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Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou

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Abstract

The grey wolf has one of the largest historic distributions of any terrestrial mammal and can disperse over great distances across imposing topographic barriers. As a result, geographical distance and physical obstacles to dispersal may not be consequential factors in the evolutionary divergence of wolf populations. However, recent studies suggest ecological features can constrain gene flow. We tested whether wolf–prey associations in uninterrupted tundra and forested regions of Canada explained differences in migratory behaviour, genetics, and coat colour of wolves. Satellite-telemetry data demonstrated that tundra wolves (n = 19) migrate annually with caribou (n = 19) from denning areas in the tundra to wintering areas south of the treeline. In contrast, nearby boreal coniferous forest wolves are territorial and associated year round with resident prey. Spatially explicit analysis of 14 autosomal microsatellite loci (n = 404 individuals) found two genetic clusters corresponding to tundra vs. boreal coniferous forest wolves. A sex bias in gene flow was inferred based on higher levels of mtDNA divergence (F_{ST} = 0.282, 0.028 and 0.033; $P < 0.0001$ for mitochondrial, nuclear autosomal and Y-chromosome markers, respectively). Phenotypic differentiation was substantial as 93% of wolves from tundra populations exhibited light colouration whereas only 38% of boreal coniferous forest wolves did (χ^2 = 64.52, $P < 0.0001$). The sharp boundary representing this discontinuity was the southern limit of the caribou migration. These findings show that substantial genetic and phenotypic differentiation in highly mobile mammals can be caused by prey–habitat specialization rather than distance or topographic barriers. The presence of a distinct wolf ecotype in the tundra of North America highlights the need to preserve migratory populations.

Keywords: *Canis lupus*, foraging ecology, gene flow, grey wolf, migratory behaviour, phylogeography

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Introduction

Grey wolves are large mammalian carnivores with an ability to disperse over long distances across substantial topographic obstacles. Individuals typically disperse 50 km before establishing territories, but dispersal distances of several hundred kilometres are not uncommon (Mech 1970; Fritts 1982; Merrill & Mech 2000). Consequently, on regional and continental scales, grey wolves exhibit only weak patterns of differentiation by distance (Roy *et al.* 1994; Vilà *et al.* 1999; Leonard *et al.* 2005; Pilot *et al.* 2006), although this pattern may be more pronounced locally because of close relatedness among neighbouring wolf packs (Forbes & Boyd 1997). Nonetheless, wolves vary

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geographically in size and coat colour and five or more subspecies co-exist in North America (Nowak 1995). Recently, genetic analysis of wolf populations separated by water barriers (Carmichael *et al.* 2001) or existing in distinct habitats (Geffen *et al.* 2004; Pilot *et al.* 2006; Carmichael *et al.* in press) suggested that habitat and prey distribution may be the primary factors explaining genetic divergence. Similar relationships between habitat and genetic differentiation were also found in California coyotes (Sacks *et al.* 2004, 2005). Studies focusing specifically on ecology and prey base differences among wolf populations are needed to better understand the evolutionary processes that lead to genetic and phenotypic divergence and reproductive isolation.

Grey wolves of the boreal coniferous forest and the tundra of North America are reported to exhibit differences in prey specialization that might influence natural history and morphology (Kelsall 1968). In northern Canada, barren-ground caribou (*Rangifer tarandus groenlandicus*) migrate from calving grounds in the tundra to wintering grounds south of the treeline (Calef 1981). During the winter, grey wolves that inhabit areas dominated by migratory caribou are thought to abandon territorial behaviour, which is exhibited during the summer, and migrate with the caribou to their breeding grounds, although the phenomenon has not been spatially documented for wolves based on tracking of the migration (Kuyt 1972; Parker 1973; Walton *et al.* 2001a). In contrast, wolves inhabiting heavily forested regions such as the boreal coniferous forests of the Northwest Territories and Alberta, Canada are believed to follow the established wolf behavioural pattern of defending permanent territories and consuming resident, nonmigratory species such as deer (*Odocoileus* spp.), elk (*Cervus elaphus*), moose (*Alces alces*) and woodland caribou (*Rangifer tarandus caribou*) (Young & Goldman 1944; Mech 1970). Studies by Cook *et al.* (1999) and Ballard *et al.* (1997) indicated that some populations or some individuals are migratory in forested regions of North America. However, there have been no studies comparing the genetics of migratory wolves identified directly through telemetry with wolves from populations without migratory prey.

The tundra, taiga and boreal coniferous forest biomes are uninterrupted habitats that grade into each other ('taiga' is used hereafter to describe the northern part of the boreal coniferous forest biome; Rowe 1972; Bliss 1988; Elliot-Fisk 1988; ESWG 1995). Human density in these regions is very low and there are no physical barriers to dispersal for wolves or caribou. The taiga was previously recognized as a boundary zone between distinct subspecies of wolves based on morphological analyses (Goldman 1944; Hall 1981). We used satellite telemetry to document migration in tundra wolves and their barren-ground caribou prey in northern Canada. Although migratory and nonmigratory wolves may overlap in distribution during the winter (Kelsall 1968; Kuyt 1972), we predict that they should be genetically differentiated because they den in different areas and are specialized on different prey. To test this hypothesis, we analysed 14 autosomal microsatellite loci and 425 bp of mitochondrial control region DNA in 404 wolves, and four Y-chromosome microsatellite loci in 202 male wolves from tundra, taiga and boreal coniferous forest environments. Furthermore, we tested for differences in coat colour from these regions to assess levels of phenotypic differentiation, and by inference, the strength of natural selection.

