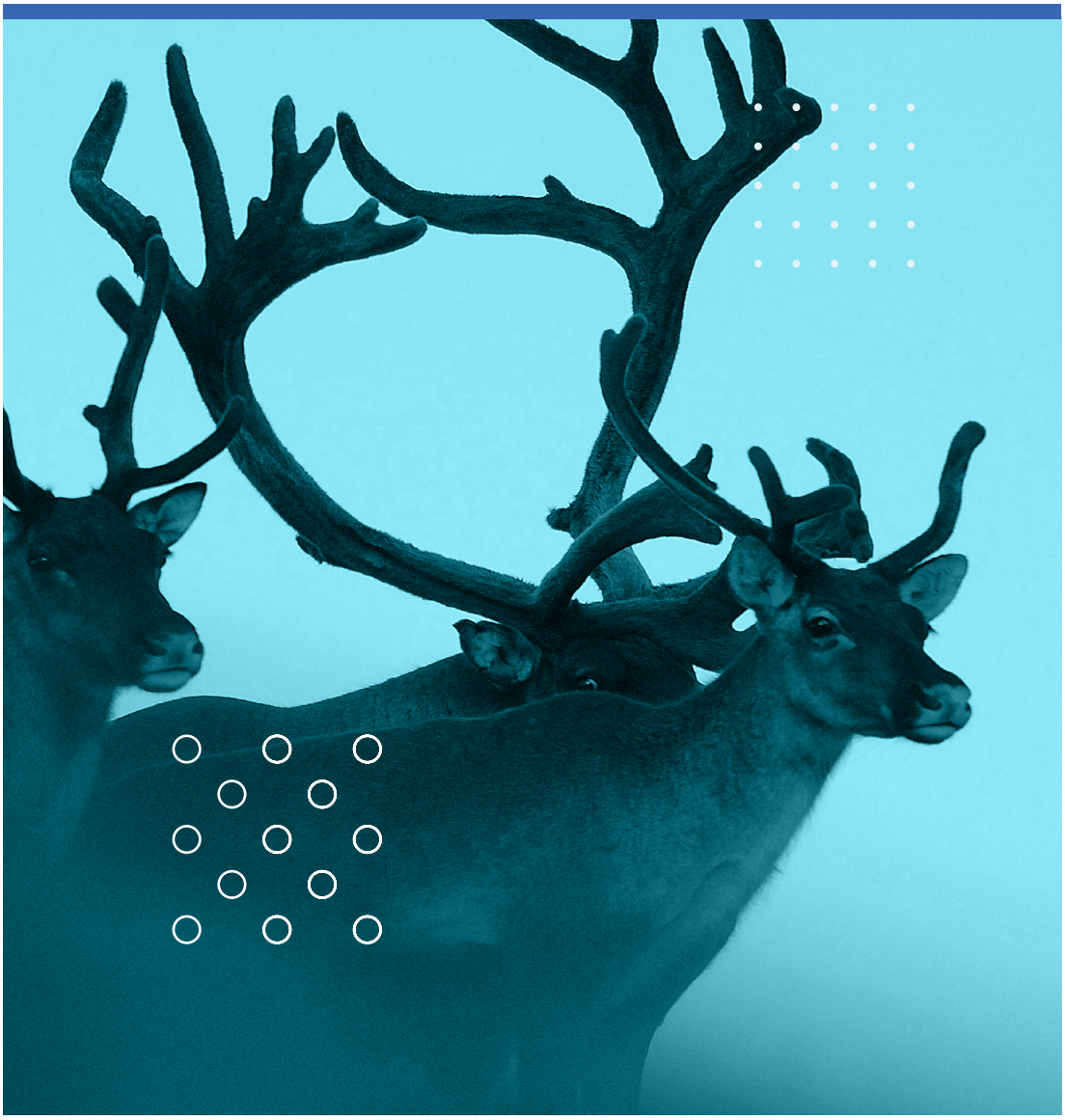
University College of Southeast Norway
Faculty of Technology, Natural Sciences and Maritime Sciences

Doctoral dissertation Nr 11
2017

Kjersti Sternang Kvie

Natural and human-induced impacts on the genetic structure of Eurasian reindeer



HSN

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Natural and human-induced impacts on the genetic structure of Eurasian reindeer

A PhD dissertation in
Ecology

Faculty of Technology, Natural Sciences, and Maritime Studies
University College of Southeast Norway
Kongsberg, 2017

Doctoral dissertations at the University College of Southeast Norway no. 11

ISBN: 978-82-7206-426-5 (print)
ISBN: 978-82-7206-427-2 (electronic)

Print: **University College of Southeast Norway**
Cover image: Olav Strand

Acknowledgements

The work presented was performed at the Department of Natural Sciences and Environmental Health, University College of Southeast Norway (HSN), Bø in Telemark, and Department of Basic Sciences and Aquatic Medicine (BasAM), Norwegian University of Life Sciences (NMBU), Oslo. Additional financial support has been provided by the Norwegian Wild Reindeer Centre and through the ERC Advanced Grant "Arctic Domus", based at the University of Aberdeen. I would like to thank BasAM for allowing me to carry out the work for this thesis at their department.

A special thanks to my supervisors Jan Heggenes and Knut Håkon Røed for guiding me through this project. You have done so with great enthusiasm, knowledge and support, and it has been a privilege to work with you. A special gratitude to Knut - thank you for your patience and for always finding the time to discuss our work.

I would like to thank all co-authors of the papers included in this thesis, as well as all of you that have contributed with samples and data for this project. Without your contributions, this thesis would not have been possible to carry out. I would also like to thank all good colleagues, both former and present. A special gratitude to Liv Midthjell for sharing your expertise- and for valuable help in the lab. It has been a great pleasure sharing an office and lab space with you.

To my family and friends, thank you for always being there. Dad, Solveig and Elisabeth, I could not have accomplished this work without your endless support. To mom, I know you would have been proud. Linéa, Tormod, Marianne, Niri, Vibeke and Jan-Tore, thank you so much for good help and for always making me smile, even when things seems a bit unmanageable.

Finally, my dearest Jonas, thank you for motivating and encouraging me every day, just by being you.

Oslo, December 2016

Kjersti Sternang Kvie

Sammendrag

Geografisk variasjon hos arter og populasjoner er basert på genetisk variasjon, som videre er grunnleggende for naturstyrt evolusjon. Genetisk variasjon og struktur er styrt av faktorer som mutasjoner, seleksjon og genetisk drift, mens genflyt kan føre til genetisk homogenisering av populasjoner. Menneskelig aktivitet kan påvirke disse prosessene i stor grad og menneskeskapt habitatødeleggelse- og fragmentering er i dag ansett som en av de største truslene mot biologisk mangfold. Det er også bred enighet om at menneskeskapt global oppvarming har forårsaket store endringer i antall og utbredelse, og dermed også genetisk struktur, hos mange arter i nyere tid. Det er derfor viktig å ha kunnskap om genetisk variasjon og struktur innen og mellom bestander, hvordan denne påvirkes av naturlige og menneskeskapte prosesser, og å vite mer om hvordan arter har respondert på miljøendringer tidligere.

I denne doktorgraden er reinsdyr (*Rangifer tarandus*) brukt som modellart for å studere genetisk struktur i og mellom populasjoner. Reinsdyr er en velegnet modellart fordi den over lengre tid har levd i relativt upåvirkede habitater, men har blitt utsatt for sterkt antropologisk press i nyere tid. For å studere genetisk struktur er det nødvendig å bruke molekylære markører som har relevant oppløselighet i forhold til spørsmålene som stilles og tidsrammen som studeres. I denne doktorgraden testet vi derfor tre mitokondrielle markører, nemlig kontroll regionen, cytochrome b og cytochrome *c* oxidase subunit I, både samlet og hver for seg. Ved å analysere det sammensatte fragmentet fikk vi høy oppløselighet og fem haplotypegrupper/undergrupper, som tidligere er beskrevet hos rein i Eurasia, ble identifisert. Vi fant også en gruppe med haplotyper identifisert hos rein utbredt i Russland, og et gruppe som bestod av haplotyper som tidligere har vært antatt å være unike for Svalbardrein (*R.t platyrhynchus*). Den mest vanlige haplotypen på Svalbard har vi deretter identifisert på Novaia Zemlia, og i gammelt materiale fra den utdødde reinsdyrbestanden på Franz Josef Land. Ved å analysere de tre markørene separat, fant vi derimot store forskjeller i oppløselighet. Minst variasjon fant vi i cytochrome b. Kontrollregionen ga derimot tilsvarende resultater som det sammensatte

fragmentet. Vi antyder derfor at kontrollregionen kan være en anvendelig enkeltmarkør med tilstrekkelig oppløselighet for å studere innen-arts genetisk variasjon hos Eurasisk rein.

Kontrollregionen ble videre brukt for å se på et mulig felles opphav og koloniseringsrute for høyarktisk arkipelagisk rein i Eurasia. Dette temaet har til nå vært uklart og mye omdiskutert. Ved å analysere materiale fra nålevende bestander på Svalbard og Novaia Zemlia, i tillegg til gammelt arkeologisk materiale fra Franz Josef Land (ca. 2000 år), fant vi genetiske likheter som tilsier at populasjonene på disse arkipelene har et felles opphav. Alderen på materialet fra Franz Josef Land viser at det er den stedegne, ville typene som er og har vært utbredt på disse arkipelagene. En genetisk kobling mellom to undersøkte ville populasjoner fra det Russiske fastlandet og populasjonene på Novaia Zemlia, Svalbard og Franz Josef Land, viser at det er sannsynlig at arkipelene har blitt kolonisert fra det Russiske fastlandet.

For å studere genetisk struktur, variasjon og opphav i Eurasiske populasjoner som i varierende grad er preget av menneskelig påvirkning, brukte vi mikrosatellittmarkører i tillegg til kontrollregionen. Effekter av menneskelig påvirkning er et viktig tema i forhold til bevaring av mange arter. Menneskelig aktivitet har hatt særlig stor innvirkning på reinsdyrs økologi og utbredelse, om dermed sannsynligvis også på genetisk struktur, i Vest Europa de siste 100 år. Særlig har tidligere sammenhengende leveområder i Sør-Norge blitt redusert og fragmentert, med en sannsynligvis påfølgende reduksjon av genflyt mellom populasjonene. Vi fant store forskjeller i grad av genetisk variasjon og struktur innen norske rein, og mellom norsk og russisk rein. I hovedsak ser vi at de små, isolerte populasjonene i Norge har mindre variasjon og viser større grad av differensiering enn de større populasjonene som er mindre preget av menneskelig påvirkning (Nordfjella, Hardangervidda og Ryfylke). Flere av de små populasjonene er også preget av founder-effekter, som kan forklares av at de har opphav i noen få utsatte eller forvillede dyr, og det er liten eller ingen vandring av dyr mellom områdene. De russiske fastlandspopulasjonene har generelt mer genetisk variasjon sammenliknet med de norske. Dette kan forklares med at russisk rein er mindre påvirket av habitatødeleggelse

og fragmentering og at de utgjør større, sammenhengende populasjoner, sammenliknet med situasjonen i Norge.

I tillegg til habitatforstyrrelser kan tamreindrift i områder nært opp mot villreinområdene ha preget villreinstammene i Eurasia. At tamrein og villrein lever tett opp til hverandre i mange områder, gir en unik mulighet til å studere hvordan denne sameksistensen påvirker populasjonene genetisk. Våre resultater viser at de norske populasjonene har et betydelig innslag av haplotyper typisk for tamrein. For flere av de mindre og mer isolerte populasjonene kan dette forklares av opphav. For de større populasjonene som Hardangervidda og Ryfylke er dette mest sannsynlig et resultat av tilfeldig innblanding av tamrein fra tamreindrift i nærliggende områder. Den omvendte situasjonen med en mer menneskelig kontrollert innblanding av gener fra villrein inn i tamreinbestander, er også et viktig tema, bl.a. annet i forhold til ulike teorier om temming av dyr. I Zaibaikal'e som ligger sør-øst i Sibir i Russland lever tamrein og villrein i nær sameksistens. I disse områdene har gjeterne over lang tid drevet kontrollert hybridisering mellom tamrein og villrein for å 'forbedre' bestemte egenskaper i tamreinstammen. Basert på analyser gjort på både mikrosatellitter og kontrollregionen, fant vi en klar genetisk differensiering mellom tamrein og villrein. Dette viser at gjeterne, uten egen genetisk kunnskap, over tid har klart å utvikle tradisjonelle teknikker hvor de holder de to gen-poolene atskilt selv om de lever tett på hverandre, og til tross for at det drives bevisst kryssing mellom enkelte ville og tamme dyr.

Nøkkelord: *Rangifer tarandus*; genetisk variasjon, genetisk struktur; kolonisering; introgresjon; genetisk drift; founder-effekt; flaskehals; fragmentering; habitattap; bevaringsgenetikk; hybridisering.

Summary

Geographic variation in species and populations is based on genetic variation, which in turn is fundamental to nature-controlled evolution. Genetic variation and structure is driven by factors such as mutations, selection and genetic drift, while gene flow may lead to genetic homogenization of populations. Human activity can influence these processes to a great extent and human-induced habitat destruction- and fragmentation is today considered one of the greatest threats to biodiversity. There is also a broad consensus that anthropogenic global warming has caused major changes in numbers and distribution, and probably genetic diversity, in many species in recent times. It is therefore important to have knowledge about genetic variation and structure within and between populations, how it is affected by natural and anthropogenic processes, and to learn more about how species have responded to climate change in the past.

In this thesis, we used reindeer (*Rangifer tarandus*) as model species for studying genetic structure within and between populations. Reindeer is a relevant model species because it over a long period have lived in relatively unaffected habitats, but has been exposed to strong anthropologic pressure in recent times. Population genetic studies requires the use of molecular markers with the appropriate resolution in relation to the questions and timeframe under study. In this thesis, we therefore tested three mitochondrial markers, namely the control region, cytochrome b and cytochrome *c* oxidase subunit I, both merged and separately. By analyzing the merged fragment, we obtained high resolution and five haplotype clusters/sub-clusters, previously described in Eurasian reindeer, were identified. We also found a sub-cluster with haplotypes found in Russian reindeer, and a sub-cluster comprising haplotypes previously thought to be unique for Svalbard reindeer (*R.t platyrhynchus*). We have later identified the most common haplotype on Svalbard in reindeer from Novaia Zemlia, and in ancient material from the extinct reindeer population on Franz Josef Land. By analyzing the three markers separately, we found large differences in resolution. Lowest resolution was found in the cytochrome b region, while the control region gave similar results as the merged fragment. Hence, we suggest

that the control region can be a useful single marker, with sufficient resolution, to study intraspecific genetic structure in Eurasian reindeer

The control region was further used to study a possible common origin and colonization route of High Arctic archipelagic reindeer in Eurasia. This topic has until now been unclear and widely debated. By analyzing material from extant populations on Svalbard and Novaia Zemlia, in addition to ancient archaeological material from Franz Josef Land (about 2000 years), we found genetic similarities implying a common origin for the three archipelagic populations. The age of the Franz Josef Land material shows that it is the indigenous, wild type that are and have been prevalent on these archipelagos. A genetic link between the two wild populations from the Russian mainland and the populations at Novaia Zemlia, Svalbard and Franz Josef Land, show that it is likely that the archipelagos has been colonized from the Russian mainland.

To study the genetic structure, variation and ancestry in Eurasian populations, which to varying degrees are influenced by human activity, we used microsatellite markers in addition to the control region. Effects of human impacts is an important issue in relation to the conservation of many species. Human activity has had a pronounced impact on reindeer ecology and distribution, and probably also on genetic structure, in Western Europe during the last 100 years. In particular, the previously continuous habitats of southern Norway have been reduced and fragmented, probably with subsequent reduction of gene flow among populations. We found large differences in the degree of genetic variation and structure in Norwegian populations, and between Norwegian and Russian populations. Mainly, we see that the small, isolated populations in Norway have lower levels of genetic variation and are more differentiated, compared to the larger populations that are less affected by human activity (i.e. Nordfjella, Hardangervidda and Ryfylke). Several of the smaller populations are also characterized by founder effects, which may be explained by their origin, comprising a few (re)introduced or straying animals, as well as low levels of migration between areas. In general, we found higher levels of genetic variation in the Russian mainland populations, compared to the Norwegian populations. This may be explained by the Russian populations being less

affected by habitat destruction and fragmentation and that they constitute larger, contiguous populations, compared with the situation in Norway.
In addition to habitat disturbance, reindeer husbandry in areas close to the wild reindeer areas may also have had an effect on the Eurasian wild reindeer populations. The fact that domestic and wild reindeer live close in many areas, provides a unique opportunity to study how this co-existence affects populations genetically. Our results show that the Norwegian wild populations contain a considerable number of haplotypes typical for domestic reindeer. For several of the smaller and more isolated populations, this can be explained by their ancestry. For the larger populations, like Hardangervidda and Ryfylke, this is probably a result of random introgression from domestic reindeer from reindeer husbandry in adjacent areas.

The reverse situation with a more human-controlled introgression of genes from wild reindeer into domestic reindeer populations is an important issue, also in relation to different theories about domestication of animals. In the Zaibaikal'e region, which is located southeast of Siberia in Russia, domestic and wild reindeer live in close co-existence. The herders in this region have a long tradition of controlled interbreeding between domestic and wild reindeer, aiming to 'improve' certain properties in the domestic herds. Based on analyzes of both microsatellites and the control region, we found a clear genetic differentiation between domestic and wild reindeer in this region. This shows that the herders, without any acquired genetic knowledge, and over time, have succeeded in developing traditional techniques where they hold the two gene pools separated, despite co-existence and deliberate interbreeding between individual wild and domestic animals.

Keywords: *Rangifer tarandus*; genetic variation, genetic structure; colonization; introgression; genetic drift; founder effect; bottleneck; fragmentation; habitat loss; conservation genetics; hybridization.

List of papers

Paper I.

Kvie, K.S., Heggenes, J., Røed, K.H. 2016. Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer. Ecology and Evolution 6(13): 4347-4358.

Paper II.

Kvie, K.S., Heggenes, J., Anderson, D.G., Kholodova, M., Sipko, T., Mizin, I., Røed, K.H. 2016. Colonizing the High Arctic: Mitochondrial DNA Reveals Common Origin of Eurasian Archipelagic Reindeer (*Rangifer tarandus*). PLoS ONE 11(1): e0165237. doi: 10.1371/journal.pone.01652.

Paper III.

Kvie, K.S., Heggenes, J., Røed, K.H. Genetic heterogeneity in a fragmented landscape despite high migratory and dispersal capacity in reindeer (*Rangifer tarandus*). Manuscript.

Paper IV.

Anderson, D.G., Kvie, K.S., Davydov, V.N., Røed, K.H. Maintaining genetic integrity of co-existing wild and domestic populations: Genetic differentiation between wild and domestic *Rangifer* with long traditions of intentional interbreeding. Manuscript.

Summary of papers

Paper I: Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer (*Rangifer tarandus*).

Phylogenetic analyses provide information that can be useful in the conservation of genetic variation by identifying intraspecific genetic structure. Reconstruction of phylogenetic relationships requires the use of markers with the appropriate amount of variation relative to the timeframe and purpose of the study. Here, genetic structure and clustering are inferred from comparative analyses of three widely used mitochondrial markers, the CR, cytb and the COI region, merged and separately, using Eurasian reindeer as a model. A Bayesian phylogeny and a MJ network, both based on the merged dataset, indicate several distinct maternal haplotype clusters within Eurasian reindeer. In addition to confirm previously described clusters, two new sub-clusters were found. When comparing the results from the merged dataset with the results from analyses of the three markers separately, similar clustering was found in the CR and COI phylogenies, whereas the cytb region showed poor resolution. Phylogenetic analyses of the merged dataset and the CR revealed congruent results, implying that single sequencing analysis of the CR is an applicable method for studying the haplotype structure in Eurasian reindeer.

Paper II: Colonizing the High Arctic: Mitochondrial DNA reveals common origin of Eurasian archipelagic reindeer (*Rangifer tarandus*).

In light of current debates on global climate change, it has become important to know more on how large, roaming species have responded to environmental change in the past. Using the highly variable mitochondrial control region, we revisit theories of *Rangifer* colonization and propose that the High Arctic archipelagos of Svalbard, Franz Josef Land, and Novaia Zemlia were colonized by reindeer from the Eurasian mainland after the last glacial maximum. Comparing mtDNA control region sequences from the

three Arctic archipelagos, showed a strong genetic connection between the populations, supporting a common origin in the past. A genetic connection between the three archipelagos and two Russian mainland populations was also found, suggesting colonization of the Eurasian high Arctic archipelagos from the Eurasian mainland. The age of the Franz Josef Land material (>2000 years before present) implies that Arctic indigenous reindeer colonized the Eurasian Arctic archipelagos through natural dispersal, before humans approached this region.

Paper III. Genetic heterogeneity in a fragmented landscape despite high migratory and dispersal capacity in reindeer (*Rangifer tarandus*)

Effects of habitat fragmentation on gene flow is a major concern within conservation genetics. Wild reindeer (*R. tarandus*) is a migratory species, highly affected by human activity. The number and continued presence of European wild reindeer has been reduced significantly in recent times, and populations are increasingly fragmented due to accelerating anthropogenic habitat modification and reduction, and also displacement in benefit of domesticated reindeer herds. In this study, we used microsatellites and a mitochondrial marker to assess genetic structure in Norwegian wild reindeer populations, subject to major habitat fragmentation during the last 100 years. It represents an unintended large-scale spatial fragmentation experiment, which we used to examine if the observed genetic structure is consistent with landscape genetics theoretical predictions, or alternatively, a result of non-natural processes associated with human activities. Our results show high levels of differentiation among most populations. However, genetic structure could not be explained by fragment size and distance. Theoretical predictions were only supported for the one main population with nearby fragments, which appear to constitute a limited core-satellite system. All the smaller and often more isolated populations seem to be highly influenced by human induced recent colonization histories, as well as isolation due to fragmentation through human-induced restriction to gene flow, distorting an expected isolation-by-distance effect.

Paper IV: Maintaining genetic integrity of co-existing wild and domestic populations: Genetic differentiation between wild and domestic *Rangifer* with long traditions of intentional interbreeding

The introgression of non-native and domesticated genes into the local wild gene pool is a conservation concern, as it is thought to reduce natural genetic diversity and may threaten local adaptations. Conversely, the need to introduce wild genes into the domestic gene pool is commonly thought to be a necessary first-step in the origins of animal domestication. For those classic domestic species which had been domesticated long-ago, this has been difficult to study due to the rareness or extinction of the wild form of the species. Here we present genetic analyses of co-existing wild and domestic herds of reindeer (*Rangifer tarandus*) in the Zaibaikal region of Siberia; a region thought to have been one of the hearths of the emergence of reindeer husbandry. Despite a tradition of holding domestic reindeer in the same range as wild reindeer, and a tradition of deliberate, but controlled interbreeding, we demonstrate strong genetic differentiation between regional wild and domestic herds. We found a stronger differentiation between pooled wild and domestic reindeer in mtDNA compared to the nuclear microsatellites, which suggests mainly male-mediated gene flow between the two gene pools. The observed differentiation persists, despite co-existence and controlled interbreeding between domestic and wild *Rangifer* by indigenous herdsmen. The genetic results, and our survey of the traditional breeding practices, indicate that the herders have an effective breeding technique which while mixing pedigrees on the level of certain individuals in the short-term, guards against wholesale introgression between wild and domestic populations over the long-term. The present study gives support to a model of domestication where wild males and domestic females are selectively interbred which nevertheless stops short of hybridizing the two populations.

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1. Introduction

Extinction of populations and species is a natural part of evolution - and usually balanced by the formation of new populations and species. However, biodiversity is reduced when extinction happens at a high rate like during mass extinctions (Frankham et al. 2010, Barnosky et al. 2011). In recent times, anthropogenic habitat destruction and fragmentation resulting from of a growing and expanding global human population, is the main reason behind the observed rapid decline in biodiversity (Bevanger 2005). Presently there is also a general consensus that anthropogenic caused climate warming will be a serious threat to global diversity. It has produced numerous shifts in distribution and abundance of species in our time (Parmesan and Yohe 2003). These changes have such an impact on the Earth that a new geological epoch have been suggested, and that we should refer to the present as the Anthropocene, rather than "within Holocene" (Crutzen 2006, Steffen et al. 2007, Lewis and Maslin 2015).

A species or populations resilience to both natural and human- induced environmental changes will vary with its genetic composition and physiological tolerance (Willi and Hoffmann 2009). Within and among population genetic diversity represents the ultimate evolutionary potential of a species, i.e. the potential to respond adequately to environmental change (Freeland 2005). Therefore, quantification of genetic variation within- and between populations is thus an important part of conservation biology. It generates information about current, but also previous demographic status, and can help us understand the natural, and in recent times also anthropogenic forces, acting on genetic variation (Begon et al. 2006). This is necessary knowledge contributing to making sustainable management plans.

