Paper III

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Consequences on genetic diversity and population performance of introducing continental red deer into the northern distribution range

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Abstract

Game management has the last centuries involved translocations of non-native individuals to reinforce local native populations of many species, but there are few quantitative studies of potential negative effects on population viability as expected when taxa with different local adaptations hybridise. The European red deer has been subject to particularly many instances. Around 1900 a total of 17 red deer of Hungarian (*Cervus elaphus hippelaphus*) and German (*C. e. germanicus*) origin were introduced onto the island Otterøya in Norway where few native red deer (*C. e. atlanticus*) remained (n~13). To assess interbreeding, the present stock on Otterøya and the indigenous Norwegian and Hungarian populations were characterised in 14 microsatellite loci and in the control region of mtDNA. An intermediate level of genetic variation in the Otterøya population and the presence of population specific alleles from either the indigenous Norwegian or the Hungarian population demonstrate that the introduced red deer interbred with the native. Even distributions of one indigenous and one non-indigenous mtDNA haplotype in the Otterøya population and two point estimates of admixture indicate similar genetic contributions from the two parental populations into the hybrid stock. Low numbers of migrants identified with Bayesian assignment tests demonstrate low recent gene flow from Otterøya into the Norwegian mainland population. Finally, the body mass of red deer on Otterøya was similar or larger than in the adjacent indigenous Norwegian stocks, demonstrating that population performance has not been reduced in the hybrid stock and that gene flow has not had any negative effects.

Keywords: Translocation, hybrid stock, introgression, admixture, dispersal

Introduction

Species are distributed along environmental gradients (Begon *et al.* 1996), and gene frequencies may change locally when adaptations develop in populations by natural selection (Endler 1992; Strickberger 1996). However, locally adapted populations admix to an increasing degree because of the range shifts in many species associated with the present use of land and recent climatic changes, especially in temperate areas (IPCC 2001; IPCC 2007). In addition, hybridisation rates increase worldwide because of human-mediated translocations and habitat modifications, causing the extinction of native species, subspecies and locally adapted populations (Allendorf *et al.* 2001). When genetically different taxa hybridise, local adaptations may be lost from the native taxa by introgression of non-indigenous alleles and loss of local alleles and co-adapted gene complexes (Rhymer & Simberloff 1996; Barton 2001; Burke & Arnold 2001). Gene flow between populations in different environments may therefore constrain local adaptation and lower the short-term fitness of native populations (Storfer 1999). Alternatively, increasing levels of genetic variation from an isolate break (Hartl & Clark 1997) 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).

The last centuries, game management has involved translocations of non-native individuals of many species into former habitats or native populations (Hartl 1991; DeYoung *et al.* 2003; Kruckenhauser & Pinsker 2004). In Europe translocations have been especially common among red deer (*Cervus elaphus*) populations to re-establish or reinforce local populations and 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). Many of these populations are morphologically different and have been described as separate subspecies (Lønnberg 1906; Whitehead 1972; Whitehead 1993), and even though some argue for one common European

78 subspecies (Groves & Grubb 1987; Polziehn & Strobeck 2002), there is genetic differentiation
79 among these populations (Gyllensten *et al.* 1983; Kuehn *et al.* 2003; Ludt *et al.* 2004). The impact
80 of such translocations should thus be evaluated considering the growing knowledge on ungulate
81 population genetics and phylogeography (Randi 2005).
82 In Norway, the red deer has existed at least since the sub-boreal period (Ahlèn 1965) and
83 low levels of genetic variation documented by allozyme and microsatellite analyses suggest long-
84 time isolation and previous bottlenecks (Gyllensten *et al.* 1983; Røed 1998; Haanes *et al.* in prep$_1$).
85 Historically, red deer were distributed across most of southern Norway (Friis 1874; Collett 1877)
86 but after 1750 a major decline limited the population to only a few locations along the west coast
87 (Collett 1909; Ingebrigtsen 1924). To our knowledge only one translocation of non-indigenous red
88 deer into the Norwegian population has occurred in recent times. On the island Otterøya in the
89 northern range of its distribution, the local red deer stock was almost extirpated in 1898, counting
90 only 12-14 individuals including three or four stags (Collett 1909; Collett 1912). Therefore, to
91 avoid local extinction, 17 red deer of a captive cross between the Hungarian (*C. e. hippalphus*) and
92 German (*C. e. germanicus*) subspecies, including at least one stag, were translocated to Otterøya
93 and into the native Norwegian subspecies (*C. e. atlanticus*) from 1900 to 1903 (Die-Woche 1902;
94 Collett 1909; Finsberg 1934). Ten years after, the Otterøya population counted 100 individuals
95 (Collett 1909) and since the 1930's culls have increased considerably to an annual cull of 319 in
96 2006. Hungarian and German red deer both reported have a larger body size and antlers compared
97 to the Norwegian subspecies (Lønnberg 1906; Collett 1909; Haigh & Hudson 1993).
98 We have quantified the level of gene flow between the native and introduced continental
99 red deer deriving from each of the two major lineages in the European red deer. The success of the
100 German / Hungarian cross was evaluated from the genetic impact on the Otterøya population, and
101 recent gene flow into the mainland population was estimated. We also aimed to assess the
102 performance of red deer from the mixed stock at the island Otterøya compared with pure stocks

from both the mainland and another island (Hitra) in the region. As a proxy for performance, we used body mass, which for Norwegian red deer is closely correlated with age of first reproduction (Langvatn *et al.* 2004) and survival during the first critical winter (Loison *et al.* 1999).