Materials and methods

Study area

The study area is located in the central sub-Arctic and high latitude forest regions of Canada (Fig. 1). Topography is gently rolling, with many lakes frozen over half the year. In these regions, the climate is characterized by short cool summers and long winters (Overpeck *et al.* 1997). The northeastern part of the study area north of the treeline consists of semi-arid low-Arctic tundra (Bliss 1988). The southwestern portion of the study area encompasses the Northern Canadian Shield Taiga (Rowe 1972; ESWG 1995) and the boreal coniferous forest (FAO 2001), where annual rainfall is relatively higher (Elliot-Fisk 1988). The taiga is a belt approximately 200-km wide south of the treeline, which is frequented by barren-ground caribou during the winter (Calef 1981; Miller 1982). For climate and vegetation, this area is transitional between tundra and boreal coniferous forest (Rowe 1972; ESWG 1995). The boreal coniferous forest extends south of the typical winter range of migratory barren-ground caribou. In addition to wolves and barren-ground caribou, muskoxen (*Ovibos moschatus*) occur only in the tundra portions of the study area. Moose occurs typically at low density throughout the boreal coniferous forest and taiga areas. Bison (*Bison bison*) and woodland caribou occur in some boreal coniferous forest portions of the study area.

Satellite telemetry

In order to document the movement patterns of wolf packs in relation to migratory barren-ground caribou, 19 migrating caribou and 19 tundra wolves were tracked by satellite telemetry. The remoteness of the study area, absence of roads, and long periods of short-day length during winter required the use of satellite telemetry to effectively track wolf and caribou movements. We also deployed VHF radiocollars (Telonics Inc.) on seven wolves to aid in relocating packs in case the satellite collars malfunctioned. In 1997–1998, grey wolves were captured within a 60 000-km^2 area of tundra centred on 64°27′N, 110°35′W (Fig. 1). This

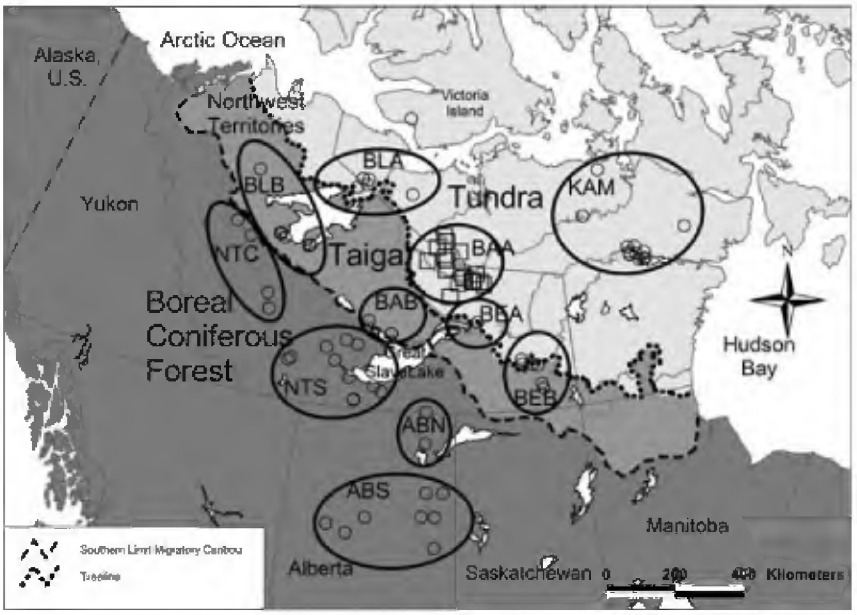

Fig. 1 Sampling locations (open circles and squares) and the 11 predefined groupings of wolf samples (large ellipses) used in the genetic analyses ($n = 404$ individuals). We employed blood from 26 live-captured individuals (squares) and tissue from pelts of 378 legally hunted wolves (circles). The study area included the Northwest Territories (NWT, $n = 309$ samples), Nunavut ($n = 49$) and the province of Alberta ($n = 46$), Canada. Main landscape features included lakes and rivers, whereas the habitat was characterized by tundra (light-grey area north of the treeline, dotted line), Northern Canadian Shield Taiga (grey area between the treeline and the southern limit of caribou migration, dashed line) and boreal coniferous forest (dark-grey area south of the limit of caribou migration).

area lies within the annual range of the Bathurst barren-ground caribou population (Calef 1981; Gunn *et al.* 2002), and during 1996 and 1998, caribou were also satellite collared. Both species were captured with net guns fired from helicopters and fitted with collars also produced by Telonics. We targeted adult wolves rather than subadult potential dispersers (Mech 1970). Furthermore, only caribou cows were captured, because their migratory behaviour is well described (Calef 1981; Miller 1982). Collars were programmed to transmit at various intervals ranging from 1 to 5 days. We generally obtained one complete year of monitoring before the power supply of a collar was exhausted. For this analysis, we selected a standard 5-day interval to generate a location data set that was consistent among seasons. Only locations with error < 1000 m were included in the analyses.

