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Refugial origin and postglacial colonization of holarctic reindeer and caribou

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Abstract: The classification and colonization of reindeer and caribou (*Rangifer tarandus*) was assessed from analysis of both proteins, nuclear DNA and mitochondrial DNA. I demonstrate that the current subspecies designations are not compatible with the differentiation at these markers, suggesting that the morphological differences among extant subspecies did not evolve in separate glacial refugia. Thus, morphological differences among extant subspecies probably evolved as adaptive responses to post-glacial environmental changes. An exception to this is the North American woodland caribou, where all three marker systems support a subspecies-specific refugium as the ancestral origin of these animals. Three major mtDNA haplogroups reported, represent three separate origins of the species during the last glaciation. The most influential origin has contributed to the gene pool of all extant subspecies, suggesting the existence of a large and continuous glacial population ranging across extensive areas of tundra in Eurasia and Beringia. The North American tundra forms (*R.t. granti* and *groenlandicus*) and the arctic forms (*R.t platyrhynchus*, *R.t pearyi* and *R.t eogroenlandicus*) almost exclusively comprise haplotypes of such an origin. Another small and isolated refugium seems to have arisen in western Eurasia in close connection to the extensive ice sheet that covered Fennoscandia. The two Eurasian subspecies *R.t. tarandus* and *R.t. fennicus* appear to have a diphyletic origin as both the putatively small and isolated Eurasian refugium and the large Beringia refugium have contributed to their gene pools. A third distinct and geographically well-defined refugial area was probably located south to the extensive North American continental ice sheet from where the ancestors of the present North American woodland caribou (*R.t. caribou*) likely originated.

Key words: interglacial refugia, microsatellites, mitochondriel DNA, phylogeography, recolonization, transferrin.

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Introduction

Reindeer and caribou (*Rangifer tarandus*) are distributed throughout the northern holarctic and are representatives of the fauna in this region. Besides serving a vital role in the sosio-economics of many present human settlements, *R. tarandus* was probably the key species for the human immigration and colonization in arctic and sub-arctic by retreat of the ice at the end of the last glacial period. An understanding of the post-glacial re-colonization of *R. tarandus* therefore contributes knowledge towards our own early immigration and colonization.

The classification of *R. tarandus* has been greatly debated. Historically, there has been almost as much confusion in vernacular names for the species as in scientific names. Alternating use of two vernacular names for this species - reindeer and caribou - has certainly contributed to the confusion. However, present view is that both refer to the same species and that caribou includes all wild specimens in North America, while reindeer refers to both wild and domestic animals in Eurasia. Domestic animals in North America of Eurasian origin are also called reindeer.

Different terms have also been used for R. *tarandus* corresponding to ecological adaptations *i.e.* the woodland or forest form, the barrenground or tundra form, and the arctic form. The woodland or forest reindeer/caribou appear to be adapted to forested areas, characterized morphologically by large body size, relatively long legs and with short distance between their antlers. The barrenground or tundra form primarily inhabits open tundra habitat or mountain regions, with a medium sized body size and larger space between their antlers. The Arctic reindeer and caribou appear to be adapted to colder and harsher environments. They are usually relatively small-bodied with short legs and rostrum and maintain thick insulating winter pelage. The distinctiveness of the different ecotypes however is not absolute, and a gradually transition between the different morphs in a mainly south-north direction appears to exist in both Eurasia and North America.

The subspecies classification of R. *tarandus* has been dominated by a high number of described subspecies without well-defined subspecific characteristics. However, Banfield's (1961) revision and summary of the classification of reindeer and caribou based mainly on craniometrical measurements, is widely used as a guideline for the now living or recently extinct subspecies of reindeer and caribou. These include (see Fig. 1 for distribution): The Eurasian tundra reindeer (R.*t. tarandus*), distributed almost continuously across the tundra region, including the three lines and mountain areas across Eurasia. It is difficult to draw a southern boundary for the distribution of this subspecies in northern Europe and Asia because of possible overlap with the Eurasian forest reindeer (R.*t. fennicus*). The most typical form of this subspecies is to be found in the taiga in the Karelia at the boarder region between Finland and Russia. However, similar forest reindeer inhabit other areas in Russia. It is debatable whether these should be classified within the same subspecies. In the New World, the Alaska tundra caribou (R.*t. granti*) are distributed throughout the Alaskan Peninsula and partly into Yukon, Canada. The Canadian barrenground caribou (R.*t. groenlandicus*) are distributed east to the Alaska tundra caribou