1.1. Factors affecting genetic variation and structure

Genetic variation can be described as naturally occurring genetic differences between individuals, populations or species (Frankham et al. 2010). Genetic variation ultimately

originates from mutations, and thereafter also recombination. A mutation is a sudden change in an allele or chromosome (Freeland 2005), and when mutations occur in a coding region, they may be lethal, deleterious or even beneficial. More often, they are neutral i.e. silent, or occurring in non-coding regions and hence, will not affect fitness (Frankham et al. 2010). Mutation rates in nature are relatively low and must accumulate over time to represent an evolutionary potential, so the significance of mutations as an evolutionary driver to environmental change is small. The usually abundant geographic variation in gene frequencies in most species is caused also by other processes; local natural selection and genetic drift will usually lead to genetic differentiation, whereas gene flow will lead to homogenization. Natural selection leads to local adaptation and thereby population differentiation. However, within populations, natural selection can alter genetic variation through different processes. Selection may reduce within-population genetic variation through stabilizing or directional selection, or increase variation through disruptive or balancing selection. Also, positive selection may increase variation temporarily, but variation will be reduced when the selected alleles reaches fixation (Freeland 2005). Genetic drift is the stochastic element to the evolution of populations. Loss of within-population variation due to stochastic drift, i.e. loss of alleles by chance, is dependent on the effective population size. The effect will be minimal in large populations but becomes more prominent as population size decreases (Hedrick 2001) and may offset the effect of selection as evolutionary driver (Frankham et al. 2010). However, in a geographic context, genetic drift will most likely lead to increased differentiation between populations. As the probability of losing a specific allele is the inverse of the allele's frequency within the population, rare alleles will be the first to be lost (Maruyama and Fuerst 1985). Rare alleles are, however, important to preserve as they may be well suited for changed future environmental conditions and hence, increase a population's ability to adapt (Begon et al. 2006). Inbreeding depression is yet another and more immediate problem (Amos and Balmford 2001). Individuals in small isolated populations are more likely to mate with close relatives, which in turn lead to reduced heterozygosity and fitness through fixation of recessive deleterious alleles within the population. This is because individuals that are forced to mate with close relatives are more likely to derive the harmful allele from both parents so that the deleterious effect

is expressed (Hedrick 2001, Begon et al. 2006,). Gene flow, i.e. migration among populations, will counteract the differentiating effects of selection, genetic drift and inbreeding by being a source to new genetic material from other populations (Frankham et al. 2010), given that the migrants contribute in reproduction (Coulon et al. 2004). There is thus a balance between the homogenizing effect of migration, depending in large part on the degree of effective gene flow between populations, and genetic drift, selection and also mutations, causing genetic differentiation of populations (Hedrick 2004). This balance determines geographic genetic variation in a species. Habitat fragmentation, caused by natural (semi)barriers or human activities and infrastructure, reduces habitat area and thereby population size, but also gene flow among habitats and hence, may separate populations into partially or ultimately completely isolated fragments. In a geographic context, reduced population size and gene flow may lead to increased population differentiation in the short term due to selection and drift, but will in the long term often also lead to overall loss of genetic diversity and fitness due to the continued negative feedback effects of loss through genetic drift and inbreeding (Frankham et al. 2010).

1.2. Genetic effects of range shifts, bottlenecks and human-induced translocations

Climatic oscillations over the Quaternary (2.4 million years ago – present) have had a major impact on the geographic distribution and genetic structure of species through population extinctions and range shifts (Hewitt 2000). The demographic impact of range shifts alters the genetic structure of populations by the elimination of populations and lineages, reduction in genetic variation due to bottlenecks and founder events (Slatkin and Excoffier 2012), as well as the spread of mutations by selection and population expansion (Hewitt 2004). Bottlenecks may be caused by natural processes, such as habitat disturbance or range expansions (Hewitt 2000) or be a consequence of recent human activity (Vila et al. 2003). The severity of a bottleneck depends on the magnitude

of the population reduction and size of the bottlenecked population, and the speed of recovery (Freeland 2005). The initial loss of genetic variation will be proportional to the reduction in population size, and the longer time it takes for a small population to recover, the more genetic variation will be lost (Freeland 2005). A founder effect is one type of bottleneck characterized by loss of allelic variation following the founding of a new population from a very small number of individuals (Nei et al. 1975). The founders will only carry a proportion of the genetic variation found in the much larger source population. The relative allelic frequencies found in the founding population may be very different from what it was originally and hence, may cause the new population to differ considerably from the source population (Dlugosch and Parker 2008).

Gene flow between populations may increase fitness by counteracting the effects of genetic drift and inbreeding (Freeland 2005, Yannic et al. 2014b). This has been a common rationale in conservation biology for human induced translocations. Obviously, translocations have also been a practice to re-establish extinct populations or to enhance reduced populations. Hybridization between distinct lineages may result in genetic rescue, if it leads to a reduction in inbreeding depression. Genetic rescue is often attributed to heterosis (hybrid vigour) which is elevated fitness in the hybrid offspring. Heterosis may be the result of either the production of relatively fit hybrid individuals or from the masking of deleterious alleles (Freeland 2005, Fitzpatrick and Shafner 2007). However, breeding between diverged populations, through natural processes or through human mediated translocations, may result in loss of locally adapted genotypes and/or loss of positive epistatic interactions, and hence decrease survival rates (Fitzpatrick and Shafner 2007, Frankham 2010). Introgression of non-native and domesticated genes into local wild gene pool, under which conditions it may occur and to which extent, and if it can be controlled or not, may therefore be a conservation concern. Also, separating between natural- or human induced translocations is an important issue in conservation genetics, as introgression from domestic animals may function as a variable for classifying protection status of populations.

1.3. The study species: *Rangifer tarandus*

Rangifer is a ruminant ungulate in the widespread deer family, Cervidae. In Eurasia, both wild and semi-domestic *Rangifer* are referred to as reindeer, while in North America, the wild, native type is called caribou. Caribou has never been successfully domesticated, and the reindeer husbandry in North America is based on reindeer introduced from Siberia during the 1890s (Jernsletten and Klokov 2002) and hence, referred to as reindeer. The distribution of reindeer and caribou is circumpolar and spans across Arctic and sub-Arctic Eurasia and North America (Banfield 1961) (Figure 1). Reindeer have also been introduced to Iceland (Thorisson 1980), Greenland (Jepsen et al. 2002) and to the sub-Antarctic island of South Georgia (Leader-Williams 1988) where an eradication attempt is now on-going.

Figure 1. The circumpolar distribution of ecotypes of reindeer and caribou (modified from Conservation of Arctic Flora & Fauna (CAFF) 2001).

As for many large mammals in northern Eurasia and America, the evolutionary history of *Rangifer* has been highly influenced by glacial and interglacial effects (Lorenzen et al. 2011, Yannic et al. 2014a). At the time of the last glaciation, glaciers covering large land areas in northern Eurasia and America repeatedly confined the distribution of reindeer to different refugia (Flagstad and Røed 2003). Sequence data from the mitochondrial control region (CR), from all extant subspecies of *R. tarandus* show three major mtDNA haplotype groups, indicating three different refugia during the last glaciation (Flagstad and Røed 2003). These refugia were putatively located in; i) Beringia, which was the largest and extended far into Eurasia, ii) a smaller refugia in south-central Europe, north of the Alps and iii) a refugial area south of the North American ice sheet (Flagstad and Røed 2003). The large Eurasian glacial population, possibly ranging from Beringia to central Europe, seems to have been the most influential source to the present gene pool and have affected all current populations to some extent (Flagstad and Røed 2003, Yannic et al. 2014a). As the ice retreated due to warmer climate during the end of the Weichselian/Wisconsin, reindeer from the Beringian population appear to have recolonized exposed areas in North America, Siberia and Fennoscandia, and during most of the Holocene, reindeer and caribou have been widely distributed in most mountain, tundra and taiga regions throughout the northern Holarctic (Banfield 1961). However, the number and the continued presence of wild reindeer throughout their range has recently been significantly reduced and fragmented due to accelerating anthropogenic habitat modification, the extensive displacement in benefit of domesticated herds of the species (Andersen and Hustad 2004), and probably also due to global warming. The decline of wild reindeer populations has been particularly dramatic in the European part of Eurasia. Today, wild European reindeer are mostly restricted to mountainous areas in the southern and central parts of south Norway and hence, Norway have a particular responsibility to protect these last remnants of the European wild reindeer, as well as to preserve their habitat.

Reindeer and caribou have had a major influence on human development and postglacial colonization of northern Eurasia and America. The close connection between humans and *Rangifer* have contributed to a great interest in its taxonomy and origin (Røed 2007).

A high number of described sub-species, primarily based on morphology but without well-defined characteristics, have dominated the taxonomy. Although outdated, the sub-species classification made by Banfield (1961) is still widely applied. However, more recent genetic studies based on mitochondrial DNA indicate discrepancies between existing ecotype and subspecies designations and genetic differentiation (Gravlund et al. 1998, Flagstad and Røed 2003, Cronin et al. 2006, McDevitt et al. 2009, Yannic et al. 2014a, Yannic et al. 2016). This may imply that the current geographical distribution of mtDNA haplotypes reflects historical demographic events, but does not reflect taxonomy as described hitherto. Due to the lack of resolution of *Rangifer* taxonomy, ecotype designations have been increasingly applied, and populations have been subdivided into various ecotypes according to their life-history strategies and ecological conditions such as the woodland or boreal/forest or sedentary form, the barren-ground or tundra or migratory form, the mountain form, and the Arctic form (Banfield 1961) (Figure 1).

1.4. Eurasian reindeer

Most reindeer in Eurasia belong to the tundra reindeer sub-species (*R.t. tarandus*) (Figure 2). Less than half of the 3-4 000 000 reindeer across Eurasia are wild, and in many areas wild and domestic populations live in close coexistence (Syroechkovskii 1995, Baskin 2005).

Figure 2. Semi-domestic tundra reindeer (*R.t tarandus*) from northern Finland. Photo S. Côté.

Norway has, in addition to about 150 000 domestic reindeer, about 40 000 wild reindeer, divided into 23 more or less isolated populations distributed in the south-central parts of the country. Due to reindeer's natural nomadic behavior and their historically continuous geographic distribution we would expect the Norwegian, wild reindeer to show a homogenous genetic structure as a result of gene flow between adjacent populations (Røed 1986). However, because of increased anthropogenic pressure and associated reduction and fragmentation of reindeer habitat during the last 100 years or so, natural migrations and probably also straying, have been restricted. This may have affected gene flow, divergence and genetic structure in and among these populations. Genetic make-up may also have been influenced by the introduction and herding of domestic reindeer in and adjacent to wild reindeer areas. There is little knowledge about genetic divergence and degree of gene flow between native wild- and domesticated animals in current wild reindeer areas in south-central Norway, although some of the larger, continuous populations have been investigated (Røed et al. 2008, Røed et al. 2011).

In addition to mainland tundra reindeer, Norway has the small sized Arctic type (*R.t platyrhynchus*) distributed on the Svalbard archipelago. This Arctic form, thought to be adapted to cold, open environments, is usually recognized by its small body size, with short rostrum and legs, as well as a thick, pale winter pelage (Banfield 1961). The total population is small and variable due to challenging winter conditions, and assumed to reach > 10000 animals in 'good' years (Villrein.no). According to pellets found in peat cores, *R.t platyrhynchus* occurred on Svalbard at least 5000 years ago (Van der Knaap 1986). There has been an extensive and long-standing debate on the colonization routes and dispersal of Arctic reindeer inhabiting the islands in the western Eurasian and North American Arctic (Banfield 1961, Gravlund et al. 1998, Flagstad and Røed 2003, Forman 2004). Different hypothesis' regarding refugial origin of Svalbard reindeer have been proposed (Gravlund et al. 1998, Flagstad and Røed 2003) either from the west trough North America, or from the east, from the Russian mainland. In contrast to most mainland reindeer, which tend to be highly migratory, island reindeer are often sedentary and more isolated (Côté et al. 2002). Hence, the sedentary Svalbard reindeer are characterized by low genetic variability indicating isolation, possible bottlenecks, and

subsequent genetic drift as important evolutionary demographic processes (Côté et al. 2002, Røed 2005).

Russian reindeer comprise tundra reindeer, which is found on Novaia Zemlia, the Kola Peninsula and in Siberia, as well as forest reindeer which is particularly distributed in Evenkia, Trans-Baikal Territory, Southern Yakutia and Far East of Russia (Baskin 1986) (Fig. 1). Both Russian wild and domestic reindeer show regional morphological- and ecological differences, which can be explained by different environmental conditions (Baskin 1986). Wild, Russian reindeer are distributed in the tundra and forest-tundra zones, the taiga zone, as well as the mountain taiga areas in South Siberia (Figure 1). Approximately 85 % of the Russian wild reindeer is found in Taimyr, Northern Yakutia and Central Chukotka (Syroechkovski 1999). However, there has been major fluctuations in the estimated number of Russian wild reindeer, depending to a large extent on the different estimations of the Taimyr population (Kholodova et al. 2011), which is considered to be the largest wild reindeer population in Eurasia (Baskin 2005, Kholodova et al. 2011). The number of domestic reindeer in Russia was reported to be 1.2 million in 2001 which shows a major decline from 1969 when the domestic reindeer population was estimated to comprise approximately 2.5 million reindeer (Baskin 2005). The large decline in many regions was mainly due to socio-economic transformations following the dissolution of the Soviet Union (Baskin 2005). The present reindeer herding communities in Russia, and elsewhere, are facing large changes in their societies due to challenges like global warming, increasing human activity and development (Oskal et al. 2009).

Domestic and wild reindeer live in close co-existence in many areas in Russia. A particularly interesting region is the Zaibaikal'e area in southeastern Siberia. Zabaikal'e has been suggested to be one possible origin point for reindeer husbandry (Wiklund 1918, Maksimov 1928, Pomishin 1990). Furthermore, the region is interesting because of its specific herding techniques. It has been documented that local Evenki herdsmen have had a traditional practice of selectively cross-breeding domestic female reindeer with wild males to produce offspring described locally as the baiunchikan, often held for

transportation. Hence, this area presents a unique opportunity to study whether the Zaibaikal'e Evenki are able to enforce a strict genetic separation of wild and domestic reindeer, despite controlled inter-breeding.

2. Objectives

The overall purpose of this thesis is to study genetic ancestry and structure in Eurasian reindeer populations, on different temporal and spatial scales, -to examine possible long-term natural and/or recent anthropogenic influences that may explain observed patterns.

Specifically, this thesis aims to study:

1) The performance of different molecular markers for studying intraspecific genetic variation and structure in Eurasian reindeer on different temporal and spatial scales (paper I, II, III and IV).

2) Origin and colonization route of the High Arctic reindeer distributed on the archipelagos of Svalbard, Novaia Zemlia and Franz Josef Land (paper II).

3) Differentiation and genetic integrity in Eurasian reindeer distributed in areas recently influenced by human activities and fragmentation (paper III and IV).

3. General Methods

3.1. DNA extraction, PCR, sequencing and microsatellite analysis

DNA was extracted from muscle samples, hair follicles, skin samples, blood samples, archaic antlers and from FTA cards designed for forensic work (Smith and Burgoyne 2004). DNA from muscle- and skin samples were extracted using DNaeasy Blood & Tissue Kit (Qiagen) following manufacturers protocol. The same kit was used for extraction from archaic antlers, following a protocol from Bjørnstad and Røed (2010). DNA extraction from EDTA blood was carried out using DNeasy Blood & Tissue Kit (Qiagen) or by using a boiling method for DNA extraction (see paper II for details). The same standard boiling method was used to extract DNA from FTA cards. DNA from hair follicles were extracted using the chelex method (Walsh 1991). Polymerase chain reactions was used for amplification in all instances (see individual papers for further details). MtDNA was sequenced using the Sanger method (Sanger et al. 1977). Capillary electrophoresis and data analysis were performed with an ABI 3130xL- or 3500xL instrument (Applied Biosystems) for both mtDNA- and microsatellite PCR products.

3.2. Population genetic analyses

We used DnaSP v5.10 (Librado and Rozas 2009) to calculate mtDNA polymorphism, and to test for neutrality by calculating Tajima's D (Tajima 1989) (paper I). A negative Tajima's D corresponds to an excess of rare polymorphisms and implies a population size expansion or positive selection, while the positive values reflect an excess of intermediate-frequency alleles, and suggests population bottlenecks or balancing selection (Akey et al. 2004). The same program was used to perform a second neutrality test, the Ramos-Onsin's and Roza's (Ramos-Onsins and Rozas 2002) R2 value (paper II), which may be more appropriate when dealing with small sample sizes. BEAST v1.8.0 (Drummond et al. 2012) was used to construct Bayesian phylogenies (paper I and II) and to calculate time since most recent common ancestor (tMRCA) (paper I) based on the

mtDNA data. Network v4.6 (ref.fluxus-engineering.com) was used to construct median-joining networks (Bandelt et al. 1999) (paper I, II and IV). A MJ network should give an appropriate presentation of the intraspecific variation found, as it takes into account the fact that intraspecific relationship tends to include extant ancestral haplotypes and multifurcations (Posada and Crandall 2001). We used Arlequin v.3.5 (Excoffier and Lischer 2010) to test for recent demographic expansion by calculating the mismatch distributions of pairwise nucleotide differences (Slatkin and Hudson 1991, Rogers and Harpending 1992) and to calculate the sum of squared deviations (SSD) to test if the observed distribution deviated significantly from the expected under the population expansion model (paper II). The Harpending Raggedness index (Harpending 1994) was calculated to check for demographic changes (paper II). A smooth morphology indicates a population expansion, whereas a ragged morphology indicates constant population size (Harpending 1994). The same program was used to calculate pairwise F_{ST} values for expressing differentiation between populations and to perform Mantel tests (paper III). The Mantel test examines if there is an association between genetic distances (F_{ST}) and geographical distances between populations (transformed to the natural logarithm of the geographical distance in kilometers), for both mtDNA and microsatellites. GenALEx v.6.5 (Peakall and Smouse 2012) was used to calculate microsatellite genetic diversity (paper III and IV). Deviations from expectations under Hardy-Weinberg equilibrium (HWE) were calculated in GENPOP v.4.4 (paper III and IV). Bayesian assignment was performed as implemented in the program STRUCTURE 2.3.4 (Pritchard et al. 2000a, Pritchard et al. 2000b) to investigate population structure in the microsatellite data (paper III and IV).

4. Results and General Discussion

4.1 Molecular markers for studying intraspecific phylogenetic- and population genetic structure

A molecular marker provides information about allelic variation at a given locus (Schlotterer 2004). A wide range of molecular markers are available and which marker to choose depends on the extent of genetic polymorphism needed to approach a particular question, as well as the time and cost required to perform the analyses (Parker et al. 1998). Inter- and intraspecific variation has traditionally been studied through analyses of protein- or DNA polymorphism. In this study, we analyzed genetic variation in mitochondrial- and in nuclear DNA, more specifically in microsatellites.

Mitochondrial DNA is a commonly used molecular marker that can provide insights about population and species history (Zhang and Hewitt 2003). MtDNA have a high mutation rate compared to nuclear genes (about 5-10 times), which makes it suitable for studying population history over a short timeframe (Avise 2000). Its applicability can further be explained by its assumed neutral, clock like and clonal fashion of inheritance (Avise 2000, Gissi et al. 2008). Clonal inheritance, in most animals maternal, is considered an advantage as the non-recombining nature of mtDNA simplifies analyses of intraspecific variation. However, maternal inheritance means that if the male and female history differ, then mtDNA will only reflect the history of the female portion, not the species or population as a whole (Galtier et al. 2009). Also, clonal inheritance means that the effective population size for mtDNA is approximately one-quarter that of nuclear genes. This means that mtDNA linages have a much faster rate of lineage sorting and allele extinction. Hence, the within population variation at equilibrium is expected to be lower, and divergence between population higher, compared to nuclear genes (Zhang and Hewitt 2003).

Nuclear DNA (nDNA) contains unique single-copy regions that usually codes for a particular gene product as well as non-unique duplicated or repetitive regions. Repetitive DNA may be made up of coding segments (e.g. ribosomal RNA genes) or non-coding tandemly repeated units like minisatellites and microsatellites (Parker et al. 1998). Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs) are usually highly polymorphic and are widely dispersed throughout eukaryotic genome (Davis and Strobeck 1998). Microsatellites consist of multiple copies of tandem arranged simple sequence repeats with a size that usually range from one to six base pairs (Selkoe and Toonen 2006). Microsatellites have the advantage of being co-dominant, multi-allelic, highly reproducible as well as hyper-variable (Oliveira et al. 2006) and hence, highly applicable for studies of contemporary population structure and dynamics (Zhang 2003). However, there are challenges that may lead to erroneous interpretation of the data as microsatellite markers can be limited by null alleles, allelic dropouts and stutter bands (Hoffman and Amos 2005).

Different mitochondrial markers, with variable evolutionary rates, have been used to study genetic structure and phylogeny in reindeer and caribou, i.e. the non-coding control region (CR) (Gravlund et al. 1998, Flagstad and Røed 2003, Kholodova et al. 2011, Røed et al. 2011, Klütsch et al. 2012, Røed et al. 2014) and the protein coding cytochrome b (cytb) region (Randi et al. 1998, Cronin et al. 2003, Yannic et al. 2014). In paper I, we wanted to test the resolution and applicability of both the CR and the cytb region for phylogenetic studies of Eurasian reindeer. To obtain a longer fragment from the coding part of the mtDNA genome, we included a fragment from the cytochrome *c* oxidase subunit I (COI) region. To take advantage of the three markers' ability to resolve relationships on different taxonomic levels, we started by merging the three markers. The merged dataset showed high resolution and several well-supported clusters/sub-clusters were identified. We found a main division between the highly variable cluster I, comprising haplotypes found in both wild and domestic Eurasian reindeer, and cluster II, which includes haplotypes commonly found in Fennoscandian domestic reindeer. Both clusters are previously described by Røed et al. (2008). Cluster I further showed

separation into six well-supported sub-clusters (denoted Ia-If), whereas four have been described in previous studies (Røed et al. 2008, Bjørnstad et al. 2012). One of the undescribed sub-clusters (Ic) includes haplotypes thought to be unique to Svalbard reindeer. However, the most common haplotype found on Svalbard have later been identified in high frequencies on Novaia Zemlia in Russia, as well as in archaic material from the extinct reindeer population previously situated on the Russian archipelago Franz Josef Land (paper II). A complex structure within the Russian samples was found, implying that a high number of samples is needed to better understand the haplotype structure within Russian reindeer. However, in addition to previously described haplotype clusters with haplotypes commonly found in Russian reindeer, we also found a new cluster, denoted If. High differences in resolution was revealed when analyzing the three markers separately. The CR dataset showed a high degree of genetic variation and clustering congruent with the results obtained from the merged dataset. The cytb fragment showed low resolution and none of the clusters/sub-clusters found in the phylogeny based on the merged data set was identified. The COI region showed intermediate levels of resolution and six of the in total seven clusters/sub-clusters were identified. Higher resolution in COI compared to cytb might suggest that the COI is a more useful addition to the CR when studying intraspecific genetic structure in reindeer, compared to the cytb region.

The most recent common ancestor (MRCA) was estimated from the CR dataset to clarify if the clusters identified in paper I diversified during- or after the last glacial maximum (LGM; 19–27 000 YBP, Clark et al. 2009). We obtained estimates that ranged between 4000-8600 YBP. The colonizing history of Eurasian reindeer is highly influenced by the LGM and the colonization routes after the retreat of the ice. The relatively young colonization history of Eurasian reindeer, show the necessity to use highly variable markers, e.g. the CR, to be able to identify haplotypes that diversified after the last glacial period. However, high mutation rates might lead to reduced resolution and underestimation of genetic structure due to homoplasy (Bradman et al. 2011). Homoplasy is sequence similarity due to convergent, parallel, or reverse evolution, rather than common ancestry (Bradman et al. 2011). We found lower resolution in one sub-cluster (If) in the CR compared to the merged dataset and the COI data. The incongruence

found between the two loci show that we should not rule out the possibility that there is some level of homoplasy in the CR dataset, and show that adding slower evolving loci may reveal phylogenetic inconsistencies, even when the data are characterized by relatively recent divergence.

However, as mentioned above, the mitochondrial genome is inherited in a clonal, non-recombining way, and is therefore unlikely to reflect complex demographic evolution correctly. Hence, care should be taken when interpreting evolutionary demographic history based on the mitochondrial genome alone (Ballard and Whitlock 2004). Particularly, using the mitochondrial genome as a single marker to study demographic processes suffers from reduced power to precisely identify or quantify degrees of admixture between populations (Larson and Burger 2013). This has been shown in several studies where phylogenetic trees inferred from mtDNA failed to recover the true phylogeny, and instead were masked by the most recent admixture episode (Miller et al. 2012, Ottoni et al. 2013, Larson and Fuller 2014). However, previous genetic screening of extant wild and domestic herds across Eurasia, using both mitochondrial and autosomal microsatellite markers, revealed a similar phylogenetic pattern for both markers with the domestic herds in Fennoscandia being clearly different from native wild reindeer in Norway, and both wild and domestic herds in Russia (Røed et al. 2008). This is further supported in this thesis, as both microsatellites and the CR showed a clear separation between the native, wild reindeer from Rondane-Dovre, the Langfjella populations and the remaining Norwegian populations with an assumed domestic ancestry (paper III). A clear distinction between wild and domestic reindeer was also evident from both microsatellite and CR analyses of reindeer herds in the Zaibaikal'e area in Russia (paper IV). The similar distinct phylogenetic pattern from both the autosomal and maternal markers implies that the mtDNA phylogeny of Eurasian reindeer mainly reflects the genome as whole, at least with regard to the main phylogenetic patterns.