We determined seasonal home ranges using ARCVIEW 3.2a Geographic Information System (GIS; ESRI, Inc.) and the ANIMAL MOVEMENT 1.1 extension to ARCVIEW (Hooge & Eichenlaub 1997). We calculated fixed-kernel home-range-use distributions using least-squares cross validation of a smoothing parameter (Seaman & Powel 1996) obtaining seasonal 50% and 95% probability polygons in two seasons (spring/summer and fall/winter defined following Walton *et al.* 2001a).

The overlap of two home ranges provides a first approximation of interaction between organisms (Macdonald *et al.* 1980). However, this index does not take into account the utilization distribution within the shared parts of each range. For example, two ranges might overlap by less than 50% although the shared area contains the most utilized parts of both ranges. Alternatively, two individuals may concentrate their activities in different parts of a largely shared range. The spatial overlap of two home ranges and congruence in at least part of the utilization distributions is termed 'static interaction' (Macdonald *et al.* 1980; Doncaster 1990). Following Tew & Macdonald (1994), we used nonparametric utilization distribution analyses to measure static interactions between wolves and caribou. In ARCVIEW, we divided the area encompassing all wolf and caribou locations into a grid. We obtained 165 grid cells measuring 50×50 km, which is an area potentially covered by a wolf or caribou individual in 1 day (Mech 1970; Miller 1982). This cell size also satisfied the conditions suggested by Doncaster (1990) that the size of grid-cells must clearly be large enough that some cells contain several fixes, but not so large as to obscure the overall configuration of the range. We calculated the visit frequency of wolves and caribou for each cell. We used Spearman correlation (2-tailed) for testing the association between these two species over all grid cells, representing correlations in range use. High correlation implies not only high overlap but also similar utilization. We used a permutation test implemented in SPSS version 10.0 to test whether the observed value of Spearman's rho was significantly different from zero.

Because of performance limitations of satellite data-loggers and limited battery power in cold environments (Walton *et al.* 2001b), we could not consistently acquire locations for all collars during each transmitting cycle. We analysed wolf–caribou distances during each calendar season (spring, summer, fall, and winter) from summer 1997 to summer 1999. We randomly selected 44 wolf and 39 caribou locations for each season from among the available data. This sample represented the minimum number of locations available within any season for all collared wolves and caribou, respectively. We also chose two

equivalent random samples of wolf (n = 44) and caribou locations (n = 39) from the whole location data set, to create a year-round data set. Finally, we calculated Euclidean distances between wolf and caribou locations within each season and for all seasons, and compared caribou–wolf distances for each season to the all-season sample pairwise with a Mann–Whitney U-test using SPSS Version 10.0.

DNA and coat colour samples

We sampled blood from 26 wolves live-captured in the Northwest Territories for telemetry observations (satellite-collared wolves of Fig. 1) and tissue from pelts of 378 legally hunted wolves for a total 404 DNA samples (Fig. 1). Hide samples were from wolves killed by hunters from 1999 to 2000 in the Northwest Territories (n = 283; of which 82, 179 and 22 were harvested in the tundra, taiga and boreal coniferous forest, respectively), Nunavut (n = 49; from tundra) and Alberta (n = 46; from boreal coniferous forests). We recorded pelt colour and sex for each hide sample and each captured wolf. We used the fur grading and pelt guide from Obbard (1987) to standardize descriptions of colour morphs. However, since pelt colour varies over the body surface of a wolf and the position of hide samples was unknown, we classified pelt colour into two general categories, 'dark' (grey through black) and 'light' (white to near white). We used chi-squared tests to compare occurrences of light wolves in forested and tundra habitats.

Molecular analyses

DNA was isolated using the QIAGEN DNeasy extraction kit. A 425-bp segment of the control region of the mitochondrial genome was amplified and sequenced (Vilà *et al.* 1999). Amplification from 1 μL of the extract was carried out in a 50-μL reaction consisting of 1× reaction buffer, 2.5 mM $MgCl_2$, 0.06 mM dNTPs, 0.5 mM of each primer and 1.6 U of *Taq* (Promega). Reactions were run for 35 cycles of 95 °C for 1 min, 47 °C for 1 min and 72 °C for 1 min in a Primus 96 plus (MWG-Biotech) PCR machine. Polymerase chain reaction (PCR) products were separated on a 2% agarose gel, the correct band was excised and purified using the UltraClean kit (MoBio). PCR products were cycle sequenced and run on a Beckman sequencer.