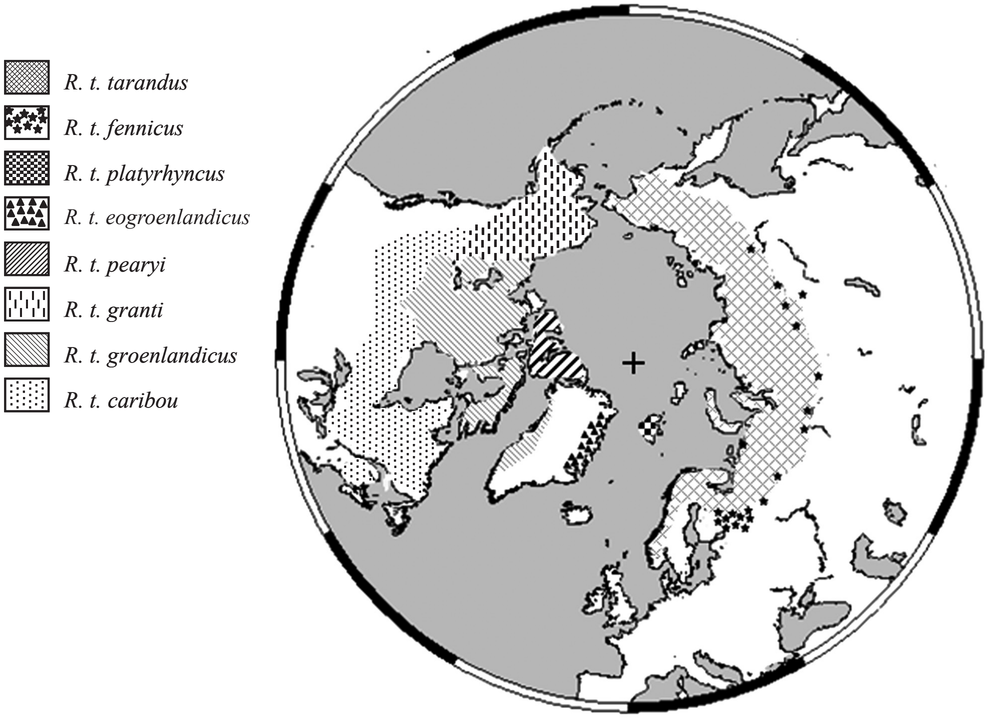

Fig. 1. Geographic distribution of the different subspecies of reindeer. Although additional subspecies have been suggested, these eight constitute the conventional taxonomic classification of the species.

across the tundra region in mainland northern Canada, including the Baffin Island and the west coast of Greenland. The American woodland caribou (*R.t. caribou*) are distributed mainly in the taiga region south to the distribution of the Canadian barrenground caribou from Newfoundland, Labrador and northern Québec in the East through British Columbia and southern Yukon in the West. Among the arctic ecotypes, there are the Peary caribou (*R.t. pearyi*) living on the Arctic Islands of northern Canada, and the Svalbard reindeer (*R.t. platyrhynchus*) isolated on the Spitsbergen Archipelago. The now extinct East-Greenland reindeer (*R.t. eogroenlandicus*) also belonged to an Arctic ecotype. This subspecies inhabited the east coast of Greenland and became extinct approximately 100 years ago. The recently extinct *R.t. dawsoni* that occupied Queen Charlotte Island of western Canada was also classified as a separate subspecies (Banfield, 1961).

To what extent the present classification of *R. tarandus* correctly represents the different refugial origin has been an open question. Here, I summa-

Table 1. Populations of reindeer and caribou analysed for serum transferrin polymorphism. The numbers quote the geographic locations of the population provided in Fig. 2.

No.	Population/location	Subspecies	Reference
1	Wild reindeer, Norway	*R.t. tarandus*	From Røed (1998a)
2	Domestic reindeer, Norway	*R.t. tarandus*	From Røed (1998a)
3	Domestic reindeer, Kola, Russia	*R.t. tarandus*	Present study
4	Domestic reindeer, Yakutia, Russia	*R.t. tarandus*	Present study
5	Domestic reindeer, Alaska	*R.t. tarandus*	Røed & Whitten (1986)
6	Forest reindeer, Kuhmo, Finland	*R.t. fennicus*	Present study
7	Svalbard reindeer, Nordenskiolland	*R.t. platyrhynchus*	Røed *et al.* (1986)
8	Western Arctic Herd, Alaska	*R.t. granti*	Present study
9	Central Arctic Herd, Alaska	*R.t. granti*	Present study
10	Porcupine Herd, Alaska/Canada	*R.t. granti*	Present study
11	Denali Herd, Alaska	*R.t. granti*	Present study
12	Nelchina Herd, Alaska	*R.t. granti*	Present study
13	Fourty-mile Herd, Alaska	*R.t. granti*	Present study
14	Jasper, eastern Alberta, Canada	*R.t. granti*	Present study
15	Central Alberta, Canada	*R.t. granti*	Present study
16	Beverly Herd, Canada	*R.t. groenlandicus*	Røed & Thomas (1990)
17	Baffin Island, Canada	*R.t. groenlandicus*	Røed *et al.* (1991)
18	Kaminuriak Herd, Canada	*R.t. groenlandicus*	Present study
19	Boothia Peninsula, Canada	*R.t. groenlandicus*	Røed *et al.* (1986)
20	Victoria Island, Canada	*R.t. groenlandicus*	Present study
21	Akia, Greenland	*R.t. groenlandicus*	Present study
22	Kangerlussaq, Grennland	*R.t. groenlandicus*	Present study
23	Peary caribou, Parry pop., Canada	*R.t. pearyi*	Røed *et al.* (1986)
24	Peary caribou, Peel pop., Canada	*R.t. pearyi*	Røed *et al.* (1986)
25	Northern Saskatchewan, Canada	*R.t. caribou*	Present study
26	Eastern Manitoba, Canada	*R.t. caribou*	Røed *et al.* (1991)
27	Slate Island, Ontario, Canada	*R.t. caribou*	Røed *et al.* (1991)
28	Leaf River Herd, Québec, Canada	*R.t. caribou*	Røed *et al.* (1991)
29	George River Herd, Québec, Canada	*R.t. caribou*	Røed *et al.* (1991)
30	Gaspe Herd, Québec, Canada	*R.t. caribou*	Røed *et al.* (1991)
31	Brunette Island, Labrador, Canada	*R.t. caribou*	Røed *et al.* (1991)
32	Newfoundland, Labrador, Canada	*R.t. caribou*	Present study