Materials and methods

Study area

The Otterøya is situated at 64.5°N and 11.3°E. It is 143 km^2 and separated from land by sounds that are mostly 1 - 2 km wide but only 500 metres in a couple of short stretches. Yearly average precipitation is 1440 mm and the average yearly temperature is 3.7°C. Much of the island is covered by boreal rainforest characterised by Norway spruce and pine, and cultivated areas are scattered (~4 km^2). The 'counting area' is the area of suitable red deer habitat (Mysterud *et al.* 2002), constituting only 98.5 km^2 as much area is elevated above the tree line (~400 m). The island constitutes winter pasture for 600 reindeer and has scarce stocks of roe deer (*Capreolus capreolus*) and moose (*Alces alces*).

Sampling and genetic analyses

From 2001 to 2003 muscle tissue was sampled from 20 Hungarian red deer, 40 red deer from Otterøya and 136 red deer from adjacent (mainly mainland) areas in Norway. Hungarian red deer samples from three locations were taken as representative of the introduced continental red deer cross. Indigenous Norwegian red deer were sampled from six localities adjacent to Otterøya (Fig. 1) at distances from 83 to 236 km (mean = 151; SE = 22). The genetic variation of the Norwegian localities except locality No2 had been previously described (Haanes *et al.* in $prep_2$).

Genomic DNA was isolated from whole blood and muscle tissue (Qiagen DNeasy KIT). We selected 14 polymorphic microsatellite loci that show Mendelian heredity in Norwegian red deer (Haanes *et al.* 2005). These were CSSM03 (Moore *et al.* 1994), OarCP26 (Ede *et al.* 1995), RT5 (Wilson *et al.* 1997), SRCRSP10 (Bhebhe *et al.* 1994), NVHRT73 and NVHRT48 (Røed & Midthjell 1998), McM58 (Hulme *et al.* 1994), OarFCB193 and OarFCB304 (Buchanan & Crawford 1993), BM5004, BM888, BMC1009, BM4208 and BM4107 (Bishop *et al.* 1994). They were amplified on a GeneAmp PCR System 9600 (Applied Biosystems) in 10µL reaction mixtures with 30–60 ng of genomic template DNA, 2 pmol of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 0.2 mM dNTP, and 0.5 U of *AmpliTaq* DNA polymerase (Applied Biosystems). After denaturation at 94ºC for 5 min, 30 cycles of amplification with 1 min at 95ºC, 30s at 55ºC and 1 min at 72ºC were followed by 10 min extention at 72ºC. The PCR products were then separated by size with capillary electrophoresis (ABI310) and electromorphs genotyped with GENOTYPER1.1.1 (both Applied Biosystems).

For a subsample of each population a 463 base pair region of the mitochondrial D-loop adjacent to the $tRNA^{pro}$ gene was amplified using the primers 5'-AATAGCCCCACTATCAGCACCC (L15394) and 5'-TATGGCCCTGAAGTAAGAACCAG (H15947) (c.f. Flagstad & Røed 2003). Thirty-five cycles of amplification with 30s at 94°C, 30s at 60°C and 45s at 72°C were preceded by a 2 min pre-denaturation step and followed by a final 7 min extension. Amplifications were performed in 10 µl volumes containing 1.5mM $MgCl_2$, 200 µM of each dNTP, 4 pmol of each primer and 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems). PCR products were purified using ExoZapit™ (Amersham Biosciences). Sequencing was performed using BigDye terminator cycle sequencing chemistry on an ABI 3100 instrument, and sequences aligned manually using SeqScape version 2.0 (Applied Biosystems).

Data on red deer body mass

The data on red deer performance derive from annual autumn harvest lasting from 10 September to 15 November in the period 1965 to 2006. The date and location (municipality) of harvest together with biological information on sex, body mass and the mandibles for each of 20161 deer were provided by the hunters. Body mass is recorded as dressed mass, which is live mass minus head, skin, viscera, bleedable blood and metapodials, constituting about 58% of live mass and highly correlated with total mass. Using the mandibles, calves and yearlings were aged based on patterns of tooth eruption (Loe *et al.* 2004), whereas older animals were aged using annuli in the cementum of the first incisor (Hamlin *et al.* 2000). The data used are a subset of a larger dataset from the whole of the southwest coast (e.g., Mysterud *et al.* 2001, Pettorelli *et al.* 2005). The subset was selected according to distance from the focal area of Otterøy. Harvest data may be prone to bias in some cases due to hunter selectivity (see in depth discussion in Mysterud *et al.* 2008). However, as the tradition for hunting is similar between our focal areas, this is unlikely to be important for the spatial contrast of focus here.

We have good knowledge of the performance of these populations, being heavily affected by density dependence and also by climate (the North Atlantic Oscillation (NAO); Mysterud *et al.* 2001). As a measure for density, we use the proxy "number of harvested animals" per km^2 of red deer habitat (Table 1). Despite this being a crude index, the increase in harvest has been 5 fold due to a huge density increase, and over time the measure has shown to correlate well with other direct measures of density (cfr. Mysterud *et al.* 2007). As an index of climate, we used the station-based winter (Dec-Mar) index of Hurrell (1995).