Fourteen autosomal microsatellites, originally developed for dogs, were amplified: c2001, c2006, c2010, c2017, c2054, c2079, c2088 and c2096 (Francisco *et al.* 1996), u250 and u253 (Ostrander *et al.* 1993), vWF (Shibuya *et al.* 1994), and PEZ01, PEZ05 and PEZ08 (PerkinElmer, Zoogen; see dog genome map at http://www.research.nhgri.nih.gov/dog_genome/) as in Vilà *et al.* (2003). For genotyping, the concentration of all DNA was measured and adjusted to 10 ng/μL. PCRs included 1× buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 32 pmol of each primer (one of which was fluorescently labelled), 0.5 U of AmpliTaq Gold (Applied-Biosystems) and 10 ng of template DNA in a 10-μL reaction. Touchdown reactions (58–52 °C) were run on a PTC-225 tetrad thermocycler (MJ Research). The males (n = 202) were also typed at four Y-chromosome microsatellite loci: MS41A, MS41B, MS34A and MS34B (Sundqvist *et al.* 2001). PCR multiplex amplification and typing were undertaken as described by Sundqvist *et al.* (2001). Autosomal and Y-chromosome PCR products were run on an ABI PRISM 377 sequencer (PerkinElmer) with a Genescan-500 Tamra size standard.

Descriptive statistics: autosomal microsatellite data

Scoring errors, large allele dropout and null alleles were assessed with the program MICRO-CHECKER (van Oosterhout *et al.* 2004). ARLEQUIN version 3.1 (Excoffier *et al.* 2005) was used to test for conformance to Hardy–Weinberg equilibrium and GENEPOP version 3.3 was used to measure linkage disequilibrium within sampling locations (Bonferroni-corrected P value corresponding to alpha = 0.05; Raymond & Rousset 1995; Sacks *et al.* 2004). We estimated these values in population units defined by the Bayesian clustering method implemented in GENELAND version 1.0.5 (Guillot *et al.* 2005). In GENELAND, as opposed to other individual-based cluster programs (e.g. STRUCTURE; Pritchard *et al.* 2000), spatially explicit information for all individuals is used along with genotypic data to deduce the best number of subdivisions (K) and assign individuals to each subdivision (see Excoffier & Heckel 2006). Guillot *et al.* (2005) suggest inferring K in a first run and then running the algorithm again with K fixed at the previously inferred value in order to estimate the other parameters such as the assignment of individuals to the inferred populations. This method also takes into account location errors (induced by measurement error) by introducing an additive noise to the coordinates, the true coordinates being treated as unknown and as parameters to be estimated. We ran the Markov chain Monte Carlo (MCMC) five times (to verify the consistency of the results), allowing K to vary, with the following parameters: 500 000 MCMC iterations, a maximum rate of Poisson process fixed to 500, uncertainty attached to spatial coordinates fixed to 1 km, minimum K fixed to 1, maximum K fixed to 30, and the maximum number of nuclei in the Poisson–Voronoi tessellation fixed to 200. We used the Dirichlet model as a model for allelic frequencies. We employed the mode for the estimated number of populations as the best approximation of the number of populations present in the data (Guillot *et al.* 2005; see Fig. S1, Supplementary material). We then ran the MCMC 100 times with K fixed. We calculated the posterior probability of population membership for each pixel of the spatial domain using a burn-in of 50 000 iterations. In general, our approach and initial conditions follow Coulon *et al.* (2004)

who addressed a similar problem of population subdivision in roe deer. This approach allowed determination of population boundaries with probability levels ranging from 0.1 to 0.9. We used standard Geographic Information System procedures to geo-reference the map produced by GENELAND. Specifically, we imported the GENELAND map as a background map in ARCVIEW and then digitized the boundaries and saved the resulting graphic as a geo-referenced layer.

Population structure: mitochondrial DNA and Y-chromosome haplotype data

We used SAMOVA (spatial analysis of molecular variance; Dupanloup *et al.* 2002) to identify genetically distinct populations based upon mitochondrial and Y-chromosome haplotype data for comparison to population units defined by GENELAND using autosomal microsatellite markers. First, sampling localities were arranged into 11 groups based on the following criteria: (i) dominant habitat, (ii) dominant prey (see Peterson & Ciucci 2003), and (iii) spatial distribution (samples inspected by government personnel in the same locality were grouped; see Appendix). The SAMOVA method employs a simulated annealing procedure and uses haplotype sequence and frequency along with geographical coordinates of the sampled 11 groups for identifying clusters that exhibit close genetic relationships. To determine the model of DNA substitution that best fitted our data, we employed FINDMODEL (available at http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html). The program implements the methods developed for MODELTEST (Posada & Crandall 1998) using scores for likelihood of trees generated under 28 compared models. The Akaike Information Criteria (AIC) estimates and the hierarchical likelihood-ratio tests (hLRT) that are implemented in MODELTEST resulted in the same models of nucleotide substitution. We measured the divergence between mitochondrial haplotypes using the best-fit model: a Tamura–Nei model of sequence evolution and a gamma distribution of the substitution rates with a value of $\alpha = 0.5$ (Tamura & Nei 1993; Wakeley 1993). The *F*-statistic (see below) analogues, F_{ST}, F_{SC} and F_{CT}, were estimated for each hypothesis stipulated on the number of genetic clusters, and their significance levels were determined with 1000 permutations (Excoffier *et al.* 1992). Clustering by SAMOVA is based on a hierarchical analysis of F_{ST} and maximizing the proportion of total genetic variance between groups (F_{CT}). SAMOVA also incorporates a geographical constraint, which is generated automatically to ensure that the units defined by the method are geographically contiguous. In SAMOVA, the user specifies the number of clusters the method should define. We used SAMOVA to generate structures containing 2, 3, 4, 5 and 6 population clusters. The simulated annealing process was repeated 250 times to ensure that the final configuration of the *K* population clusters was not affected by a given initial configuration. In SAMOVA analyses, F_{CT} is expected to increase with increasing number of populations (*K*) because of the reduction of the proportion of variance due to differences between populations within each group (F_{SC}; Dupanloup *et al.* 2002).