4.2 Origin and colonization history of high Arctic reindeer as inferred from mtDNA

By recovering ancient DNA, it is possible to go back in time and study the genetic relationship between current and ancient populations and species. However, the degradation processes of DNA obtained from animal remains may cause some technical challenges. Depending on how the material is conserved, little and often no DNA survives in ancient tissue. Multicopy DNA sequences are more likely to survive, probably because they occur in large numbers in the cell, hence, ancient DNA have mainly been retrieved from the mitochondrial genome (Hofreiter et al. 2001). Different refugia and postglacial colonization routes have been suggested for several roaming, terrestrial species through analyses of both contemporary and ancient mtDNA (Fedorov and Stenseth 2002, Flagstad and Røed 2003, Dalén et al. 2005, Zigouris et al. 2013). In paper II we used both modern and ancient mtDNA to study ancestry and a possible colonization route for high Arctic reindeer. For Svalbard reindeer, two possible colonization routes have previously been suggested based on CR data. Flagstad and Røed (2003) found the most common haplotype on Svalbard, thought to be unique for the archipelago, in northern Quebec – implying a colonization route to Svalbard through North America. However, Gravlund et al. (1998) identified a haplotype common on Svalbard in a sample from the wild Taimyr population, suggesting colonization of Svalbard from the Eurasian mainland. By analyzing CR sequence data from contemporary reindeer populations on Svalbard and Novaia Zemlia, and ancient samples from the now extinct population on the Franz Josef Land, we found that the most common haplotype on Svalbard (cf. paper I) is also present in high frequencies in the material from Novaia Zemlia and Franz Josef Land, implying common ancestry (paper II). We also found a genetic link between the three archipelagos and two wild mainland populations in Russia (Pechora River, Komi Republic and Peza River, Arkhangelsk Oblast) – supporting the theory that Svalbard, Novaia Zemlia and Franz Josef Land was colonized from the Eurasian mainland.

In paper II, we also wanted to resolve the question of possible human induced translocation to the high Arctic archipelagos. After the last glaciation, natural processes

of recolonization of new habitable areas occurred as the ice retreated. This process has historically accelerated by human intervention due to an increased interest in game species and their husbandry (Acevedo and Cassinello 2009). There is literature documenting the human interest in wild reindeer, as well as the movement of reindeer between islands in the Eastern Barents Sea in historic times (Khakhin 2001, Zhitkov 1903), pointing towards hunting of wild reindeer by Pomor or Vikings coastal dwellers on Novaia Zemlia from the 12th century (Beliaev 2004). There are also documented attempts of the early Soviet authorities to translocate approximately 600 domestic reindeer to Novaia Zemlia from Kolguev Island, which is the nearest population of domestic reindeer, between 1928 and 1933 (Zubkov 1935). By including CR data from Kolguev, we were able test for introgression from domestic reindeer into the high Arctic populations. Only one individual on Novaia Zemlia had a haplotype that is common on Kolguev, which may be explained by the translocation of domestic reindeer from Kolguev to Novaia Zemlia, as mentioned above. However, the lack of a clear genetic connection between Kolguev and the high Arctic populations, indicate that the maternal genetic structure of the high Arctic reindeer populations under study, is mainly indigenous. A wild, native origin of these populations is further supported by previously published radiocarbon dates from ancient material from Franz Josef Land, suggesting that reindeer populated the archipelago 6400-1300 years before present (YBP) (Forman 2004). This is congruent with our results from radiocarbon dating of archaic antlers from Franz Josef Land, which gave an age of more than 2000 YBP. Also, date since expansion for the sub-cluster comprising haplotypes mainly found on the Eurasian high Arctic archipelagos (Ic) was calculated to 5862 YBP. This is relatively close to the estimated time since most recent common ancestor (tMRCA) for sub-cluster Ic (mean tMRCA: 4823 YBP) (paper I) and well within the timeframe when reindeer is suggested to have populated the region. These results all imply that Arctic indigenous reindeer colonized the Eurasian Arctic archipelagos through natural dispersal, before humans approach this region.

4.3 Genetic variation, differentiation, and integrity in Eurasian reindeer populations

Despite its limitations, mtDNA have shown to be a powerful tool for population genetic studies. However, analyses of nuclear markers like the highly variable and biparentally inherited microsatellites, may add a necessary compliment and provide more complete and accurate results (Simonsen et al. 1998). In paper III, we studied genetic structure in 17 Norwegian wild reindeer populations, by analyzing 12 reindeer specific microsatellite loci as well as the CR.

Norwegian wild reindeer are highly fragmented and currently managed as 23 separate sub-populations (http://nvs.villrein.no). These populations are defined as wild, however, based on historical evidence, they may also be influenced in varying degree by herds of domestic and mixed origin. Due to the reindeer's natural nomadic behavior, we would expect wild, migrating reindeer to show a large-scale homogenous genetic structure due to gene flow among adjacent populations (Jackson and Fahrig 2016, Miguet et al. 2016). However, we found highly varying levels of genetic variation and differentiation, particularly in the microsatellite loci (paper III). High levels of variation were found in the relatively large Langfjella populations (Nordfjella, Hardangervidda, Setesdal), whereas low levels of variation were observed in the smaller populations e.g. Svartebotnen, Førdefjella, Sunnfjord, Blefjell and Våmur- Roan. Both population-based analyses (F_{ST} and R_{ST}), and individual cluster assignment analysis, suggests a main pattern of limited levels of gene flow between most populations and high levels of differentiation, particularly in the smaller populations. Reindeer inhabiting Langfjella, as well as some adjacent populations with an assumed origin from Langfjella, showed less differentiation, which might imply some level of genetic exchange among these areas, conforming to a core-satellite model. A clear genetic distinction between native wild reindeer and the populations with an assumed domestic ancestry was evident from both microsatellite and the CR data. This is congruent with the results from Røed et al. (2008), showing Norwegian reindeer to differentiate into three main clusters separating between the populations in Rondane/Dovre assumed to represent native wild population,

Hardangervidda with a history of considerably mixing of domestic native wild herds, and domestic reindeer. In paper III further sub-structuring was found and the individual cluster assignment analysis implied as many as eight to nine sub-clusters within the dataset. The high level of differentiation among Norwegian reindeer populations seems to be highly affected by founder effects and genetic drift due to small effective population sizes, as well as isolation due to fragmentation through human induced restriction to gene flow, distorting an expected isolation-by-distance effect.

We would expect the biparentally inherited microsatellite markers to show a more homogeneous genetic structure compared to the mtDNA markers, due to low effective population size of the mitochondrial genome in addition to strong female philopatry in reindeer (Roffler et al. 2012). However, we found higher levels of differentiation among populations in the microsatellite markers. This pattern could likely reflect higher resolution in microsatellites compared to mtDNA, the recent landscape fragmentation, and relatively recent origin of several of the populations. Also, reindeer is a highly polygynous species (Roffler et al. 2012), resulting in low effective population sizes, especially in the smaller populations. This effect is enhanced by the fact that Norwegian wild reindeer populations are managed with a highly skewed sex ratio, containing very few males. Hence, we can expect high levels of genetic drift also in the microsatellite markers, especially when gene flow is limited.

In general, Norwegian reindeer populations showed less variation in the CR compared to the Russian mainland populations (paper I, II, III and IV) (Table 1). Reduced genetic variation and high levels of differentiation is expected to be found in marginal populations compared to core populations, as fragmentation and isolation in marginal regions result in higher levels of genetic drift (Lesica and Allendorf 1995, Yannic et al. 2014a). Norwegian reindeer is distributed at the margins of reindeer's distribution area, while Russian reindeer may be considered to live in the core area, and hence, lower genetic variation in Norwegian reindeer is expected. The structure found within Norwegian reindeer, particularly the smaller and more fragmented areas, seems to be highly

influenced by demographic events as well as current isolation due to fragmentation. In contrast, many of the wild reindeer populations in Russia have shown to be less affected by human influence, probably due to the vast territories they occupy, sparse human population in these regions as well as poorly equipped hunters (Baskin 2005). A clear difference in genetic variability was also evident among the wild island populations studied in paper II. We observed lower levels of genetic variation on Svalbard compared to the wild populations on Novaia Zemlia and Belyi Island - probably reflecting high levels of genetic drift due to isolation and possibly bottlenecks, in the Svalbard population (Côté et al. 2002, Røed 2005).

Table 1. Study populations and assumed ancestry from domestic herds (D), native wild herds (W) or a mix of the two (M), and sample size (n). Genetic variation is given as number of haplotypes (Nh), haplotype diversity (Hd) and nucleotide diversity (π) for the CR in a 468 bp long fragment. Genetic variation calculated for populations marked with an asterisk is based on a 400 bp long fragment.

Population (ancestry)	n	Nh	Hd	
Hardangervidda (M)	32	10	0.760	0.016
Brattefjell-Vindeggen (M)	25	4	0.597	0.017
Blefjell (M)	19	3	0.556	0.014
Norefjell-Reinsjøfjell (D)	21	2	0.324	0.010
Nordfjella (M)	38	5	0.656	0.016
Lærdal Årdal (M)	31	3	0.617	0.015
Fjellheimen (D)	38	4	0.508	0.014
Sunnfjord (D)	14	3	0.560	0.011
Førdefjella (D)	9	3	0.556	0.011
Svartebotnen (D)	8	3	0.464	0.008
Skaulen Etnefjell (D)	18	4	0.669	0.016
Setesdal Ryfylke (M)	18	10	0.876	0.018
Setesdal Austhei (M)	33	9	0.777	0.019
Våmur-Roan (D)	48	4	0.334	0.007
Reinheimen-Breheimen (D)	17	3	0.544	0.014

Rondane-Dovre (W)	40	4	0.421
Forollhogna (D)	17	5	0.625
Domestic South (D)	42	8	0.670
Domestic North (D)	27	10	0.752
Taimyr (W)	26	20	0.971
Svalbard (W)	27	3	0.501
Novaia Zemlia (W)*	20	5	0.632
Belyi Island (W)*	22	6	0.814
Kolguev (D)*	24	6	0.728
Pechora River (W)*	14	12	0.978
Peza River (W)*	15	6	0.800
Nomama (D)	10	3	0.380
Lake Nichatka (D)	18	10	0.870
Tiania (D)	24	11	0.850
Chapo-Ologo (D)	36	9	0.840
Nomama (W)	24	10	0.910
Lake Nichatka (W)	10	6	0.780
Tiania (W)	7	4	0.720
Chapo-Ologo (W)	5	3	0.800

In Norway, wild and domestic herds have been present for several centuries and are usually kept separated in different mountain areas, with enhanced migration barriers due to increasing human activity (Røed et al. 2014). In paper III, we show that all populations, except Rondane-Dovre, contain a substantial fraction of mitochondrial haplotypes that are characteristic for domestic reindeer. Historically, several of the smaller populations under study have a known influence from straying or reintroduced domestic reindeer. Also the large populations in Langfjella, is highly affected by introgression from domestic reindeer as a result of extensive reindeer husbandry in these areas (Bjørnstad and Røed 2010). In the Zaibaikal'e area in southeastern Russia the situation is very different. Wild and domestic reindeer are assumed to have same ancestry, have co-existed for a long time, and are geographically sympatric. There is also a tradition of deliberate, but

controlled interbreeding between wild and domestic reindeer in this region. Nevertheless, by analyzing the CR and 13 reindeer specific microsatellites we found significant differentiation between four wild and four domestic populations in the Zaibaikal'e region (paper IV). We also found a pattern of stronger differentiation between wild and domestic reindeer in mtDNA compared to the nuclear microsatellites, suggesting a sex-biased genetic structure. The low CR haplotype sharing between wild and domestic reindeer, where only two among 36 haplotypes were shared, indicate very low levels of female mediated gene flow. This is consistent with female philopatry and male-biased dispersal commonly seen among most mammal species (Greenwood 1980, Pusey 1987, Goudet et al. 2002). However, the sex-biased genetic structure observed in Zaibaikal'e appears to be aggravated by a sex-biased and tightly controlled breeding practice. Our results suggest that the reindeer herders in south-eastern Zaibaikal'e, without any prior genetic knowledge, have developed herding techniques that effectively maintain the genetic integrity of co-existing and overlapping populations of wild and domestic reindeer, even in the presence of intentional interbreeding. These practices have made it possible for both populations to co-exist, selectively interbred, but with little trace of long-term genetic mixture between them. The local people have been able to develop such effective practices based on how the wanted traits obtained by interbreeding could best be maintained over time, i.e. be maintaining the differences, and thereby preserve the genetic integrity of wild and domestic populations.

5. Conclusions and Future Perspectives

Both the mitochondrial control region and the microsatellite markers have shown to be highly useful tools to study intraspecific genetic variation and structure in Eurasian reindeer. Microsatellites are particularly suitable for studying recent population history and stochastic processes, while mtDNA is an appropriate molecular marker for examining deeper divergence.

Genetic structure of Eurasian reindeer is now relatively well documented, especially in Norway. In general, Eurasian reindeer seems to be highly structured due to both demographic processes and adaptation. A clear distinction between wild and domestic reindeer is evident in Norway, among the Eurasian high Arctic archipelagos/islands, as well as in the Zaibaikal'e area in southeast Siberia. The mitochondrial haplotypes that characterize domestic reindeer seems to be highly diverged, which may give the impression of a large evolutionary difference between wild and domestic reindeer. Several of the Norwegian wild reindeer populations contain high frequencies of mtDNA haplotypes typical for domestic reindeer, due to human induced translocations and introgression. An important question for the future is whether the major genetic differences we find between populations with wild and domestic ancestry, and for the highly genetic structured Norwegian wild reindeer, using assumed neutral markers, have a functional significance.

New techniques will allow research to focus more on adaptability and not solely on effects of demographic processes. Whole genome sequencing of the reindeer genome have now been performed (Kantanen, pers.com) which will give a wider genetic coverage and provide valuable information on molecular evolution in reindeer. This opens for studies using both whole genome sequencing (HGS) and single nucleotide polymorphisms (SNPs) to better understand possible physiological and ecological effects of the genetic structuring of *Rangifer* populations.

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Paper I.

Kvie, K.S., Heggenes, J., Røed, K.H. 2016. Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer. Ecology and Evolution 6(13): 4347-4358.

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Merging and comparing three mitochondrial markers for phylogenetic studies of Eurasian reindeer (*Rangifer tarandus*)

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Keywords
COI, CR, cytb, mitochondrial markers, phylogeny, *Rangifer tarandus*.

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Funding Information
Norwegian Wild Reindeer Centre.

Received: 11 November 2015; Revised: 25 April 2016; Accepted: 2 May 2016

Ecology and Evolution **2016; 6(13): 4347–4358**

doi: 10.1002/ece3.2199

Abstract

Phylogenetic analyses provide information that can be useful in the conservation of genetic variation by identifying intraspecific genetic structure. Reconstruction of phylogenetic relationships requires the use of markers with the appropriate amount of variation relative to the timeframe and purpose of the study. Here, genetic structure and clustering are inferred from comparative analyses of three widely used mitochondrial markers, the CR, cytb and the COI region, merged and separately, using Eurasian reindeer as a model. A Bayesian phylogeny and a MJ network, both based on the merged dataset, indicate several distinct maternal haplotype clusters within Eurasian reindeer. In addition to confirm previously described clusters, two new subclusters were found. When comparing the results from the merged dataset with the results from analyses of the three markers separately, similar clustering was found in the CR and COI phylogenies, whereas the cytb region showed poor resolution. Phylogenetic analyses of the merged dataset and the CR revealed congruent results, implying that single sequencing analysis of the CR is an applicable method for studying the haplotype structure in Eurasian reindeer.

Introduction

Genetic diversity is the raw material for evolution which allows species to adapt to environmental change and to evolve local adaptations (Conner and Hartl 2004). Identification of intraspecific genetic structure and variation through phylogenetic analyses is therefore an important area in species conservation (Avise 2000). A wide range of molecular markers are presently available, and the loci of choice for most phylogenetic studies are located on the mitochondrial genome (Gissi et al. 2008). Mitochondrial DNA (mtDNA) is a popular genetic marker due to qualities such as high mutation rate, maternal inheritance and high prevalence in the cells, making it easy to amplify (Avise 2000; Gissi et al. 2008). MtDNA show high intragenomic variability, and substitution rates depend on which region that is considered (Pesole et al. 1999). Variation within the regions is also found; for example, the mitochondrial control region (CR), also called the displacement loop (D-loop), consists of selectively neutral blocks showing high mutation rates in addition to a more conserved central domain (Reyes et al. 2003). The highly variable regions are frequently used for intraspecific studies, whereas the conserved region is used for studies of more diverged taxa (Reyes et al. 2003). Other commonly used mitochondrial markers are the protein-coding cytochrome b (cytb) and cytochrome *c* oxidase subunit 1 (COI) regions. These markers are often used for examining deeper splits within species (Kurose et al. 1999; Ursenbacher et al. 2006; Kvie et al. 2013), in addition to being popular markers in species delimitation and in phylogenetic studies above species level (Hebert et al. 2003; Tobe et al. 2010). A potential problem associated with markers showing elevated substitution rates is the loss of information and underestimation of relationships between populations due to homoplasy, that is, sequence similarities due to convergent, parallel, or reverse evolution rather than common ancestry (Ballard and Rand 2005). Using additional, protein-coding markers that are more conserved as control might be a solution to avoid potential bias due to homoplasy. However, a shortcoming of using protein-coding mtDNA as a phylogenetic marker is possible selection owing to metabolic requirements (Foote et al. 2011). Although assumed to evolve in a neutral

manner, recent studies have questioned this assumption and selection on mtDNA regions has been detected in several species (Castoe et al. 2008; da Fonseca et al. 2008; Foote et al. 2011).

Reindeer (*Rangifer tarandus*) (Fig. 1), a migratory ungulate in the family Cervidae, have been widely distributed in most mountain, tundra and taiga areas throughout the northern Holarctic during most of the Holocene. Reindeer have been classified into three ecological groups: woodland, tundra and high arctic island forms, based on morphological and historical data (Banfield 1961). These ecological groups include nine subspecies, of which seven are extant. However, previous genetic studies based on mitochondrial DNA indicate discrepancies between current ecotype and subspecies designations and genetic differentiation (Gravlund et al. 1998; Flagstad and Røed 2003; Cronin et al. 2006; McDevitt et al. 2009). This may imply that the current geographical distribution of mtDNA haplotypes reflects historical demographic events rather than taxonomy as it is described today. Previous studies on reindeer haplotype structure are mainly based on single sequencing data from either the CR (Flagstad and Røed 2003; Røed et al. 2008; Kholodova et al. 2011; Klütsch et al. 2012; Weckworth et al. 2012) or the cytb region (Cronin et al. 2006; Yannic et al. 2014), but we are not aware of any combined studies. Phylogeographical analyses of reindeer based on the CR show three main haplotype clusters, probably originating in three separate glacial populations. The largest cluster comprises reindeer from Eurasia as well as North America, pointing toward a Beringian origin. The North American and sedentary woodland caribou (*R.t. caribou*) constitutes a second main CR cluster with haplotypes probably originating south of the Wisconsinan ice sheet (Flagstad and Røed 2003). Finally, a third cluster comprising haplotypes with a purely Eurasian origin is suggested to have undergone recent isolation, probably in connection with ice expansion in Eurasia during the Weichselian (Flagstad and Røed 2003). Two of the three clusters above have in later studies been further subdivided. The Beringian cluster shows six CR subclusters within Eurasian reindeer (Røed et al. 2008; Bjørnstad et al. 2012), while three lines within the North American woodland caribou have been described (Klütsch et al. 2012). The Beringian cluster and the North American cluster have also been identified through the phylogenetic reconstruction of the cytb region (Yannic et al. 2014).

Figure 1. Wild tundra reindeer (*Rangifer tarandus tarandus*) on Hardangervidda, Norway. Photograph: Arvid Haga.

Reindeer, as well as other Holarctic species, may be threatened by climate change and other anthropological impacts, and therefore, clear identification of phylogenetic structure below the species level is important in order to protect genetic variation (Vogler and Desalle 1994; Bloor et al. 2015). Here, we test the phylogenetic performance of three mitochondrial markers, merged and separately, using Eurasian reindeer as a model species. In addition to the much used CR and cytb region, a fragment from the COI region is included to obtain a longer fragment from the coding part of the mitochondrial genome. First, we want to merge the sequence data from the CR, cytb and the COI region to take advantage of the three markers' ability to resolve relationships on different taxonomic levels, as this may lead to increased resolution and clearer delineation of phylogenetic groups (Knaus et al. 2011). Second, we want to see how the three markers perform individually by comparing the variability found in each marker. It would be reasonable to expect these markers to show compatible results, as the mitochondrial genome can be considered a single locus and hence the three markers are principally linked (Avise 2000). However, properties of neutral variation in the CR compared to the functional gene parts of the mtDNA genome may result in different phylogenetic patterns due to different levels of resolution (Avise 2000).

Material and Methods

Study material

DNA from a total of 183 skin, muscle and blood samples were extracted and analyzed. The skin samples were stored dry and cold, the muscle samples in $\geq$ 80% ethanol, and the blood samples in EDTA. The samples were collected in Norway/Svalbard (n = 137) and Russia (n = 46), covering the northwestern parts of Eurasia (Table 2). A major part of the samples were collected in Norway to include the variation and structure found in the margins of the species distribution area. The sample set comprises wild tundra reindeer from south-central

parts of Norway (Hardangervidda and Rondane/Dovre), reindeer from the Svalbard archipelago, representing the wild high arctic type, and reindeer from northern Norway ($n = 27$), representing the domestic Scandinavian tundra type. Wild reindeer from western Taimyr as well as domesticated reindeer from Kola/Yamal were included representing Russian tundra reindeer.

DNA extraction and amplification

DNA was extracted using DNAeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA) following the manufacturer's protocol. A 503-base pair-long fragment from the mitochondrial control region was amplified using the forward primer RtCRF-(0-) (5′-AAT AGC CCC ACT ATG AGC ACC-3′) (Flagstad and Røed 2003) and the reverse primer RtCR-(528) (5′-TAG GTG AGA TGG CCC TGA AGA AA-3′) (Bjørnstad and Røed 2010). Amplification was performed using the following program: 95°C for 2 min, 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min (steps 2–4 cycled 30 times) and finally 72°C for 10 min. Amplification of a 308-bp-long fragment from the cytb region was performed using the primers cytb F (5′-CCATCCAACATCTCAGCATGATGAAA-3′) and cytb R (5′-GCCCCTCAGAATGATATTTGTCCTCA-3′). The PCR program for this primer pair was as follows: 95°C for 5 min, 95°C for 45 sec, 53–55°C for 40 sec, and 72°C for 2 min (steps 2–4 cycled 32 times) and finally 72°C for 10 min. For amplification of a 657-bp-long COI fragment, minor modifications were made to a primer pair originally used for antelopes, COIbF/COIbR (Bitanyi et al. 2011), using the primer design program Primer3Plus (Untergasser et al. 2007). Amplification was performed with the primer pair Rt_COIF (5′-TCACAAAGA-CATTGGCACCT-3′) and Rt_COIR (5′-TGATTCTTTG-GACACCCTGA-3′). The PCR profile used was as follows: 94°C for 1 min, 94°C for 30 sec, 50°C for 40 sec, 72°C for 1 min (steps 2–4 cycled 5 times), 94°C for 30 sec, 54°C for 40 sec, 72°C for 1 min (steps 5–7 cycled 35 times), and 72°C for 10 min. PCRs were performed using reaction mixture containing 1–2 μL DNA template, 1X buffer, 1.5 mmol/L $MgCl_2$, 0.8 mmol/L dNTPs, 5 pmol of each primer, 0.5 μg/μL bovine serum albumin (BSA), 0.5 U/μL AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) and dH_2O to make up the total volume of 20 μL.

Sequencing

The samples were cleaned for unincorporated primers and nucleotides using Illustra ExoProStar (GE Healthcare, Buckinghamshire, UK) diluted 10 times. Cycle sequencing was performed in a 10-μL reaction volume, using BigDye v3.1 sequencing kit (Applied Biosystems) following the manufacturer's recommendations. Purification was carried out using standard EDTA/EtOH precipitation. Capillary electrophoresis and data analysis were performed with an ABI 3130xL or 3500xL instrument (Applied Biosystems). All sequences were sequenced in both directions, and the consensus sequences were aligned by ClustalW (Thompson et al. 1994) and edited in MEGA v.5.2 (Tamura et al. 2011). We used Mega v.5.2 to manually concatenate the sequenced fragments from the three regions (CR, cytb, and COI).

Using highly conserved primers increases the chance of amplifying nonfunctional copies of mtDNA (numts), which might be a source for erroneous interpretation of the data (Gelissen et al. 1983; Galtier et al. 2009). Alignments produced from the coding parts of the mitochondrial genome (cytb and COI) were controlled for the presence of numts by translating the alignments from nucleotide to amino acid sequences to check for stop codons and frameshift mutations.

