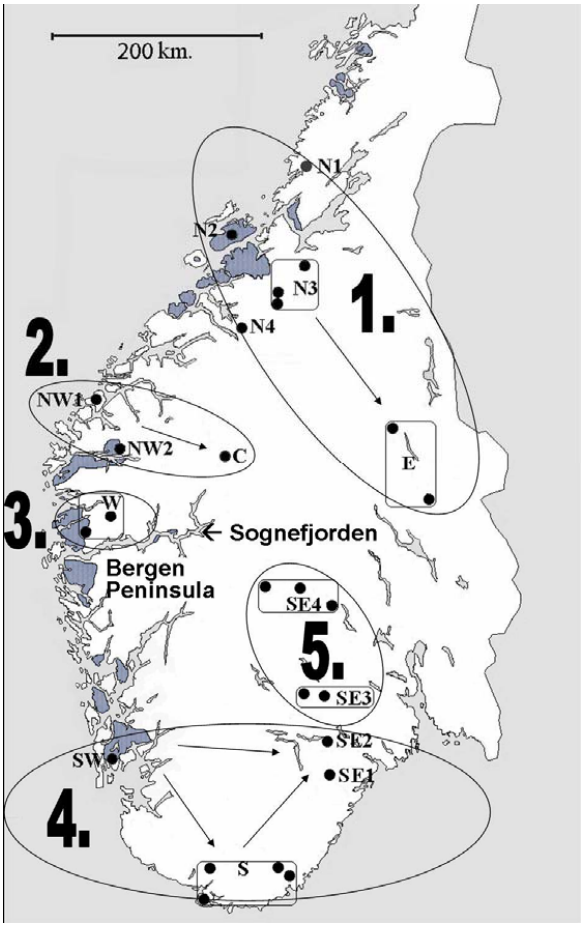

Figure 4. Baysian clustering (ellipses) of Norwegian red deer sampled at 15 localities (dots for separate localities and rectangles for combined localities). Distribution prior to 2000th century in grey shade (Collett 1909; Ingebrigtsen 1924; Langvatn 1998).

In the localities constituting cluster one to three, most individuals were unambiguously assigned to the same cluster, indicating a strong Hardy-Weinberg signal and low recent dispersal. However, in the localities comprising cluster four and five many individuals were assigned to other clusters, demonstrating dispersal and admixture. Of the 145 individuals sampled from the localities comprising cluster four, 37 were assigned to cluster five and 5 to cluster three. Correspondingly for cluster five (n = 54), eleven and nine individuals were assigned to cluster four and three, respectively. Gene flow is obviously high in this area, but dispersers may also come from the west coast. Some individuals sampled in the eastern area may for example have been assigned to the not sampled relict area on the Bergen peninsula. Overall the genetic structure in Norwegian red deer seems to be more broken down in the eastern and newly established areas than among the relict areas along the west coast.

Sex-biased dispersal, philopatry and polygony in Norwegian red deer

To investigate for sex-biased dispersal in the spatially expanding Norwegian red deer population, the genetic variation of males and females was investigated separately for the 14 microsatellite loci (Paper V). The division of genetic variation among five identified subpopulations (Paper IV) was used to assess differences in genetic structure between the sexes and applied as prior information in full Bayesian analysis (STRUCTURE) for identification of any first – generation dispersers of each sex. Significant differences between the sexes in the F_{st} values among subpopulations demonstrated a stronger structure of genetic variation among females than males in the Norwegian red deer population and together with a much higher number of identified male than female first generation dispersers indicate that dispersal is male-biased (Paper V). Bayesian analysis with prior information on sampling thus revealed a higher number of male than female dispersers among the subpopulations. Depending on the criteria set for assignment, a significant difference was however only found when using relaxed levels of assignment ($p<0.6$). The male distance for dispersal varied from 30 (n = 2), to 100-150 (n = 5) and 200-300 (n = 6) kilometres, most of which can be termed as long-distance.

Since the degree of polygony has a large effect on genetic differentiation of social groups (Chesser 1991b; Chesser 1991a), we also assessed the degree of polygony in Norwegian red deer. Individual reproduction success can be measured by assigning the paternity of all offspring in a population and controlled experimental settings are ideal to estimate such

variation among males. In two Norwegian red deer farms the skewness of male reproduction was confirmed under an experimental setup with a varying stag segment in different hind densities. Different numbers of stags with various ages were in three enclosures allowed to range freely with a number of females during the rut, and the following spring (year after) calves were sampled to estimate male reproductive success (Table 2). The 21 microsatellites screened in the heredity study were used for genetic parentage assignment of 103 calves and in all cases traditional paternity by exclusion was used.

Table 2. Experimental setup of captive red deer in three enclosures in two Norwegian farms during two years used to estimate reproductive success in males. Stags are listed in order of hierarchical dominance, subscripts indicate reproducing individuals, the number of females per enclosure and births the successive year are given below.

