Paper IV

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

1 ***Genetic structure in an expanding cervid population after population***

2 ***reduction***

3 H. Haanes[1], K. H. Røed[1], Ø. Flagstad[2] and O. Rosef[3]

4 1. *Norwegian School of Veterinary Science, Dep of Basic Sciences and Aquatic Medicine, PO-8146 Dep, N-0033 Oslo,*

5 *Norway.*

6 2. *Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway*

7 3. *Telemark University College, Dep of Environmental and Health Studies, N-3800 Bø in Telemark, Norway.*

8

9 *Corresponde; H.Haanes, address 1, Email: hallvard.haanaes@veths.no, Phone: 004722964788, Fax: 004722964786*

10

13

14 *Wordcount main text: 3437*

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

Abstract

The Norwegian red population (*Cervus elaphus*) was from the mid 1800th to the early 2000th century drastically reduced in size and distribution but has the last century expanded both demographically and spatially. We have investigated genetic variation, differentiation and admixture in this spatially expanding ungulate population, using 14 microsatellites. The present genetic structure is moderate to strong and the average F_{st}-value 0.08. Low M-ratios indicate loss of genetic variation in all localities and signals of a recent bottleneck was identified in 14 of 15 localities. Genetic distances between the localities indicate two main routes of dispersal during expansion, one from the north-west and one from south-west. Bayesian assignment tests verify a break of the dataset in two, and demonstrate 99.9 % probability for the presence of five sub-populations, which coincide well with five relict populations from before the onset of expansion.

Introduction

The demography and distribution of species vary through time and space (Begon et al. 1996) and greatly affect levels of genetic variation and population structure (Hartl and Clark 1997; Hedrick 2000). Many species have a history of reduced or fragmented population size, often followed by demographic growth and spatial expansion. During the Pleistocene, extensive climatic oscillations and rapid changes in the distribution of continental ice sheets resulted in successive shifts in the demography and geographical range of many species. Founder events and isolation after successive leading edge expansions involved loss of genetic variation and increased homozygosity in many of the newly colonised areas (Hewitt 2000; Hewitt 2001). Recently, scientists have established significant climatic changes since pre-industrial times that also have involved population fluctuations and range shifts for many species, especially in temperate areas (IPCC 2001 2007).

Loss of genetic variation during bottlenecks (Nei and et al. 1975; Chakraborty and Nei 1977), may in very small and fragmented populations involve reduced adaptability and increased risk of extinction (Lande 1988; Soulé and Mills 1992). The genetic effects of demographic population expansions have been well examined (Slatkin and Hudson 1991; Beaumont 1999; Chakraborty and Kimmel 1999), but recently attention has been drawn to the effects of spatial population expansion on genetic structure (Ray et al. 2003; Excoffier 2004). With a limited number of dispersing individuals genetic variation may be lost during colonisation because of founder effects and subsequent bottlenecks (Hedrick 2000). In expanding populations new demes may become genetically differentiated because of genetic drift depending on the migration rates (Austerlitz et al. 1997; Excoffier 2004), especially when dispersers move long distances and become isolated (Nichols and Hewitt 1994; Ibrahim et al. 1996). However, the homogenising effect of migration on genetic structure is large and when genetically different subpopulations merge, the level of genetic variation can increase as a result of the isolate break (Hartl and Clark 1997). Thus, under a spatial range expansion, genetic variation may be lost from founder effects and subsequent

64 bottlenecks, but may also increase due to merging of genetically different demes. Genetic structure

65 is one of the parameters for estimation of effective population size and is thus important for making

66 management and conservation plans (Wang and Caballero 1999; Nunney 2000).

67   The red deer (*Cervus elaphus*) is an ungulate species and a highly priced game and trophy

68 for hunting. In Norway the red deer (*Cervus elaphus atlanticus*) has existed at least since the sub-

69 boreal period (Collett 1909; Ahlèn 1965) and written records document an abundant population

70 distributed throughout most of Southern Norway until approximately year 1750 (Friis 1874; Collett

71 1877). In the mid 1800th century the Norwegian red deer population declined drastically and until

72 the beginning of the last century it was confined to only five or six locations along the western coast

73 (Fig.1) counting a few hundred individuals in total at the most extreme (Collett 1909; Ingebrigtsen

74 1924). In southernmost Sweden a separate red deer population was reduced even more (Lønnberg

75 1906) and has for the last 150 years been confined to a very small population (Ahlèn 1965). Since

76 the beginning of last century, and especially after 1950, the Norwegian red deer population has

77 expanded from the western coast localities, demographically as well as spatially. It is now common

78 in most parts of southern and central Norway with a total population size ranging from 100 000 to

79 120 000 individuals in 1997 (Langvatn 1988; Forchhammer et al. 1998; Langvatn 1998; Fig. 1).

80 Many reasons have been suggested for these population fluctuations, including high pressures of

81 predation and hunting from the middle of the 1800th century (Collett 1877; Collett 1909), as well as

82 temporal changes in the use of agricultural land (Ahlèn 1965; Mysterud et al. 2002). We have

83 assessed the present genetic variation of the Norwegian red deer population to investigate for any

84 recent bottlenecks and to address the effect of spatial population expansion on genetic structure.

85

Methods and materials

Sampling and laboratory procedures

88 Between 2000 and 2004 we sampled blood or tissue from 419 wild Norwegian red deer from 24

89 municipalities across Norway (Fig.1). Samples from some of the municipalities were pooled to

90 obtain a minimum of 15 individuals in each of totally 15 localities (Table1). In general, the western

91 localities are distributed within the area where the Norwegian red deer population was confined

92 from the mid 1800th to the early 2000th century, whereas the eastern localities are recently

93 established populations outside this area (Table 1; Fig. 1).