Statistical analyses of genetic data

Random mating within populations was assessed by exact tests of Hardy-Weinberg equilibrium across the 14 microsatellite loci using GENEPOP 3.4 with default settings (Raymond & Rousset

1995). Significance levels were sequentially Bonferroni adjusted for repeated tests (Rice 1989). To investigate genetic variation we calculated the number of private alleles, allele richness (El Mousadik & Petit 1996) and gene diversity (Nei 1987) in each population across loci using FSTAT 2.9.3 (Goudet 2001). Genetic variation in the control region of mtDNA was calculated using ARLEQUIN 2.000 (Schneider *et al.* 2000).

For the microsatellite data, genetic structure within (F_{is}) and among (F_{st}) populations (Weir 1996) was assessed using FSTAT with sequential Bonferroni adjustment. Genetic distances D_A (Nei *et al.* 1983) among the populations (with Norwegian localities separated) were calculated and a Neighbour joining tree built with 1000 bootstraps on loci using POPULATIONS (available at http://www.pge.cnrs-gif.fr/bioinfo/populations/index.php). The tree was visualised using TREEVIEW1.6.6 (Page 1996).

To address the degree of interbreeding between the continental red deer and the native island population at the time of the introduction, we used the microsatellite data and estimated the proportionate admixture from two of the parental populations into the Otterøya population using present day Norwegian and Hungarian populations as representatives. We used ADMIX1.0 (Bertorelle & Excoffier 1998) to calculate bootstrap estimates of two admixture estimators (1000 replicates), the allele frequency based m_C (Chakraborty *et al.* 1992) and the coalescent based m_Y (Bertorelle & Excoffier 1998). To include the possible affect of genetic drift on the admixture estimates we also estimated admixture using LEA (Chikhi *et al.* 2001) with 200 000 Monte Carlo Marcov Chain (MCMC) iterations.

To assess recent gene flow between the Norwegian mainland and Otterøya we used the microsatellite data and Bayesian individual assignment as implemented in STRUCTURE2.0 (Pritchard *et al.* 2000) with uniform priors, an admixture model (α=1, α_{max}=50), three clusters (K=3), correlated allele frequencies (Falush *et al.* 2003), 100000 burnins cycles and 500000 MCMC iterations.

Statistical analyses of body mass data

We analysed variation in (ln) body mass of red deer with a combination of additive and linear models. Based on previous results, we ran separate models on males and females due to do their strongly different life histories (Mysterud *et al.* 2001). In addition, it is particularly important to model the age effect correctly, since so much of the variation is found in that parameter. Therefore, and since we are not directly interested in the age effect, we tried both age as a class variable (0, 1, 2, 3, 4, ≥5 yrs; providing good fit for females, Mysterud *et al.* 2001) and also modelled the age effect with smoothing splines (for males; Yoccoz *et al.* 2002) that are very flexible using the library (mgcv) in R (Wood 2006). Similarly, for any growth or decay during the autumn, we used a spline for the "date of harvest" effect so we can make sure this do not bias our results. Due to age-dependent effort in the rutting of males, we ran this as an interaction between age and date of harvest (cfr. Yoccoz *et al.* 2002).

Our focal factor is the spatial contrasts between Otterøy and adjacent areas. We therefore used "Treatment" contrasts, i.e., comparing levels of a factor with one specific level – a reference level (the Otterøy stock). However, environmental conditions may not be comparable, so that any spatial difference may not be due to genetic effects, but rather reflect either density or habitat quality. We therefore entered the density index (described above) and the NAO (climate index) to control for annual fluctuations.

Results

Genetic variation

In each investigated population all microsatellite loci were in Hardy-Weinberg equilibrium after sequential Bonferroni adjustment, except BM4208 in the Hungarian population and BM5004 in the

Hungarian and Otterøya populations. Across the 14 microsatellite loci 151 alleles were found in the three investigated populations. Among these, 73 alleles were population specific for either the indigenous Norwegian (7), Otterøya (13) or Hungarian (56) population (Tables 2 & 3). Another 25 microsatellite alleles found in the Otterøya population were conspecific with either the Hungarian (16) or the Norwegian (9) population (Table 2), strongly suggesting inheritage from both populations. The Hungarian red deer had by far the highest gene diversity, allele richness and number of private alleles, the Otterøya population was intermediate, while the indigenous Norwegian population was the least genetically variable (Table 3). Five mtDNA haplotypes were found in each of the indigenous Norwegian population and the Hungarian population. Genetic divergence for all the Hungarian haplotypes except one was demonstrated by from one to three inserts. The two mtDNA haplotypes in the Otterøya population were evenly distributed ($n_A = 9$, $n_B = 7$). One was a Norwegian haplotype, previously reported (Genbank AF291888), whereas the other was indigenous to Otterøya.

The microsatellite data demonstrated limited gene flow and strong genetic structure from significant F_{st} values between the Otterøya population and both the Hungarian (0.13) and the indigenous Norwegian population (0.19). Equivalently, long genetic distances (D_A) of respectively 0.40 and 0.33 showed that the Otterøya population was genetically different and intermediate of both investigated parental populations (Fig. 2). By comparison a higher F_{st} value (0.23) and a longer genetic distance (0.48) was found between the Norwegian and Hungarian populations. Within the indigenous Norwegian population much shorter genetic distances (Fig. 2) and intermediate F_{st} values (0.08) indicated moderate genetic structure. A very low inbreeding coefficient ($F_{is} = 0.001$) indicated little genetic structure within the Otterøya population.