rize previous studies together with new data, using both proteins, nuclear DNA and mitochondrial DNA as genetic markers to assess the classification and post-glacial colonization of reindeer and caribou.

Transferrin variability.

The populations of reindeer and caribou analysed for serum transferrin polymorphism, both here and in previous studies, are provided in Table 1. All presently living subspecies are included, covering most of *R. tarandus*'s distribution in North America and Eurasia. The methods for analysis of the trasferrin polymorphism can be found in Røed *et al.* (1991) and references therein. Altogether, 30 transferrin alleles were detected. The genetic patterns revealed considerable heterogeneity among the populations analysed, both with regard to the number of alleles detected and the allele frequency distribution. However, the main pattern of the frequency distribution previously detected, with a major dichotomy between the *R.t. caribou* on the one hand and various populations of all the other subspecies on the other (Røed *et al.*, 1991), was also detected after including several more populations. This is illustrated by the frequency distribution of pooled frequencies of three alleles that was almost exclusively found among populations of American woodland caribou (Fig. 2). This supports the hypothesis that during the Wisconsin glaciation, the ancestral populations of *R.t. caribou* survived in a refugium separate from that of the ancestral populations of the other subspecies. However, precaution is required when interpreting evolutionary events from genetic patterns at one locus only. The various transferrin alleles may also have different adaptive values (*cf.* Zhurkevich & Fomicheva, 1976; Røed, 1987) with the possibility that the allele frequency distribution reflects selective forces rather than phylogenetic events.

Microsatellite variation

Over the last decade, new DNA-based methods have become available, offering a greater potential than earlier marker systems. Microsatellites, a class of simple tandemly repeated sequences (Litt &

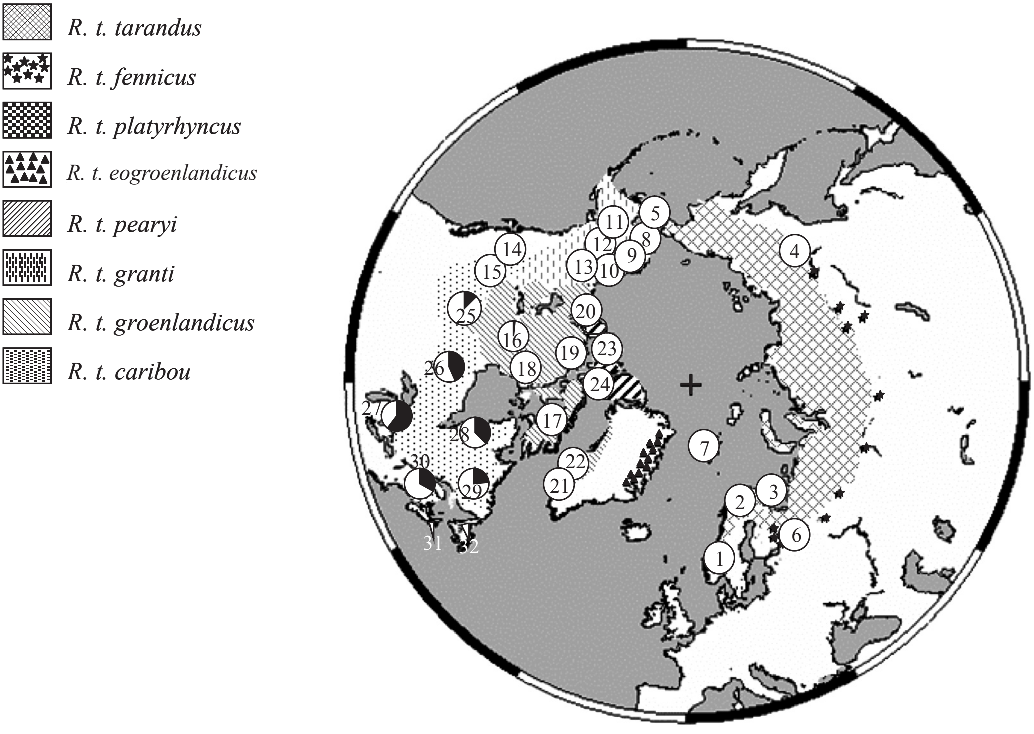

Fig. 2. Frequency distribution of three pooled transferrin alleles (Tf^{A-3}, Tf^{A-2} and Tf^{A-1}) given as section of pies. Numbers refer to the sampled population provided in Table 1. The indicated subspecies distribution is as for Fig. 1.