	Farm A		Farm B			
	2001	2002	2001	2001	2002	2002
	7.5	8.5	V_1	V_2	V_1	V_2
Stag age	3.5	4.5	2.5_3	3.5_4	3.5_3	4.5_4
	1.5	2.5			1.5	1.5
	1.5	2.5			1.5	1.5
		1.5				1.5
		1.5				
Nos ♀	40	49	16	14	8	23
Births	**25**	**21**	**14**	**8**	**13**	**20**

Our results clearly confirmed that reproduction is skewed among male red deer, even in situations with very high hind densities. In one of the enclosures a 3.5 year stag was the

dominant while in the two other enclosures an older adult stag was the dominant. In all three enclosures the dominant male in both years achieved most of the reproduction (Table 3). In the two enclosures with an adult dominant stag, it performed from 67 to 95 % of the reproduction, while the dominant 3.5 to 4.5 years old stag in the third enclosure achieved 92 % the first year and 57 % the second year.

Table 3. Number of paternities per reproducing male red deer per year (n = 2) in an experimental setup of three enclosures with various numbers of males and females performed in two Norwegian farms.

Year	Farm A		Farm B			
Stag age	7,5	3,5	V_1	$2,5_3$	V_2	$3,5_4$
2002	22	3	13	1	1	12
2003	19	2	6	2	6	14
Totalt	**41**	**5**	**19**	**3**	**7**	**28**

Discussion

Genetic variation in a previously reduced cervid population

Establishment of the genetic tool to assess genetic variation in Norwegian red deer

The apparently overall low level of genetic variation in Norwegian red deer (Paper I) was in agreement with previous reports of microsatellite variation (Røed 1998; Røed & Midthjell 1998) and the comparisons with German and Scottish red deer in some loci (Slate *et al.* 2000b; Poetsch *et al.* 2001) provided a basis for further comparative investigations. Non-Mendelian heredity may post a problem in population genetics (Callen *et al.* 1993; Jones *et al.* 1998; Ardren *et al.* 1999) and size calling often differs between laboratories (Pasqualotto *et al.* 2007). We therefore studied the heredity of applied microsatellite markers (Paper I) before conducting a comparative study addressing genetic variation and differentiation (Paper II).

Heterozygous genotypes with a null allele will be incorrectly genotyped as homozygous and may involve false offspring-parent mismatches (Dakin & Avise 2004). Repeatedly negative PCRs' in the locus NVHRT21 for nine individuals with otherwise good products for other microsatellites (same isolate) and the mismatches of Mendelian heredity in NVHRT21 and MCM104 were strong indications of null alleles (Paper I). The mismatching individuals were all homozygous, and in McM104 two hinds with repeated mismatches and the two-generation genealogy provided further support. Null alleles are common both within and among species (Jarne & Lagoda 1996; Dakin & Avise 2004) and can in population studies create an apparent excessive homozygosity that may produce serious problems (Callen *et al.* 1993; Ardren *et al.* 1999). The two microsatellite loci NVHRT21 and McM104 were therefore not recommended for population genetic and parentage studies of Norwegian red deer (paper I).

Genetic variation in Norwegian compared to European red deer indicates bottlenecks

The unambiguously lower levels of genetic variation estimated for microsatellite loci in Norwegian and Swedish red deer compared to in the other investigated European populations suggest that genetic variation has been lost from the Scandinavian populations (Paper I & II). Microsatellites are generally neutral and not under selection (Li *et al.* 2002), and the lower allele diversity and heterozygosity strongly indicate that these populations have been exposed to comparably longer lasting or stronger random genetic drift than the other investigated

European populations. The 50 mutational size repeats that were missing from across the size ranges of microsatellite loci in all the investigated populations indicate an older and common European bottleneck (Paper II). The additionally missing alleles in the Scandinavian populations indicate other and more recent bottlenecks, a suggestion which was supported by their genetic differentiation and by separate bottleneck analyses (Paper II). Genetic differentiation is rapid during bottlenecks (Chakraborty & Nei 1977), and the stronger differentiation of microsatellite variation suggests that the Scandinavian populations have been exposed to strong genetic drift during additional population reduction(s).

Strong previous genetic drift has earlier been suggested from an absent to low allozyme variation in the Norwegian and Swedish populations, respectively (Gyllensten *et al.* 1983). The single unique mtDNA haplotype observed in Swedish red deer provide support for a severe and long lasting bottleneck (Paper II), but the similar level of microsatellite variation as in the Norwegian population is striking. One explanation may be that nuclear DNA, which is bi-parentally inherited, has been introduced by males. Our results suggest that males disperse to a higher degree and over longer distances than females (Paper V), and microsatellite variation may have been introduced by males while maternally inherited mtDNA not have been introduced by females. Red deer of non-indigenous origin has been introduced the last century at localities well separated from the distribution of the indigenous population in the southernmost part of Sweden (Ahlèn 1965; Gyllensten *et al.* 1983; Gill 1990), and the lack of agreement between the levels of variation in mtDNA and microsatellites suggests male immigration from these introduced localities (Paper II). If such new microsatellite variation has been brought in through male immigration, it also means that the magnitude of the Swedish bottleneck has been underestimated.