94   Genomic DNA was isolated from whole blood and muscle tissue (Qiagen, DNeasy KIT).

95 Previous investigations have indicated a generally low level of genetic variation in Norwegian red

96 deer (Baccus et al. 1983; Gyllensten et al. 1983; Røed 1998; Røed and Midthjell 1998). We selected

97 14 polymorphic microsatellite loci that show Mendelian heredity in Norwegian red deer (Haanes et

98 al. 2005). These were CSSM03 (Moore et al. 1994), OarCP26 (Ede et al. 1995), RT5 (Wilson et al.

99 1997), SRCRSP10 (Bhebhe et al. 1994), NVHRT73 and NVHRT48 (Røed and Midthjell 1998),

100 McM58 (Hulme et al. 1994), OarFCB193 and OarFCB304 (Buchanan and Crawford 1993),

101 BM5004, BM888, BMC1009, BM4208 and BM4107 (Bishop et al. 1994). The microsatellites were

102 amplified on a GeneAmp PCR System 9600 (Applied Biosystems) in 10μL reaction mixtures with

103 30–60 ng of genomic template DNA, 2 pmol of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM

104 Tris-HCl, 0.2 mM dNTP, and 0.5 U of *AmpliTaq* DNA polymerase (Applied Biosystems).

105 Thermocycling parameters after denaturation at 94ºC for 5 min were 30 cycles with 95ºC for 1 min,

106 55ºC for 30 sec and 72ºC for 1 min, followed by an additional 10 min at 72ºC. The PCR products

107 were then separated by size with capillary electrophoresis (ABI310, Applied Biosystems) and

108 electromorphs were genotyped with GENOTYPER1.1.1 (Applied Biosystems).

109

Population genetics analysis

Each of the 15 localities was assessed through exact tests of Hardy-Weinberg equilibrium across the

14 loci using GENEPOP 3.4 with the default settings (Raymond and Rousset 1995). Sequential

Bonferroni correction was used to adjust for repeated tests (Rice 1989). To assess differences in

genetic variation among localities we used FSTAT 2.9.3 (Goudet 2001) to calculate the allelic

richness (El Mousadik and Petit 1996) and the gene diversity (Nei 1987) for each locality across

loci. To assess possible impact on genetic variation, recent bottlenecks were addressed using a one-

tailed Wilcoxon test (10,000 iterations) as implemented in the software BOTTLENECK (Cornuet

and Luikart 1996), which tests if the observed gene diversity is higher than expected at mutation-

drift equilibrium from the number of observed alleles in each locality across loci. Most

microsatellites fit a two-phase model of mutation (TPM) better than a strict stepwise mutation

model (Di Rienzo et al. 1994) and we therefore used a TPM model with the default settings of 30%

variation from the infinite allele model (IAM) and 70% from the stepwise-mutation-model (SMM).

Secondly, the M-ratio (Garza and Williamson 2001) was calculated for each locality as the ratio

between the observed number of alleles and the number of repeats in the allele size range of each

locus, averaged across loci. This would give an indication of any loss of alleles during any recent

population reductions.

   F-statistics (e.g., Weir 1996) as implemented in FSTAT, with Bonferroni adjusted

significance tests, were used to assess genetic structure within (F_{IS}) and among (F_{ST}) localities.

Pairwise geographical distances among localities (km) were calculated from longitude and latitude

(http://jan.ucc.nau.edu/~cvm/latlongdist.html), and isolation-by-distance was assessed in

GENEPOP by testing the correlation between geographical distances and pairwise $F_{ST}/(1 - F_{ST})$

values. We used a Mantel test (Mantel 1967) in the implemented program ISOLDE (10000

permutations) to test for significance. To further assess genetic differentiation we used the genetic

distance D_A (Nei et al. 1983), which is based on the geometric distances of populations on a

135 multidimensional hypersphere independently of any mutation models (Nei 1987; Nei 2000).

136 Distances (D_A) were calculated between each pair of localities and a neighbour joining (NJ) tree

137 built with 1000 bootstrap replicates across loci with the software POPULATIONS (available at

138 http://www.pge.cnrs-gif.fr/bioinfo/populations/index.php). The tree was visualised by the software

139 TREEVIEW (Page 1996).

140   To assess genetic structure without prior knowledge of sampling locations, we used

141 Bayesian assignment as implemented in STRUCTURE (Pritchard et al. 2000). The log likelihood of

142 our data set ($\ln \Pr(X \mid K)$) was estimated given different numbers of genetic clusters ($K \in [1,7]$ using

143 an admixture model ($\alpha=1$, $\alpha_{max}=50$) with uniform priors, correlated allele frequencies (Falush et al.

144 2003), 100000 burnin cycles and 500000 MCMC iterations. For each K-value, STRUCTURE

145 estimates the mean log likelihood of the data set ($\ln \Pr(D \mid K)$) from several runs and uses Bayes'

146 theorem to compute the probability of each K-value. Since higher K values often involve runs with

147 higher posterior probabilities but a higher variance among runs (Evanno et al. 2005), we also

148 identified K from the marked increase of variance among runs and calculated delta K to identify

149 breakpoints in the data set. Genetic structure and the degree of admixture among the 15 localities

150 were then interpreted from their membership in each of the K clusters and from the probabilities of

151 individual assignment to these clusters.