Statistical analyses

DNA polymorphism calculations (the number of haplotypes, gene diversity, and nucleotide diversity) were performed in DnaSP v5.10 (Librado and Rozas 2009) for each locus and for the different geographical regions**.** DnaSP was also used to test for neutrality by calculating Tajima's *D* (Tajima 1989) as selectively neutral markers will give more precise estimates of genetic variation and structure, compared to a marker under selection (Avise 2000). A negative Tajima's *D* corresponds to an excess of rare polymorphisms and implies a population size expansion or positive selection, while the positive values reflect an excess of intermediate-frequency alleles, and suggests population bottlenecks or balancing selection (Akey et al. 2004). All four alignments were reduced to include only one sequence of each haplotype for the phylogenetic analyses. We used BEAST v1.8.0 (Drummond et al. 2012) to construct Bayesian phylogenies for the three regions combined and separately. For the combined analyses, a single gene tree was constructed as all three markers are mitochondrial and therefore linked. We partitioned the datasets into three regions (cytb, COI and CR) to allow for different substitution models and rates for each locus. We used PartitionFinder v.1.1 (Lanfear et al. 2012) to identify the optimum partition scheme and substitution models for the two coding regions. The cytb region was partitioned into 1st, 2nd, 3rd codon position, while the COI region was partitioned into two partitions, (1st + 2nd) and 3rd. We used the HKY and the HKY + I + G substitution model for the cytb/COI regions and the CR, respectively. The substitution rate was set to 2.23%/Myr for the cytb region (Yannic et al. 2014) and 58.9%/Myr

for the CR (Ho et al. 2008). Based on the settings above, the substitution rate for the COI region was estimated in BEAST and subsequently set to 7.6%/Myr. Sequences (n = 11) from previously described CR haplotype clusters were included in the CR Bayesian analysis to designate sequences from the current study to previously described haplotype clusters (Røed et al. 2008; Bjørnstad et al. 2012). The analyses were run for 100 000 000 generations and 10% of the initial samples removed as burn-in. Convergence for the phylogenies generated in BEAST was assessed in TRACER (Rambaut et al. 2014), and the effective sample size for all parameters was above the general recommendation (ESS > 200). We also used BEAST v1.8.0 to calculate time since most recent common ancestor (tMRCA) to test whether the different CR haplotype clusters/subclusters diverged during or after the last glacial maximum (LGM). The molecular clock, model of substitution, and the number of generations were set as described above for the CR. The stem option was not implemented so that the time reported reflects tMRCA of the taxon set (the haplotype cluster). A median-joining network (Bandelt et al. 1999) based on the full, concatenated dataset (n = 183) was constructed using Network v4.6 (ref.fluxus-engineering.com). A MJ network should give an appropriate presentation of the intraspecific variation found, as it, unlike bifurcating phylogenetic methods, takes into account the fact that intraspecific relationship tends to include extant ancestral haplotypes and multifurcations (Posada and Crandall 2001).

Results

Sequence data and genetic variation

A total of 1284 bp from 183 individuals were analyzed for the CR, cytb and the COI region (GenBank accession numbers CR: KX094568-KX094750, cytb: KX066893-KX067075, and COI: KX085230-KX085412). To include as many sequences as possible, the CR alignment was trimmed from 503 or 504 bp (depending on the presence of a thymine indel) to 467 or 468 bp. The cytb dataset was analyzed in its full length of 308 bp, while the COI dataset was trimmed from 657 bp to 508 bp. Translating sequences from the two coding mtDNA regions revealed no stop codons or frameshift mutations in the alignments. All substitutions were synonymous except from one individual, with a single nonsynonymous substitution in the cytb region.

Standard estimates of DNA polymorphism in the merged dataset showed a high degree of variation with 54 haplotypes and haplotype (hd) and nucleotide (π) diversity equal to 0.917 and 0.010, respectively (Table 1). The CR dataset, analyzed separately, also showed a high degree of haplotype and nucleotide diversity (hd = 0.908, π = 0.020) and comprised 46 haplotypes (Table 1). In the cytb fragment, 13 haplotypes were found (hd = 0.731 and π = 0.005), whereas the COI region identified 18 haplotypes (hd = 0.856 and a π = 0.004) (Table 1). None of the datasets deviate from a neutral model with a constant population size; that is, none of the Tajima's D estimates are significantly different from zero (Table 1). Standard estimates of DNA polymorphism for each locus show large differences in variability in the geographical regions under study (Table 2). Haplotype diversity and nucleotide diversity are lowest within the wild populations from Svalbard/Norway (Svalbard and Rondane/Dovre), while the highest levels of DNA polymorphisms are found in wild reindeer from Russia (Taimyr). The wild population from Hardangervidda and the domestic populations from Norway (Finnmark) and Russia (Kola/Yamal) show variation within a more intermediate range (Table 2).

Table 1. Tajima's D and genetic variability in Eurasian reindeer (n = 183) based on the three mtDNA markers analyzed individually and merged.

	N[1]	L[2]	Tajima's D[3]	H[4]	Hd[5]	π[6]
CR	183	468	0.170	46	0.908	0.020
cytb	183	308	−0.860	13	0.731	0.005
COI	183	508	−1.011	18	0.856	0.004
Merged	183	1284	−0.344	54	0.917	0.010

[1]The number of individuals.
[2]Sequence length.
[3]Neutrality test to measure whether the data deviate from the expectations under a neutral model with the constant population size. None of the Tajima's D values are significant (P > 0.10).
[4]The Number of haplotypes.
[5]Haplotype (gene) diversity.
[6]Nucleotide diversity.

Haplotype structure based on the merged dataset

The Bayesian phylogeny and the MJ network indicated a high degree of variation, and several well-supported clusters/subclusters were identified (Fig. 2A and B), some of which have been described in previous studies based on the CR (Røed et al. 2008; Bjørnstad et al. 2012). In the current study, new subclusters were defined when showing high support (≥90), comprising ≥4 haplotypes in addition to showing some degree of geographical structure. Clusters that have been described in previous studies were included when showing ≥2 haplotypes. The merged dataset shows a main division between clusters **I** and **II,** with six subclusters identified within cluster **I**. Subcluster **Ia** includes 4 haplotypes (n = 43) found in wild reindeer

from Rondane/Dovre, except for two samples from Hardangervidda. Subcluster **Ib** comprises five haplotypes ($n = 14$) found in both wild and domestic reindeer from Hardangervidda, Finnmark, and Kola/Yamal. Haplotype cluster **Ic** includes 4 haplotypes ($n = 27$) that are all unique for Svalbard reindeer. Two smaller subclusters denoted **Id** and **Ie**, both comprising two haplotypes, were also identified. **Id** haplotypes ($n = 5$) were found in domesticated reindeer from Kola/Yamal and Finnmark, and in wild reindeer from Taimyr, whereas haplotypes in subcluster **Ie** ($n = 10$) were found in domesticated reindeer from Kola/Yamal. The highly variable subcluster **If** comprises 10 haplotypes ($n = 12$) found in wild reindeer from Taimyr and in domesticated reindeer from Kola/Yamal. Finally, cluster **II** includes the most diverged haplotypes and is separated from other clusters with a minimum of 11 mutations in the MJ network (Fig. 2B, mutations not shown). This cluster includes 11 haplotypes ($n = 42$) found mainly in domesticated reindeer from Finnmark, in addition to samples from Kola/Yamal, Hardangervidda, Rondane/Dovre, and the Taimyr area.

Haplotype structure based on the three markers analyzed separately

We included 11 haplotypes downloaded from GenBank to the single marker analyses of the CR, in order to identify previously described clusters. The Bayesian phylogeny generated from the 57 CR haplotypes shows several well-defined clusters (Fig. 3). Subclusters **Ia–If** and cluster **II** obtain high support (posterior probability ≥ 0.96) and are congruent with the results from the Bayesian phylogeny based on the merged dataset and from previous studies of the CR (Figs. 2A, 3). Subcluster **If**, based on the CR, has structure similar to **If** derived from the merged dataset; however, only four of the CR haplotypes obtain support (Figs. 2A, 3). The Bayesian phylogeny of the 13 cytb haplotypes shows low resolution and none of the clusters/subcluster found in the merged and the CR phylogenies are identified (Fig. A1). The COI dataset, comprising 18 haplotypes, shows higher resolution and subclusters **Ia**, **Ib**, **Ic**, **Id**, **If** and cluster **II** are identified. Subclusters **Ia**, **Ib**, **Id** and **II** are identified as having only one haplotype. High support (≥95) is obtained for subclusters **Ic** and **If** (Fig. A2). Subcluster **If** includes the same samples (except from one individual from Finnmark, Norway) as subcluster **If** based on the merged dataset. Calculations of tMRCA for the CR subclusters **Ia**–**If** and cluster **II** gave estimates ranging from 4008 to 8603 years before present (YBP). The oldest dates are estimated for cluster **II** (8603 YBP, HPD interval: 2899–15,000) and for subcluster **If** (7848 YBP, HPD interval: 1332–16,300). The youngest dates are estimated for subcluster **Ie** (4008 YBP, HPD interval: 5170–8764) and for subcluster **Ic** (4823 YBP, HPD interval: 497–10,600) (Table 3).

Discussion

Haplotype structure as inferred from the merged dataset

The merged dataset indicates a main division between the highly diverse cluster **I**, comprising six subclusters (**Ia**–**If**), and the well-supported cluster **II.** Cluster **Ia** comprises the ancient, wild and native haplotypes from Rondane/Dovre, which are still the dominating types in these populations (Røed et al. 2014). Subclusters **Id** and **Ie** both show only two haplotypes in the merged dataset. These are subclusters previously described from analyses of the CR and are dominated by haplotypes commonly found in domesticated Russian reindeer (Røed et al. 2008). The four haplotypes in subcluster **Ic** are unique for the

Table 2. DNA polymorphism in the CR, cytb, and the COI region for the different geographical regions.

		CR			cytb			COI			Merged		
Area	N^1	H^2	Hd[3]	π^4	H^2	Hd[3]	π^4	H^2	Hd[3]	π^4	H^2	Hd[3]	π^4
Hardangervidda	30	10	0.811	0.017	5	0.639	0.004	5	0.644	0.003	11	0.818	0.008
Rondane/Dovre	53	4	0.437	0.008	2	0.142	0.001	3	0.380	0.001	4	0.437	0.004
Svalbard	27	3	0.501	0.001	1	0.000	0.000	2	0.205	0.000	4	0.630	0.001
Finnmark	27	8	0.695	0.014	4	0.533	0.003	4	0.573	0.003	11	0.798	0.007
Taimyr	26	20	0.972	0.017	10	0.843	0.006	10	0.898	0.006	20	0.972	0.009
Kola/Yamal	20	10	0.758	0.015	5	0.616	0.004	8	0.742	0.005	11	0.842	0.009

[1]Sample size.
[2]Number of haplotypes.
[3]Haplotype (gene) diversity.
[4]Nucleotide diversity.

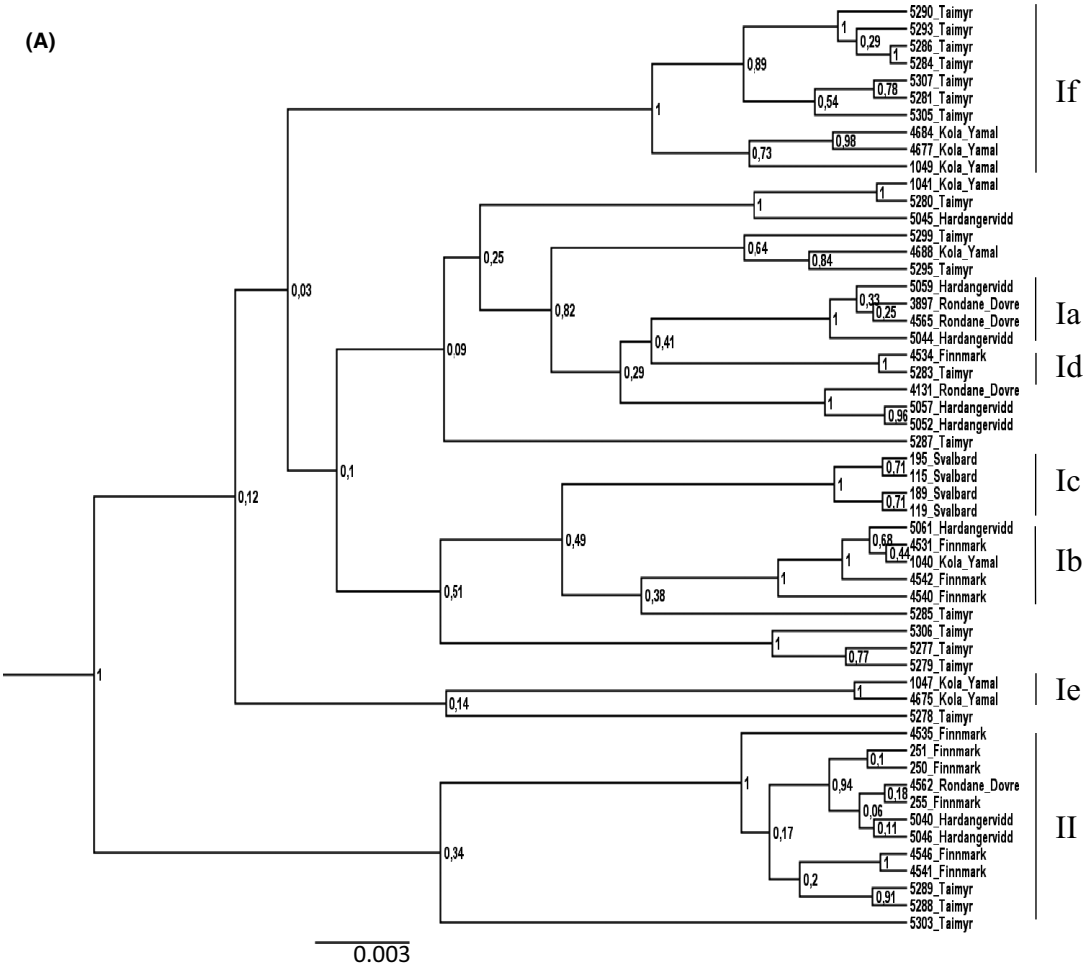

Figure 2. (A) Bayesian phylogeny of the three datasets combined, showing 54 haplotypes and well-supported subclusters **Ia–If** and cluster **II** (posterior probability values shown at each node). We found a main division between clusters **I** and **II** as well as six subclusters within cluster **I**. (B) Median-joining network of the concatenated dataset (1284 bp) from the CR, cytb, and COI region (n = 183) showing subclusters **Ia–If** and cluster **II.** The size of the circles corresponds to the number of individuals with the same haplotype. Pink: domesticated reindeer from Yamal/Kola, Russia. Purple: Wild reindeer from Taimyr, Russia. Green: Wild reindeer from the Svalbard archipelago, Norway. Yellow: Domesticated reindeer from Finnmark, northern Norway. Blue: Wild reindeer from Rondane/Dovre, central parts of Norway. Red: Wild reindeer from Hardangervidda, southern parts of Norway. The gray circles show the clusters/subclusters that obtained high support in the Bayesian phylogeny based on the three regions combined (A).

Svalbard archipelago and have not previously been described to constitute a separate subcluster. Svalbard reindeer is an isolated and sedentary population of approximately 9000–11,000 animals (Øritsland 1986), showing low degree of genetic variation (hd = 0.501, π = 0.001 in the CR). Reduced levels of genetic variation in the Svalbard population are also reported from studies based on other markers like transferrin (Soldal and Staaland 1980; Røed 1985) and microsatellites (Côté et al. 2002; Yannic et al. 2014). In the present study, we obtained support for the Svalbard haplotypes to comprise a separate subcluster, suggesting that Svalbard reindeer have been isolated from other Beringian populations for a substantial amount of time. This finding is also supported by the tMRCA mean estimate, which indicates isolation of the Svalbard population 4823 YBP (HPD interval: 497–10,600 YBP). Haplotypes in subcluster **Ib** are in combination with haplotypes from cluster **II**, the most commonly found haplotypes in Scandinavian domestic reindeer. Subcluster **Ib** has also previously been suggested to split off from the other Beringian subclusters and to constitute a separate group (Bjørnstad et al. 2012). This finding is supported by the Bayesian phylogeny in the current study.

The merged dataset shows that the Russian haplotypes are highly diverse and appear in all clusters except **Ia** (south-central Norway) and **Ic** (Svalbard), as well as being dominating in clusters **Ie** and **If** (Russia). The Taimyr samples seem to be especially variable (hd = 0.972, π = 0.017 in the CR) as shown by others (Kholodova et al. 2011; Baranova et al. 2012). This may be explained by the size of the Taimyr population, which is the largest extant Eurasian wild reindeer population, constituting 700 000–750 000 animals (Kholodova et al. 2011). The Taimyr population is probably also a historically united population which has maintained a high effective population size over a long period of time (Kholodova et al. 2011). Our results point in the direction of a separate Russian subcluster (**If**). However, due to the size and complexity of the Russian populations, more extensive sampling should be conducted before we draw conclusions regarding genetic structure within Russian reindeer.

Comparing the results from the merged dataset and the three mitochondrial markers analyzed separately

Comparing the results derived from the merged dataset with the results from the three markers analyzed separately showed similar maternal clustering in the phylogenies, except in the phylogeny inferred from the cytb fragment. None of the subclusters found in the merged dataset could be identified by analyzing the cytb region. Low levels of genetic variation could possibly be explained by selection and the replacement of preexisting variation by selective sweeps (Avise 2000; Foote et al. 2011). However, a negative and nonsignificant Tajima's D estimate indicates that positive selection is an unlikely source for the low genetic variation found in this marker. Low levels of variation in this marker can probably be explained by high levels of functional constrains reflecting the central role of cytb in energy production in the mitochondria (da Fonseca et al. 2008). A longer fragment from the cytb region could have been sequenced and analyzed (see Cronin et al. 2006; Yannic et al. 2014). However, our results are congruent with the results from a study performed by Yannic et al. 2014; showing little differentiation in the cytb region within Eurasian reindeer. More structure is found when analyzing the COI region, but with low support for some of the clusters. Nevertheless, this implies that the COI region might be a more appropriate addition to the CR than the cytb region. The merged data as well as the CR dataset show high degree of structure within Eurasian reindeer. Congruence between the two phylogenies may be explained by the difference in substitution rates between the CR and the two protein-coding markers, making the CR the most influential contributor of genetic variation. While the evolutionary rate for the cytb region is calculated to be 2.23% /MY (caribou and reindeer, Yannic et al. 2014) and 7.6% /MY for the COI region (reindeer), a substitution rate as high as 72.46% / MY has been calculated for the CR (reindeer, Røed et al. 2014). The high substitution rate in the CR is also consistent with the results from a study on the bovine control region where a substitution rate of 58.9% /MY is proposed (Ho et al. 2008).

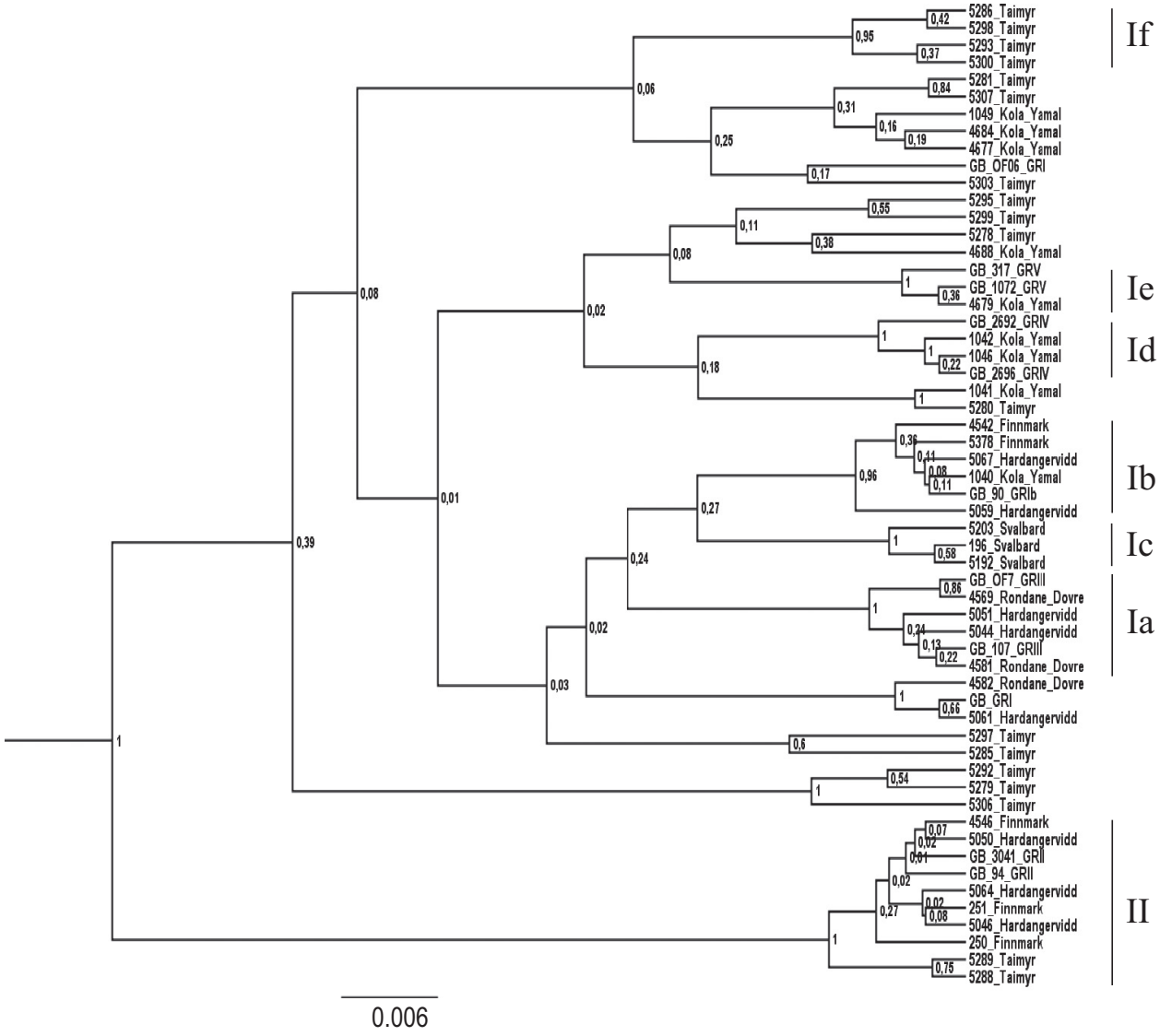

Figure 3. Bayesian phylogeny of the 46 CR haplotypes and subclusters **Ia**–**If** and cluster **II**. To assign haplotypes from the current study to previously described haplotype clusters, 11 haplotypes, downloaded from GenBank, were included in the analysis. A main division between clusters **I** and **II**, and six subclusters within cluster **I** was found (posterior probability values shown at each node). Subclusters **Ic** and **If** are not previously described as separate subclusters. A suggestion for renaming of CR clusters and subclusters is presented in the Appendix, due to inconsistent labeling in previous publications (Fig. A3).

Table 3. Mean and median tMRCA and 95% HPD interval for the different subclusters and cluster **II.**

Haplotype clusters	tMRCA YBP (mean)	tMRCA YBP (median)	95% HPD interval
Ia	6042	5554	1753–11,400
Ib	7058	6425	2065–13,400
Ic	4823	4134	497–10,600
Id	5542	4950	48–11,400
Ie	4008	3490	517–8764
If	7848	6577	1332–16,300
II	8603	7958	2899–15,000

The most recent common ancestor (MRCA) estimates obtained from the CR subclusters were all within a relatively short time period (4008 - 8603 YBP). This implies that the subclusters under study diversified after the last glacial period and hence that postglacial colonization routes and isolation events have had a great impact on present-day maternal haplotype structure. The relatively young colonization history of Eurasian reindeer makes it necessary to use highly variable markers (e.g., the CR), as the variation in more conserved markers probably reflects events from a more distant past, rather than postglacial

diversification of haplotypes (Hewitt 2000). However, the high substitution rate, which makes the CR a popular marker for intraspecific phylogenetic studies, may also lead to ambiguous phylogenetic patterns and the misinterpretation of the data. Reduced resolution and genetic structure due to homoplasy have shown to be a possible methodological problem when using highly variable markers (Vandewoestijne et al. 2004; Bulgarella et al. 2010; Bradman et al. 2011). In the present study, high variability and well-supported subclusters were found in the CR phylogeny. Several of the subclusters were also identified in the COI phylogeny, indicating that the CR has an appropriate amount of variation for studying Eurasian reindeer haplotype structure. However, subcluster **If** (Russia) showed lower resolution in the CR compared to the COI phylogeny and the phylogeny based on the merged dataset. The incongruence found between the two markers leaves subcluster **If** unresolved, and we cannot rule out the possibility of there being some degree of homoplasy within the CR dataset. As such, including slower-evolving markers may be a useful method to reveal phylogenetic inconsistencies, even when the data are characterized by a main pattern of recent divergence.

Concluding remarks

Bayesian phylogeny and a MJ network derived from the merged data revealed high degree of structure within Eurasian reindeer and nine clusters/subclusters were identified. We found Svalbard reindeer to constitute a separate subcluster (denoted **Ic**), implying that Svalbard reindeer have been isolated from the large continuous Beringian population for a substantial amount of time. We also found a complex structure within the Russian samples, implying that more extensive sampling is needed in order to obtain a better understanding of haplotype structure within Russian reindeer. Comparing results from the merged dataset with the results from analyses of the three markers separately shows similar clustering in all phylogenies except in the cytb phylogeny. Analyses based on the merged dataset showed topology and clustering, congruent with the results from analyzing the CR separately. However, reduced resolution was found in one of the CR subclusters (**If**) compared to the result based on the merged dataset, demonstrating the value of adding slower-evolving loci, when dealing with highly variable markers. Nonetheless, our results imply that the CR is an appropriate marker for studying intraspecific genetic structure in Eurasian reindeer and possibly for the identification of significant phylogenetic or population units in need of separate management considerations.

Acknowledgments

We would like to thank the Norwegian Wild Reindeer Centre, S. D. Mathisen, S. Côté, L. Kolpashikov, and V. Mikhailov for providing the samples and L. Midthjell for assistance in the laboratory.

Conflict of Interest

None declared.