The three different bottleneck analyses all suggest population reductions. Low M-ratios suggest previous bottlenecks in all populations except the Hungarian, and support an old and common bottleneck, while the lower values in the Scandinavian populations also suggest other and more recent bottlenecks (Paper II). A signal of a recent population bottleneck of low severity or duration was thus identified in the Norwegian population, which corresponds very well with the known historical population reduction from 300 to 100 years ago (Paper II & IV). By comparison, the contemporary and longer lasting population reduction in the Swedish population was much more severe (Lønnberg 1906; Ahlèn 1965; Lavsund 1975; Lavsund 1990) and is not expected to yield such a signal since loss of both common and rare gene

copies during very severe bottlenecks will involve both reduced heterozygosity and allelic diversity (Maruyama & Fuerst 1985; Allendorf 1986; Amos & Balmford 2001). The hierarchical Bayesian analyses supported population reduction in all populations (Paper II), but suggested onsets of decline from 5000 to 10 000 years ago. This corresponds with the period around and after postglacial colonisation, and the different estimates of decline among the populations could reflect founder events of various magnitudes. However, the model assumes only one change in population size and the estimates may be biased by additional bottlenecks, like the more recent ones indicated by the other analyses.

Likely time periods for genetic bottlenecks in the Norwegian population were during colonisation after the last ice age around 8000 BP, during the known population reduction from the mid 1800th to the beginning of the 2000th century (Collett 1909; Ingebrigtsen 1924), and before the historic records of the 1600th century (Claussøn Friis 1599). In the BOTTLENECK analysis, the bottleneck signal is only expected to be detectable for a short period of time. Counted in generations this period is approximately 0.2 to 4 times the size of the bottleneck effective population size (Luikart & Cornuet 1998). During the decline from 300 to 100 years ago the Norwegian population counted only a few hundred individuals at the most extreme (Collett 1909; Ingebrigtsen 1924), but considering the reducing effects of harem polygony (50 - 70 %; Nunney 1993), age-structure and a skewed sex ratio, the effective population size would be much lower. A signal from a bottleneck with an effective size of around 100 would last for 20 to 400 generations. The generation time of male red deer have been estimated to 8 years (Gaillard 1992; Kruuk *et al.* 2002), and estimates would correspond with a time frame from 100 to 3200 years ago. However, females in good condition may reproduce at ages as young as 1.5 or 2.5 years (Haigh & Hudson 1993) and with an average generation time for both sexes of 6 years or less the bottleneck signal would last 2400 years or less. Other bottlenecks with a lower effective population size would last even shorter. Archaeological finds of red deer in Norway have been dated as far back as 6700 to 8000 BP (Mikkelsen & Høeg 1976; Hufthammer 2006; Rosvold 2006), and it seems reasonable that the identified signal of a recent bottleneck is not from the period of postglacial colonisation of Norway. Given the coincidence of present genetic structure with the population subdivisions during the period of decline from 300 to 100 years ago, which indicate strong genetic drift (Paper IV), this period seems likely to account for the signal.

Contemporary population reductions have been reported in many cervid species (Gill 1990), and has in the red deer been explained by increased pressures of predation and hunting (Collett 1877; Collett 1909; Jedrzejewska *et al.* 1997), climatic variation (Forchhammer *et al.* 1998; Mysterud *et al.* 2001b) and alterations in use of agricultural land (Ahlèn 1965; Mysterud *et al.* 2002). Such reductions may also have occurred during other periods after post-glacial colonisation, and seem especially likely at the start of the Iron Age and when modern fire arms were introduced in hunting. However, the severe bottleneck indicated by the single and unique observed haplotype in the Swedish population (Paper II), coincides well with the very low population numbers reported from this population the last four centuries (Lønnberg 1906; Ahlèn 1965; Lavsund 1975; Lavsund 1990). This underlines the importance of the common cervid population reductions from 300 years ago, as strong genetic drift also may involve loss of adaptive genetic variation important to present or future adaptation.

Empirical studies have indicated that heterozygosity is related to the capability of immediate adaptive response after a bottleneck while allelic diversity is expected to be important for the long-term response to selection (Allendorf 1986). During bottlenecks rare alleles are lost first and allelic diversity reduced, while the slower drift of common alleles entails a much slower reduction of heterozygosity (Nei & et al. 1975; Maruyama & Fuerst 1985). Genetic drift therefore tends to first remove variability that is of least current importance and loss of immediately important (adaptive) variation will always tend to lag behind loss of neutral variability (Amos & Balmford 2001). Genetic variation is usually assayed using neutral genetic markers, which reflect the passive loss from genetic drift, but are less informative about variation affecting fitness (Amos & Balmford 2001). Even though neutral variation might be an indicator of adaptive variation at high levels of variation, with low neutral levels populations may be either well or poorly adapted to their present environment (Hedrick 2001). Under the neutral theory of evolution most genetic variation is not under selection but maintained mainly by mutation and genetic drift (Kimura 1968; Kimura 1979; Kimura 1991). In really small populations where genetic drift is strong all gene variants may therefore be effectively neutral and even adaptations can potentially be lost (Hedrick 2000). Among several reviewed studies, genetic diversity was thus correlated with different fitness parameters when measured both as heterozygosity and allelic diversity by neutral markers (Reed & Frankham 2003). Considering the recent demographic and spatial expansion (Paper IV), the lost heterozygosity in the Norwegian population (Paper II) does not seem to have involved loss of any present adaptations. However, the last few centuries habitats and

environment have substantially changed, especially in temperate areas (IPCC 2001; IPCC 2007), and the reduced heterozygosity and allelic diversity could reflect loss of variation that was adaptive in a past environment or that may become adaptive with future changes.