The bootstrap estimate of admixture based on microsatellite allele frequencies indicated an even admixture between indigenous Norwegian and Hungarian red deer into the Otterøya population with proportions of $m_C = 0.55$ (SE = 0.06) and $m_C = 0.45$ (SE = 0.06) admixed from the

Norwegian and Hungarian populations respectively. The coalescent based estimator indicated a higher proportion admixed from the Norwegian population with $m_Y = 0.69$ (SE = 0.05) and a lower proportion from the Hungarian population with $m_Y = 0.31$ (SE = 0.04). The admixture estimate including genetic drift showed more skewed proportions with 0.85 from the Norwegian (P1) and a 0.1 from the Hungarian population (Fig. 3).

The individual assignment tests showed very limited recent gene flow between the Norwegian mainland and Otterøya populations (Fig. 5). All three populations had high memberships (> 0.9) to each of the three predefined clusters and all individuals had high corresponding membership coefficients ($q > 0.90$), except nine. Three indigenous Norwegian and four Otterøya individuals were assigned with a lower admixture coefficient ($0.69 < q < 0.90$), indicating migration by more ancestral generations. Two individuals sampled on Otterøya were assigned to the "Norwegian cluster" with q values of 0.99 and 0.61, indicating first or second generation migration.

Body mass variation

The models of female ($r^2 = 0.853$) and male ($r^2 = 0.724$) body mass variation gave largely similar results concerning the spatial contrast, after controlling for strong effects of age, date of harvest, density and the NAO (Table 5). Deer from Otterøya were larger than deer from coastal municipalities in Sør-Trøndelag (population P3; municipalities 1612, 1613 and 1622) including the island of Hitra (population P5), but not to all of the inland municipalities (1635 and 1636). Body mass of red deer from Otterøy was of comparable body mass to those from the mainland municipalities (1714, 1721) in Nord-Trøndelag (population P4). The interaction term between age and municipality could not be entered due to unbalanced data, however, similar differences between municipalities was obtained when only analysing variation for a specific age class (yearlings).

Discussion and conclusions

This study demonstrates that the German / Hungarian red deer introduced onto Otterøya around 1900 interbred with the resident native Norwegian population. Variation in both microsatellite and mtDNA demonstrate that it is a genetic intermediate with heritage from both the native Norwegian and the Hungarian population. Extensive interbreeding between these presumed subspecies (Lønnberg 1906; Whitehead 1972; Whitehead 1993) was evident from the estimators of admixture and from the similar frequencies of the two haplotypes found on Otterøya. However, none of the negative effects of introgression that could be expected were observed. Rather the body mass of red deer on Otterøya was similar or larger than those of indigenous Norwegian inland and coastal localities.

Potential limitations and biases on the admixture estimates

The allele frequency-based estimator yielded close to even proportions of admixture from the indigenous Norwegian and Hungarian populations. However, frequency-based estimators are often biased towards even proportions compared to the coalescent based estimator M_Y, which also incorporates molecular divergence between alleles and parental populations (Bertorelle & Excoffier 1998; Wang 2003). The coalescence-based estimator yielded skew proportions of admixture, as could be expected from influence by the unaccounted German part of the introduced red deer cross. Considering that the resident population on Otterøya had a similar size and sex ratio as the group of introduced German / Hungarian red deer (Collett 1909; Ingebrigtsen 1924; Finsberg 1934), the German part would constitute one quarter of admixture with free interbreeding. This is roughly proportionate with and could help explain the deviations from even admixture in the coalescence-based estimator and the genetic drift model (LEA). A genetically distinct German contribution was also apparent from the private alleles and the mitochondrial

haplotype observed in the Otterøya stock but not in neither the Norwegian nor the Hungarian population. Further, also the similar frequencies of the two haplotypes on Otterøya, which were of Norwegian and non-indigenous origin, provide support for an even admixture. The even admixture in both mtDNA and microsatellite data further indicates similar contributions from both sexes. In spite of high F_{st} values and long genetic distances between the parental populations, our data support an even admixture and free interbreeding between the introduced German / Hungarian red deer and the native Norwegian population.

Performance of the hybrid stock

The Norwegian, German and Hungarian red deer populations are located at different latitudes (northern boundaries at 64.5, 54 and 48 °N) and genetic variation indicates that the indigenous Norwegian population has been isolated for a long time (Gyllensten *et al.* 1983; Røed 1998). It therefore seems a reasonable hypothesis to assume that these populations may have developed different local adaptations. However, the negative effects on population viability expected when taxa with different local adaptations hybridise (Rhymer & Simberloff 1996; Allendorf *et al.* 2001; Burke & Arnold 2001), were in spite of its mixed origin not observed in the Otterøya hybrid stock. Even though this natural experiment does not offer an adequate evolutionary time frame, our results seem to support the high phenotypic plasticity suggested for red deer (Geist 1998; Lister 2004), rather than different local adaptations in different environments. Ten years after the introduction the Otterøya population counted 100 individuals (Collett 1909) and since then annual culls have increased considerably, reaching 319 in 2005 and 2006. This follows the general trend of expansion in the Norwegian population (Forchhammer *et al.* 1998; Langvatn, 1998), which is partly explained by climatic variation (Forchhammer *et al.* 1998; Mysterud *et al.* 2001) and the altered use of agricultural land (Ahlèn 1965; Mysterud *et al.* 2002). The increase in population size of the hybrid stock could also reflect positive effects on population viability, as could be expected