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Appendix:

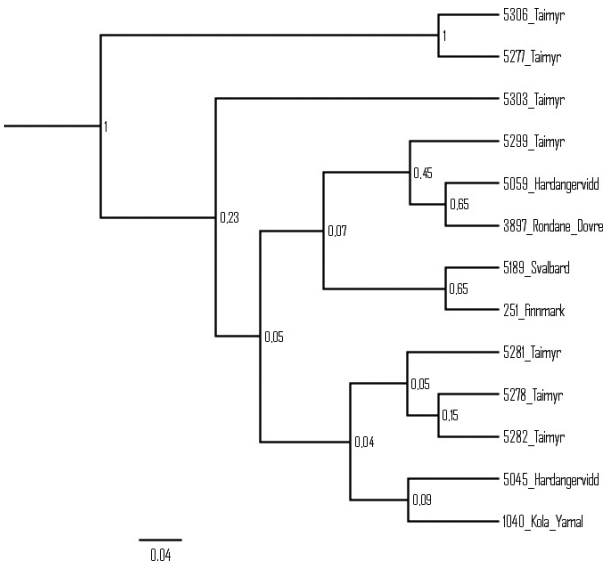

Figure A1. Bayesian phylogeny of the 13 cytb haplotypes (posterior probability values shown at each node). None of the clusters/subclusters identified based on the merged and CR dataset were found in the cytb phylogeny.

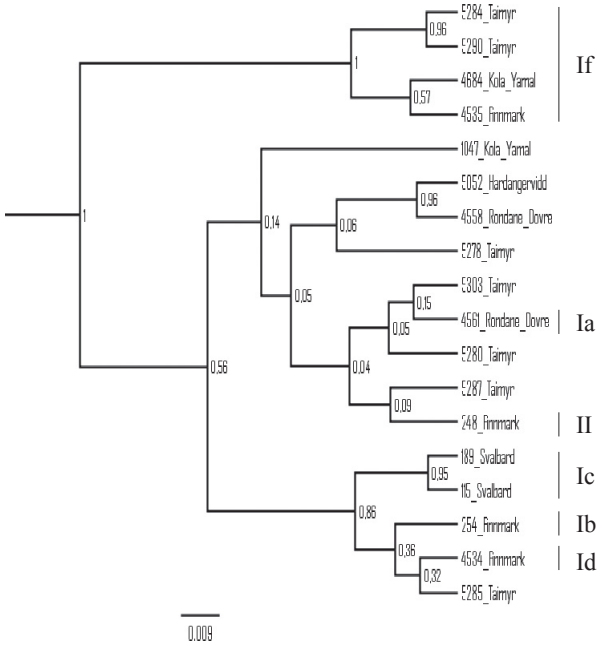

Figure A2. Bayesian phylogeny of the 18 COI haplotypes (posterior probability values shown at each node). Subclusters **Ia** – **Id**, **If**, and cluster **II** are identified. Support is obtained for subclusters **Ic** and **If.**

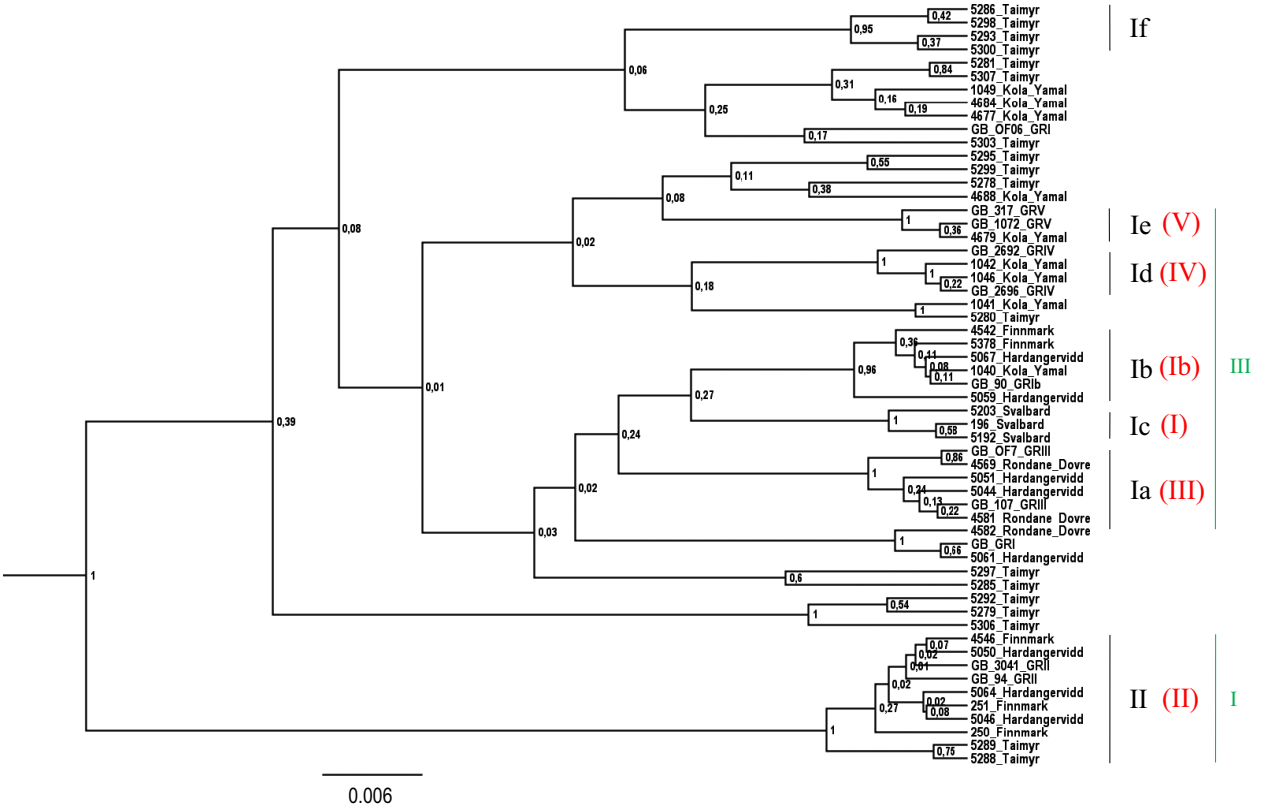

Figure A3. Bayesian phylogeny of the 46 CR haplotypes, in addition to 11 haplotypes representing previously described haplotypes (downloaded from GenBank). Subclusters **Ia**–**If** and cluster **II** are identified. This figure gives an overview of how the clusters/subclusters have been labeled in previous studies and in the current study. Flagstad and Røed (2003) described three main haplotype clusters, **I**–**III**, including all extant subspecies. Clusters **I** and **III** (shown in green) are further discussed in the current study. Cluster **I**, with a suggested origin from southern Europe (Flagstad and Røed 2003), has in later publications and in the current study been denoted as cluster **II** (Røed et al. 2008; Bjørnstad et al. 2012). Cluster **III** is the large variable Beringian cluster, which has been divided into five subclusters. These subclusters have in previous publications been denoted **I**, **Ib**, **III**, **IV,** and **V** (shown in red, in brackets) (Røed et al. 2008; Bjørnstad et al. 2012). Due to the inconsistent labeling of clusters and subclusters, we suggest that all haplotypes with a Beringian origin should be placed in cluster **I**. Subclusters within cluster **I** are further labeled as **Ia**–**If**, as shown in the figure (in black).

Paper II.

Kvie, K.S., Heggenes, J., Anderson, D.G., Kholodova, M., Sipko, T., Mizin, I., Røed, K.H. 2016. Colonizing the High Arctic: Mitochondrial DNA Reveals Common Origin of Eurasian Archipelagic Reindeer (*Rangifer tarandus*). PLoS ONE 11(1): e0165237. doi:10.1371/journal.pone.01652.

PLOS ONE

RESEARCH ARTICLE

Colonizing the High Arctic: Mitochondrial DNA Reveals Common Origin of Eurasian Archipelagic Reindeer (*Rangifer tarandus*)

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Citation: Kvie KS, Heggenes J, Anderson DG, Kholodova MV, Sipko T, Mizin I, et al. (2016) Colonizing the High Arctic: Mitochondrial DNA Reveals Common Origin of Eurasian Archipelagic Reindeer (*Rangifer tarandus*). PLoS ONE 11(11): e0165237. doi:10.1371/journal.pone.0165237

Editor: William Barendse, CSIRO, AUSTRALIA

Received: June 21, 2016

Accepted: September 15, 2016

Published: November 23, 2016

Data Availability Statement: Accession numbers for the sequence data used in this study are available from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) (accession numbers: KX844728-KX844796, KX844797-KX844811, KX094698, KX094701-KX094705, KX094707-KX094724, JQ073832-JQ073847). For previously published sequences (from the Peza- and Pechora River populations), only the different haplotypes are deposited in GenBank. Samples with the same accession numbers have identical haplotypes (S2 Table).

Abstract

In light of current debates on global climate change it has become important to know more on how large, roaming species have responded to environmental change in the past. Using the highly variable mitochondrial control region, we revisit theories of *Rangifer* colonization and propose that the High Arctic archipelagos of Svalbard, Franz Josef Land, and Novaia Zemlia were colonized by reindeer from the Eurasian mainland after the last glacial maximum. Comparing mtDNA control region sequences from the three Arctic archipelagos showed a strong genetic connection between the populations, supporting a common origin in the past. A genetic connection between the three archipelagos and two Russian mainland populations was also found, suggesting colonization of the Eurasian high Arctic archipelagos from the Eurasian mainland. The age of the Franz Josef Land material (>2000 years before present) implies that Arctic indigenous reindeer colonized the Eurasian Arctic archipelagos through natural dispersal, before humans approached this region.

Introduction

Climatic oscillations over the Quaternary (2.4 million years ago–present) have had a major impact on the geographic distribution and genetic structure of species through population extinctions and range shifts [1]. The demographic impact of range shifts alters the genetic structure of populations by the elimination of populations and lineages, reduction in genetic variation due to bottlenecks and founder events, as well as the spread of mutations by selection and population expansion [2]. How individual populations respond to such changes varies with their environmental tolerance, their ability to adapt [3] and their capacity to disperse to accommodate the rate of environmental change [4]. Arctic landscapes pose a particular challenge for terrestrial mammals due to the vastness and the way that intermittent ice-cover, oceans, and topography fragment the landscape. Furthermore, Arctic species are considered particularly vulnerable to climate changes, as even small changes may result in immediate and long-lasting effects [5].

Funding: The study was supported by the ERC Advanced Grant "Arctic Domus" ERC AdG 295458 based at the University of Aberdeen (http://www.arcticdomus.org/). Funding was recieved by DGA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Reindeer and caribou (*Rangifer tarandus*) is a keystone species in the circumpolar North, not only ecologically through the way they impact upon the plant cover [6], but also as a source of subsistence to local residents and more recently as a focus for defining protected areas [7]. Fossil evidence shows that during the Pleistocene, *Rangifer* was distributed south of the ice sheet in both Eurasia and in North America, and in the Beringia refugium encompassing the Bering land bridge, Alaska, as well as large parts of Siberia [8]. *Rangifer* exhibit distinct morphological adaptations to different environments, and populations have been subdivided into various ecotypes according to their life-history strategies and ecological conditions such as the woodland or boreal forest or sedentary form, the barren-ground or tundra or migratory form, the mountain form, and the Arctic form [8]. The Arctic form, thought to be better adapted to cold, open environments, is usually recognized by its small body size, with short rostrum and legs, as well as a thicker, paler winter pelage [9]. The morphologically-based Arctic type is made up of several populations: Svalbard reindeer (*R.t platyrhynchus*), distributed on the Svalbard archipelago, the North American Peary caribou (*R.t pearyi*) primarily distributed on the Canadian Arctic archipelagos, and the recently extinct *R.t eogroenlandicus*, formerly distributed on Eastern parts of Greenland [10]. In the Arctic, also less morphologically distinct reindeer exists as those on Novaia Zemlia in northern Russia. Russian taxonomists classify the reindeer inhabiting the Novaia Zemlia archipelago as members of the tundra type [11–15] and have since 2001 been registered by the Russian Federation as a geographically isolated subspecies (*R.t. pearsoni*) with a view to restoration [16, 17].

There has been a wide-ranging debate on the colonization routes and dispersal of Arctic reindeer inhabiting the islands in the western Eurasian and North American Arctic [8, 9, 18, 19]. Mitochondrial DNA (mtDNA) has shown to be a highly useful marker to describe past extinction and range expansions on near-present evolutionary time scales [20]. Based on contemporary- and ancient mtDNA, different refugia and colonization routes have been suggested for a range of roaming terrestrial arctic species like the collared lemming (*Dicrostonyx groenlandicus*) [21], reindeer (*Rangifer tarandus*) [19], the Arctic fox (*Alopex lagopus*) [22] and the wolverine (*Gulo gulo*) [23]. Svalbard reindeer, Peary caribou and *R.t eogroenlandicus* have been shown to comprise of mtDNA haplotypes signalling a common origin in an ancient Beringian and/or Eurasian pre-glacial population [19]. Recent genetic studies of Novaia Zemlia reindeer have demonstrated the same [14]. The fact that Peary caribou and *R.t eogroenlandicus* shared certain mtDNA haplotypes, morphological similarities, as well as have been observed to migrate from Ellesmere Island to North Greenland, provides convincing evidence for a North American colonization route for these two subspecies [9]. However, the colonization route of the existing archipelagic Svalbard and Novaia Zemlia reindeer populations has remained an open question.

Svalbard reindeer are characterized by low genetic variability indicating isolation, possible bottlenecks, and subsequent genetic drift as important population processes [24, 25]. The Svalbard population is characterized by three control region (CR) haplotypes which were previously thought to be unique to Svalbard [26]. However, the most common of these was also found in northern Québec, supporting the idea that these reindeer might have colonized Svalbard from North America [19]. This idea found support in the previously reported similarities in transferrin polymorphism between Svalbard reindeer and Peary caribou and with both having some similarities with the American woodland caribou [27, 28]. A recent genetic survey of wild reindeer populations on Novaia Zemlia have identified nine distinct CR haplotypes, with six thought to be unique to the archipelago [29] and the others showing links both Westward and Eastward. The population is further characterized to show low levels of variation compared to mainland populations [29]. Grinevetskii[30] in 1883 first recorded two different types of wild reindeer distributed on the Novaia Zemlia archipelago–one on the South Island

and one on the North Island–and reported that local hunters identified a morphological similarity between the latter and animals living on Svalbard. The existence of two separate "races" on the archipelago was repeated by Sokolov [31]. These earlier observations spoke to a common colonization route for all High Arctic reindeer from Eurasia.

The idea that the wild reindeer on some of the Arctic archipelagos might have been genetically linked to the Eurasian mainland was first raised by Gravlund et al. [9] who found a CR haplotype common on Svalbard in a sample from the wild reindeer population on western part of the Taimyr peninsula. The Taimyr population is considered to be the largest wild reindeer population in Eurasia, covering the northern parts of central and partly western Siberia [32]. An early published observation of a possible contemporary migration of a single reindeer bull from Novaia Zemlia to Svalbard over the winter ice presented a possible route. Nøis [33] speculated that Svalbard may have been colonized by reindeer from the Novaia Zemlia archipelago, situated 770 km south east of Svalbard, using the Franz Josef Land group of islands as a stepping stone [34]. The distance from the Franz Josef Land archipelago to Svalbard is approximately 400 km. Although there are no reindeer surviving on Franz Josef Land today, archaic bones and antlers are widely distributed [9, 35]. Previous radiocarbon dating of reindeer antlers sampled on the archipelago indicates that reindeer occupied Franz Josef Land as early as ~6000 years before present (YBP) [36].

A third account of the colonization of some of the High Arctic islands focusses on human-instigated translocations. Resolving the question of possible human induced translocation is important for the issue of introgression from domestic herds, a key parameter for classifying protection status of populations. There is a relatively broad Russian language literature documenting the human interest in wild reindeer, and the movement of reindeer herds between islands in the Eastern Barents Sea in historic times [16, 37, 38]. These sources point to the hunting of wild reindeer by Pomor or Viking coastal dwellers in Novaia Zemlia archipelago from the 12th century onwards [39]. There is one record of domestic reindeer being translocated to the islands by an academic expedition in 1896 [40], although local Nenets families living on the South Island of Novaia Zemlia in the 19th century were recorded as living without domestic reindeer [38]. There are well-documented attempts of the early Soviet authorities to translocate up to 604 head of domestic reindeer from Kolguev Island between 1928 and 1933 [41]. There are also scattered references to wild reindeer migrating over the ice from the Siberian mainland to Novaia Zemlia [16, 37, 38]. Finally, translocation of domestic reindeer from the Norwegian mainland to Svalbard, during expeditions taking place in 1872 and 1913, are also documented [42]. However, there are no domestic reindeer on Novaia Zemlia or on Svalbard today.

Here, we use the highly variable CR to compare sequence data from contemporary reindeer populations on Svalbard and Novaia Zemlia, with ancient samples from the now extinct population on the Franz Josef Land archipelago. Despite the work done comparing Svalbard reindeer to the mainland *Rangifer* populations in both North America and Eurasia, there has been a conspicuous lack of studies comparing the reindeer populations on each of these three neighbouring archipelagos to each other–the clearest approach to discussing possible common colonization routes. A genetic link between Svalbard, Novaia Zemlia and Franz Josef Land would tell us if these archipelagic populations have a common origin and also help to answer the question of whether or not the current distribution of the Arctic type is caused by natural dispersal or recent human induced dispersal of domestic reindeer. We also included sequence data from reindeer on the nearest population of domestic reindeer situated on Kolguev Island to test for introgression from domestic reindeer. Two wild mainland- and one wild island population from Russia were included to help answer the question of a possible biographical link between the Arctic archipelagos and the Eurasian mainland.

Material and Methods

Study populations

Blood-, muscle- and archaic antler samples were obtained from arctic populations on Svalbard (n = 3, in addition to 24 sequences downloaded from GenBank), Novaia Zemlia (n = 20) and Franz Josef Land (n = 15.) The Svalbard population was sampled at Nordenskiöld Land on Spitsbergen and on Nordaustlandet. Wild reindeer samples from Novaia Zemlia were collected on the South Island (Fig 1A, S2 Table). Archaic antlers were collected on Hooker- and Hays islands on the Franz Josef Land group. Four of these samples were ^{14}C dated (S1 Table). Skin- and velvet samples were collected from wild reindeer on Belyi Island (n = 22), and from domestic reindeer from Kolguev Island (n = 24). Belyi Island lies 430 km to the south and east of Novaia Zemlia and directly North of the Iamal peninsula and has a distinct population of wild reindeer [43, 44]. Kolguev Island lies 254 km Southwest of Novaia Zemlia and has supported Nenets domestic reindeer breeders [45] as well as a history of provisioning Novaia Zemlia with domestic stock in the 19th and early 20th century. Sequences from two continental wild reindeer populations, the Peza River Basin, Peza district, Arkhangelsk oblast' (n = 6) and the headwaters of the Pechora River in the Pechro-Ilychskii Nature Reserve, Komi Republic (n = 10), were downloaded from GenBank and included in the analyses in order to test for possible gene flow between the mainland and the archipelagos (S2 Table). Additional sequences from these populations were provided by the investigators (n = 13) [46, 47] (S2 Table). The

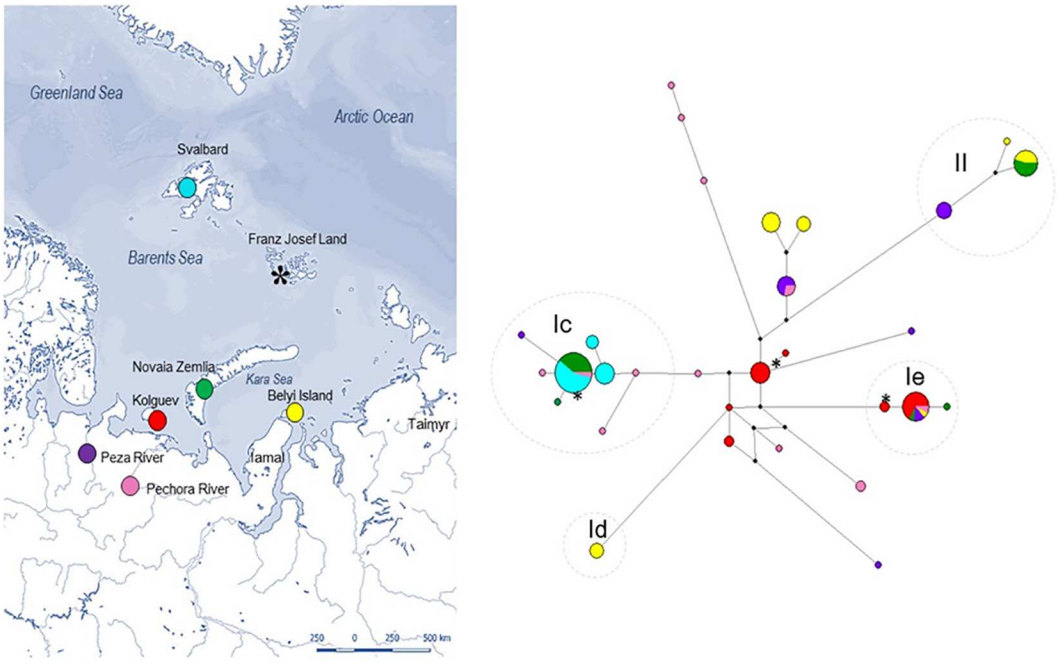

Fig 1. Sampling locations and phylogenetic network showing genealogical relationships in the CR between reindeer populations. Map of Northern Eurasia, with focus on the Eurasian Arctic archipelagos, showing the geographic origin of the samples (a) and a MJ network of the 122 CR sequences (400 bp) (b). Five previously described haplotype clusters (**Ic**, **Id**, **Ie**, and **II**) [26, 69] are identified. The MJ network show haplotype sharing between Svalbard (turquoise), Novaia Zemlia (green) and Pechora River (pink) within sub-cluster **Ic**. Including the Franz Josef Land samples (asterisk) show that 13 of the 15 ancient samples sequenced were identical to the most common haplotype found on Svalbard and on Novaia Zemlia. We also found one individual with a haplotype belonging to sub-cluster **Ie**, and one haplotype that is unique for Franz Josef Land. The map (a) is printed here for the first time under a CC BY license, with permission of the cartographer Allessandro Pasquini.

doi:10.1371/journal.pone.0165237.g001

Peza River population is classified as a forest reindeer ecotype and the Pechora River population as a forest-mountain reindeer ecotype [47]. To our knowledge, these populations have not previously been compared with Eurasian arctic archipelagic populations.

Ethics statement

Blood and muscle samples from Svalbard were collected as part of the Man and the Biosphere (MAB) project which started in 1978. The Svalbard MAB field project was coordinated by the Norwegian Polar Institute (Norway's central governmental institution for scientific research, mapping and environmental monitoring in the Arctic and the Antarctic). Hunting permits of reindeer within the Svalbard MAB field project was approved by the Governor of Svalbard. Sampling of muscle-, skin- and velvet samples from Novaia Zemlia, Belyi Island and Kolguev required no specific permits and was done under an ethics review for the ERC Arctic Domus and performed under the ERC Arctic Domus ethics annex.

Muscle and skin samples of wild reindeer from Novaia Zemlia and Belyi Island were collected from dead animals via subsistence hunting. The sampling was conducted by the authorized managers of these populations, according to the regulations stated by the Ministry of Nature Protection of the Russian Federation. Velvet samples from domestic reindeer on Kolguev Island was collected from dead animals during industrial slaughter. No animals were sacrificed for this study and the field work did not involve endangered or protected species.

DNA analyses of contemporary samples

Tissue samples were stored in ethanol ($\geq$ 80%) or kept frozen until analysed, blood samples were stored in EDTA. DNA extraction of muscle-, velvet- and skin samples was performed using DNeasy Blood & Tissue Kit (Qiagen) following the manufactures protocol. DNA extraction from EDTA blood was carried out using DNeasy Blood & Tissue Kit (Qiagen) or by using a boiling method for DNA extraction (SI).

A 503 base pair (bp) long fragment from the mitochondrial control region was amplified using the forward primer RtCRF (5'-AAT AGC CCC ACT ATG AGC ACCC-3') [19] and the reverse primer RtCR-528 (5'-TAG GTG AGA TGG CCC TGA AGA AA-3') [48]. Amplification was performed using the following program: 95°C for 2 min, 95°C for 30 sec, 55°C for 30 sec and 72 °C for 1 min (step 2–4 cycled 30 times) and finally 72 °C for 10 min. PCR reactions were performed in 20 μl total volume using 1–2 μl DNA template, and with the following final concentrations; 1X buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 5 pmol of each primer, 0.5 μg/μl Bovine Serum Albumin (BSA), 0.5 U/μl AmpliTaq DNA polymerase (Applied Biosystems), and dH_2O to make up the remaining volume.

The samples were cleaned for unincorporated primers and nucleotides using Illustra Exo-ProStar (GE Healthcare) diluted 10 times. Cycle sequencing was performed in a 10 μl reaction volume, using BigDye v3.1 sequencing kit (Applied Biosystems) following manufacturer's recommendations. Purification was carried out using standard EDTA/EtOH precipitation. Capillary electrophoresis and data analysis were performed with an ABI 3130xL- or 3500xL instrument (Applied Biosystems). All sequences were sequenced in both directions and the consensus sequences were aligned by ClustalW [49] and edited in MEGA v5.2 [50]. The sequence alignment was trimmed to 400 bp to be aligned with sequences downloaded from GenBank.

DNA analyses of ancient samples

DNA was extracted from antler powder using DNeasy Blood & Tissue kit (Qiagen) following Bjørnstad and Røed [48]. Standard precautions for working with ancient samples were

undertaken [51, 52]. All equipment and working surfaces were cleaned using sodium hypochlorite, ethanol or UV-light. Samples were mechanically cleaned and the outer surface was removed before drilling out the powder. To test for contamination, blank extraction and PCR controls were used in each PCR reaction and only DNA sequences which could be replicated from at least two independent amplifications of each primer pair were accepted.