The impact of translocating cervids among populations

Interbreeding and admixture between translocated and indigenous red deer

This study shows that red deer translocated from a continental environment one century ago has interbred with native Norwegian red deer in a temperate coastal climate and that population performance not was reduced in the subsequent hybrid stock (Paper III). Support for the interbreeding was gained from the genetically intermediate position of the hybrid stock and its possession of gene copies that were private to each of the two investigated parental populations. A high population growth since the translocation and an equal or larger average body mass of hybrids than in adjacent indigenous localities indicated that the population performance of the hybrid stock not has been reduced. Hybridisation and introgression seemingly have not had any negative effects, but this assessment also depends on the degree of admixture.

Our analyses demonstrate interbreeding but with various degrees of admixture between the introduced and native red deer (Paper III). Frequency-based estimators do not incorporate molecular information about the divergence of the observed alleles and are often biased towards even proportions compared to coalescence-based estimators, especially when using highly variable loci (Bertorelle & Excoffier 1998; Wang 2003). The higher admixed Norwegian proportion (69 %) with the coalescence-based estimator may therefore be more accurate than the proportion indicated by the frequency-based estimator (55 %). However, these moment estimators does not account for the effect of genetic drift since the time of the translocation, which may have been significant for several decades on Otterøya. One decade after the translocation the Otterøya stock only counted 100 individuals (Collett 1909) and because of very high culling it declined in the second and third decades (Ingebrigtsen 1924). The LEA analysis of admixture incorporates the effect of genetic drift (Chikhi *et al.* 2001) and showed an even higher Norwegian admixed proportion (85 %).

The translocated red deer were of Hungarian / German origin, and the German part of this cross has not been accounted for in our analyses (Paper III). If the German population at the time was genetically different from the Norwegian and Hungarian populations, this part of the blood cross may have introduced bias into our analyses. Within North-west Europe the present German population is genetically differentiated from both the Norwegian and Danish populations (Gyllensten *et al.* 1983; Strandgaard & Simonsen 1993) but has been much affected by genetic drift the last century (Kuehn *et al.* 2003). German red deer are also differentiated from the present Hungarian population in two enzyme coding genes along a geographic cline (Herzog & Gehle 2001). The Hungarian (Balkan) population is considered a separate subspecies with mtDNA variation distinct from North-west European red deer (Ludt *et al.* 2004) and the unaccounted German part was probably more similar to the Norwegian population. This would introduce a bias in our analyses involving underestimation of the Hungarian admixture proportion. However, translocations of red deer have been common within Europe (Hartl 1991; Hartl *et al.* 1991) and may obscure natural distributions (Hartl *et al.* 2003). The exact genetic differentiation within North-west Europe at the time is therefore uncertain, and the magnitude of any bias from the unaccounted German part of the translocated red deer on the admixture estimates can not be exactly determined.

Impact on the population performance of a hybrid stock

Hybridisation among closely related taxa may have many different outcomes. In some species hybridisation and introgression among subspecies rarely occur (Hansen 2002; Storfer *et al.* 2004), like between common and blackface impala (*Aepyceros melampus melampus & A. m. peters*; Lorenzen & Siegismund 2004). While in other species various degrees of hybridisation among subspecies have been documented (Jensen *et al.* 2005). Hybridisation is common within the red deer species complex (Hartl *et al.* 1995; Tate *et al.* 1998; Hartl *et al.* 2003) and negative effects are known between genetically and morphologically different taxa like the wapiti and the red deer (Asher 2000). Examples of negative consequences from other species are hybrid swamping between closely related taxa (Allendorf *et al.* 2004) and loss of local native genetic variation (Shaw *et al.* 1992; Garcia-Marin *et al.* 1999; Susnik *et al.* 2004; Latch *et al.* 2006).

Hybridisation events may also have important evolutionary consequences like speciation (Barton 2001; Burke & Arnold 2001) and one proposed conservational guideline has been to

conserve natural hybridisation events while trying to prevent anthropogenic ones (Allendorf *et al.* 2001). Intentional translocation of species within their ranges for conservation and management reasons have been common and have yielded positive results in many species (Griffith *et al.* 1989; Fischer & Lindenmayer 2000). The best source for such reinforcement of genetic variability is the most divergent and variable population possible without being adaptive differentiated (Storfer 1999; Maudet *et al.* 2002). Good examples are provided by the Scandinavian wolf (Vila *et al.* 2003), song sparrows (Keller *et al.* 2001), wild boar (Vernesi *et al.* 2003) and the Florida Panther (Keller & Waller 2002; Vila *et al.* 2003).