152

153 **Results**

154 For each of the sampled localities, all loci were in Hardy-Weinberg equilibrium after sequential

155 Bonferroni adjustment, except for the locus BM4208 in locality SE3 (p=0.0004). We found a total

156 of 74 alleles, an average gene diversity of H=0.61 (SE=0.02), an allelic richness of A=4.1 (SE=0.3),

157 a F_{is} value of 0.018 (SE=0.01) and an overall F_{st} value of 0.08 (SE=0.02) (Table 1). We found

158 significantly higher gene diversities than expected from the observed number of alleles in all of the

159 localities except N1 (p=0.09; Table1), suggesting deviations from mutation-drift equilibrium after

7

160   loss of alleles during recent bottlenecks. This was verified by the low M-ratio values in all

161   localities, as a M-ratio smaller than 0.68 can be assumed to represent a recent reduction in

162   population size (Garza and Williamson 2001). However, the M-ratio varied little between the

163   localities, which had quite similar amounts of genetic variation (Table1), with no differences in

164   either allelic richness or gene diversity (One-way ANOVA; F=0.24, F=0.32, respectively; p=0.99

165   for both parameters). This, together with the low number of observed alleles in the whole data set

166   (74) compared to the number of alleles which could be expected from the allele size range

167   combined for all loci (142), suggests a general loss of alleles from the whole data set.

168   Among the 120 pairwise F_{ST}-values between localities, 102 were significant after sequential

169   Bonferroni correction, ranging from 0.004 to 0.188 (Table 2). Many of these indicated moderate

170   (0.05-0.15) to strong (0.15-0.25) genetic structure (Wright 1978; Hartl and Clark 1997).

171   Differentiation was particularly strong between the southernmost and northernmost localities along

172   the coastline, and isolation-by-distance was highly significant (p<0.0001). The NJ tree (Fig. 2)

173   showed a main dichotomy between localities north and south of Sognefjorden, the largest fjord in

174   Norway. The locality at Sognefjorden (W) showed an intermediate position in the NJ tree and was

175   moderately, albeit significantly, differentiated from all other populations (Table 2). The south and

176   south-eastern localities (S, SE 1-4) clustered with the south-western locality (SW) with high

177   bootstrap values, indicating that these newly established localities were founded by dispersers from

178   the southern part of the coastline. Similarly, the recently established eastern locality (E) clustered

179   with the localities north on the coastline (N 1-4), indicating that its founders originated from the

180   northern area.

181   The STRUCTURE algorithm showed that a partitioning of the genetic variation into five

182   clusters was most probable (P(K=5|D)=0.999). Moreover, a much higher variance among runs with

183   K> 5, indicate that five clusters represent the main genetic structure (Table 3). This was supported

184   by a high delta value for K=5. Another high delta value demonstrated a major break in the data set

185 with K=2, reflecting a dichotomy of genetic divergence between localities north and south of the

186 Sognefjorden (W; Fig.3), as could be expected from spatial expansion from the most differentiated

187 localities. With K=5, the proportionate cluster membership was for most of the localities much

188 higher in one of the clusters (Table 4) and divides the data geographically into three clusters of

189 localities along the north-western coast (clusters 1, 2, 3), one cluster from the south-western to the

190 south-eastern coast (cluster 4) and one cluster in south-eastern to central Norway (cluster 5).

191 Localities E and C, both newly established localities, had a strong affinity towards the northern and

192 north-western areas. Fig. 3 shows the probabilities of individual assignment to each of the five

193 clusters and visualises their geographic distribution. A large proportion of the individuals as well as

194 localities have a genetic signature typical for one specific cluster. However, some individuals and

195 localities have a divided membership between two or three clusters, indicating a mixed origin from

196 different sources. Such a pattern is particularly pronounced in the south-eastern localities.

197

Discussion

199 Our analysis clearly showed that the Norwegian red deer is not a panmictic population. The many

200 significant F_{st} values indicated limited gene flow among most of the sampled localities, especially

201 between the northern and southern localities, and demonstrated the presence of moderate to strong

202 genetic structure. We found that isolation-by-distance was significant among the localities, a pattern

203 compatible with limited gene flow and random genetic drift within the localities. The STRUCTURE

204 algorithm showed that a partitioning of the genetic variability into five clusters was most probable

205 (99.9%), even though also indicating a higher hierarchical dichotomous breakpoint between

206 localities north and south of Sognefjorden. Thus, we interpreted the expanding Norwegian red deer

207 population to consist of five sub-populations, four distributed from north to south along the coast

208 and the fifth situated in the central and south-eastern part of the sampled area.

209   Generally speaking, genetic structure in a spatially expanding population may result from

210   both long distance dispersal and limited migration among demes (Nichols and Hewitt 1994; Ibrahim

211   et al. 1996; Austerlitz et al. 1997; Excoffier 2004). In our particular case, however, the demographic

212   history of the Norwegian red deer population may also have played a significant role. After the

213   population size had been reduced from the mid 1800th the Norwegian red deer were in the early

214   2000th century distributed among five or six main locations along the coast from the north to the

215   south-west (Collett 1909; Ingebrigtsen 1924; Langvatn 1988). Four of these are concurrent with the

216   four subpopulations we identified along the coastline, indicating that the observed genetic structure

217   was formed by genetic drift during the population decline after the mid 1800th century.