323 from heterosis and reduced inbreeding involved with hybridisation of more closely related taxa
324 (Haig 1998; Freeland 2005). The increased level of genetic variation in the small initial founding
325 population on the Otterøya may thus have prevented the negative effects of inbreeding and
326 counteracted loss of genetic variation from random genetic drift. In red deer heterosis effects have
327 been documented as both increased lifetime reproductive success and calf body mass (Coulson *et*
328 *al.* 1998; Slate *et al.* 2000), and could explain the heavier body mass on Otterøya compared to
329 most indigenous localities. However, German and Hungarian red deer have a relatively larger body
330 mass than Norwegian red deer (Lønnberg 1906; Collett 1909; Haigh & Hudson 1993) and may
331 indicate effects from additive genetic variation. On the other hand, much geographic variation in
332 body size is attributable to phenotypic plasticity affected by habitat and nutrition (Lister 1984;
333 Geist 1998), as demonstrated by the huge increase in body size and antlers of west European red
334 deer after translocation to New Zealand (Huxley 1931). Further, red deer body mass is generally
335 strongly negatively related to density (Mysterud *et al.* 2001) and the higher density on Otterøya
336 may have obscured differences to the inland localities with apparently similar body mass. These
337 comparisons were difficult because of the lack of adequate data on habitat quality, as density
338 relative to resource levels is expected to determine body mass.
339
340 **Implications for management**
341 Generally, management is concerned with conservation of local biodiversity and indigenous
342 genetic variation (Rhymer & Simberloff 1996; Storfer 1999; Allendorf *et al.* 2001). Even though
343 no first-generation migrants from the Otterøya population were detected in the mainland
344 population in 2001 and 2002, we observed very low frequencies of some alleles that were only
345 common in the Hungarian and Otterøya populations. Otterøya is separated from the mainland by
346 only a 200-300 meter wide sound, and these alleles are probably the result of introgression into the
347 mainland population during 30 generations. Considering the recent range shifts of many species

348 (IPCC 2001; IPCC 2007), and the population expansion of the Norwegian red deer last century
349 (Forchhammer *et al.* 1998; Langvatn 1998), some dispersal from the Otterøya seems very likely.
350 Until the effects of heterosis (positive) on Otterøya have been further addressed, the question at
351 hand is whether the genetically different hybrid population on Otterøya, with its higher genetic
352 diversity, should be allowed to expand and interbreed with the native mainland population.
353

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361

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Figure legends

Figure 1. Sampling locations of indigenous Norwegian red deer (*Cervus elaphus atlanticus*) for investigation of the suspected hybrid population on the island Otterøya after introduction of Hungarian red deer a century ago.

Figure 2. Unrooted Neighbour Joining tree based on pairwise D_A-distances between Hungarian, indigenous Norwegian and red deer of a possible hybrid population at the Otterøya. Bootstrap value of main branching is 100 (1000 replicates).

Figure 3. Frequency historgram of LEA results of proportion of admixture (P1) of Hungarian red deer (a) and Norwegian red deer (b) into the hybrid population on Otterøya with 500000 iterations.

Figure 5. Individual posterior probabilities (y-axis) of Bayesian assignment to three clusters (K=3; different colours) among Hungarian (1), Norwegian (3) and Otterøya hybrid (2) population (separated by vertical lines) analysed with uniform priors.

Table 1. An overview of mean density and sample size of red deer body mass deriving from 1965-2006 in Sør-Trøndelag (termed population "P3") and Nord-Trøndelag (termed population "P4") counties and the islands of Otterøy ("P4-Otterøy) and Hitra ("P5"). Pop = Population; mun=municipality. Density = mean density index (No harvested per km^2 of red deer habitat)

Pop-mun	Density	1965-69	1970-74	1975-79	1980-84	1985-89	1990-94	1995-99	2000-04	2005-06	Sum
P3-1612	0.42	0	67	55	251	238	527	697	946	605	3386
P3-1613	0.92	0	70	51	121	246	378	1015	1226	597	3704
P3-1622	0.35	0	22	10	13	13	110	182	346	222	918
P3-1635	0.08	0	7	0	23	24	76	131	137	148	546
P3-1636	0.23	0	23	18	27	103	280	360	500	217	1528
P3-1638	0.39	62	58	64	147	149	241	502	929	517	2669
P4-1714	0.05	0	0	0	0	1	5	3	45	64	118
P4-1721	0.02	0	0	1	1	0	0	0	15	28	45
P4-Otterøy	0.68	76	134	36	53	48	39	0	46	145	577
P5 (Hitra)	1.19	274	504	61	24	716	879	831	2091	1290	6670
Sum		412	885	296	660	1538	2535	3721	6281	3833	20161

546 Table 2. Allele frequencies (a-r) for 14 microsatellite loci (L) in Hungarian (H), a hybrid
547 population at Otterøya (O) and in the indigenous Norwegian red deer (N). Private
548 alleles are *italic* and con-specific alleles in **bold**.