The genetic divergence between the taxa that hybridised on Otterøya was characterised by long genetic distances in microsatellite markers and mutational differences in mtDNA (Paper III). F_{st} values between the Hungarian and Norwegian populations for microsatellites and mtDNA sequences (Paper II) indicated great genetic differentiation (Hartl & Clark 1997). The translocated Hungarian / German deer originated from continental European environments below 48 and 54 °N respectively, and it seems likely they have different adaptations than the Norwegian population living in a coastal climate at 64.5 °N (Otterøya). However, any negative effects of hybridisation as expected between populations with different adaptations (Rhymer & Simberloff 1996; Storfer 1999; Burke & Arnold 2001) have not been observed in the hybrid stock on Otterøya. Rather, population growth seems to support the high phenotypic plasticity suggested for red deer (Geist 1998; Lister 2004). One decade after the translocation event the approximately initial 30 individuals had tripled to a population size of around 100 individuals (Collett 1909). Major culls involved a population decline in the second and third decade after the translocation (Ingebrigtsen 1924), but since then the Otterøya population has grown in size. The total cull *per decade* has since the 1930'ies developed from 134, 147, 223, 322, 536, 784 to 1071 in the 90'ies, and culminated with *annual culls* of 319 in 2005 and 2006 (pers.com. Aursand,T. Head of Forestry, Municipality of Namsos, North Trondheim County). The translocation event does thus *not* seem to have caused any loss of present local adaptations through replacement of native gene copies or break-up of co-adapted gene complexes, as could be expected after introgression from taxa with different adaptive variation (Rhymer & Simberloff 1996; Barton 2001; Burke & Arnold 2001). The genetic divergence between native and translocated red deer may rather have prevented local inbreeding and had positive effects in the almost diminished Otterøya population. Such results have for example been reported from translocations of white-tailed deer (*Odocoileus*

virginianus) in North America followed by population growth and expansion (DeYoung *et al.* 2003).

On Otterøya the interbreeding between genetically differentiated red deer resulted in a hybrid stock with a population performance that expressed by body mass is larger or similar to in adjacent indigenous Norwegian localities (Paper III). Moreover, the apparently similar body masses of the North Trønderlag localities were represented by unbalanced data, making discrimination of any weight differences to Otterøya difficult. Red deer body mass is generally strongly negatively related to density (Mysterud *et al.* 2001c) and the scarce data points did not allow an adequate comparison controlling for red deer density and habitat quality. In red deer positive effects of heterosis have been documented after interbreeding between distantly related individuals as increased lifetime reproduction and increased calf weights (Coulson *et al.* 1998; Slate *et al.* 2000a). A prolonged positive effect on body mass after an initial first hybrid generation heterosis effect could be explained by positive epistatic interactions among genes associated with heterosis or by increased heterozygosity involving hetrozygote overdominance. Heterosis could therefore be one reason for the heavier body mass on Otterøya compared to most indigenous localities. In addition, German and Hungarian red deer are relatively larger than Norwegian red deer (Lønnberg 1906; Collett 1909; Haigh & Hudson 1993) and additive effects of genetic variation expressing body size may also explain the intermediate size of Otterøya red deer. Moreover, much geographic variation in morphological traits like body size, antlers and scull length may be attributable to phenotypic plasticity related to differences in habitat and nutrition (Lister 1984; Geist 1998), as demonstrated by the huge increase in body size and antlers of west European red deer after being translocated to New Zealand (Huxley 1931). It is therefore difficult to separate the effects of additive and dominance genetic variance on the seemingly larger body mass of the hybrid population, but both explanations supports that genetic variation has been lost from the Norwegian red deer population.

Gene flow between a hybrid stock and the indigenous Norwegian population

The spreading and introgression of genetic variation from the hybrid stock on Otterøya into the adjacent indigenous Norwegian population has been low prior to the sampling years 2001 and 2002 (Paper III). For management and conservation purposes it was important to estimate this gene flow. In small isolated populations even a single migrant can have a huge genetic

effect (Vila *et al.* 2003) and male red deer can disperse very long distances (Paper V). Single cases of long-distance dispersal from Otterøya after the translocation event thus seem likely. The applied Bayesian method has been used by many others and allows identification of first and second generation dispersers (Berry *et al.* 2004; Evanno *et al.* 2005). Any introgression during previous generations may be difficult to detect and some gene flow from Otterøya during last century seems likely, especially during the last decades with rapid population growth. Experiments have shown that an increased red deer density involves increased aggression (Blanc & Thériez 1998) and with increasing density on Otterøya the probability of dispersal will increase. Considering the most recent population growth on Otterøya, dispersal after the years 2001 and 2002 should therefore be monitored closely, and management actions could be considered to avoid gene flow and introgression into the mainland indigenous population.

Whether or not the Otterøya hybrid population should be conserved or not is a difficult question. Considering the reduced genetic variation in the Norwegian mainland population (Paper II), one alternative could be to allow gene flow and enrichment from the more genetically variable and viable hybrid population on Otterøya (Paper III). European red deer does after all seem to possess enough phenotypic plasticity to meet environmental changes as great as from when translocated from the continent to the Norwegian coastal temperate climate. However, considering the recent climatic changes (IPCC 2001; IPCC 2007), genetic variation that once was adaptive in a past Norwegian climate may still exist in the Norwegian population, and may some day again become adaptive because of future changes. On Otterøya such gene variants may have been lost by introgression. As a precautionary rule one could argue that the indigenous gene pool of the Norwegian red deer population therefore should be conserved and gene flow prevented. Nevertheless, the situation offers an interesting experiment for studies of introgression from a genetically different population into an indigenous population and for the effects on fitness and population performance.