218   Unfortunately, we did not sample the last one or two locations from this period. One of these,

219   situated at the Bergen Peninsula, could be concurrent with our fifth STRUCTURE cluster. Indeed,

220   three of the south-eastern localities (SE2, SE3, SE4) that are located close to the Bergen Peninsula

221   had a high membership in this cluster suggesting partial foundation from this area. We conclude

222   that Norwegian localities became genetically differentiated through genetic drift during the major

223   decline, and that gene flow during the subsequent range expansion has not been large enough to

224   erase this genetic structure.

225   The significant deviations from the heterozygosity expected with the observed number of

226   alleles in 14 out of 15 localities indicated a recent bottleneck (Cornuet and Luikart 1996). Loss of

227   alleles from a bottleneck was also indicated by the low M-ratio's in all the localities. The

228   Norwegian red deer population was abundant prior to the 1800th century (Friis 1874; Collett 1877;

229   Collett 1909) and one likely period for a recent bottleneck was during the decline between the mid

230   1800th and early 2000th century. Since we did not record any difference in genetic variation between

231   old and recently established populations, the recent bottlenecks recorded in the young localities is

232   probably not due to separate founder events, but may be the same signal following the founding

233   individuals. However, the low number of observed alleles compared to possible repeats in the allele

234   size range of microsatellite loci may also suggest older and more severe bottlenecks prior to the

235   abundant period in the 1500th and 1600th centuries, probably more severe than during the population

236   reductions after the mid 1800th century (Haanes et al in prep).

237   The Norwegian red deer population has recently expanded drastically both demographically

238   and spatially, especially the last fifty years (Langvatn 1988; Forchhammer et al. 1998). By

239   comparison, the neighbouring Swedish red deer population has not recovered to the same degree

240   after the 1800th and 1900th century decline and still does not count more than 1200 to 1500

241   individuals in central Scania (pers.com. Anders Jarnemo, Swedish University of Agricultural

242   Sciences). Analyses of the Swedish population (Vänersborg) indicated very low gene flow into the

243   Norwegian population and assignment of individuals showed no admixture across the border

244   (Haanes et al. in prep). With the major population expansion the last century, we expected more

245   gene flow and less present genetic structure in the primary area around the five relict populations.

246   However, Southern Norway is divided by a central mountain range, which may constitute a barrier

247   for dispersal from the west to the east. Moreover, the coastline is deeply punctuated by broad fiords

248   with steep edges, constituting possible barriers for north-south dispersal along the coast.

249   Accordingly, the significant F_{st} values between localities separated by fiords and inlets, like the

250   island locality N2 and adjacent coastal localities (N3 and N4), indicated that water constitutes a

251   barrier for red deer dispersal. Thus, even though long distance dispersal is common and red deer are

252   frequently observed swimming (Collett 1909; Ingebrigtsen 1924; Langvatn 1988), our results

253   showed that migration and dispersal along the coast have been limited. Similarly, the pattern of

254   dispersal into the areas of new establishment seems to indicate that the massive mountain ranges of

255   Norway have constituted barriers for red deer dispersal. The low genetic divergence between the

256   south-western (SW) and south-eastern (SE) localities as well as between the north-western (NW)

257   and eastern (E) localities demonstrate two main routes of range expansion, one from the area on the

258   north-western coast towards south-east and one from the area on the south-western coast around the

259   coastline and into south-eastern Norway. In addition, the close relationship between the central (C)

260   and north-western (NW1, NW2) localities indicated foundation from the north-western coast by a

261   third dispersal route across the northern part of the central Norwegian mountain range

262   (Jotunheimen), where mountains are less alpine than further to the south. Dispersal from the area

263   adjacent to Sognefjorden (W) seems to have been limited, presumably because the massive alpine

264   mountains in the central parts of Jotunheimen have functioned as a major barrier. The low degree of

265   admixture between localities in the north, the south and the Sognefjord locality (W) further

266   supported our interpretation of limited gene flow along the coast and across the highest mountain

267   ranges of Norway. Equivalently, the high degree of admixture in the south-east and the central

268   localities probably has been the result of higher migration and dispersal in this area which has fewer

269   barriers of massive mountains and no large fiords with steep sides. Management could therefore

270   take the identified barriers to dispersal into consideration and attempt to avoid genetic drift in the

271   more isolated subpopulations on the west coast.

272   Finally, the population density of Norwegian red deer is positively correlated to the North

273   Atlantic Oscillations (Forchhammer et al. 1998; Forchhammer et al. 2001; Mysterud et al. 2001).

274   Thus, under a scenario of continued climatic change (IPCC 2001, 2007), we anticipate that the red

275   deer population in Norway will continue to expand both demographically and spatially. The

276   expanding parts from the north-western and the southern coast most likely will meet in the near

277   future. Future studies of Norwegian red deer could include modelling of divergence times and may

278   contribute even further to our understanding of the genetic effects of a spatial population expansion,

279   where the whole process from complete isolation in small relict populations, through the expansion

280   phase to a large population can be observed and analysed.