Association with distance and ecology: comparing autosomal microsatellite, mitochondrial and Y-chromosome data

To assess the association of genetic distance with geographical distance and environmental variables, we followed an analytical design similar to that used in Roy *et al.* (1994), Geffen *et al.* (2004), and Pilot *et al.* (2006). To visualize any apparent relationship with distance or environment, Nei's (1978) unbiased distance among the 11 wolf groups defined above was used to construct neighbour-joining (NJ) trees with the program MICROSAT (Minch 1997). As a measure of the support for tree topologies, 1000 bootstrapped-across-individuals distance matrices were generated and a consensus tree of all resulting NJ trees was built with the program PHYLIP 3.572 (Felsenstein 1995). Genetic differentiation between wolf groups was examined with conventional *F*-statistics calculated with the program GENETIX version 4.05 (Wright 1951; Weir & Cockerham 1984; Belkhir *et al.* 2004). To visualize F_{ST} values among the 11 groups, we performed nonmetric multidimensional scaling (MDS) analysis (Mardia *et al.* 1979; Lessa 1990). This analysis collapses variation on a two-dimensional plane, such that the Euclidean distances among these points match the genetic distance matrix as closely as possible. A stress value for MDS, which evaluates the fit on two dimensions, was obtained with the PROXSCAL formula (SPSS 14.0).

We also performed a partitioning of genetic distance matrices among the 11 wolf groups using distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999; McArdle & Anderson 2001). This is a form of multivariate multiple regression which can be carried out directly on a distance or dissimilarity response matrix of choice. There were four response matrices of interest: (i) F_{ST} distances; and (ii) Nei's unbiased distances obtained using autosomal microsatellite data; (iii) F_{ST} distances obtained using mitochondrial DNA data; and (iv) F_{ST} distances obtained using Y-chromosome microsatellite data. We estimated the central location for each group of wolf samples by averaging latitude and longitude values of all samples in that group, and Euclidean distances were calculated among central locations. We used both standard and log-transformed distances. Distance-based redundancy analysis was used to test the effects on genetic distances of Euclidean geographical distances and of latitudinal and longitudinal

distances (i.e. difference among locations in just latitude or just longitude). We also tested the effect of the categorical variables habitat (i.e. whether tundra, taiga or boreal coniferous forest) and prey movements (i.e. whether wolf prey in the area was migratory or resident). Similar to Pilot *et al.* (2006), we used the program DISTLM version 5 (Anderson 2004) to test whether the predictor variables listed above were correlated with genetic distances. In such 'marginal tests', the P values were obtained using 999 unrestricted, simultaneous permutations of the rows and columns of the distance matrices for all variables. We also used DISTLM to perform 'conditional tests', in which latitude and longitude were included as covariates into the model. The conditional tests allowed us to examine the extent to which any predictor variable explained genetic diversification among the wolf groups beyond that explained by geographical distances alone. In conditional tests, P values were obtained using 999 permutations of the rows and columns of the multivariate residual matrix under the reduced model (Freedman & Lane 1983; Anderson & Legendre 1999).

We chose the exact test of population differentiation as implemented in ARLEQUIN to test for differences between populations in the distribution of mtDNA haplotypes, Y-chromosome haplotypes and autosomal microsatellite alleles. We calculated the mean and standard deviation of gene diversity for mitochondrial and Y-chromosome data and nucleotide diversity for mitochondrial data. For autosomal microsatellite data, we calculated the observed and expected heterozygosities (H_O and H_E) for each wolf grouping according to Nei (1987). These analyses were performed on all samples and on female and male wolves separately, to assist in evaluating sex-biased dispersal (Prugnolle & de Meeus 2002). We performed assignment tests to determine the composition of subdivisions and identify migrants (Paetkau *et al.* 1997; Waser & Strobeck 1998). The program ARLEQUIN was employed to compute the log-likelihood of the genotype of each individual in every sample, as if it was drawn from a population sample having allele frequencies equal to those estimated for each sample. We also interpreted assignment tests with the methods first described by Favre *et al.* (1997), who predicted that the more dispersing sex should have, on average, lower assignment probabilities than the philopatric sex. Finally, we calculated the variance of assignment indices, as the variance is expected to be larger for the sex dispersing most (Favre *et al.* 1997).