L		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
CSSM 03	H	.06	*.27*	.00	*.03*	*.03*	.18	.09	**.15**	*.21*	.00								
	O	.00	.00	**.13**	.00	.00	.15	.20	**.53**	.00	.00								
	N	.01	.00	**.11**	.00	.00	*.47*	.41	.00	.00	*.01*								
OarCP 26	H	.05	*.18*	.30	.03	*.03*	.00	.40	*.03*	.00									
	O	.34	.00	.04	.25	.00	*.03*	.11	.00	*.24*									
	N	.51	.00	.01	.35	.00	.00	.13	.00	.00									
RT5	H	.00	.15	*.05*	**.13**	.15	*.18*	*.03*	*.15*	*.03*	.10	*.03*	*.03*						
	O	*.13*	.60	.00	**.18**	.09	.00	.00	.00	.00	.01	.00	.00						
	N	.00	.41	.00	.00	.59	.00	.00	.00	.00	.01	.00	.00						
NVH RT73	H	.25	*.03*	*.08*	*.05*	*.03*	.13	.03	*.13*	.00	.08	*.03*	*.08*	*.03*	.00	**.08**	*.03*		
	O	.00	.00	.00	.00	.00	*.26*	.00	.00	*.30*	.03	.00	.00	.00	*.31*	**.12**	.00		
	N	.04	.00	.00	.00	.00	*.61*	.23	.00	.00	.12	.00	.00	.00	.00	.00	.00		
McM 58	H	*.05*	*.05*	*.05*	**.13**	.25	.18	.13	*.05*	.05	.00	.03	.03	*.03*					
	O	.00	.00	.00	**.19**	.04	.50	.09	.00	.13	*.04*	.00	.01	.00					
	N	.00	.00	.00	.00	.09	.46	.36	.00	.03	.00	.07	.01	.00					
BM 5004	H	*.05*	.30	.13	.50	.03													
	O	.00	.12	.37	.51	.00													
	N	.00	.26	.26	.48	.01													
OarFC B193	H	.25	.23	.13	*.03*	.03	*.05*	*.10*	**.05**	.03	.10	*.03*	.00						
	O	.26	.07	.00	.00	.00	.00	.00	**.21**	.03	.11	.00	**.33**						
	N	.01	.11	.01	.00	.21	.00	.00	.00	.14	.07	.00	**.46**						
OarFC B304	H	.00	.13	.05	.28	**.15**	*.05*	.13	**.15**	.08									
	O	.00	.45	.13	.24	**.03**	.00	.01	**.13**	.01									
	N	*.23*	.31	.01	.24	.00	.00	.08	.00	.14									
BM 888	H	*.10*	*.03*	.10	*.03*	**.10**	**.15**	*.30*	.00	.10	.00	.00	*.03*	.05	.00	.03	.00	.00	.00
	O	.00	.00	.22	.00	**.08**	**.26**	.00	**.01**	.01	**.05**	.00	.00	.00	**.09**	.00	.00	**.03**	**.26**
	N	.00	.00	.01	.00	.00	.00	.00	**.01**	.17	**.17**	*.03*	.00	.10	**.07**	.01	*.28*	**.17**	**.01**
NVH RT48	H	.00	.00	.33	.03	.00	**.43**	.00	*.10*	.08	*.03*	.00	.00	*.03*					
	O	.00	*.09*	.49	.01	.00	**.01**	*.07*	.00	.00	.00	*.32*	*.01*	.00					
	N	*.10*	.00	.55	.13	*.01*	.00	.00	.00	.21	.00	.00	.00	.00					
BMC 1009	H	.00	*.18*	**.10**	.00	.18	*.18*	.15	**.03**	*.15*	**.05**								
	O	.00	.00	**.08**	**.41**	.20	.00	.05	**.01**	.00	**.25**								
	N	*.14*	.00	.00	**.49**	.01	.00	.36	.00	.00	.00								
BM 4208	H	**.05**	.33	.00	.00	.03	.10	.10	*.38*	**.03**									
	O	**.01**	.29	*.18*	*.05*	.10	.00	.19	.00	**.19**									
	N	.00	.19	.00	.00	.46	.25	.10	.00	.00									
BM 4107	H	*.08*	.00	.38	*.05*	**.13**	*.05*	.00	*.03*	*.08*	.10	*.13*							
	O	.00	*.11*	.18	.00	**.09**	.00	**.18**	.00	.00	.43	.00							
	N	.00	.00	.13	.00	.00	.00	**.87**	.00	.00	.01	.00							
SRCR SP10	H	.31	*.17*	.53	.00														
	O	.00	.00	.72	***.28***														
	N	.04	.00	.13	***.84***														

Table 3. Genetic variation in microsatellites and mtDNA from red deer of the Hungarian, indigenous Norwegian (No) and the island Otterøya populations. The number of private alleles (A_{Pr}), allelic richness (A_R) and Nei's (1987) unbiased gene diversity (H) are give for 14 microsatellites and the number of haplotypes (nh) and haplotype diversity (h) are given for the mtDNA control region. The number of analysed individuals (n) is given and standard errors are in brackets (SE).