Gene flow and structure in a spatially expanding cervid population

Genetic drift and limitations to gene flow in the Norwegian red deer population

Within the Norwegian red deer population genetic variation in the 14 selected microsatellite loci showed a pronounced genetic structure after one century of demographic population growth and spatial expansion (paper IV). Genetic structure indicates that mating is not random within a population (Hartl & Clark 1997) and may result from genetic drift in areas among which gene flow is limited through isolation by geographical distance or physical barriers to dispersal. During spatial population expansion genetic differentiation may result from founder events and limited migration within the population (Austerlitz *et al.* 1997; Excoffier 2004), especially when dispersal is long distance (leptokurtic) and followed by isolation (Nichols & Hewitt 1994; Ibrahim *et al.* 1996). However, in the Norwegian red deer population the F_{st} values were higher and the genetic distances longer between the localities on the west coast than among the eastern localities, demonstrating a stronger genetic structure in the areas relict from the period before the recent population expansion than in areas that are newly established.

The similar levels of genetic variation between all localities indicate that genetic variation not has been lost during any founder events, or that subsequent gene flow has replaced such losses. Genetic differentiation from spatial expansion is observed in species like snails (Schweiger *et al.* 2004) and hamsters (Neumann *et al.* 2005), probably because of their low potential for within population migration. In other species with high motility like the Australian rabbit, such genetic differentiation is not observed (Zenger *et al.* 2003). Red deer are highly motile and during the Norwegian population expansion dispersal over long distances have both been observed (Collett 1912; Ahlèn 1965) and identified genetically (Paper III - V). However, some limitation to dispersal is indicated by the significant isolation by distance (Paper IV), and may be explained by a male-biased dispersal and female philopatry (Paper V). Long distance female dispersal into isolated new areas (Leptocurtic dispersal) may thus have been limited and / or migration among newly established areas has been too high for founder events to have incurred any genetic differentiation.

During the drastic population decline from 300 to 100 years ago the Norwegian red deer distribution was limited to a few areas along the western coastline (Fig. 4; Melchior 1834; Collett 1909; Ingebrigtsen 1924) and genetic differentiation during this period, followed by

spatial population expansion, may explain much of the observed overall genetic structure (Paper IV). First, the bottleneck signals identified in the Norwegian population suggest strong previous genetic drift (paper II and IV). During bottlenecks genetic drift is strong and genetic differentiation rapid (Nei & et al. 1975; Chakraborty & Nei 1977) and one likely period for such a bottleneck was during the major decline from 300 to 100 years ago. The significant excessive heterozygosity in both the pooled Norwegian sample (paper II) and in the separate localities (paper IV) shows that rare alleles are missing and thus suggests such *a recent bottleneck* (Cornuet & Luikart 1996). Secondly, the main genetic structure identified through Bayesian clustering of individuals corresponded well with the distribution of five areas relict from the decline from 300 to 100 years ago (Collett 1909; Ingebrigtsen 1924).

The division of localities along the west coast in separate and well defined clusters identified by Bayesian assignment indicates previous strong genetic drift and limited subsequent gene flow along the west coast (Paper IV). The extension of the clusters eastwards to localities in newly established areas shows the most probable dispersal routes during spatial population expansion, as for example in the second cluster stretching eastwards across a low mountain pass to a central and newly established locality situated in a valley facing eastwards (Fig. 4). By comparison the third cluster comprised only one locality isolated to the north by the Sognefjorden and by high mountain passes to the east and north. This picture is supported by the stronger genetic structure among separate localities along the west coast and by the short genetic distances among relict localities on the west coast and eastern localities in newly established areas. Previous strong and rapid genetic differentiation within areas relict from the population decline 300 to 100 years ago may thus explain much of the observed genetic structure, but to maintain this pattern subsequent gene flow must also have been limited through one century of population growth and spatial expansion.

Limitations to present gene flow were indicated by both the significant isolation by distance and the coincidence of genetic structure with geographical features (paper IV). Among the localities on the west coast, the genetic structure was stronger than anticipated from isolation by geographical distance, suggesting physical barriers to gene flow. The Norwegian coastline is deeply punctuated by steep-sided fiords, and divided by mountain ranges. These physical features coincided very well with the observed genetic dissimilarities, especially across the widest and steepest-sided of these fiords, the Sognefjorden (Fig. 4). Similar landscape features like Scottish lochs and steep mountain slopes have been found to significantly influence red

deer dispersal (Perez-Espona *et al.* 2008). Limitations of gene flow by Norwegian fiords and mountain ranges may thus both have entailed isolation and genetic drift during the decline from 300 to 100 years ago, and may explain how genetic structure have been maintained through one century of population growth and expansion.

The dispersal routes indicated by short genetic distances and Bayesian clusters also correspond very well with the distribution of high mountain ranges in southern Norway, which probably have dictated the pattern of dispersal during spatial expansion (Paper IV). The two main dispersal routes follow the northern and southern boundaries of central mountain ranges in southern Norway, one across low mountain passes and through valleys, and the other along the relatively flat southern coastline before ending up in the flat parts of the south-east. Here, the higher numbers of identified dispersers suggest a higher degree of admixture compared to among localities in the relict areas on the west coast. High elevation thus clearly constitutes a major barrier for gene flow, both for dispersal from western localities across central mountain ranges to the east, and together with fiords for the migration along the west coast. Physical barriers and significant isolation by distance have thus limited gene flow from breaking down the genetic structure from genetic drift during previous population contraction.