Table 2. Genetic variability in 13 microsatellite loci in subspecies of reindeer and caribou.

Subspecies	Population	Mean sample size per locus	Mean no. of alleles per locus	Mean heterozygosity
R.t. tarandus	Norway, domestic	201	9.2	0.73
R.t. tarandus	Norway, wild	181	9.7	0.76
R.t. tarandus	Finland, domestic	57	8.2	0.77
R.t. fennicus	Finland, wild	33	7.4	0.71
R.t. platyrhynchus	Svalbard reindeer	20	1.8	0.26
R.t. granti	Alaska caribou	36	10.2	0.77
R.t. groenlandicus	Canada, NWT	20	8.8	0.80
R.t. groenlandicus	Canada, Victoria Isl.	25	8.4	0.78
R.t. groenlandicus	West Greenland	18	4.9	0.46
R.t. pearyi	Peary caribou	11	6.0	0.68
R.t. caribou	Canada, Ontario	13	5.0	0.61
R.t. caribou	Canada, Québec	25	6.5	0.61

Luty, 1989; Tautz, 1989; Weber & May, 1989) have been described as ideal markers since they are highly polymorphic, codominant, abundant throughout the genome and amenable to polymerase chain reaction (PCR) technology. Most microsatellites are effectively neutral loci and thus, their geographical distribution will reflect the patterns of genetic drift and gene flow in populations. One major advantage of using the microsatellites are their rapid evolutionary rate, evolving 10-100 times faster than much of the coding nuclear DNA. Their high evolutionary rate provides an ideal genetic tool for population studies covering relatively short evolutionary time spans.

Microsatellites are increasingly used as genetic markers in reindeer and caribou (Kushny *et al.*, 1996; Wilson *et al.*, 1997; Røed, 1998b; Røed & Midthjell, 1998; Zittlau *et al.*, 2000; Côté *et al.*, 2002; Jepsen *et al.*, 2002; Røed *et al.*, 2002; Courtois *et al.*, 2003; Cronin *et al.*, 2003; McLoughlin *et al.*, 2004). I will present microsatellite analyses for different populations of *R. tarandus* representing all present living subspecies (Table 2).

Template DNA was extracted by standard procedures including proteinase K digestion and phenol/chloroform extraction. Thirteen reindeer-specific microsatellite loci were analysed for polymorphism (NVHRT-01, -03, -16, -24, -31, -48, -73 and -76, Røed & Midthjell, 1998, and RT-1, -5, -6, -9 and –27, Wilson *et al.*, 1997). All forward primers were end-labelled with fluorescent tags and the PCR products were electrophoresed using an ABI Prism 310 Genetic-Analyzer.

Altogether, 184 alleles were detected among the thirteen markers. All loci were polymorphic in all populations except for Svalbard reindeer (*R.t. platyrhynchus*), where five loci were monomorphic. Both mean number of alleles per locus and mean heterozygosity were low for this subspecies compared with all the other populations analysed (Table 2). Reduced genetic variation in this subspecies is in accordance with previously studies, both on protein polymorphism (Storset *et al.*, 1978; Soldal & Staaland 1980; Røed, 1985; Røed *et al.*, 1986) and microsatellite variation (Côté *et al.*, 2002). Svalbard reindeer have apparently been isolated for a long period, during which genetic drift and loss of genetic variability occurred. Similar processes likely occured for West Greenland caribou (*R.t. groenlandicus*). This subspecies also has relatively low levels of genetic variability (Table 2). Reduced genetic variability for caribou on Greenland, as also detected by Gravlund *et al.* (1998) and by Jepsen *et al.* (2002), could be a result of the many natural barriers as glaciers and wide fjords in this area together with extreme fluctuations in population size (Jepsen *et al.*, 2002). Somewhat reduced variability was also indicated in the two populations of woodland caribou (*R.t. caribou*), and to some extent also in the Peary caribou (*R.t. pearyi*) (Table 2). However, caution is needed

Table 3. Genetic distance (Nei, 1978) based on microsatellite variation in 13 loci in subspecies of reindeer and caribou.