From the ancient material a 266 bp fragment of the mtDNA control region was amplified using the primer pair 259F/524R (5'–TGCCCCATGCTTATAAGCAAG–3'/ 5'–GTGAGATG GCCCTGAAGAAA–'3), or by amplifying two overlapping amplicons of respectively 140 bp with primers 259F and 398R (5'- CCTTTCTTGTCAACATGCGTA– 3') and 178 bp with primers 347 F (5'–TGCCCCATGCTTATAAGCAAG–3') and 524R. PCR amplification and sequencing were performed as in Bjørnstad and Røed [48]. The sequences were aligned by ClustalW and edited in MEGA v5.2. The sequence alignment was trimmed down to 190 bp.

^{14}C dating of ancient samples

For a verification of the time horizon, we ^{14}C dated 4 antler samples from Franz Josef Land which also amplified successful DNA (S1 Table). All ^{14}C dates were calibrated using CALIB 6.1.1 [53] based on the data set IntCal13 [54]. The ^{14}C dating of 4 of the 15 ancient samples from Franz Josef Land all revealed an age of more than 2000 years (2468–3835 YBP, S1 Table), suggesting that these samples are from wild, indigenous reindeer.

Statistical analyses

DNA polymorphism estimates (number of haplotypes, gene diversity and nucleotide diversity) were calculated in DnaSP [55] for the contemporary populations, and for the data set including the ancient antler samples. Genealogical relationships were examined by constructing a Median Joining (MJ) network [56] using Network v4.6 (fluxus-engineering.com). BEAST v1.8.0 [57] was used to construct a Bayesian phylogeny based on the haplotypes identified in the dataset comprising the contemporary populations. We used the HKY G+I substitution model and the substitution rate was set to 58.9%/Myr [58]. The analyses were run for 100 000 000 generations and 10% of the initial samples was removed as burn-in. Convergence was assessed in TRACER [59] and the effective sample size for all parameters were above the general recommendation (ESS> 200). We used Arlequin v.3.5 [60] to test for recent demographic expansion of the sub-cluster dominating on the Arctic archipelagos by calculating the mismatch distributions of pairwise nucleotide differences [61, 62], as implemented in Arlequin and with 10 000 bootstrap replicates. For the same sub-cluster, Arlequin was used to calculate the sum of squared deviations (SSD) to test if the observed distribution deviated significantly from the expected under the population expansion model. The Harpending Raggedness index [63] was calculated to check for demographic changes. A smooth morphology indicates a population expansion, whereas a ragged morphology indicates constant population size [63]. Arlequin was used to calculate Fu's Fs [64] and Tajima's D [65] to check for deviations from neutrality. We used DnaSP to estimate a third neutrality test, the Ramos-Onsin's and Roza's [66] R_2 value, which may be more appropriate when dealing with small sample sizes. Haplotype frequencies in each of the seven populations were calculated in Arlequin.

Results

A total of 137 samples from seven populations were analyzed for the mitochondrial control region, including 122 contemporary- and 15 ancient samples. The 400 bp long alignment, comprising the contemporary populations, varied from low to relatively high levels of genetic diversity with a total of 30 haplotypes, overall haplotype diversity (Hd) = 0.910, and nucleotide

Table 1. Geographic origin, status (wild/domestic) and polymorphism in the CR in the sampled reindeer populations. Sample size (N), number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) in the CR region for the 400 bp long fragment, and for the 190 bp long fragment including all populations.

Geographic origin	Status	400 bp				190 bp			
		N	H	Hd	π	N	H	Hd	π
Svalbard, Norway	Wild	27	3	0.570	0.002	27	1	0.000	0.000
Kolguev, Russia	Domestic	24	6	0.728	0.009	24	6	0.728	0.013
Novaia Zemlia, Russia	Wild	20	5	0.632	0.018	20	4	0.626	0.029
Belyi Island, Russia	Wild	22	6	0.814	0.018	22	6	0.814	0.026
Pechora River, Russia	Wild	14	12	0.978	0.019	14	10	0.956	0.027
Peza River, Russia	Wild	15	6	0.800	0.018	15	6	0.800	0.030
Franz Josef Land, Russia	Wild	-	-	-	-	15	3	0.257	0.009
Total	-	122	30	0.910	0.019	137	24	0.820	0.027

doi:10.1371/journal.pone.0165237.t001

diversity (π) = 0.019 (Table 1). The Svalbard population exhibited the lowest level of variation (Hd = 0.570, π = 0.002) showing 3 haplotypes. The Novaia Zemlia population showed an intermediate level of genetic diversity (Hd = 0.632, π = 0.018) and in our study 5 haplotypes were identified. The domestic population from Kolguev Island showed an intermediate level of haplotype diversity and low levels of nucleotide diversity (Hd = 0.728, π = 0.009) and 6 haplotypes were found. The wild population from Belyi Island exhibited relatively high levels of variation (Hd = 0.814, π = 0.018) with 6 haplotypes identified. The two wild mainland populations from the Peza and Pechora river basins also showed high levels of variation (Hd = 0.800, π = 0.018 and Hd = 0.978 π = 0.019, respectively) with 6 haplotypes found in the Peza population and 12 in the Pechora population (Table 1).

We identified four previously defined haplotype clusters denoted sub-cluster **Ic**, **Id**, **Ie** and cluster **II** [26, 67, 68] (Fig 1B), all showing high support in the Bayesian phylogeny (posterior probability $\geq$ 99, S1 Fig), except sub-cluster **Ic** showing an intermediate level of support (posterior probability = 71). In the present study, we found sub-cluster **Ic** to comprise the 3 haplotypes previously found in Svalbard reindeer (n = 27), but also to include haplotypes found on Novaia Zemlia (n = 12), the Pechora River (n = 3), the Peza River (n = 1) and Franz Josef Land (n = 13) (Figs 1B and 2). Haplotypes in sub-cluster **Id** are commonly found in Russian domestic reindeer, but have also been identified in wild reindeer from Taimyr [26]. In the current study, we identified one haplotype belonging to sub-cluster **Id** in four wild reindeer from Belyi Island (Figs 1B and 2). **Ie** haplotypes are also commonly found in Russian domestic reindeer [26]. However, in the present study, **Ie** haplotypes were found in samples from Kolguev (n = 12), Belyi Island (n = 1), Peza River (n = 2), Pechora River (n = 1), Novaia Zemlia (n = 2) and Franz Josef Land (n = 1) (Figs 1B and 2). Finally, haplotypes in cluster **II** have previously been known to dominate in Scandinavian domestic reindeer [69]. We found cluster **II** haplotypes in wild reindeer from Peza River (n = 5), Novaia Zemlia (n = 6) and on Belyi Island (n = 6) (Figs 1B and 2). One of the three cluster **II** haplotypes found in the current study is identical to a cluster **II** haplotype commonly found in Scandinavia [26, 69].

Sub-cluster **Ic** appear to have experienced a recent population expansion as the mismatch distribution analysis showed no significant deviation from the expected distribution under the sudden expansion model (see S2 Fig). An expansion was further supported by a non-significant Harpending Raggedness index (0.071, p = 0.600) and SSD value (0.010, p = 0.710). Finally, Fu's Fs and Ramos-Onsins R_2 were highly significant with Fs = 5.948 (p = 0.000) and R_2 = 0.103 (p = 0.002) adding support for a demographic expansion for this sub-cluster. Tajima's D was negative (-1.018), but not significant (p = 0.173). Date since expansion for sub-cluster **Ic**

was calculated to 5862 years before present (YBP) (95% CI: 535–10375) based on the mean number of pairwise differences, τ (τ = 2.762).

The 190 bp long fragment, including all seven populations, showed high levels of variation (Hd = 0.820, π = 0.027) and 24 haplotypes were identified. However, low levels of genetic variation was found in the ancient Franz Josef Land population (Hd = 0.257, π = 0.009) (Table 1). Three haplotypes were found here, with one individual in sub-cluster **Ie,** 13 in sub-cluster **Ic**, and one sample with a haplotype unique for Franz Josef Land (Figs 1B and 2).

Discussion

Our results show that the most common haplotype found on Svalbard is also the most common haplotype found among the contemporary wild population on the South Islands of Novaia Zemlia and the extinct population on Franz Josef Land–suggesting that the population history of wild reindeer on these High Arctic islands was tightly linked. The genetic similarity between these archipelagic reindeer populations indicate gene flow and dispersal from a common source population, and supports the theory that all of these islands were likely colonized from the Eurasian mainland. The hypothesis of an eastern colonization of the Arctic archipelagoes is further supported by a genetic connection between the three archipelagic populations and one individual from Pechora River (Komi Republic), who shared the most common

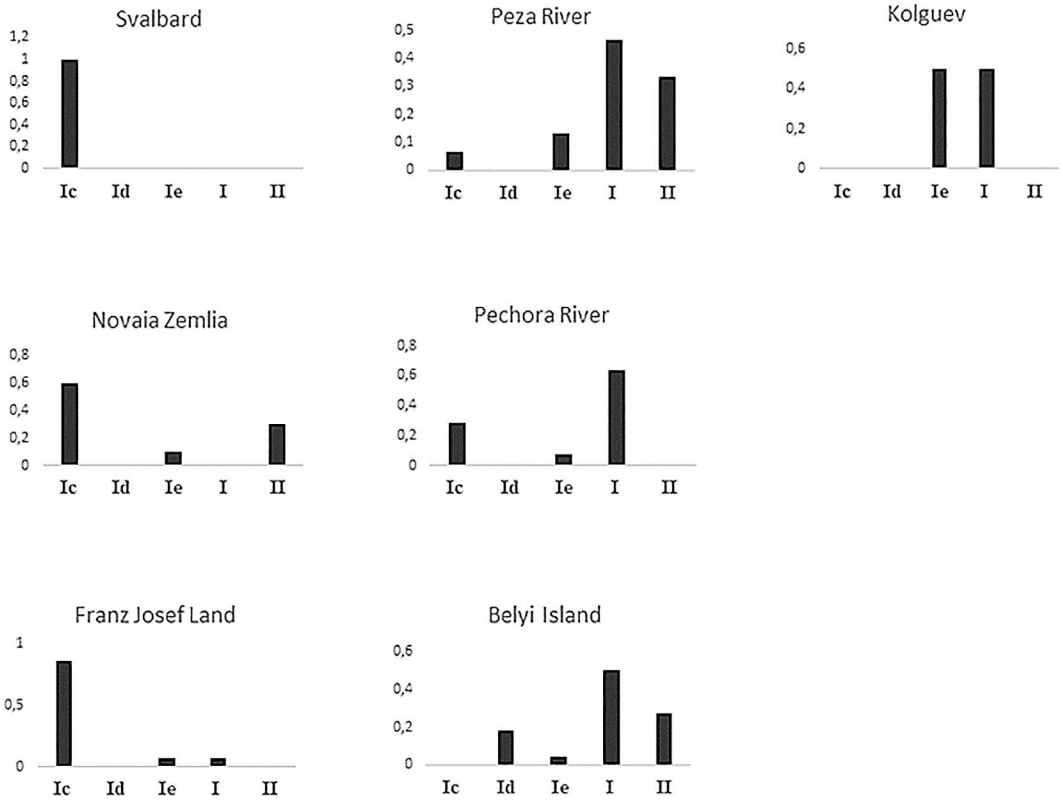

Fig 2. Frequencies of CR haplotype clusters in the sampled reindeer populations. Frequencies of haplotypes belonging to sub-cluster **Ic**, **Id**, **Ie** and cluster **II** in all seven populations. Haplotypes that did not cluster with any of the previously described clusters were placed in cluster **I**. Haplotype frequencies are calculated from the 400 bp long fragment for all populations, except haplotype frequencies in the ancient material from Franz Josef Land, which were calculated from the 190 bp long fragment. Haplotype frequencies show that **Ic** haplotypes are common on Svalbard, Novaia Zemlia and in the ancient material from Franz Josef Land. **Ic** haplotypes are also found in the Pechora- and Peza River populations, but are absent in the domestic reindeer population sampled on Kolguev.

doi:10.1371/journal.pone.0165237.g002

haplotype found on Svalbard, Novaia Zemlia and Franz Josef Land. Also, three similar haplotypes were observed in single samples from wild reindeer in both Pechora river basin and in the Peza river basin. However, the presence of **Ic** haplotypes in the two mainland populations could be the result of random haplotype survival from a common refugial population. More extensive sampling of Russian wild reindeer, with a special emphasis on islands in northern Siberia, would be necessary to clarify this further.

Natural- or human induced dispersal

Published radiocarbon dates of archaic bones collected on Franz Josef Land suggest that wild reindeer populated the archipelago 6400–1300 YBP [36]. These results fit with the dates we obtained for our own radiocarbon dated antler samples from the same islands (>2000 years). Pollen studies of archaic reindeer pellets found in peat cores on Svalbard indicate that reindeer colonized this archipelago between 6700 and 5000 YBP [70, 71]. The history of colonization of Novaia Zemlia by reindeer for much of Holocene remains to be documented. However, geomorphologic studies suggest that by the time wild reindeer were present on Svalbard and Franz Josef Land, Novaia Zemlia was also de-glaciated and therefore open for natural colonization as well [72, 73]. The early colonization of Svalbard and Franz Josef Land, and the strong genetic link found between the ancient Franz Josef Land samples and the contemporary populations on Svalbard and Novaia Zemlia, both imply that the Arctic reindeer type lived on all of these archipelagos long before humans approached the region [74].

We did find one individual on Novaia Zemlia with a haplotype also found in reindeer on Kolguev Island (sub-cluster **Ie**). This finding can be explained by the recent translocation of a small number of domestic reindeer from Kolguev to Novaia Zemlia, between 1928 and 1931 [41]. The weak genetic connection found between the wild populations on Novaia Zemlia and the present-day domestic population on Kolguev Island, together with the genetic similarities found between Svalbard, Novaia Zemlia and Franz Josef Land, imply that the maternal genetic structure of northern archipelagic reindeer populations, including present populations on Novaia Zemlia and Svalbard, is mainly indigenous.

Post glacial colonization of Eurasian arctic archipelagos

Our results indicate an eastern colonization route of the Eurasian arctic archipelagos. The Bering land refuge has traditionally been most widely discussed as a single origin point for various continental distributions of *Rangifer* [19, 75] as well as several other circumpolar species [76]. However, recently there has been a discussion of the importance of the role of a lesser-known set of refugia in the High Arctic of Western Siberia. Fedorov et al. [77] performed a circumpolar phylogeographic analysis of lemmings (*Lemmus*) questioning the centrality of the traditional Beringian refuge for the post-glacial re-colonization of the Arctic. They demonstrate how four different mtDNA linages of the circumpolar lemming (*Lemmus*) indicate separation by glacial barriers, followed by post glacial colonization from refugia other than Beringia. Shaefer et al. [78] in their recent review of North American mtDNA phylogeographic analyses for multiple circumpolar species point to additional complexity of multiple "refugia within refugia" within and between ice sheets. Salonen, Seppä [79] in their review of the palynological literature for Western Siberia point to possible refuge located in the Pechora River basin. The existence of alternative refugia, separated from Beringia, might be one explanation for the unique genetic composition observed in Eurasian archipelagic reindeer. On the other hand, our results suggest that there was a population expansion of sub-cluster **Ic** as recently as 5000–6000 YBP. This implies that the unique genetic composition of these Arctic reindeer populations may have resulted from bottlenecking, isolation, and then subsequent expansion

in the High Arctic well after the retreat of the ice, rather than isolation in alternative refugia during the last glacial maximum (LGM). Further studies of the genetic structure in Russian reindeer populations would help to answer this problem.

Post-glacial range shifts and the expansion of reindeer populations, as well as those of other cold-adapted species, is probably connected to the major environmental changes taking place in northern Eurasia during the Holocene [80]. Sea surface conditions in the southeastern Barents Sea region reconstructed from dinoflagellate cyst assemblages, indicate a warm and stable climate between 8000–5000 YBP [81]. These correlate well with other terrestrial and marine records of climate conditions during this period [81], as well as estimates indicating that the spruce (*Picea*) and birch (*Betula*) tree lines in northern Eurasia were located at least 150 km further North from their present location, and in the case of birch, may have reached the seacoast [36, 79]. The expanding forest may have driven Arctic-adapted reindeer populations to migrate further north to seek open tundra landscapes. The warmer climate would have facilitated growth of various vascular plants in the high arctic, thus expanding the food base for reindeer on the Eurasian arctic archipelagos [71]. As mentioned above, there are anecdotal accounts of the movement of large-bodied reindeer from the Eurasian mainland to Svalbard in historic times [34], suggesting that such migrations between islands over the ice are possible. This scenario is supported by the shared haplotypes held by wild reindeer in Taimyr and in Svalbard by Gravlund and colleagues [9], and our own discovery of shared haplotypes between the three arctic archipelagos and the mainland populations from the Peza and Pechora River basins in the current study.

Concluding Remarks

Rangifer are in many ways a classic circumpolar species providing an important anchor to the environmental history of the High Arctic, and also to the lives of local people. Contemporary climate change would be expected to alter the distribution and demography of *Rangifer* today as has been the case in the past. Mammals living in the High Arctic have limited opportunities to migrate further north. To survive, they will have to depend on their ability to adapt where they are. Therefore they are under particular risk. This necessitates having proper management plans with an emphasis on conserving genetic variability for indigenous archipelagic reindeer.

This study has established certain strong genetic similarities found between wild reindeer populations on Svalbard, Novaia Zemlia and Franz Josef Land implying that the maternal genetic structure of these archipelagic reindeer populations is indigenous and unique. The study lends considerable weight to the hypothesis that these islands may have colonized from the Eurasian mainland via an eastern route. Moreover the study strongly disproves the suggestion that populations for example on Novaia Zemlia are feral populations of introduced domestic reindeer. It is our hope that these important results will help clarify existing conservation plans for wild reindeer on Novaia Zemlia in the Russian Arctic Strict Nature Reserve and its aim at conserving this important and unique population.

Supporting Information

S1 Fig. Bayesian phylogeny based on the 400 bp long fragment, excluding the ancient samples from Franz Josef Land. The Bayesian phylogeny shows 30 control region haplotypes and support for sub-cluster **Ic**, **Id**, **Ie** and **II** (posterior probability values $\geq$70 is shown at each node).
(TIF)

S2 Fig. Mismatch distribution. The observed pairwise difference (blue bars) and the expected mismatch distribution (red bars) under the sudden expansion model among individuals in sub-cluster **Ic**. The mismatch analyses show a unimodal distribution, which is characteristic for a recently expanded population [63].
(TIF)

S1 Table. ^{14}C and calibrated radiocarbon dates on antler samples from Franz Josef Land. The ^{14}C dates were calibrated using CALIB 6.1.1 [53], based on the data set IntCal13 [54] with 2σ ranges.
(PDF)

S2 Table. Sample information and NCBI GenBank accession numbers.
(PDF)

S1 Text. Boiling method for DNA extraction.
(PDF)

Acknowledgments

We would like to thank Dr. Elena A. Volzhanina for her historical research on the history of domestic reindeer translocation to Novaia Zemlia.

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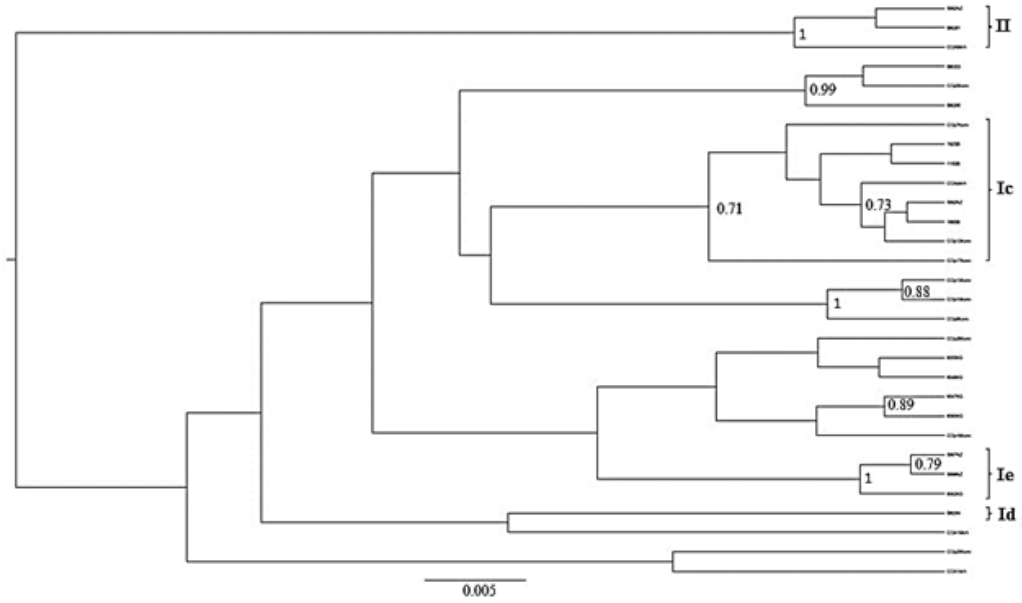
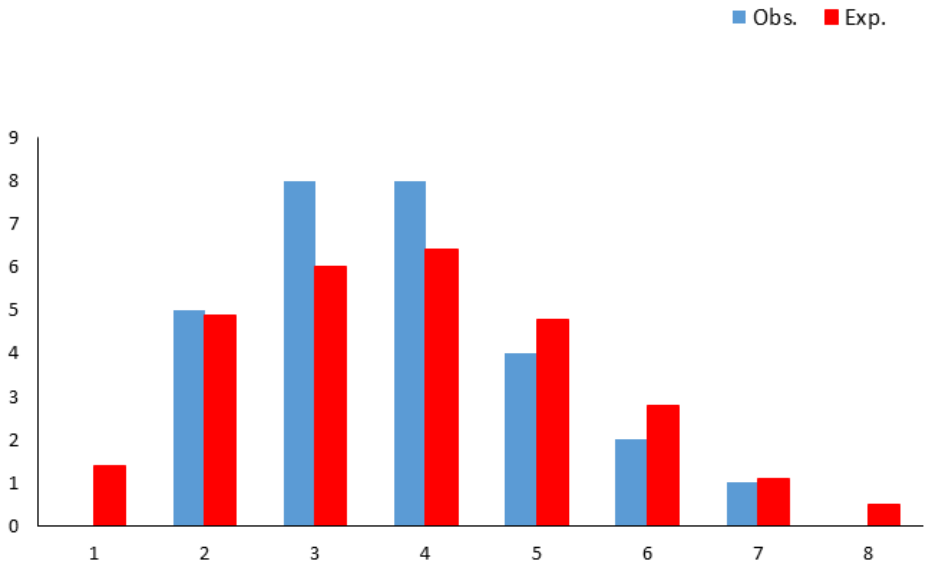
Supporting Information

S1 Fig. Bayesian phylogeny based on the 400 bp long fragment, excluding the ancient samples from Franz Josef Land. The Bayesian phylogeny shows 30 control region haplotypes and support for sub-cluster Ic, Id, Ie and II (posterior probability values ≥70 is shown at each node).

doi:10.1371/journal.pone.0165237.s001



S2 Fig. Mismatch distribution. The observed pairwise difference (blue bars) and the expected mismatch distribution (red bars) under the sudden expansion model among individuals in sub-cluster Ic. The mismatch analyses show a unimodal distribution, which is characteristic for a recently expanded population [63].doi:10.1371/journal.pone.0165237.s002



S1 Table. ^{14}C and calibrated radiocarbon dates on antler samples from Franz Josef Land.