281

References

Ahlèn I (1965) Studies on the red deer, *Cervus elaphus* L. Scandinavia. III. Ecological investigations. Viltrevy 3: 177-376

Austerlitz F, JungMuller B, Godelle B, Gouyon PH (1997) Evolution of coalescence times, genetic diversity and structure during colonization. Theor Popul Biol 51: 148-164

Baccus R, Ryman N, Smith MH, Reuterwall C, Cameron D (1983) Genetic variability and differentiation of large grazing mammals. J Mammal 64: 109-120

Beaumont MA (1999) Detecting population expansion and decline using microsatellites. Genetics 153: 2013

Begon M, Harper JL, Townsend CR (1996) Ecology, individuals, populations and communities, third edn. Blackwell, Oxford

Bhebhe E, Kogi J, Holder DA, et al. (1994) Caprine microsatellite dinucleotide repeat polymorphism at the SR-CRSP-6, SR-CRSP-7, SR-CRSP-8, SR-CRSP-9 and SR-CRSP-10. Anim Genet 25: 203

Bishop MD, Kappes SM, Keele JW, et al. (1994) A genetic linkage map for cattle. Genetics 136: 619-639

Buchanan FC, Crawford AM (1993). Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB226 and OarFCB304 loci. Anim Genet 24: 145

Chakraborty R, Nei M (1977) Bottleneck Effects on Average Heterozygosity and Genetic Distance with Stepwise Mutation Model. Evolution 31: 347-356

Chakraborty R, Kimmel M (1999) Statistics of microsatellite loci: estimation of mutation rate and pattern of population expansion. In: Goldstein DB, Schlotterer C (eds.) Microsatellites; evolution and applications, Oxford University Press, Oxford, pp. 139-150

Collett R (1877) Bemerkninger til Norges Pattedyrfauna (in Norwegian). Nyt Magazin for Naturvidenskaberne 22

310   Collett R (1909) Hjorten i Norge (*Cervus elaphus atlanticus*), nogle biologiske meddelelser (in Norwegian). Bergens

311   museums Aarbok 6

312   Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks

313   from allele frequency data. Genetics 144: 2001-2014

314   Di Rienzo A, Peterson AC, Garza JC, et al. (1994) Mutational processes of simple-sequence repeat loci in human

315   populations. Proc Natl Acad Sci USA 91: 3166-3170

316   Ede AJ, Pierson CA, Crawford AM (1995) Ovine microsatellites at the OarCP9, OarCP16, OarCP20, OarCP21,

317   OarCP23 and OarCP26 loci. Anim Genet 25: 129-130

318   El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan

319   tree [*Argania spinosa* (L) Skeels] endemic to Morocco. Theor Appl Gen 92: 832-839

320   Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software

321   STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

322   Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the

323   infinite-island model. Mol Ecol 13: 853-864

324   Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked

325   loci and correlated allele frequencies. Genetics 164: 1567-1587

326   Forchhammer MC, Clutton-Brock TH, Lindstrøm J, Albon SD (2001) Climate and population density induce long-term

327   cohort variation in a northern ungulate. J Anim Ecol 70: 721-729

328   Forchhammer MC, Stenseth NC, Post E, Langvatn R (1998) Population dynamics of Norwegian red deer: density-

329   dependence and climatic variation. Proc R Soc Lond B Biol Sci 265: 341-350

330   Friis JA (1874) Tilfjelds i ferierne (in Norwegian). Cammermeyer, Christiania, No

331   Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol

332   10: 305-318

333   Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices. Release 2.9.3.2. Available

334   from http://www.unil.ch/izea/softwares/fstat.html.

335   Gyllensten U, Ryman N, Reuterwall C, Dratch P (1983) Genetic differentiation in four European subspecies of red deer

336   (*Cervus elaphus* L.). Heredity 51: 561-580

337   Haanes H, Rosef O, Veiberg V, Røed KH (2005) Microsatellites with variation and heredity applicable to parentage and

338   population studies of Norwegian red deer (*Cervus elaphus atlanticus*). Anim Genet 36: 454-455

339   Hartl DL, Clark AG (1997) Principles of population genetics, 3rd edn. Sinauer, Sunderland, US

340   Hedrick PW (2000) Genetics of populations. Jones and Bartlett, Boston

341   Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405: 907-913

342   Hewitt GM (2001) Speciation, hybrid zones and phylogeography - or seeing genes in space and time. Mol Ecol 10: 537-

343   549

344   Hulme DJ, Silk JP, Redwin JM, Barendse W, Beh KJ (1994) Ten polymorphic ovine microsatellites. Anim Genet 25:

345   434-435

346   Ibrahim KM, Nichols RA, Hewitt GM (1996) Spatial patterns of genetic variation generated by different forms of

347   dispersal during range expansion. Heredity 77: 282-291

348   Ingebrigtsen O (1924) Hjortens utbredelse i Norge (in Norwegian). Bergens Museums Aarbok 1922-1923 Naturvitensk.

349   Række 6: 1-58

350   IPCC (2001) Third assessment report of the Intergovernmental Panel on Climate Change. Cambridge Univ Press,

351   Cambridge

352   IPCC (2007) Fourth assessment report of the Intergovernmental Panel on Climate Change. http://www.ipcc.ch

353   Lande R (1988) Genetics and demography in biological conservation. Science 241: 1455-1460

354   Langvatn R (1988) Hjortens utbredelse i Norge - en oversigt (in Norwegian). Villreinen 1: 1-8

355   Langvatn R (1998) Hjortens erobring av Norge. In: Brox KH (ed) Brennpunkt natur (in Norwegian), Tapir. Cop,

356   Trondheim, No, pp.49-71

357   Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27: 209-220

358   Moore SS, Byrne K, Berger KT, et al. (1994) Characterization of 65 bovine microsatellites. Mamm genome 5: 84-90

359   Mysterud A, Stenseth NC, Yoccoz NG, Langvatn R, Steinheim G (2001) Nonlinear effects of large-scale climatic

360   variability on wild and domestic herbivores. Nature 410: 1096-1099

361   Mysterud A, Langvatn R, Yoccoz NG, Stenseth NC (2002) Large-scale habitat variability, delayed density effects and

362   red deer populations in Norway. J Anim Ecol 71: 569-580

363   Nei M, et al. (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1-10

364   Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency

365   data. J Mol Evol 19: 153-170

366   Nei M (1987) Molecular evolutionary genetics. Columbia University Press, NY

367   Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency

368   data. J Mol Evol 19: 153-170

369 Nichols RA, Hewitt GM (1994) The Genetic Consequences of Long-Distance Dispersal During Colonization. Heredity
370   72: 312-317