Subspecies-Population	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
1. R.t. tarandus (Norway, domestic)	****										
2. R.t. tarandus (Norway, wild)	0.15	****									
3. R.t. tarandus (Finland, domestic)	0.09	0.15	****								
4. R.t. fennicus (Finland, wild)	0.21	0.14	0.09	****							
5. R.t. platyrhynchus (Svalbard)	0.93	0.83	0.99	0.99	****						
6. R.t. granti (Alaska)	0.29	0.17	0.23	0.14	0.74	****					
7. R.t. groenlandicus (Canada, NWT)	0.27	0.20	0.19	0.16	0.62	0.05	****				
8. R.t. groenlandicus (Canada, Victoria)	0.33	0.29	0.21	0.21	0.88	0.13	0.12	****			
9. R.t. groenlandicus (West Greenland)	0.97	0.83	0.81	0.76	2.21	0.75	0.69	0.83	****		
10. R.t. pearyi (Peary caribou)	0.35	0.42	0.30	0.37	0.70	0.65	0.18	0.31	0.79	****	
11. R.t. caribou (Canada, Ontario)	0.59	0.35	0.45	0.38	0.97	0.19	0.26	0.32	1.54	0.65	****
12. R.t. caribou (Canada, Québec)	0.59	0.39	0.46	0.36	1.06	0.14	0.20	0.28	1.24	0.46	0.14

when interpreting the relatively few individuals analysed for the Peary caribou. Besides, the microsatellite variation pattern among these samples showed an excess of homozygotes as compared to Hardy Weinberg expectations, a pattern also previously seen in the transferrin variation (Røed *et al.*, 1986). This possibly reflects genetic structuring within the sampled material.

The microsatellite allele frequency distribution comparison among the sampled populations was performed by estimates of the genetic distance (Nei, 1978) (Table 3). The genetic distances ranged from 0.05 between the two subspecies Alaska caribou (*R.t. granti*) and Canadian barrenground caribou from the North West Territories (*R.t. groenlandicus*), to 2.21 between the Svalbard reindeer (*R.t. platyrhynchus*) and caribou from West Greenland (*R.t. groenlandicus*). The West Greenland caribou showed generally large distance to all the analysed populations. Relatively low genetic distance was detected among the Scandinavian samples, including both the Eurasian tundra reindeer (*R.t. tarandus*) and the Finish forest reindeer (*R.t. fennicus*). In North America, the genetic distance between the woodland caribou (*R.t. caribou*) and the other populations was generally large (Table 3).

A dendrogram (Fig. 3) summarizing the genetic distances was constructed using the UPGMA cluster method (Sneath & Sokal, 1973). This analysis illustrates the genetic distinctiveness of the caribou on West Greenland (*R.t. groenlandicus*) and Svalbard reindeer (*R.t. platyrhynchus*) that both clustered out from the others. However, the low genetic variation detected in these populations suggests that their distinctive genetic pattern reflects some relatively recent bottleneck effects rather than phylogenetic events. Among all the other populations, the two woodland caribou (*R.t. caribou*) populations clustered away from the others supporting a separate origin for this subspecies. Also Peary caribou (*R.t. pearyi*) separated out from the others (Fig. 3). However, this subspecies was clearly most genetically similar to the Canadian barrenground caribou *(R.t. groenlandicus*) from North West Territories (Table 3), suggesting common origin of the ancestors of these populations. The European populations and the three samples of barrenground caribou in Alaska and Canada clustered away from each other, possibly reflecting relatively recent isolation processes. The opening of the Bering Strait in late Weichselian / Wisconsin might be such a process.

Mitochondrial DNA sequences

Mitochondria are specialised organelles located in the cytoplasm. Most somatic cells have thousands of copies of mitochondrial DNA (mtDNA) that provides a large number of starting copies for amplification. However the cytoplasmatic contribution of the male and the female parent is generally unequal; the egg contributes the bulk of the cytoplasm and the sperm essentially none. The mitochondrial genes are therefore strictly maternal