On a larger and longer lasting scale, the European red deer may conform to a model of genetic differentiation by contraction and spatial expansion. The reduced level of genetic variation in the Norwegian compared to other European red deer populations (Paper II), accordingly seem to verify that genetic variation can be differentiated and even lost during expansion phases (postglacial). The European red deer population may through time have gone trough several cycles of contraction and expansion, but on a scale too large to investigate for this study. That would have required a much more extensive sampling and additional genetic markers with slower mutation. A meta-population study of the common vole showed that such cycles may involve differentiation by expansion but that the level of global genetic variation is maintained (Berthier *et al.* 2006). This may also be the case for European red deer and would support how a high level of plasticity (Paper III) may have been maintained in the European population through contraction and expansion cycles.

The reasons for population contractions and expansions in red deer can be many. Red deer densities are influenced by predation and hunting (Collett 1877; Collett 1909; Jedrzejewska *et al.* 1997), climatic variation (Forchhammer *et al.* 1998; Mysterud *et al.* 2001b) and altered

uses of agricultural land (Ahlèn 1965; Mysterud *et al.* 2002). Climatic variation may affect population densities either directly through survival or indirectly by altering habitats or forage quality, both affecting body weights (Mysterud *et al.* 2001a; Mysterud *et al.* 2002). The recent climatic changes may thus be one of several plausible and synergistic explanations for the continuously increasing red deer distributions. What triggered the recent spatial expansion in the Norwegian population may be a combination of factors, but most probably also involved increased competition within the area of distribution at the beginning of last century when the population began to grow. Competition increases among hinds with increasing densities (Blanc & Thériez 1998) and we suggest that spatial expansion started when the population density reached a level where competition became too intense not to disperse. Continued population expansion may in the future break down the observed genetic structure in flat areas like eastern and south eastern Norway with high levels of admixture. However, the significant isolation by distance in the population also suggests limitations for the dispersal potential of red deer, which may be related to differences in dispersal between the sexes.

Sex-biased dispersal, philopatry and polygony in Norwegian red deer

Most of the genetic variation of the Norwegian red deer population could be divided into five subpopulations (Paper IV) and among these the sex-related differences in genetic structure and numbers of first generation dispersers demonstrated that dispersal is male biased in this spatially expanding ungulate population (Paper V). With female philopatry limitations to female dispersal may after reproduction translate to limited gene flow in the next generation, and theoretical studies show that philopatry in concert with polygony can involve genetic differentiation among social groups (Chesser 1991b; Chesser 1991a). Small-scale genetic structure has thus been observed in many philopatric and polygynous ungulate species (Mathews & Porter 1993; Petit *et al.* 1997; Coltman *et al.* 2003) and to the extreme in white-tailed and red deer (Purdue *et al.* 2000; Nussey *et al.* 2005). Other genetic consequences of sex-biased dispersal at the subpopulation and meta-population levels have been poorly documented (Prugnolle & de Meeus 2002) but recently large scale genetic structure in a colonial bat species was suggested to reflect female philopatry during stepwise colonisation of new areas (Chen *et al.* 2008).

Natal faithfulness towards mobile social units may have a similar function as philopatry on genetic differentiation, especially with polygynous mating systems (Chesser 1991a). With

females dispersing in matrilineal groups their dispersal among subpopulations may be more reduced than in males, and a reduced gene flow (after reproduction) may help explain the significant isolation by geographic distance demonstrated in paper IV, especially in the flat south-eastern areas of Norway without physical barriers for dispersal. We have explained the main genetic structure of the Norwegian red deer by previous genetic differentiation during population decline that has been maintained by limitations to gene flow during one century of spatial expansion (Paper IV). Generally, increasing dispersal with population growth and spatial expansion would be expected to break down such genetic structure, but limitations to gene flow by females dispersing in matrilineal groups (Paper V) may in addition to physical barriers (Paper IV) also explain how genetic structure has been maintained among the Norwegian red deer subpopulations.

Increasing aggression among hinds with density (Blanc & Thériez 1998) and increased competition and reduced reproduction success (Clutton-Brock *et al.* 1982a), probably involved an increased fitness in hinds that started to disperse when population densities became high at the beginning of last century. It is thus likely that the spatial expansion in Norway started because of an increasing population density in the localities along the west coast, and hinds started to disperse into new areas. By comparison, in the population on the Scottish island of Rhum, increased density has apparently not affected the dispersal rates of neither males nor females (Nussey *et al.* 2005), but this may be due to the lack of any new and un-habited areas to colonise. Within social lineages cooperation between relatives allow better exploitation of scars or heterogeneously distributed resources (Chesser *et al.* 1993) and on the onset of spatial population expansion from dense areas with high competition it seems likely that hinds would start to disperse in social groups.