	Microsatellites				**mtDNA**		
Pop	n	A_{pr}	A_R (SE)	H (SE)	n	nh	h (SE)
No	136	7	3.9 (.4)	0.59 (.04)	17	5	0.76 (.07)
Otterøya	40	14	5.0 (.4)	0.69 (.03)	16	2	0.53 (.06)
Hungary	20	56	8.1 (.7)	0.81 (.03)	14	5	0.86 (.07)

565 Table 5. Analysis of body mass of red deer from Sør-Trøndelag (mainland-P3; island Hitra-P5)

566 and Nord-Trøndelag (mainland P4, island Otterøy) using a model with both linear and additive

567 (spline; df = 3) terms. Baseline level for age class are calves (age = 0), and for spatial variation it is

568 Otterøy.

	Estimate	Std. Error	t	p
A. Females				
Intercept	3.3253	0.0102	327.605	<0.001
Age (1 vs 0)	0.5735	0.0050	114.434	<0.001
Age (2 vs 0)	0.7583	0.0057	133.256	<0.001
Age (3 vs 0)	0.8187	0.0060	135.652	<0.001
Age (4 vs 0)	0.8623	0.0076	113.655	<0.001
Age (≥5 vs 0)	0.9022	0.0049	185.933	<0.001
Space (P3-1612 vs. Otterøy)	-0.0427	0.0094	-4.544	<0.001
Space (P3-1613 vs. Otterøy)	-0.0120	0.0090	-1.330	0.184
Space (P3-1622 vs. Otterøy)	-0.0588	0.0121	-4.845	0.000
Space (P3-1635 vs. Otterøy)	-0.0053	0.0146	-0.366	0.714
Space (P3-1636 vs. Otterøy)	-0.0082	0.0110	-0.750	0.453
Space (P3-1638 vs. Otterøy)	-0.0318	0.0097	-3.274	0.001
Space (P4-1714 vs. Otterøy)	0.0085	0.0219	0.388	0.698
Space (P4-1721 vs. Otterøy)	-0.0317	0.0445	-0.713	0.476
Space (P5 vs. Otterøy)	-0.1709	0.0087	-19.667	<0.001
Density	-0.0495	0.0051	-9.627	
			F	p
spline (Date of harvest)			78.532	<0.001
spline (NAO)			9.808	<0.001
B. Males	Estimate	Std. Error	t	p
Intercept	4.1569	0.0152	273.435	<0.001
Space (P3-1612 vs. Otterøy)	-0.0472	0.0160	-2.956	0.003
Space (P3-1613 vs. Otterøy)	-0.0395	0.0166	-2.379	0.017
Space (P3-1622 vs. Otterøy)	-0.0689	0.0177	-3.891	<0.001
Space (P3-1635 vs. Otterøy)	-0.0242	0.0194	-1.252	0.211
Space (P3-1636 vs. Otterøy)	-0.0065	0.0168	-0.384	0.701
Space (P3-1638 vs. Otterøy)	-0.0552	0.0162	-3.409	0.001
Space (P4-1714 vs. Otterøy)	-0.0305	0.0351	-0.869	0.385
Space (P4-1721 vs. Otterøy)	-0.0303	0.0426	-0.711	0.477
Space (P5 vs. Otterøy)	-0.2356	0.0168	-13.998	<0.001
Density	-0.0601	0.0070	-8.555	<0.001
			F	p
spline(Age*Date of harvest)			3728.350	<0.001
spline (NAO)			56.390	<0.001

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Figure 1.