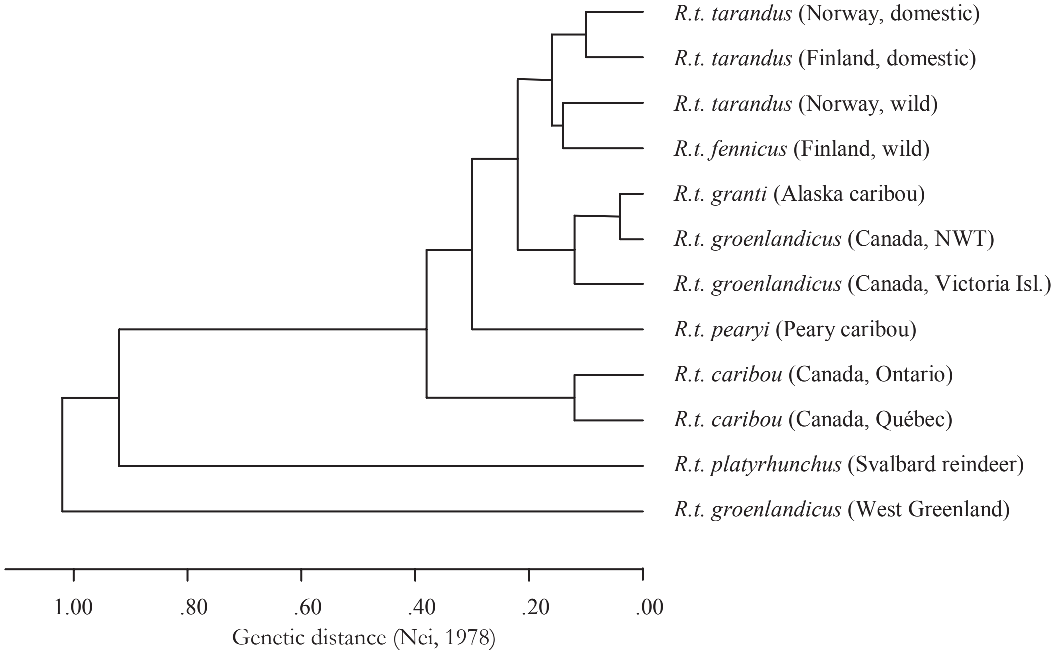

Fig. 3. Cluster analysis of the genetic distance (Nei, 1978) between subspecies of reindeer and caribou based on variability in 13 microsatellite loci.

inherited. This indicates simple heredity and no interpretation problems due to recombination. The rate at which the mitochondrial genome evolves through mutations is faster than most of the coding regions of the nuclear DNA, but slower than microsatellite DNA. It is an ideal genetic tool for inter-population phylogeographical studies covering intermediate evolutionary time span. Another advantage of using the mtDNA is that much of the analysis is based on the presence of haplotypes and haplogroups, and less dependent on frequencies of haplotypes. This makes the interpretation of such data more independent of recent bottleneck effects.

MtDNA sequence variation in the control region have recently been reported for all the extant subspecies of reindeer and caribou (Flagstad & Røed, 2003). The phylogenetic relationships among the different haplotypes were estimated and three distinct major haplogroups were indicated, presumably representing three separate populations during the last glaciation. These three haplogroups are illustrated in Fig. 4, providing a minimum spanning network (MSN) using the statistical parsimony approach described by Templeton *et al.* (1992). As indicated, haplogroup I exclusively consisted of haplotypes found among the Fennoscandian individuals represented by both *R.t. tarandus* and *R.t. fennicus*. Haplogroup II comprised haplotypes found mainly among the North American woodland caribou (*R.t. caribou*) together with two haplotypes found in two individuals of barrenground origin (Gro7 and Gro101). Haplogroup III constitutes a wide sub-network of cross-linked haplotypes located between haplogroups I and II in the network. All the subspecies were well represented in this haplogroup (Fig. 4).

The mtDNA sequences were also analysed for molecular variance to examine the amount of genetic variability partitioned within and among populations, as well as groups of populations (Flagstad & Røed, 2003). There was no relationship between the current subspecies designations and differentiation at the mtDNA level (Table 4). The current subspecies designations are therefore not compatible with the differentiation at the mtDNA level. In fact, 0% of the variability was explained at the subspecies level, strongly suggesting that the morphological differences among extant subspecies did not evolve in separate glacial refugia. Similarly, weak

Table 4. Analysis of molecular variance of mtDNA haplotypes of reindeer and caribou based on several possible groupings of the populations examined.

Grouping criteria	Groups of populations	Proportion of total variation assigned to groups	Significance level of grouping (*P*)
Subspecies	[*tarandus*] [*fennicus*] [*platyrhynchus*] [*pearyi*] [*caribou*] [*groenlandicus*] [*granti*]	0%	0.52
Ecotype	[*tarandus*, *groenlandicus*, *granti*] [*platyrhynchus*, *pearyi*] [*fennicus*, *caribou*]	0.2%	0.43
Geographic distribution (Eurasia *vs*. North America)	[*tarandus*, *fennicus*, *platyrhynchus*] [*pearyi*, *granti*, *groenlandicus*, *caribou*]	1.4%	0.34
Northern *vs*. southern haplogroups	[haplogroup I, haplogroup III] [haplogroup II]	32.8%	0.09
Eurasian *vs*. North American haplogroups	[haplogroup I] [haplogroup II, haplogroup III]	41.9%	< 0.001
Three main haplogroups	[haplogroup I] [haplogroup II] [haplogroup III]	49.0%	< 0.001

relationships were found when grouping the populations according to the ecotypes of tundra, arctic and woodland types, or geographic distribution of Eurasia and North America. The only convincing figures appeared when populations were grouped in various ways according to the main haplogroups as suggested from the phylogenetic analyses. When considering the three groups as separate entities, almost 50% of the total variability was explained at the group level (Table 4), supporting the actual existence of three main mtDNA lineages.

In the same study, the three mtDNA lineages, as revealed from the phylogenetic analysis, were also tested for sudden population expansion using the mismatch distribution approach as given by Slatkin & Hudson (1991) and by Rogers & Harpending (1992). The mismatch distribution is the distribution of the observed number of differences between pairs of haplotypes. The mismatch distributions of haplogroups I and III were clearly unimodal, a pattern compatible with a historical population expansion. Contrastingly, haplogroup II showed a multimodal mismatch distribution, suggesting stable population size through time or that this haplogroup comprised several sub-groups. The average number of nucleotide differences was much higher for haplogroup III as compared to that of haplogroup I, indicating that the ancestral populations of the two haplogroups expanded at different times. Assuming a divergence rate of 16% per million years, the putative expansion of haplogroup I was dated to 15 000 years before present (BP), while that of haplogroup III was estimated to have occurred at approximately 115 000 years BP (Flagstad & Røed, 2003).