Results

Migratory behaviour and association of barren-ground caribou and wolves

We satellite-collared 19 adult grey wolves (12 females, 7 males) and 19 barren-ground caribou (all females) and monitored them from summer 1997 to summer 1999. During the observation period, pooled locations of all caribou covered a triangular area originating southwest of Bathurst Inlet and spreading south and west (350 973-km^2 95% probability polygon). This area is historically occupied by the Bathurst caribou herd (Calef 1981; Gunn *et al.* 2002). Pooled locations of all wolves covered a smaller area within the area occupied by the Bathurst caribou (172 601-km^2 95% probability polygon). These results showed that the wolves monitored in this study also inhabited the historical range of the Bathurst caribou.

For grey wolves, during two full years of observations, individual spring/summer core use areas (50% probability polygons; $n = 19$, 319 ± 425 km^2) were smaller than fall/winter core areas ($n = 19$, 14 144 ± 12 701 km^2; $t = 4.986$, $P < 0.001$). During this period, spring/summer core areas of barren-ground caribou individuals ($n = 19$, 12 529 ± 10 170 km^2) were similar in size to fall/winter core areas ($n = 19$, 10 958 ± 9089 km^2; $t = 0.687$, $P = 0.495$; Fig. 2). The 19 wolves that we collared frequented 15 separate spring/summer core use areas, which were all located in the tundra. In these areas, our field observations confirmed denning activity (M.M. and H.D.C., unpublished data). Caribou also frequented the tundra during the spring/summer season. During fall/winter, wolves were more widely dispersed than in spring/summer and core areas for both species were centred in the taiga.

Static interaction analysis suggested association between wolves and caribou over grid cells covering these species' yearly range ($n = 165$). Visit frequencies of wolves and caribou for each cell were positively correlated (Spearman's rho 0.30, $P < 0.001$), which suggested correspondence in range use and high overlap (i.e. similar utilization). Furthermore, in six of the nine seasons for which interspecies spatial associations were analysed, there were significantly shorter distances between wolves and caribou compared with distances from a random sample of locations across seasons ($t > 4.24$, $P < 0.001$, Table 1). These results establish tundra grey wolves as migratory for the first time and show a coincident pattern of migration for both species. Wolves appeared to follow migrating caribou (see example in Fig. 3).

Population units based on autosomal microsatellite loci

Two wolf populations, consisting of 337 and 67 individuals, were inferred using the program GENELAND (Fig. 4; Fig. S1). The general location of the two wolf populations was consistent among the 100 runs we completed, and > 90% individuals were assigned to the same units in all runs. The northeastern population included 169 female and 170 male wolves sampled in the tundra and taiga, and the southwestern population consisted of 35 female and 32 male wolves in boreal coniferous forest areas. The boundary zone between these populations coincided with the southern