371 Nunney L (2000) The limits to knowledge in conservation genetics; The value of effective population size. In: Clegg
372   MT, Hecht MK, MacIntyre RJ (eds) The limits to knowledge in conservation genetics, Kluwer, NY, pp. 179-194

373 Page RDM (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. Comput Appl
374   Biosci 12: 357-358

375 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data.
376   Genetics 155: 945-959

377 Ray N, Currat M, Excoffier L (2003) Intra-deme molecular diversity in spatially expanding populations. Mol Biol Evol
378   20: 76-86

379 Raymond M, Rousset F (1995) GENEPOP (Version-1.2) - Population-Genetics Software for Exact Tests and
380   Ecumenicism. J Hered 86: 248-249

381 Rice WR (1989) Analyzing Tables of Statistical Tests. Evolution 43: 223-225

382 Røed KH (1998) Microsatellite variation in Scandinavian Cervidae using primers derived from Bovidae. Hereditas 129:
383   19-25

384 Røed KH, Midthjell L (1998) Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. Mol Ecol 7:
385   1773-1778

386 Slatkin M, Hudson RR (1991) Pairwise Comparisons of Mitochondrial-DNA Sequences in Stable and Exponentially
387   Growing Populations. Genetics 129: 555-562

388 Soulé ME, Mills S (1992) Conservation genetics and conservation biology: a troubled marriage. In: Sandlund OT,
389   Hindar K, Brown AHD (eds) Conservation of biodiversity for sustainable development, Scandinavian University
390   Press, Oslo, pp. 55-69

391 Wang JL, Caballero A (1999) Developments in predicting the effective size of subdivided populations. Heredity 82:
392   212-226

393 Weir BS (1996) Genetic data analysis II: methods for discrete population genetic data. Sinauer, Sunderland, US

394 Wilson GA, Strobeck C, Wu L, Coffin J (1997) Characterization of microsatellite loci in caribou *Rangifer tarandus*, and
395   their use in other artiodactyls. Mol Ecol 6: 697-699

396 Wright S (1978) Evolution and the genetics of populations. University of Chicago Press, Chicago

Figure legends

399   Figure 1.   Sampling localities of Norwegian red deer. The rectangles show combinations to obtain

400   at least 15 individuals per locality and shaded areas the approximate distribution of the

401   population around 1900 (Collett 1909; Langvatn 1998).

402

403   Figure 2.   Unrooted Neighbour Joining tree based on pairwise D_A–distances among the 15 sampled

404   localities. Bootstrap values above 50 are indicated (1000 replicates).

405

406   Figure 3.   Individual posterior probabilities of Bayesian assignment to each of two to five clusters

407   (different colours) among 419 red deer in each of 15 localities (separated by vertical

408   lines) analysed by STRUCTURE with $K \in [2,5]$.

409

Table 1. Sample size (n), allelic richness (A_R), unbiased gene diversity (H) and inbreeding coefficient (F_{is}) for each of 15 Norwegian red deer localities, arranged relative to the central mountain range. Probabilities of no deviation from mutation-drift equilibrium assuming two-phase mutation (TPM) in a Wilcoxon test are also given in additon to M-ratios' for each locality. Standard errors (SE) in brackets.

Area	Locality	n	A_R	H	F_{is}	p │ TPM	M-ratio
North							
N1	Åfjord	16	4.1 (.4)	0.62 (.04)	0.014	0.097	0.47 (.05)
N2	Hitra	37	3.7 (.4)	0.56 (.05)	0.017	0.029	0.53 (,06)
N3	Skaun, Meldal, Rennebu	27	3.5 (.3)	0.58 (.05)	-0.032	0.029	0.46 (.06)
N4	Sunndal	32	3.6 (.3)	0.58 (.05)	0.031	0.007	0.47 (.06)
North-West							
NW1	Hareid	20	3.8 (.4)	0.59 (.05)	0.052	0.003	0.47 (.06)
NW2	Eid	17	3.9 (.4)	0.63 (.04)	0.030	0.000	0.45 (.06)
Central							
C	Skjåk	23	4.0 (.3)	0.64 (.03)	0.007	0.002	0.49 (.05)
West							
W	Fjalar, Gaular	32	3.8 (.3)	0.61 (.04)	-0.039	0.000	0.48 (.05)
South-West							
SW	Tysvær	23	3.6 (.4)	0.59 (.04)	0.012	0.008	0.57 (.07)
South							
S	Farsund, Hægebostad, Birkenes, Evje	25	3.7 (.3)	0.61 (.03)	0.071	0.002	0.50 (.05)
South-East							
SE1	Drangedal	30	3.7 (.2)	0.62 (.03)	-0.042	0.000	0.49 (.05)
SE2	Nome	68	3.6 (.2)	0.61 (.03)	0.027	0.000	0.48 (.05)
SE3	Hjartdal, Notodden	25	3.6 (.2)	0.60 (.04)	0.073	0.025	0.49 (.05)
SE4	Flå, Hol, Gol	29	3.6 (.2)	0.61 (.04)	0.065	0.000	0.50 (.05)
East							
E	Rendal, Elverum	15	3.8 (.3)	0.65 (.03)	0.006	0.001	0.47 (.05)

Table 2. Population differentiation among 15 red deer localities as measured by pairwise F-st

values. Levels of significance are given after sequential Bonferroni correction (NS=not

significant, * p<0.1, ** p<0.05, *** p<0.01).