Genetic differentiation from philopatry becomes maximal without inbreeding when a group of philopatric and related females breeds with one unrelated male (Chesser 1991a). Skew in reproduction success involves a decreased effective population size with regard to maintain the genetic variation expected from the observed population size (Nunney 1993; Hedrick 2000), explaining how polygony may increase genetic differentiation. The red deer has a polygamous mating system and most reproduction is performed by dominant males (Clutton-Brock *et al.* 1982b; Clutton-Brock *et al.* 1988; Pemberton *et al.* 1992). Earlier, paternity in red deer was decided by observing which stag the mothering hind was with at the time of conception calculated backwards from the time of birth (Gibson & Guinness 1980b; Gibson &

Guinness 1980a; Clutton-Brock *et al.* 1984). Genetic fingerprinting has confirmed that dominant stags stand for most of the reproduction, but solved only 30 percent of the paternities (Pemberton *et al.* 1992). Our results (Table 2) clearly confirm this skewness and demonstrate strong polygony in Norwegian red deer, which in concert with female philopatry are expected to involve genetic differentiation.

Several hypotheses have been proposed to explain why sex-biased dispersal have evolved, ranging from avoidance of competition for local resources (Clark 1978; Greenwood 1980) or mates (Hamilton 1967) and avoiding inbreeding (Bengtsson 1978; Dobson 1982), to an increased cooperation and enhanced use of local resources (Perrin & Lehmann 2001; Le Galliard *et al.* 2006). All concern local relationships, most often among relatives, and in a recent review of the interactions of these evolutionary models the key factor identified for the direction and intensity of sex-biased dispersal was the social system of each species (Lawson Handley & Perrin 2007). In the expanding Norwegian red deer population dispersing matrilineal groups would have arrived in new areas with un-depleted resources and any competition for local resources does not seem likely as a drive for sex-biased dispersal. Correspondingly, considering the female biased sex-ratio (1:9) of the population (Langvatn & Loison 1999), the increasing numbers of males reproducing with such a skew (Clutton-Brock *et al.* 1997) and that only 2.93 % of the males in the Norwegian population reach ages of more than four years (pers.com Rolf Langvatn Norwegian, Institute for Nature Research, Trondheim, Norway), male competition for females seems unlikely. By comparison, inbreeding avoidance and the benefits of social cooperation are more reasonable. During spatial population expansion an increasing distance from any dispersing matrilineal groups to core areas and unrelated males could involve an increased risk of inbreeding. Generally, inbreeding is avoided when one unrelated male breeds with a group of philopatric and related females (Chesser 1991a) and in philopatric species inbreeding avoidance probably have played an important role for the evolution of male-biased dispersal. Such a connection is found among polygynous sciurid mammals where the rate of male-bias increase with the level of sociality (Devillard *et al.* 2004). Dispersal of hinds in matrilineal groups during spatial population expansion could thus explain the observed long-distance male dispersal, as males would have to disperse further from their matrilineal group to avoid inbreeding.

Conclusions

The main conclusions of this study of genetic variation within the Norwegian red deer population and in comparison to other European populations, are:

- Genetic variation has been lost from the Norwegian red deer through strong genetic drift during previous population reduction(s). Bottleneck signals and absence of expected gene copies indicate an old bottleneck common to the European populations, but the level of genetic variation is comparably lower in the Scandinavian populations, suggesting additional loss during post-glacial colonisation events or during more recent bottlenecks. Historical records and genetic time estimates imply that probable periods include both postglacial colonisation and during a population decline from 300 to 100 years ago. Finally, genetic drift makes it difficult to assess the genetic origin of Norwegian red deer.

- Translocation of genetically distinct Hungarian / German red deer of presumably separate subspecies involved hybridisation and full admixture with indigenous Norwegian red deer. The lack of negative effects on the hybrid population performance after one century suggest that genetic structure within Europe does not reflect adaptive differentiation and support the high phenotypic plasticity suggested for European red deer. A similar to larger body size of hybrids compared to indigenous Norwegian red deer suggests introduction of additive genetic variation or heterosis associated effects, supporting that genetic variation has been lost from the Norwegian population. A low present gene flow from the hybrid population has involved limited introgression into the Norwegian mainland population, but considering the ongoing growth in both populations this picture may change.

- The expanding Norwegian red deer population has a significant genetic structure, but not from founder events during expansion. Division of genetic variation within the Norwegian population coincides very well with its distribution from 300 to 100 years ago, providing support for the timing of the recent bottleneck and explaining the present genetic differentiation. Genetic structure is presently being maintained by limitation of gene flow by geographic distance and physical barriers like mountain ranges and the broad and steep west coast fiords. Limited female dispersal from philopatry probably affect gene flow though isolation by distance, and may and in concert with male polygony help maintain genetic structure on a small and large scale among subpopulations.

Perspectives

To better assess the demographic history of the Norwegian red deer population and achieve more precise estimates of the magnitude and time of previous bottlenecks, present genetic variation could in further studies be compared with archaeological material from different time periods. Further, in the still growing and spatially expanding Norwegian red deer population new studies on how the genetic structure change with time may give further insight into limitations to dispersal by philopatry compared to by physical barriers. More importantly, the status of the indigenous Norwegian population should be further evaluated through continued monitoring of dispersal and introgression from the growing hybrid population on Otterøya, as well as from Swedish immigrants with a translocated non-indigenous origin. Considering the previously lost genetic variation from Norwegian and Swedish red deer, it seems important to assess the role of introgression on the genetic variation for the adaptability of these populations, especially considering the future potential changes of the environment. To determine how introgression may affect individual fitness and population performance in hybrid red deer, the effects of ancestry of hybrids could be related to body size through additional sampling and recording of weight of Otterøya deer.

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