Discussion

As shown for both the microsatellite frequency variation and the mtDNA haplotype distribution, the current subspecies designations are not compatible with the differentiation at these markers. This suggests that the morphological differences among extant subspecies did not evolve in separate glacial refugia. Thus, it appears that morphological differences among extant subspecies have evolved as adaptive responses to post-glacial environmental change. A possible exception to this is the North American woodland caribou, where all the three marker systems support an origin of the ancestors of these animals in a subspecies-specific refugium.

As given by the mtDNA variation, three main haplogroups were reported and approximately 50% of the total sequence variability was assigned to variation among these groups. These results support the existence of three separate glacial populations during the Weichselian / Wisconsin. The largest and most influential origin to the gene pool was, as discussed by Flagstad & Røed (2003), represented by mtDNA haplogroup III. All North-American samples except for those of the southerly-distributed woodland caribou (+ two *R.t groenlandicus* specimens) belonged to this haplogroup, which points towards a Beringian origin. This haplogroup appears to represent an ancestral glacial population

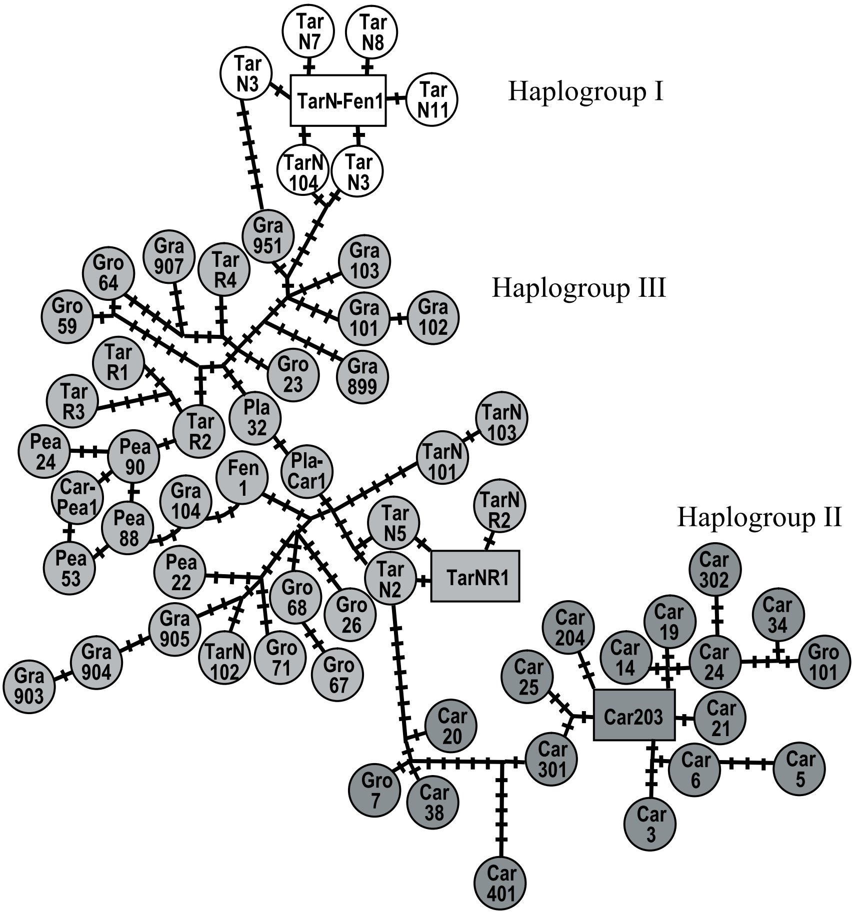

Fig. 4. Minimum spanning network (MSN) of mtDNA haplotypes of reindeer and caribou (*cf.* Flagstad & Røed, 2003). Each haplotype is given a three-letter code according to subspecies origin. Norwegian and/or Russian origin of *tarandus* (Tar) haplotypes is indicated by capital letter N and R, respectively. Bars displayed perpendicular onto the lines connecting the haplotypes represent mutations. Haplogroups I, II and III are indicated with white, dark grey and ligther grey, respectively. Haplotypes estimated to be the most likely common ancestor within each of the three haplogroups are displayed as squares.

ranging across vast areas of tundra in Eurasia and extending into North America across the Beringian land bridge. The large sequence diversity and unimodal distribution of sequence differences suggest that the population remained large and continuous after an initial demographic expansion dated to approximately 115 000 years BP. As ice cover retreated by the end of the Weichselian / Wisconsin, representatives from this population appear to have recolonized exposed habitat on the continental mainland in North America, Siberia and Fennoscandia. All current subspecies are to some extent affected by this origin. The North American tundra forms (*R.t granti* and *R.t groenlandicus*) and the Arctic forms (*R.t platyrhynchus*, *R.t pearyi* and *R.t eogroenlandicus*) almost exclusively comprise haplotypes of such an origin.