	N1	N2	N3	N4	NW1	NW2	C	W	SW	S	SE3	SE2	SE1	SE4	E
N1		.032	.020	.007	.030	.016	.021	.078	.166	.148	.117	.095	.132	.095	.006
N2	**		.061	.054	.078	.050	.030	.076	.163	.147	.134	.098	.133	.077	.041
N3	NS	***		.005	.041	.062	.055	.122	.188	.175	.154	.121	.169	.136	.021
N4	NS	***	NS		.033	.045	.039	.121	.178	.161	.133	.107	.150	.124	.026
NW1	**	***	***	***		.015	.028	.090	.167	.160	.122	.091	.128	.102	.022
NW2	NS	***	***	***	NS		.009	.045	.161	.138	.119	.085	.110	.081	.032
C	NS	***	***	***	NS	NS		.056	.122	.104	.086	.053	.078	.044	.013
W	***	***	***	***	***	***	***		.114	.105	.087	.077	.088	.046	.072
SW	***	***	***	***	***	***	***	***		.008	.032	.025	.009	.060	.101
S	***	***	***	***	***	***	***	***	NS		.014	.023	.004	.041	.099
SE3	***	***	***	***	***	***	***	***	**	NS		.013	.009	.027	.073
SE2	***	***	***	***	***	***	***	***	***	***	NS		.008	.021	.042
SE1	***	***	***	***	***	***	***	***	NS	NS	NS	NS		.039	.080
SE4	***	***	***	***	***	***	***	***	***	***	*	***	***		.053
E	NS	***	NS	***	**	***	NS	***	***	***	***	***	***	***	

5   Table 3. Mean posterior probabilities averaged across n runs for the data set comprising 419

6   Norwegian red deer, given different numbers of subpopulations in the dataset ($K \in [1,7]$).

7   The most likely number of clusters (K=5; p>0.99 according to Baye's theorem) is marked

8   in boldface and delta K values given.

9

K	n	Ln Pr (D│ K)	SD	ΔK
1	10	-14034.7	0.4	
2	10	-13223.8	1.8	332.3
3	10	-13013.9	2.5	65.1
4	10	-12926.6	3.4	28.8
5	10	**-12868.5**	2.6	156.1
6	10	-13175.6	147.3	2.8
7	10	-13244.1	215.6	4.1

Table 4. Proportion of membership for the 15 sampled localities of Norwegian red deer to each of five clusters in a Bayesian assignment test using uniform priors and an admixture model. The highest proportion for each locality is underlined.