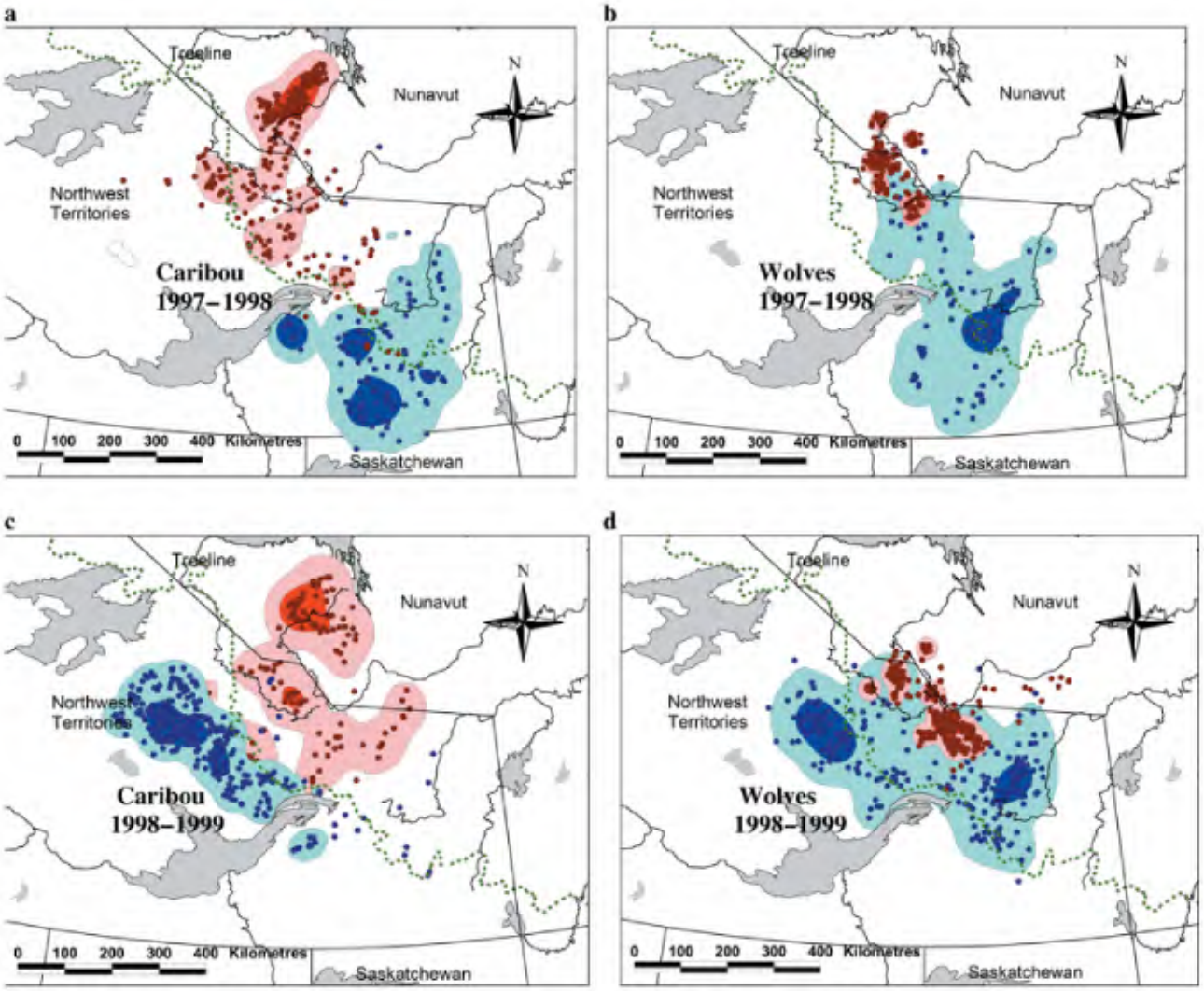

Fig. 2 Satellite-telemetry locations (dots) for a 2-year observation period for 19 wolves and 19 caribou (1997/1998, panels a and b; 1998/1999, panels c and d). Seasonal use areas indicated as 50% (dark colour) and 95% (light colour) kernel probability polygons in two seasons (spring/summer in red tones and fall/winter in blue tones, defined following Walton *et al.* 2001a, b).

Table 1 Distances in kilometres between tundra/taiga wolf and caribou locations within each season and within an equivalent random sample of locations from all seasons from summer 1997 to summer 1999. The 'all season' group of distances was employed to test differences with distances within seasons pairwise with Mann–Whitney *U*-test

	Summer 1997	Fall 1997	Winter 1997–1998	Spring 1998	Summer 1998	Fall 1998	Winter 1998–1999	Spring 1999	Summer 1999	All seasons random sample
Wolves–caribou distances (km)										
Mean	143	266	156	258	157	247	283	275	144	275
SD	59	168	88	99	80	105	181	104	64	135
P value	0.001	0.001	0.001	0.062	0.001	0.001	0.636	0.041	0.001	

limit reached by the caribou winter migration. The only exception was for two wolves (< 1%), which were assigned by GENELAND to the southwestern population despite their occurrence in the taiga environment (Fig. 4). These individuals were sampled less than 100 km from the taiga–boreal coniferous forest boundary and were males, and their coat colour was dark, consistent with a boreal coniferous forest origin (see below). Such 'outlier' animals may have

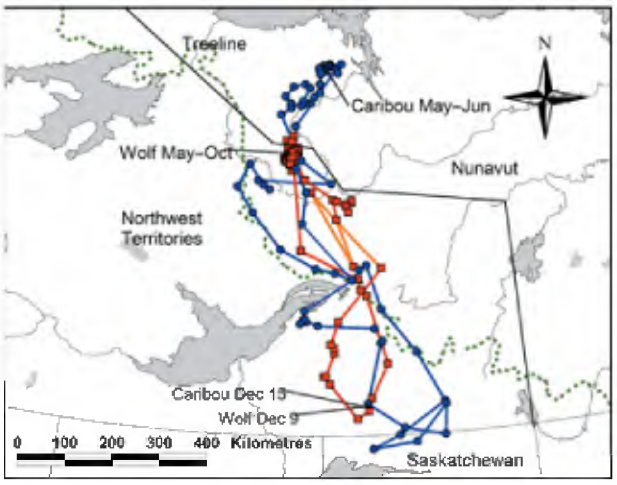

Fig. 3 Straight-line distances between consecutive locations of a collared caribou (blue circles connected by blue lines) and a collared wolf (red squares connected by red lines) during the period June 1997–June 1998. Caribou locations form a distinctive cluster during the months of May and June, corresponding to the calving ground, whereas wolf locations form a distinctive cluster throughout the period from May to October, around the den location. Locations of the caribou and wolves coincided in mid-December.

been migrants. Ninety and 92 percent of tundra/taiga and boreal coniferous forest wolves, respectively, were correctly assigned by assignment tests to their population of origin. These results strongly suggest two distinct populations are represented in our study area. The tundra/taiga sample includes principally migratory wolves. Migratory barren-ground caribou are the dominant prey there, and our telemetry results establish that these tundra wolves have adopted the migratory lifestyle of their prey. In contrast, the boreal coniferous forest population is territorial and sedentary, as is their prey (Young & Goldman 1944; Mech 1970; Hall 1981).