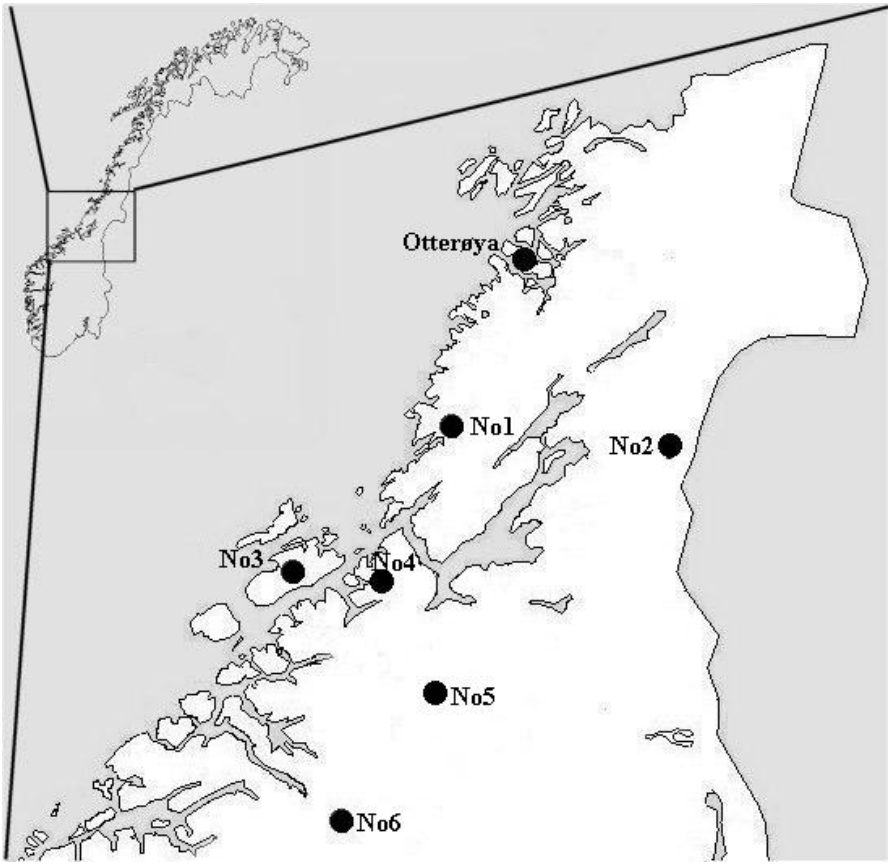
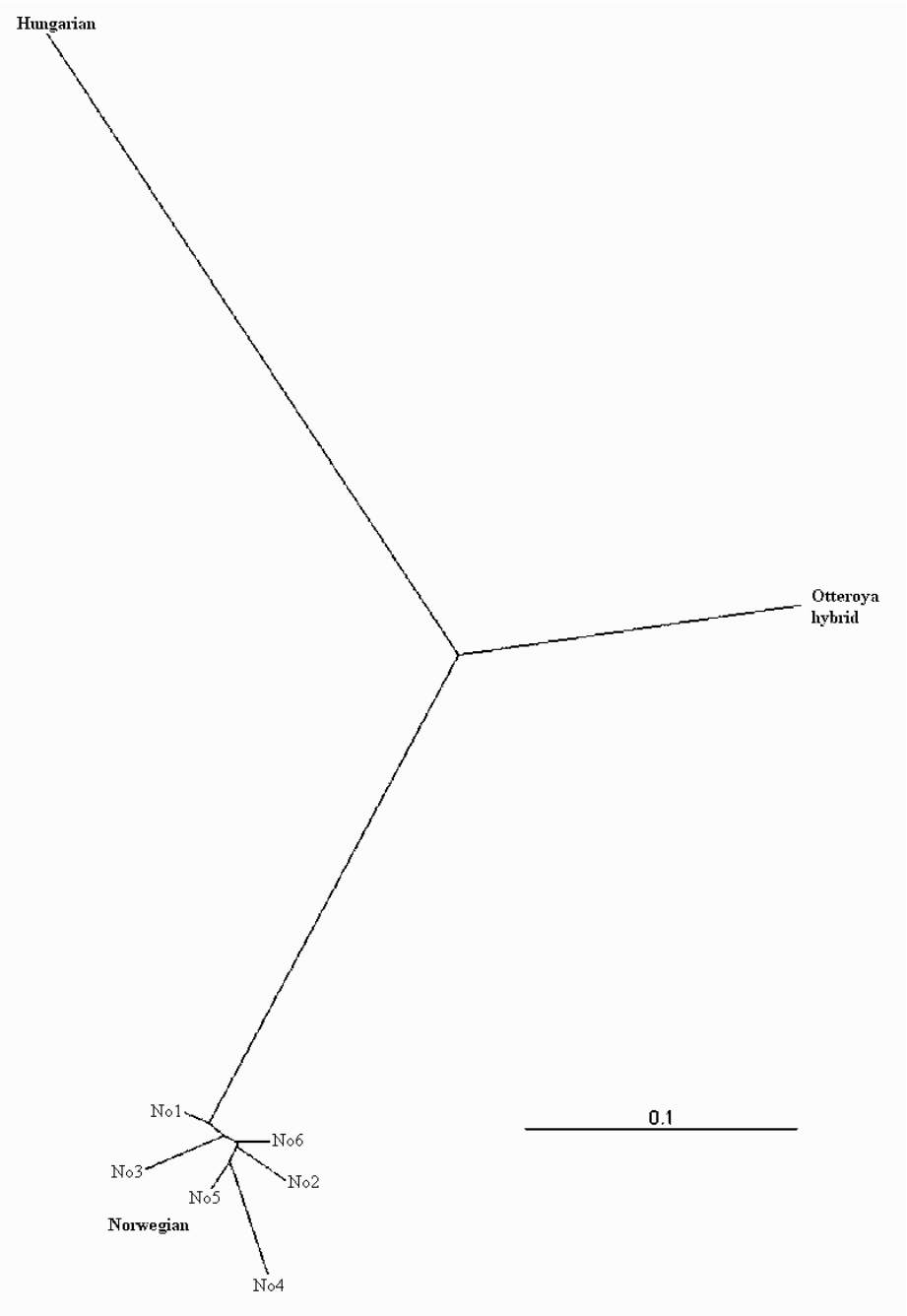


Figure 2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Otteroya hybrid
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No6
No3
No2
No5
Norwegian
No4
0.1

Figure 3

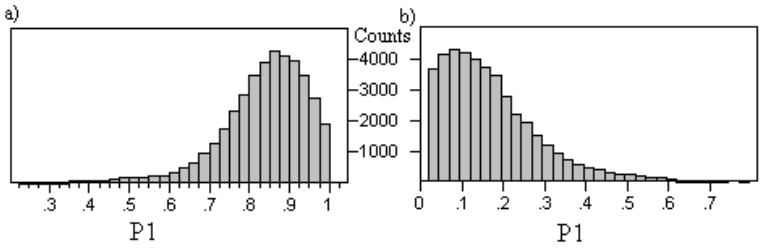

Figure 4