The mtDNA haplogroup I was suggested to have been established as a result of a rather different evolutionary history, characterized by high degrees of recent isolation (Flagstad & Røed, 2003). As haplotypes belonging to this group were not represented among any of the North American samples, a pure Eurasian origin was indicated. A recent origin in a small refugium probably isolated in connection with ice expansion in Eurasia during the Weichselian was discussed. The two Eurasian subspecies *R.t. tarandus* and *R.t. fennicus* appear to have a diphyletic origin, as both the putatively small and isolated Eurasian refugium and the large Beringia refugium have contributed to their gene pools. Accordingly, adaptation to woodland conditions for *R.t. fennicus* seems to be a recent phenomenon, possibly taking place in connection with post-glacial forest expansion.

In North America, another distinct and geographically well-defined refugial area is indicated by all three marker systems. Virtually all haplotypes belonging to mtDNA haplogroup II were found among the southerly distributed woodland caribou. This, together with the dichotomy in both the transferrin and the microsatellite variability pattern, suggests that this subspecies has its main origin in refugia located south to the Wisconsin ice sheet. The multimodal mismatch distribution of this mtDNA haplogroup provides evidence of a large stable population size through time or that this haplogroup comprised several subgroups (*cf.* Flagstad & Røed, 2003).

For the Arctic forms, a common North American origin is particularly well supported for *eogroenlandicus* and *pearyi,* as these subspecies shared a common mtDNA haplotype as reported by Gravlund *et al.* (1998). When the ice retreated, colonizers appear to have migrated across the Canadian archipelago and eventually reached Eastern Greenland. Gravlund *et al.* (1998) reported similar mtDNA haplotype among Svalbard reindeer and a specimen from the Taimyr peninsula in northern Russia and suggested a diphyletic origin of the Arctic ecotype with an Eurasian origin of the Svalbard reindeer. However, in the extensive study by Flagstad & Røed (2003), the most common mtDNA haplotype found on Svalbard was identical to the only haplotype found more than once in the Quebec region in Canada, supporting a North American colonization route towards Svalbard. This was in accordance with the previously reported distinct similarities at the transferrin locus between Svalbard reindeer and Peary caribou and with both having some similarities with the American woodland caribou (Røed *et al.*, 1986; 1991). The microsatellite variability pattern in the Svalbard reindeer (*R.t platyrhynchus*) was characterized by reduced genetic variability. However, the genetic distance estimates were slightly higher against the Eurasian subspecies than against the North American subspecies, although the distances were generally high towards all other populations (Table 3). It appears therefore that the available data based on genetic variability in both transferrin, microsatellites and mtDNA points toward an origin of the Svalbard reindeer from ancestors in North America.

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Abstract in Norwegian / Sammendrag:

Systematisk inndeling og kolonisering av rein (*Rangifer tarandus*) ble bestemt ved å analysere for variasjon i genetiske markører som proteiner, kjerneDNA og mitokondrieDNA. Dagens oppdeling av rein i underarter viser liten overensstemmelse med variasjonsmønsteret i de undersøkte markørene, noe som viser at de morfologiske forskjellene som karakteriserer dagens underarter ikke har utviklet seg i atskilte refugier i løpet av siste istid. Unntak fra dette er nordamerikansk skogsrein (woodland caribou-*R.t. caribou*) hvor alle tre markørsystemene indikerer at denne har utviklet seg i et refugium forskjellig fra andre underarter. De tre registrerte hovedhaplogruppene i mitokondrie-DNA representerer tre atskilte opprinnelser av rein i løpet av siste istid. Den mest innflytelsesrike av disse bidro vesentlig til genbanken til alle dagens underarter av rein, noe som tyder på at det under siste istid eksisterte en stor reinpopulasjon med kontinuerlig utbredelse gjennom store deler av tundraen i Eurasia og Beringia. De nordamerikanske tundrareintypene (*R.t. granti* og *R.t. groenlandicus*), samt de arktiske typene (*R.t. platyrhunchus*, *R.t. pearyi* og *R.t. eogroenlandicus*) består nærmest utelukkende av haplotyper med denne opprinnelse. Et annet lite og isolert refugium syntes å ha oppstått i Vest-Europa i nærheten av den omfattende isbreen som dekket Fennoskandia. De to europeiske underarter, *R.t. tarandus* og *R.t. fennicus,* syntes å ha en todelt opprinnelse med genetisk påvirkning fra både det antatt lille og isolerte refugiet i Eurasia samt fra det store Beringia refugiet. Et tredje geografisk distinkt refugium var antagelig lokalisert sør for den omfattende isbreen i Nord Amerika hvorfra forfedrene til dagens nordamerikanske skogsrein (*R.t. caribou*) har sin mest sannsynlige opprinnelse.