The ^{14}C dates were calibrated using CALIB 6.1.1 [53], based on the data set IntCal13 [54] with 2σ ranges. doi:10.1371/journal.pone.0165237.s003

Sample ID	Site	Calibrated date	Lab code
T-2580	Franz Josef Land	BP 2 468 ± 31	Ua-49137
T-2594	Franz Josef Land	BP 3 806 ± 32	Ua-49138
T2598	Franz Josef Land	BP 2 390 ± 30	Ua4-9139
T-2600	Franz Josef Land	BP 3 835 ± 32	Ua-49140

S2 Table. Sample information and NCBI GenBank accession numbers. doi:10.1371/journal.pone.0165237.s004

Sampling location	Federal subject	Country	Status	Id. nb.	Accession nb.
Nordenskiöld Land, Svalbard		Norway	Wild	Re115	KX094711
Nordenskiöld Land, Svalbard		Norway	Wild	Re116	KX094712
Nordenskiöld Land, Svalbard		Norway	Wild	Re117	KX094713
Nordenskiöld Land, Svalbard		Norway	Wild	Re118	KX094714
Nordenskiöld Land, Svalbard		Norway	Wild	Re119	KX094715
Nordaustlandet Svalbard		Norway	Wild	Re189	KX094716
Nordaustlandet Svalbard		Norway	Wild	Re190	KX094717
Nordaustlandet Svalbard		Norway	Wild	Re191	KX094718
Nordaustlandet Svalbard		Norway	Wild	Re192	KX844748
Nordaustlandet Svalbard		Norway	Wild	Re193	KX094719
Nordaustlandet Svalbard		Norway	Wild	Re194	KX844749
Nordaustlandet Svalbard		Norway	Wild	Re195	KX094720
Nordaustlandet Svalbard		Norway	Wild	Re196	KX094721
Nordaustlandet Svalbard		Norway	Wild	Re197	KX094722

Nordenskiöld Land, Svalbard		Norway	Wild	Re344	KX094723
Nordenskiöld Land, Svalbard		Norway	Wild	Re346	KX094724
Nordenskiöld Land, Svalbard		Norway	Wild	Re5189	KX094698
Nordenskiöld Land, Svalbard		Norway	Wild	Re5192	KX094701
Nordenskiöld Land, Svalbard		Norway	Wild	Re5193	KX094702
Nordenskiöld Land, Svalbard		Norway	Wild	Re5194	KX094703
Nordenskiöld Land, Svalbard		Norway	Wild	Re5195	KX094704
Nordenskiöld Land, Svalbard		Norway	Wild	Re5196	KX094705
Nordenskiöld Land, Svalbard		Norway	Wild	Re5198	KX094707
Nordenskiöld Land, Svalbard		Norway	Wild	Re5199	KX094708
Nordenskiöld Land, Svalbard		Norway	Wild	Re5202	KX094709
Nordenskiöld Land, Svalbard		Norway	Wild	Re5203	KX094710
Nordenskiöld Land, Svalbard		Norway	Wild	Re5205	KX844750
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5977	KX844728
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5979	KX844729
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5980	KX844730

Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5982	KX844731
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5984	KX844732
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5985	KX844733
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5986	KX844734
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5987	KX844735
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5988	KX844736
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5990	KX844737
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5992	KX844738
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5994	KX844739
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5995	KX844740
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5996	KX844741
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5997	KX844742
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5998	KX844743
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re5999	KX844744
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re6000	KX844745
Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re6002	KX844746

Novaia Zemlia, South Island	Arkhangelsk oblast	Russia	Wild	Re6003	KX844747
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6044	KX844751
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6045	KX844752
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6046	KX844753
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6047	KX844754
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6048	KX844755
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6049	KX844756
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6050	KX844757
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6051	KX844758
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6052	KX844759
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6053	KX844760
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6054	KX844761
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6055	KX844762
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6056	KX844763
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6057	KX844764
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6058	KX844765

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Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6060	KX844767
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6061	KX844768
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6062	KX844769
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Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6065	KX844771
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6066	KX844772
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6067	KX844773
Kolguev Island	Nenets Autonomous Okrug	Russia	Domestic	Re6068	KX844774
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Pechora River	Komi Republic	Russia	Wild	COp7-Komi	JQ073843
Pechora River	Komi Republic	Russia	Wild	COp8-Komi	JQ073844
Pechora River	Komi Republic	Russia	Wild	COp10-Komi	JQ073840
Pechora River	Komi Republic	Russia	Wild	COp11-Komi	JQ073833
Pechora River	Komi Republic	Russia	Wild	COp12-Komi	JQ073842
Pechora River	Komi Republic	Russia	Wild	COp13-Komi	JQ073841

Pechora River	Komi Republic	Russia	Wild	COp16-Komi	JQ073845
Pechora River	Komi Republic	Russia	Wild	COp17-Komi	JQ073847
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Pechora River	Komi Republic	Russia	Wild	COp23-Komi	JQ073832
Pechora River	Komi Republic	Russia	Wild	COp24-Komi	JQ073838
Pechora River	Komi Republic	Russia	Wild	COp25-Komi	JQ073838
Pechora River	Komi Republic	Russia	Wild	COp26-Komi	JQ073839
Mezen River	Arkhangelsk oblast	Russia	Wild	COA1-Arh	JQ073835
Mezen River	Arkhangelsk oblast	Russia	Wild	COA4-Arh	JQ073836
Mezen River	Arkhangelsk oblast	Russia	Wild	COA6-Arh	JQ073833
Mezen River	Arkhangelsk oblast	Russia	Wild	COA7-Arh	JQ073832
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Mezen River	Arkhangelsk oblast	Russia	Wild	COA9-Arh	JQ073834
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Mezen River	Arkhangelsk oblast	Russia	Wild	11a-Arh	JQ073832
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Mezen River	Arkhangelsk oblast	Russia	Wild	15a-Arh	JQ073833
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Mezen River	Arkhangelsk oblast	Russia	Wild	17a-Arh	JQ073834
Mezen River	Arkhangelsk oblast	Russia	Wild	18a-Arh	JQ073834
Mezen River	Arkhangelsk oblast	Russia	Wild	COA19-Arh	JQ073837
Mezen River	Arkhangelsk oblast	Russia	Wild	22a-Arh	JQ073834
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6281	KX844796
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Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6283	KX844794
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6284	KX844793
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6285	KX844792
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6286	KX844791
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Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6289	KX844788
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6290	KX844787

Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6291	KX844786
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Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6294	KX844784
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Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6296	KX844782
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6297	KX844781
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6299	KX844780
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6300	KX844779
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Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6302	KX844777
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6303	KX844776
Belyi Island, Yamal Nenets	Tiumen Oblast	Russia	Wild	Re6304	KX844775
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2580	KX844797
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2587	KX844798
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2594	KX844799
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2598	KX844800

Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2600	KX844801
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2601	KX844802
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2602	KX844803
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2603	KX844804
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2604	KX844805
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2605	KX844806
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2609	KX844807
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2676	KX844808
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2677	KX844809
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2678	KX844810
Hooker/Hays islands, Franz Josef Land	Arkhangelsk oblast	Russia	Wild	F2679	KX844811

S1 Text. Boiling method for DNA extraction

100 µl EDTA blood was added 1000 µl lysis buffer. The samples were vortexed before they were centrifuged at 13000 revolutions per minute (rpm) for 1 minute and the supernatant was removed. This step was repeated once before washing the pellet with 1000 µl Tris-EDTA (TE) buffer (pH 8) and centrifuged at 13000 rpm for 1 minute. The supernatant was removed and the pellet dissolved with 100µl PCR buffer with detergent containing 1 µl proteinase K (10 mg/ml) pr. 100 µl buffer. The samples were incubated on a thermomixer for 2 hours at 56 degrees and 700 rpm before inactivation of proteinase K by incubation for 10 minutes at 95 degrees.

Paper III.

Kvie, K.S., Heggenes, J., Røed, K.H. Genetic heterogeneity in a fragmented landscape despite high migratory and dispersal capacity in reindeer (*Rangifer tarandus*). Manuscript.

Paper IV.

Anderson, D.G., Kvie, K.S., Davydov, V.N., Røed, K.H. Maintaining genetic integrity of coexisting wild and domestic populations: Genetic differentiation between wild and domestic *Rangifer* with long traditions of intentional interbreeding. Manuscript.

Maintaining genetic integrity of co-existing wild and domestic populations: Genetic differentiation between wild and domestic *Rangifer* with long traditions of intentional interbreeding

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Key words: domestication; reindeer husbandry; interbreeding; introgression; reproductive isolation; male-mediated gene flow

Short title: Genetic differentiation of wild and domestic reindeer

Abstract

The introgression of non-native and domesticated genes into the local wild gene pool is a conservation concern, as it is thought to reduce natural genetic diversity and may threaten local adaptations. Conversely, the need to introduce wild genes into the domestic gene pool is commonly thought to be a necessary first-step in the origins of animal domestication. For those classic domestic species which had been domesticated long-ago, this has been difficult to study due to the rareness or extinction of the wild form of the species. Here we present genetic analyses of co-existing wild and domestic herds of reindeer (*Rangifer tarandus*) in the Zabaikal region of Siberia; a region thought to have been one of the hearths of the emergence of reindeer husbandry. Despite a tradition of holding domestic reindeer in the same range as wild reindeer, and a tradition of deliberate, but controlled interbreeding, we demonstrate strong genetic differentiation between regional wild and domestic herds. We found a stronger differentiation between pooled wild and domestic reindeer in mtDNA compared to the nuclear microsatellites, which suggests mainly male-mediated gene flow between the two gene pools. The observed differentiation persists, despite co-existence and controlled interbreeding between domestic and wild *Rangifer* by indigenous herdsmen. The genetic results, and our survey of the traditional breeding practices, indicate that the herders have an effective breeding technique which while mixing pedigrees on the level of certain individuals in the short-term, guards against wholesale introgression between wild and domestic populations over the long-term. The

present study gives support to a model of domestication where wild males and domestic females are selectively interbred which nevertheless stops short of hybridizing the two populations.

Introduction

Nature conservation aims to maintain biological diversity which is often defined as a high level of genetic diversity thought to improve the evolutionary potential for any species (Frankham 2010, Slatkin 1987). Genetic introgression from non-native, translocated representatives of the same species, or from domesticated versions of a species, is often represented as a key concern or even a threat in the conservation literature (Abbott et al. 2013, Mager et al. 2013, Wayne et al. 2016). On the other hand, studies on the origin of domestication often assume that domestic types originated from wild prototypes, and thereby imply genetic introgression from wild to tame as a necessary condition (Vigne 2011, Zeder 2012). However, the techniques whereby people manage domestic herds in conditions where wild populations may pose a risk or a benefit are largely unknown and undocumented. Such management techniques are particularly interesting, because they would necessarily have the effect on the genetic integrity of both domesticated and wild populations, and thereby effectively increase the total evolutionary potential of a species. Traditionally, biologists have assumed that herdsmen enforced absolute reproductive isolation between wild and domestic forms in order to maintain strong demographic bottlenecks in the managed populations (Driscoll et al. 2009, Price 1984). This strategy would also maintain the genetic integrity of wild populations unintentionally. However, new models of domestication suggest that while spatial separation of wild and domestic types is often sought, the domesticators of various species ranging from pigs to horses, have also sought-out cross-breeding for specific characteristics by allowing controlled introgression from wild herds. (Frantz et al. 2015, Jónsson et al. 2014, Ottoni et al. 2013). For most domestic breeds this idea remains a hypothesis since the original free-ranging wild forms have become largely extinct or restricted to a few isolated areas (Clutton-Brock 1987). The exact husbandry techniques which may

have been employed in the Neolithic can only be guessed at. However, this is not the case for reindeer.

Reindeer, *Rangifer tarandus*, are a species commonly considered to be in an early phase of domestication and which often co-exist with wild forms (Baskin 2000, Reimers et al. 2009). Today, almost 50% of the approximate 3 000 000 reindeer in the Old World are wild animals, and wild and domestic herds are managed in close co-existence in many areas of Eurasia and in Alaska (Baskin 2005, Syroechkovskiĭ 1995). Therefore, this species provides a rare opportunity to link techniques of interbreeding of domestic herds and their wild relatives to their genetic signatures. Varying degrees of gene flow between wild and domestic reindeer has been reported in several recent studies (Colson et al. 2014, Cronin et al. 2003, Jepsen et al. 2002, Mager et al. 2013, Røed et al. 2014), primarily focussing on degree of introgression of domestic lineages into the native wild gene pool, and which population and environmental factors that may affect this. However, there is a complementary side to this, which may shed more light on the important process of introgression, and we have little knowledge about the levels of introgression from wild lineages into the domestic gene pool.

In a previous study of genetic structure in *Rangifer* across Eurasia, Røed et al. (2008) reported on striking genetic differences between wild and domestic reindeer within one locality in Zaibaikal'e in southeastern Russia. Zaibaikal'e constitutes an area of approximately 132 000 square kilometers characterized by an alteration of alpine, taiga and meadow ecosystems. Both wild and domestic reindeer herds co-exist today in the region, although wild *Rangifer* are reported to be by far the more populous of the two types (www.gps.ru). It is likely that this co-existence has persisted for a long time. Zabaikal'e has been suggested to be one possible origin point for reindeer husbandry (Maksimov 1928, Pomishin 1990, Wiklund 1918), with Okladnikov et al. (1976, 116) speculating that its roots go back to the second millennium BCE. Furthermore, the region is particularly interesting because of its specific herding techniques. It has been documented that local Evenki herdsmen have had a traditional practice of selectively cross-breeding domestic female *Rangifer* with wild males (*sakzhoi*)

92 to produce offspring described locally as the *baɪunchikan* [*baɪunchikar* -pl], often held for
93 transportation (Davydov 2014, Shirokogoroff 1929, 30-31, Vodopʲianov 1970b). One influential
94 cultural-historical study used this practice of interbreeding as a criterion for distinguishing the style
95 of reindeer husbandry in Zabaikal'e from neighbouring regions (Vasilevich 1964). Evenki reindeer
96 husbandry in Zabaikal'e thereby presents itself as a unique case for studying how introgression of wild
97 genes into the domestic gene pool may have been managed during the early processes of
98 domestication. To shed light on the potential for introgression and relevant circumstances, we
99 therefore needed to combine ethnographic and genetic methods.

100 In this article we test the hypothesis that Zabaikal Evenki able to enforce a strict genetic separation
101 of wild and tame *Rangifer,* despite provisions to accept or even to encourage controlled inter-
102 breeding, by the use of strict criteria to control genetic introgression through culling or castration. A
103 strong genetic difference between the wild and domestic populations, in spite of regional traditions
104 of interbreeding, would document their pastoral skill in maintaining and controlling specific reindeer
105 pedigrees. Establishing the identity and level of distinctiveness of wild and domestic herds is further
106 important to settle an old debate on whether or not domestic reindeer held for harness constitute a
107 distinct type of reindeer, compared to wild reindeer.

108

109 Material and methods

110 *Study populations*

111 The reindeer in this study were sampled between 2012 and 2014 from wild and domestic regional
112 populations around the settlements of Chapo-Ologo and Tianɪa and the encampment at Nomama
113 (Figure 1). We have included also data from a previous study of an Evenki herd at Lake Nichatka
114 sampled in 2001 and 2002 (Røed et al. 2008, Røv et al. 2002). The name of each population
115 corresponds to the residential base for the constellation of families holding local herds around that

site, and hunt wild reindeer in that same region (cf. Figure 1). During the Soviet period, some of these residential bases would have been the headquarters for one centrally organized state farm, which would have exercised great influence on the reproduction of each regional domestic population. We understand a regional domestic population to be a larger grouping of animals, which for historic and geographic reasons probably have been in constant interchange.

The Chapo-Ologo regional domestic population consists of samples obtained from local herds with biographies suggesting they were offspring of the original Charskii state farm, which in turn combined the smaller herds of dozens of indigenous families living in the region before collectivization. The domestic data set represents herds, or portions of herds, kept by four extended families. The set of wild reindeer associated with the Chapo-Ologo domestic herds was hunted primarily in the Verkhnii Sakukan valley with a few samples from the Amudisy Lakes. The T͡ɪanɪa region captures the territory of two clan communities T͡ɪanɪa and Tokko which have restored reindeer husbandry after it nearly disappeared in 1993 when the last state farm was disbanded. The contemporary domestic regional population was assembled from a small population of a few dozen local domestic reindeer with influxes of five to fifty reindeer from Lake Nichatka, Chapo-Ologo, Perevoz, and Ust'-N͡iuzhka and another five regional encampments between 1993 and 2010. Domestic animals born in this territory were grouped together as the T͡ɪanɪa regional population. It represents the herds or portions of herds kept by five extended families. The wild reindeer samples were from animals hunted on the Urius-Milele and Usu Rivers. The domestic Nomama samples were from domestic reindeer held by the clan community corporation "Ulutki" – essentially one extended family – which is headquartered at the ice-covered headwaters of the Lena River. This region was once the home to the state farm "Severnyi" that was disbanded in 1976. The region was left empty of domestic reindeer for 15 years, albeit with a large population of feral reindeer. Domestic reindeer husbandry was reintroduced in the region first in 1992-93, according to informants by first lassoing the local feral reindeer, and more substantially by the purchase of thirty head of reindeer from the Chapo-Ologo region. The wild reindeer hunted in the Nomama region is likely separated from all the other wild herds by significant mountain ranges

over large geographic distances (more than 500 km). In contrast to all the other sites, the extended family at Lake Nichatka never had participated in collectivization. Their kinship networks for the most part looked northwards the Bodaibo region of Irkutsk *oblast'* (Anderson et al. 2014) which they would have used to exchange small groups of 5-10 head of reindeer. The wild reindeer from this setting were hunted within 25km of the main residential camp and could be considered to be part of a wild population which moves freely between this site and sites across the border to T͡Ian͡ia region.

Rangifer Life Histories

For this study, we documented wild and domestic *Rangifer* at Chapo-Ologo, T͡Ian͡ia, and Nomama. A total of 117 domestic animals were documented of which 101 were used in this study. Fifty-six wild Rangifer were documented of which 49 samples were used here. The documentation was structured around a set of open-ended questions eliciting the life history of a particular animal. The interviews, at minimum, affixed the age, sex, place of birth, and ownership status of each animal. Beyond this we photographed each animal and recorded detailed observations of the reindeer type, its pelage, its role in the social organization of the herd, peculiarities of its behaviour, and how it might have been harnessed or tamed for other activities. We recorded information in the Russian, I͡Akut, and Evenki languages. Information on wild *Rangifer* was confined to visual assessments of the age, sex and qualities of the hunted wild animal.

The previously published set from Lake Nichatka are documented only by sex and type (wild/domestic) with some specific biographical information on individual animals available in Abe (2005). Of the 35 domestic and 15 wild samples originally gathered, 31 domestic and 13 wild samples were used in this study. In 2012 we interviewed one Evenki herder who worked with this local herd for further details on this regional population.

DNA isolation

DNA were obtained from hair follicles, skin samples or from FTA cards designed for forensic work (Smith et al. 2004). DNA extraction of skin samples was performed using DNaeasy Blood & Tissue Kit (Qiagen) following the manufactures protocol. DNA from hair follicles were extracted using the chelex method (Walsh et al. 1991). DNA from FTA cards was extracted from a 0.4 x 0.4 cm piece of the FTA cards using the boiling method, as described in Kvie et al. (2016).

Mitochondrial DNA analyses

A 503 base pair (bp) long fragment from the mitochondrial control region (CR) was amplified using the forward primer RtCRF (5`-AAT AGC CCC ACT ATG AGC ACCC-3`) (Flagstad et al. 2003) and the reverse primer RtCR-528 (5`-TAG GTG AGA TGG CCC TGA AGA AA-3`) (Bjørnstad et al. 2010). Amplification was performed using the following program: 95^{o}C for 2 min, 95^{o}C for 30 sec, 55^{o}C for 30 sec and 72 ^{o}C for 1 min (step 2-4 cycled 30 times) and finally, 72 ^{o}C for 10 min. PCR reactions were performed in 20 µl total volume using 1-2 µl DNA template, and with the following final concentrations: 1X buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 5 pmol of each primer, 0.5 µg/µl Bovine Serum Albumin (BSA), 0.5 U/µl AmpliTaq DNA polymerase (Applied Biosystems), and dH_2O to make up the remaining volume. The samples were cleaned for unincorporated primers and nucleotides using Illustra ExoProStar (GE Healthcare) diluted 10 times. Cycle sequencing was performed in a 10 µl reaction volume, using BigDye v3.1 sequencing kit (Applied Biosystems) following manufacturer`s recommendations. Purification was carried out using standard EDTA/EtOH precipitation. Capillary electrophoresis and data analysis were performed with an ABI 3130xL- or 3500xL instrument (Applied Biosystems). All sequences were sequenced in both directions and the consensus sequences were aligned by ClustalW (Thompson et al. 1994) and edited in MEGA5 (Tamura et al. 2011).

190 *Microsatellite analyses*

191 All samples were analysed for 13 reindeer-specific microsatellites (NVHRT-01, NVHRT-03, NVHRT-16,
192 NVHRT-21, NVHRT-31, NVHRT-48, NVHRT-73, NVHRT-76 (Røed et al. 1998), RT-1, RT-5, RT-6, RT-9,
193 RT-27 (Wilson et al. 1997)). The amplification was performed on a GeneAmp PCR System 9700
194 (Applied Biosystems) as previously described (see methods described in Røed et al. (2002)). PCR
195 products were electrophoresed using an ABI Prism 3500xl Genetic Analyzer (Applied Biosystems). Use
196 of these markers in a previous study has given evidence of low scoring errors (< 5 %) due to stutter
197 bands, allelic dropout or null alleles (Røed et al. 2008).

198

199 *Statistical genetic analyses*

200 CR polymorphism estimates in terms of number of haplotypes (Nh), gene diversity and nucleotide
201 diversity, and pairwise haplotype differentiation (F_{ST}) were calculated in DnaSP (Librado et al. 2009)
202 and Arlequin v.3.5 (Excoffier et al. 2010), respectively. Genealogical relationships were examined by
203 constructing a median-joining network (Bandelt et al. 1999) using Network v4.6 (ref.fluxus-
204 engineering.com). We used GenALEx v.6.5 (Peakall et al. 2012) to calculate microsatellite genetic
205 diversity in terms of number of different alleles (Na), number of effective alleles (Ne), observed
206 heterozygosity (Hobs) and expected heterozygosity (Hexp).

207 Bayesian assignment, as implemented in the programme STRUCTURE 2.3.4 (Pritchard et al. 2000),
208 was used to assess whether discontinuities existed in the distribution of genetic variation within the
209 data set. For each number of genetic clusters ($K \in [1,8]$), a model with uniform priors, admixture,
210 correlated allele frequencies, 20 000 burn-ins and 200 000 Markov Chain Monte Carlo (MCMC)
211 iterations was run ten times. For each *K*-value, average posterior probability among runs and standard
212 deviation (SD) was calculated.

Results

Genetic analyses

We identified 18 unique CR haplotypes among the domestic populations and 20 unique CR haplotypes among the wild populations. However, only two haplotypes are shared between the two gene pools, with two wild individuals, both from Tiania, having shared haplotype with local domestic reindeer (Figure 2). In addition, the F_{ST} estimate for genetic differentiation between pooled wild and domestic in the CR was highly significant (F_{ST} = 0.832 $p<0.0001$) and with a significant level higher than for the microsatellites (F_{ST} = 0.032, $0.010<p<0.05$). We further identified nine to ten alleles in each of the two pooled microsatellite sets, and approximately six alleles in each regional population, noting high diversity in all populations (Table 1). High levels of pairwise genetic differences among the eight regional populations of wild and domestic *Rangifer*, respectively, was evident from the microsatellite markers (Table 2). Significant differences were found among all populations ($p<0.05$), except between the regional domestic herd from Tiania and the domestic herds from Nomama and Nichatka,($p=0.06$), and between the wild herd from Chapo-Ologo and the wild herds from Lake Nichatka ($p=0.09$) and Tiania ($p=0.208$). Pairwise genetic differentiations calculated from the CR data show no- or low levels of significance, except for the regional population of Nichatka wild reindeer which is significantly different from all other populations ($p<0.001$) (Table 3). For the CR, these tests involving the wild herds are not very informative due to the high nucleotide diversity and relatively low sample sizes.

The STRUCTURE analysis gives an increase of mean likelihood from K=1 to K=5 with an apparent main structure at K=2 (Fig 4a). Individual assignment at K=2 gives support to a substantial genetic split between the wild and domestic herds (Fig 4b). The structure at K=3 is characterized by a separation of most domestic Nomama reindeer from the other domestic herds, while at K=4 it appears as a third domestic population cluster itself around the Lake Nichatka population (Fig 4b). At K=5, although with

237 higher error margins, there is some evidence that the regional population of wild reindeer at Nomama
238 distinguishes itself from other wild populations.

239 To further test the genetic structure among the domestic reindeer, we re-ran the STRUCTURE analysis
240 with the 101 domestic samples alone. This analysis supported a separation into three genetic clusters
241 (Fig 5a) with each of Nomama, Lake Nichatka and Chapo-Ologo regional herds characterizing the
242 three clusters. The Tiana population appears to be made up of a mix of all three groups (Fig. 5b).

243

244 *Traditional Evenki herding practices for controlling introgression*

245 Reindeer are traditionally held in small local herds scattered across the region ranging in size from a
246 dozen to two hundred head by extended families of four to twelve individuals (Anderson 1991,
247 Brandišauskas 2009, Fondahl 1989, Orlov 1858, Shirokogoroff 1929). The herd structure would vary,
248 by family needs and by season, but approximately up to 40% of the herd might be breeding females,
249 the balance would be non-breeding juveniles or calves, with a handful of males kept as breeding bulls
250 and another dozen males castrated and kept for use as transport reindeer. The local herds are
251 generally kept within the confines of one watershed and are typically not moved more than 50km
252 over the course of the year. According to our own ethnographic survey, every five or ten years a herd
253 might be split and relocated into a neighbouring valley forming a new local population. Individual
254 domestic reindeer from one to twenty head, typically bulls, might occasionally be sold and exchanged
255 across regions over a distance of 500km or more. The highly trained domestic reindeer are mainly
256 used for milking and saddling (for transport) and are rarely slaughtered for food (Shnirel'man 1977,
257 Vasilevich 1964, 31). This restriction does not apply to the *baiunchikar.* Therefore, the local herdsmen
258 are also highly dependent of the presence of interbred wild/tame reindeer and wild reindeer, which
259 are an important source of food and skins for clothing and equipment. The wild populations are
260 generally distributed close up to the herding societies and are described as scattered, small, localized

groups of ten to twenty animals with a range use similar to that of the domestic populations. They do not migrate more than 30 km over the course of a year and alternate their pastures between high alpine meadows and taiga meadows in between mountain ridges (Ovdin et al. 2007, Vodop'i͡anov 1970a). An important detail in the interrelationships between these two populations is that the breeding cycle of each is offset. In this region wild *Rangifer* drop their calves two to three weeks later (in May) than domestic *Rangifer*.

The practice of tolerating interbreeding and observing *bai͡unchikar* is noted in the literature but has never been described in detail. The first European to note the practice was Leopol'd Shrenk (1883). One clue to the possible ancient nature of the practice is the fact that all the terminology is in Evenki and not in Russian (or Latin). On the basis of our interviews, we documented an "ideal" procedure as practiced in post-Soviet conditions, which might differ from how this practice was done in Soviet or Imperial times. It was explained that during the autumn rut, the majority of the domestic female herd is sequestered to avoid interbreeding with migratory wild reindeer. In the case of a small herd, they might be kept overnight in a specially built corral. In the case of a larger herd, they would be herded into an easily monitored valley. In both cases herdsmen would keep shifts night and day to monitor the herd. However, one or two breeding females - i.e. much less than 10% of the female domestic population - might be tethered for cross-breeding with a wild male. The interbreeding happens under close observation of the herders. The wild male is often shot after mating, so that he will not harm the female – or, worse, return to fission-off a group of other domestic cows under his protection by fighting off the other domestic breeding males with his superior size and antlers. The act of shooting the wild male is a pure management decision since neither the autumn meat nor the thick hide of the male in rut can be used in subsistence. The unfortunate male is not selected as such. He presents himself opportunistically.