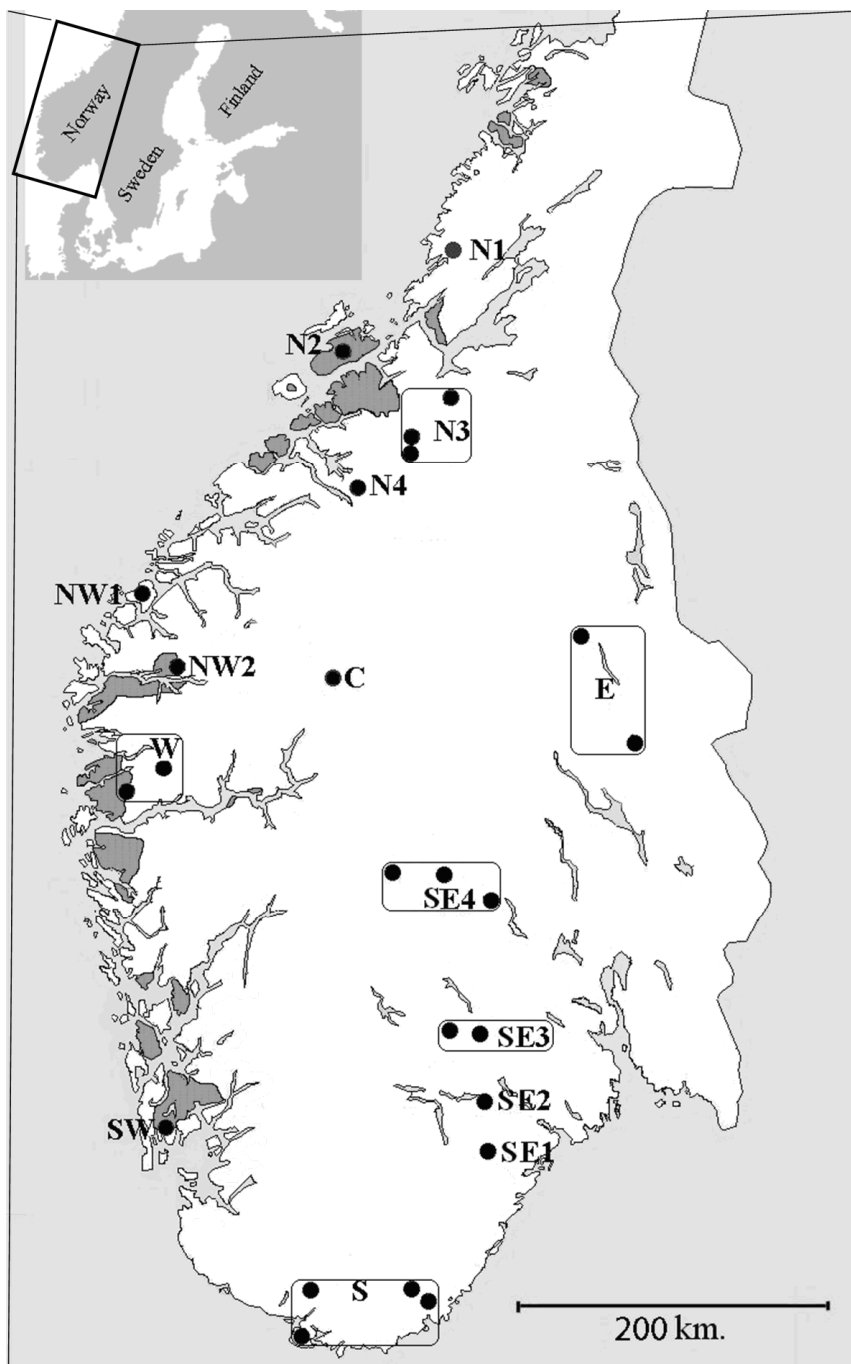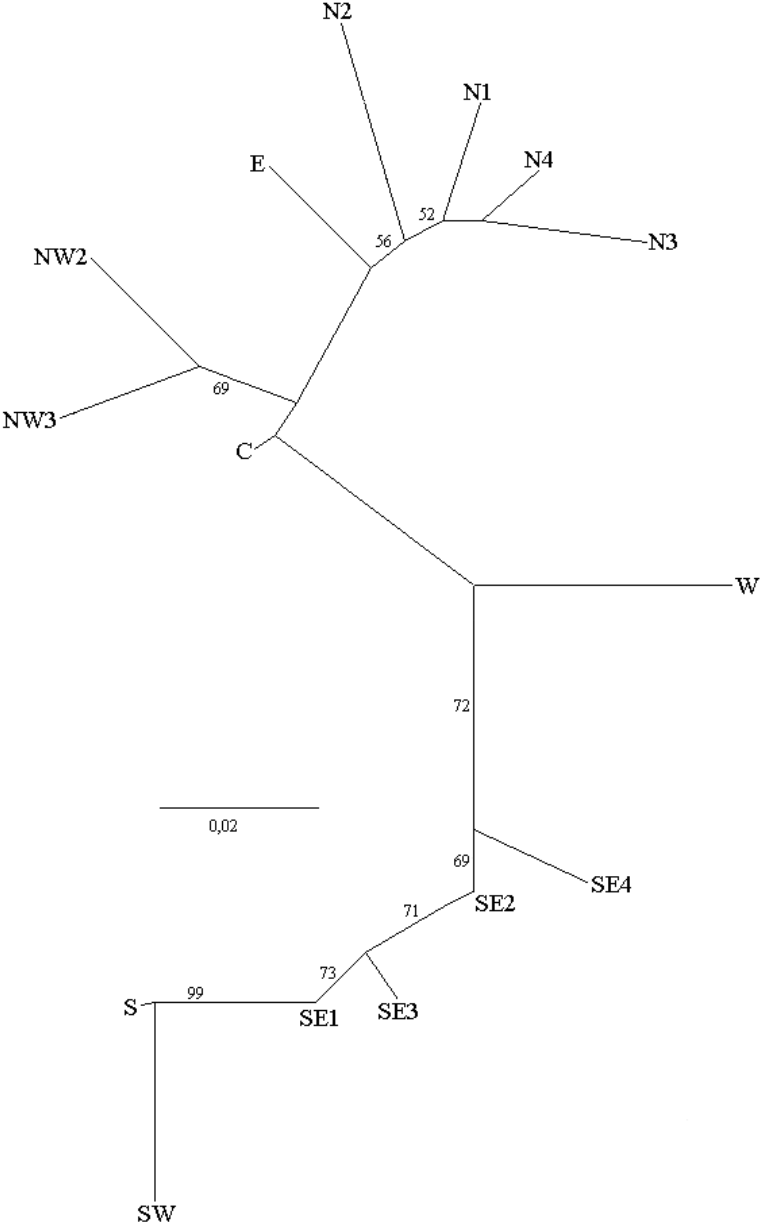|	1	2	3	4	5
N1	0.502	0.384	0.078	0.018	0.019
N2	0.646	0.194	0.081	0.023	0.056
N3	0.755	0.195	0.024	0.013	0.013
N4	0.703	0.249	0.018	0.013	0.018
NW1	0.162	0.633	0.170	0.015	0.021
NW2	0.079	0.572	0.287	0.033	0.029
C	0.136	0.525	0.118	0.056	0.165
W	0.030	0.053	0.801	0.024	0.093
SW	0.015	0.015	0.046	0.773	0.151
S	0.018	0.021	0.062	0.645	0.255
SE1	0.016	0.029	0.131	0.567	0.257
SE2	0.044	0.077	0.104	0.396	0.380
SE3	0.038	0.040	0.127	0.390	0.405
SE4	0.039	0.036	0.237	0.102	0.587
E	0.355	0.289	0.073	0.088	0.196

Figure 1.

Figure 2.

Figure 3.