The mixed *bai͡unchikan* calves are closely monitored as they grow for a set of behavioural signs that suggest if they are compatible with the rest of the herd. The herders literally describe this as watching

to see if the “domestic blood predominates”. There are many qualities that would please a herder such as gregariousness, a lack of aggression, a calm dispossession. Phenotypical qualities are also observed such as the length of the legs, the gait of the calf and the strength and resilience of the animal. A female *baiunchikan* would be monitored to see if there was a risk she would drop her calves too late in the spring for the calves to survive. The *baiunchikan* calves are not sequestered but are kept with the entire herd, which itself moves through periods of being free-ranging and free-foraging to being kept under constant watch, or even being enclosed, depending on the season. If any comment is made about the mixed-blood calves it is that they often resist walking together with the herd as a whole, that they keep to the edge of the forest, and become unruly when enclosed. It is the process of observation and selection which controls genetic introgression. Calves, which present themselves of “wild”, may be allowed to survive one or two years as juveniles but would be culled before they reach breeding age. It would be fair to say that the majority of the *baiunchikar* in normal economic conditions are not allowed to breed and are kept only as calves. Female *baikunchar* might be slaughtered for meat before they reach reproductive age. Male crossbreeds may be slaughtered, or might be prevented from breeding by castration or by trimming their antlers to prevent them from successfully competing with other males. Some male *baiunchikan* castrates are trained to harness to be cargo-carrying reindeer. Those few mixed breeds allowed to interbreed would be monitored year by year and could always be castrated or cull over the next breeding season. Their calves in the next generation would also be monitored for desirable qualities. In our field research, the *baiunchikar* presented to us were primarily calves. The two adults were castrated males, one of which was “one-quarter wild”. None of the declared mixed-breeds at the time of our research could lead to genetic introgression.

The degree to which introgression proceeds depends on local conditions. In our field research the two adult castrates were born in 2005, one from a male reindeer who was himself a *baiunchikan* logically born around 2000. The same dates would apply to the herd sampled at Nichatka and the one *baiunchikan* adult female documented there. This was a time of severe economic crisis in the region.

Subsidies to state farms had been cut resulting in a cut to funding to hire mechanised transport to bring breeding bulls in from afar, necessitating recruiting the wild population to ensure that all of their cows were covered. In a dire situation like this one would assume that a higher proportion of the interbred calves would be allowed to survive. This precise situation repeated itself before us in the Nomama local herd the summer we visited when bears had killed the last breeding domestic male in that herd. The herders were making ready to interbreed the remaining domestic females with wild *Rangifer* that same autumn. However, conditions can change. There is one published estimate that in 1970 the Soviet state farms of Zabaikal'e held between 750-800 "hybrid" reindeer out of a total domestic population of 25,000 (Vodop'ianov 1970b). That article makes clear that the peculiar economic conditions of Soviet state socialism – the use of head-counts and calf-weights to monitor productivity – encouraged herders to wildly increase the size of the herds through the use of interbreeding. The article also makes clear that the calves are rarely kept beyond a year and a half of age. Finally, all herders will admit candidly that some *baiunchikan* appear spontaneously when a small group of domestic females is covered by a wild male unintentionally.

Discussion

Our results suggest that the reindeer herders in south-eastern Zabaikal'e, without any prior genetic knowledge, have developed herding techniques that effectively maintain the genetic integrity of co-existing and overlapping populations of wild and domestic reindeer, over time and even in the presence of intentional interbreeding. This implies that the marked genetic differentiation between wild and domestic reindeer previously reported for the Lake Nichatka region (Røed et al 2008) reflects a general pattern applicable for other herds across the region as well. The distinctly different genetic cluster recorded at Nomama was a surprising result since we had assumed that the population markers would be dominated by the domestic *Rangifer* imported to the area from Chapo-Ologo.

Significant genetic differences between wild and domestic reindeer have also been reported from Alaska (Colson et al. 2014, Cronin et al. 2003, Mager et al. 2013), Norway (Røed et al. 2014) and Greenland (Jepsen et al. 2002). In Norway, wild and domestic herds have been present for several centuries and are usually kept separated in different mountain areas, with enhanced migration barriers due to increasing infrastructure (Røed et al. 2014). Therefore, classic spatial segregation likely explains the genetic differentiation (Slatkin 1987). Alaska and Greenland have a relatively recent history of co-existence of wild and domestic herds, which also have different geographic origins. Domestic reindeer were introduced to Alaska from Russia in 1890s (Ellanna et al. 2005, Simon 1998), and to Greenland from Norway in 1952 (Jepsen et al. 2002). Differences in ancestry, and presumably associated local/regional adaptability, likely have contributed to reproductive isolation, as indicated by their distinct morphological and behavioural differences (Jepsen et al. 2002, Mager et al. 2013). For Zaibaikal'e the situation is entirely different. Wild and domestic reindeer have the same ancestry, have co-existed for a long time, and are geographically sympatric. The genetic isolation observed between the wild and domestic populations appears rest solely on apparently very effective cultural practices, i.e. how the indigenous herders tightly control breeding between wild and domestic individuals, and herd the different offspring groups differently. These practices have made it possible for both populations to co-exist, selectively interbred, but with little trace of long-term genetic mixture between them. The local people have been able to develop such effective practices without any prior scientific knowledge about genetics, but rather likely based on how the wanted traits obtained by interbreeding could best be maintained over time, i.e. be maintaining the differences, and thereby preserve the genetic integrity of wild and domestic populations. This observation is clearly supported by our ethnographic work with the local herders. They assert the radical somatic and behavioural differences between wild and domestic types, and the need to carefully keep the two populations reproductively isolated while at the same time ensuring that wild *Rangifer* are close to hand for subsistence (Shirokogoroff 1929, Anderson 2000). Furthermore, it is interesting that the selective interbreeding of any remaining hybrids is allowed only if those hybrids present desirable behavioural traits.

363 This behavioural factor in selection addresses the recent hypothesis of the existence of "islands of
364 domestication" within the genome wherein selection for desirable behavioural traits allows breeders
365 to maintain domestic behaviour within an interbred population (Frantz et al. 2015). Our results
366 suggest that behavioural selection may indeed be important at the level of individual selection, but
367 there is no evidence in this study suggesting that it can lead to hybridizing entire populations. At the
368 level of single individual cases in each herd, behaviour selection is used to permit a small number of
369 hybrid bloodlines into a breeding population. Hence, our data is closer to the Eurasianist model of
370 Warmuth et al. (2012) of the gradual improvement of herds through the controlled introgression of
371 wild genes along the male line.

372 The general pattern of stronger differentiation between wild and domestic reindeer in mtDNA
373 compared to the nuclear microsatellites, which tend to be much more sensitive to population
374 changes, suggests a pattern of sex-biased genetic structure. The notably low CR haplotype sharing
375 between wild and domestic reindeer, where only two among 36 haplotypes were shared, indicate
376 very low levels of female mediated gene flow. This is consistent with female philopatry and male-
377 biased dispersal commonly seen among most mammal species (Goudet et al. 2002, Greenwood 1980,
378 Pusey 1987). This sex-biased structure has also been documented for wild *Rangifer* in Alaska (Roffler
379 et al. 2012). In Zaibaikal'e, however, this appears to be much aggravated by the strongly sex-biased
380 and tightly controlled breeding practice. Our ethnographic work further suggests that this significant
381 sex-bias may have been augmented by the preference for exchanging or importing male domestic
382 reindeer to improve a herd. These small taiga herds are often assembled through kinship. Thus,
383 particular reindeer might be gifted to one family or another when their paths cross in the forest. In
384 our work on reindeer biographies, we discovered that male reindeer in particular, might be
385 exchanged several times over between friends and relatives in different locations across hundreds of
386 kilometres with the animal spending one or two years in various camps (Davydov 2014, 365). Female
387 animals, on the other hand, are more likely to be kept within the family. Female reindeer would be
388 more likely to be translocated when a herd would grow beyond a certain threshold and relocated to

389 a new valley. This was the case for the local population at Amudisy (Chapo-Ologo region) which had
390 been fissioned twice as the herd approached 300 head. Female reindeer might be exchanged within
391 large transfers of twenty to fifty head designed to augment a herd, such as the oral accounts of the
392 building of the regional population at T͡ian͡ia. There are published ethnographic accounts of female
393 reindeer being given in exchange as bride-price in the 19th Century (Vasilevich 1969).

394 This study was originally designed to test the hypothesis that careful husbandry could successfully
395 keep wild and domestic populations genetically separated despite living in close proximity and despite
396 deliberate attempts to let certain female reindeer to interbreed. The genetic results indicate this is
397 indeed the case. Our results further suggest that it is possible to distinguish local domestic clusters,
398 which may serve as signatures of particular breeding traditions. Up until now, the classic literature on
399 the origin of reindeer husbandry and of reindeer pedigrees has relied upon the classification of
400 saddling and harnessing technologies as a proxy for grouping together distinct indigenous pastoral
401 traditions. The reindeer bodies themselves, be they wild or tame were assumed to be standard. This
402 study suggests that it is possible to distinguish regional domestic herds genetically despite complex
403 social histories of collectivization and privatization. The status of the Nomama population is a
404 particularly interesting case in this point. Despite significant influxes of reindeer purchased from
405 neighbouring regions, the Nomama population continues to distinguish itself from other groups by
406 its low level of genetic diversity within the mtDNA and a strongly different microsatellite signature.
407 The mtDNA signature, suggests a founding group i.e. that the population was established by a small
408 number of females – perhaps those that oral accounts suggested were lassoed from the free-ranging
409 feral remnants of the defunct state farm. Their distinct microsatellite signature suggests that the
410 founding population may have been augmented by hybridization with local wild reindeer. In the older
411 cultural-historical literature, the North Baikal region, where Nomama is located, corresponds to a
412 distinctly different tradition of reindeer herding technique than that found further East at Chapo-
413 Ologo, T͡ian͡ia, and Lake Nichatka (Vasilevich 1964). In this case, decollectivation and the restoration
414 of family-based reindeer husbandry between 1976 and 2012 may have produced a small-scale case

415 study of origin of local reindeer husbandry within a distinct regional population of wild and feral
416 *Rangifer* stressing the importance of strict selection and introgression along the male line.

417

418 Acknowledgements

419 The funding for the fieldwork and laboratory work for this study was provided by the ERC Advanced
420 Grant 295458 (University of Aberdeen). The sample set for Lake Nichatka was collected and deposited
421 under a research programme of the Norwegian Institute for Nature Research. We thank Liv Midthjell
422 for skilful laboratory analyses and Jan Heggennes for useful comments on an earlier version of this
423 paper.

424
425

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Table 1: Amount of genetic variation in the microsatellite loci and in the mitochondrial CR in wild and domestic reindeer herds from northeastern Zabaikal'e. N=number of individuals. For microsatellites, Na gives number of different alleles and Hobs and Hexp gives observed and expected heterozygosity. For the CR Nh gives number of different haplotypes.

		Microsatellites				MtDNA			
Status	Location	N	Na	Hobs	Hexp	N	Nh	Haplotype diversity	Nucleotide diversity
Domestic									
	Nomama	10	5.54	0.652 (0.145)	0.725 (0.092)	10	3	0.38 (0.18)	0.004 (0.003)
	Lake Nichatka	31	6.69	0.669 (0.131)	0.744 (0.073)	18	10	0.87 (0.06)	0.012 (0.007)
	T͡ian͡ia	23	6.77	0.701 (0.134)	0.774 (0.082)	24	11	0.85 (0.06)	0.013 (0.007)
	Chapo-Ologo	37	7.46	0.707 (0.109)	0.748 (0.053)	36	9	0.84 (0.04)	0.016 (0.009)
Domestic pooled		*101*	*9.62*	*0.680 (0.062)*	*0.767 (0.062)*	*88*	*18*	*0.87 (0.02)*	*0.014 (0.008)*
Wild									
	Nomama	24	8.08	0.736 (0.095)	0.808 (0.071)	24	10	0.91 (0.03)	0.020 (0.010)
	Lake Nichatka	13	6.39	0.724 (0.168)	0.760 (0.131)	10	6	0.78 (0.14)	0.009 (0.006)
	T͡ian͡ia	7	5.77	0.700 (0.226)	0.804 (0.108)	7	4	0.72 (0.18)	0.007 (0.005)
	Chapo-Ologo	5	4.07	0.636 (0.180)	0.725 (0.128)	5	3	0.80 (0.16)	0.012 (0.008)
Wild pooled		*49*	*9.69*	*0.717 (0.064)*	*0.815 (0.070)*	*46*	*20*	*0.94 (0.02)*	*0.017 (0.009)*

Table 2. Pairwise genetic differences (F_{ST}) based on 13 reindeer specific microsatellite loci, among regional populations of domestic (D) and wild (W) herds of reindeer across Zabaikal'e. Population numbers are coded to the abbreviated list in the first column. Significance levels given as ns = P > 0.05, * = 0.01< P <0.05, ** = 0.001<P<0.01, *** = P <0.001.

Code	Population	1	2	3	4	5	6	7
1	Nomama (D)							
2	Lake Nichatka (D)	0.202*						
3	T͡ian͡ia (D)	0.074^{ns}	0.008 ^{ns}					
4	Chapo-Olgo (D)	0.224*	0.133**	0.062*				
5	Nomama (W)	0.213***	0.162***	0.127***	0.114***			
6	Lake Nichatka (W)	0.407***	0.244***	0.200***	0.187**	0.165**		
7	T͡ian͡ia (W)	0.426**	0.217**	0.163**	0.203**	0.199**	0.223**	
8	Chapo-Olgo (W)	0.381***	0.197*	0.154*	0.175*	0.118*	0.111 ^{ns}	0.095 ^{ns}

Table 3. Pairwise genetic differences (F_{ST}) in the mitochondrial CR among regional populations of wild (W) and domestic (D) reindeer across Zabaikal'e. Population numbers are coded to the abbreviated list in the first column. Significant levels given as ns = P> 0.05, * = 0.01< P <0.05, ** = 0.001<P<0.01, *** = P <0.001.

Code	Population	1	2	3	4	5	6	7
1	Nomama (D)							
2	Lake Nichatka (D)	0.027 ns						
3	T͡ian͡ia (D)	0.057 ns	0.000 ns					
4	Chapo-Olgo (D)	0.066*	0.000 ns	0.008 ns				
5	Nomama (W)	0.000 ns	0.004 ns	0.017 ns	0.028*			
6	Lake Nichatka (W)	0.194***	0.151***	0.145***	0.166***	0.118***		
7	T͡ian͡ia (W)	0.109*	0.047 ns	0.059 ns	0.041 ns	0.042 ns	0.000 ns	
8	Chapo-Ologo (W)	0.000 ns	0.000 ns	0.038 ns	0.000 ns	0.000 ns	0.154*	0.017 ns

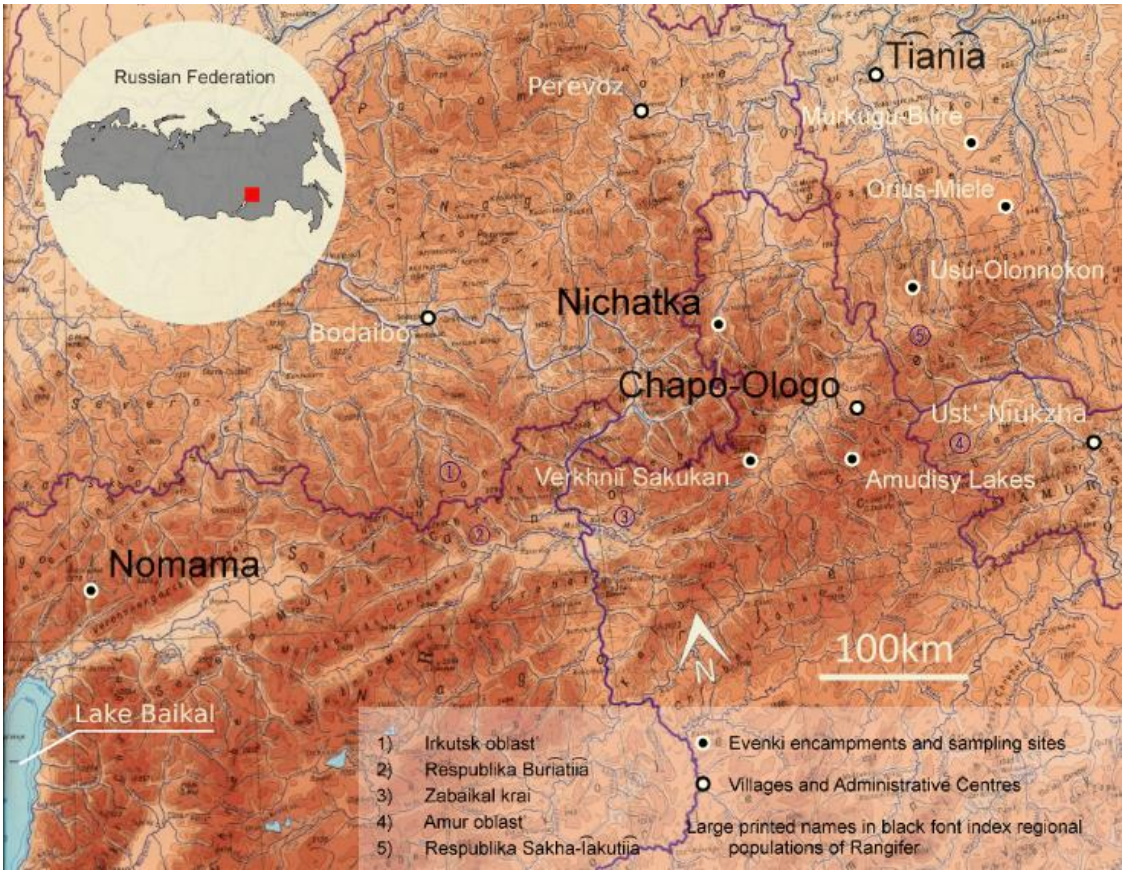


Figure 1. The locations of the local encampments and sampling sites, as well as regional populations, for wild and domestic *Rangifer* in Zabaikal'e in southeastern Siberia. The boundaries of the local political districts, which bisect the region, are indicated with numbers.

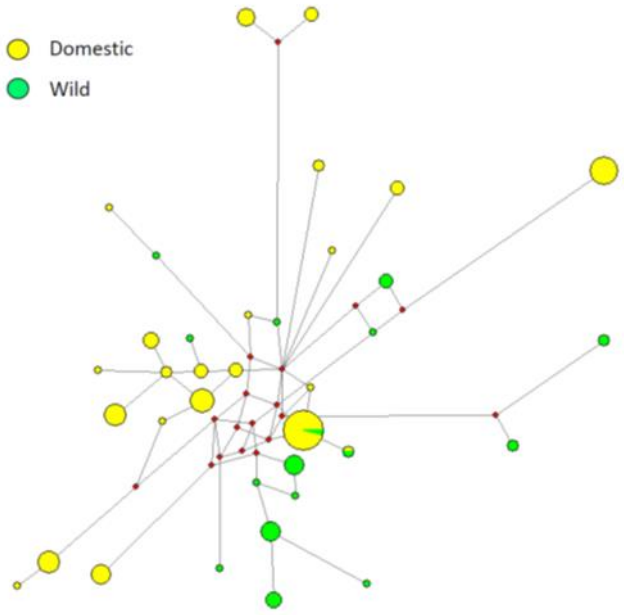
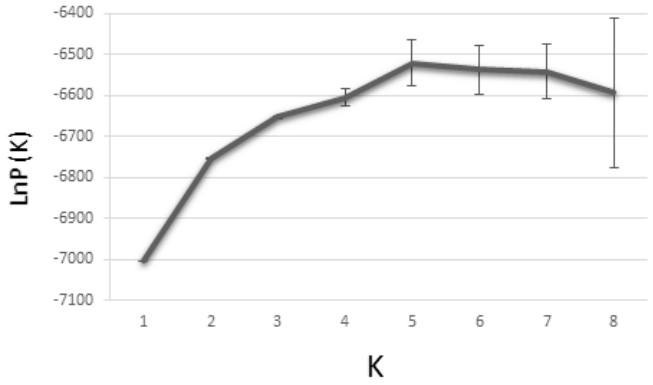


Figure 2. Median-joining network of mtDNA haplotypes in wild and domestic *Rangifer* in northeastern Zabaikal'e. Each circle represents unique haplotypes with area proportional to the number of reindeer sharing a haplotype, and with the "status" (wild or domestic) each colour coded with wild in green and domestic in yellow.

a)



b)

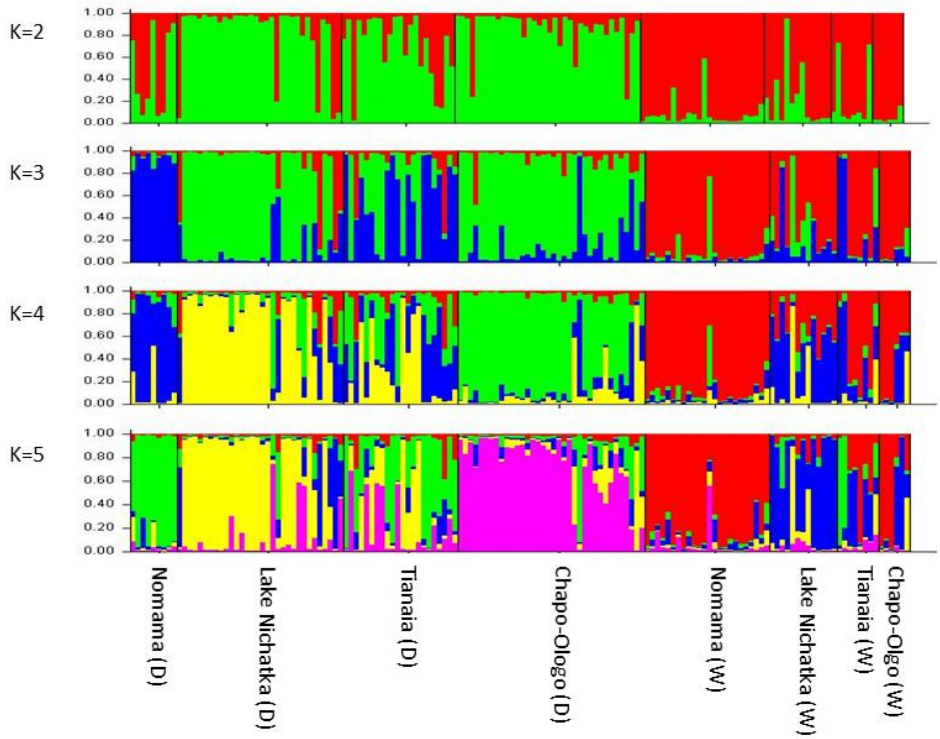
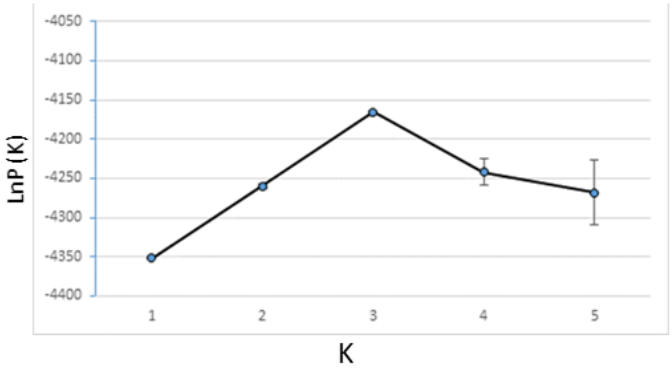


Figure 3. Bayesian clustering analyses of wild and domestic reindeer in Zaibaikal'e. a) Mean likelihood LnP (K) over 10 runs dividing the entire dataset into K = 1-8 populations, b) Individual assignment of individual reindeer to each cluster at K = 2-5.

a)



b)

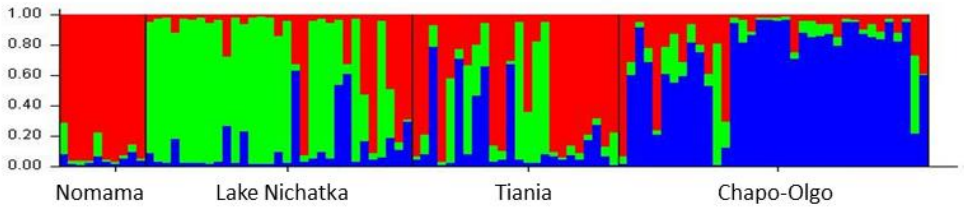


Figure 4. Figure 3. Bayesian clustering analyses Zaibaikal'e including only the domestic herd. a) Mean likelihood LnP (K) over 10 runs dividing the entire dataset into K = 1-5 populations, b) Individual assignment of each individual reindeer to each cluster at K = 3.

Doctoral dissertation Nr 11
2017
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Natural and human-induced impacts on the genetic structure of Eurasian reindeer

Dissertation for the degree of Ph.D

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Kjersti Sternang Kvie

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ISBN 978-82-7206-426-5 (trykt utgave)
ISBN 978-82-7206-427-2 (elektronisk utgave)

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