Among the 14 loci tested in the two inferred populations, three and two loci deviated significantly from Hardy–Weinberg equilibrium in tundra/taiga and boreal coniferous forest populations, respectively. No locus was found to deviate significantly in both populations. Of the 91 locus pairs tested, 10 and 4 pairs exhibited significant linkage disequilibrium in tundra/taiga and boreal coniferous forest populations, respectively. However, no locus pair was significantly associated in both populations. These estimates of Hardy–Weinberg and linkage disequilibrium were comparable to those in other studies of wild canids (Roy *et al.* 1994; Sacks *et al.* 2004). Given that deviations

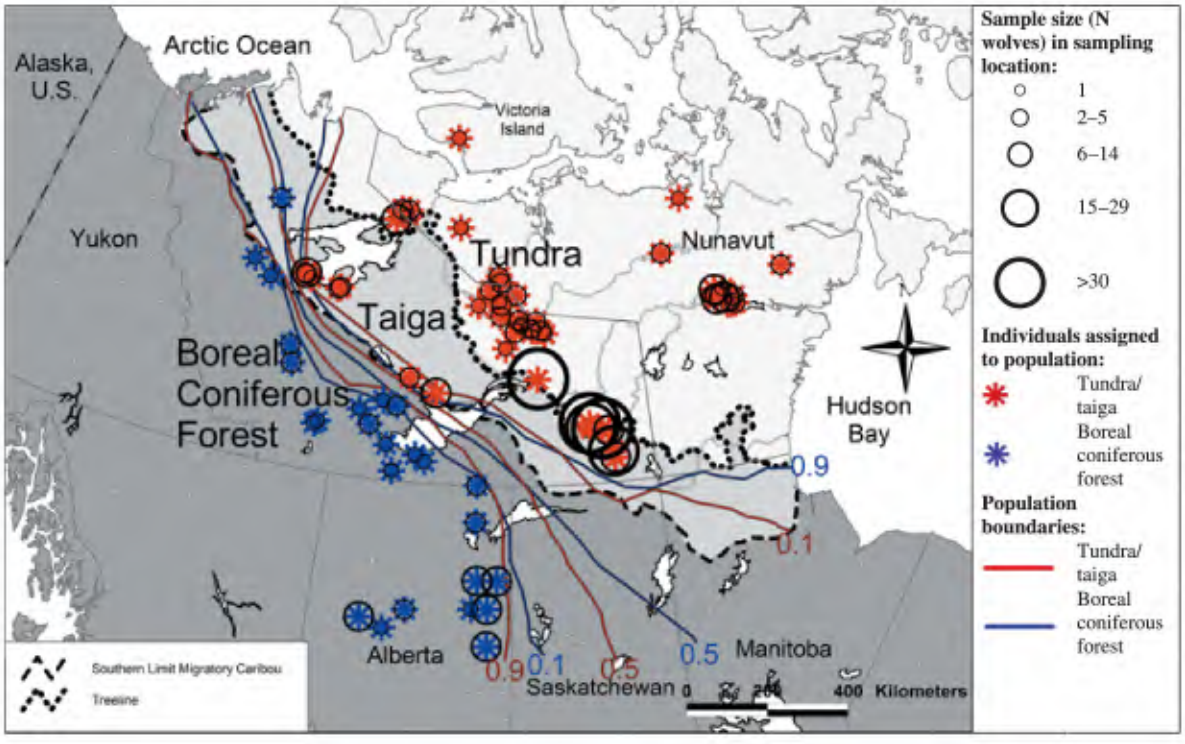

Fig. 4 GENELAND results based on data from 14 microsatellite loci and modal assignment of individuals inferred in 100 independent runs. Sampling locations for individuals assigned to the northeastern tundra/taiga population are marked with red asterisks and to a southwestern boreal coniferous forest population with blue asterisks. GENELAND also determined population boundaries with given probability levels ranging from 0.1 to 0.9 (red and blue lines for tundra/taiga and boreal coniferous forest populations, respectively).

from Hardy–Weinberg and linkage disequilibrium were inconsistent across populations, we utilized information from all 14 loci. Finally, we found no statistical support for occurrence of null alleles, large allele dropout or stuttering in the studied groups with the program MICRO-CHECKER. Alleles failing to amplify during PCR were estimated to be 5% and 4% in tundra/taiga and boreal coniferous forest populations, respectively.

Heterozygosity and allele diversity values were similar for the two populations of wolves ($H_E = 0.67 \pm 0.05$ and 0.68 ± 0.04; $H_O = 0.62 \pm 0.01$ and 0.62 ± 0.02; mean number of alleles = 7.93 ± 4.70 and 6.64 ± 3.73, for tundra/taiga and boreal coniferous forest wolves, respectively). We found significant genetic differentiation between tundra/taiga and boreal coniferous forest GENELAND grouping ($F_{ST} = 0.028$; $P < 0.0001$). Furthermore, there was a significant difference in the allele distribution of tundra/taiga and boreal coniferous forest individuals. The exact test of population differentiation showed that the two populations were heterogeneous for microsatellite frequencies ($P < 0.001$).

When analyses were conducted separately for wolves of different sex, genetic differentiation between tundra/taiga and boreal coniferous forest wolves was similar for females ($F_{ST} = 0.032$; $P < 0.0001$) and for males ($F_{ST} = 0.025$; $P < 0.0001$). Finally, we analysed the individual expected frequencies obtained with assignment tests (see above) and found no difference between female and male wolves, indicating no bias in dispersal. In addition, no difference was found between female and male wolves in the variance of corrected assignment indices further supporting lack of sex bias dispersal, as the variance is expected to be larger for the sex dispersing most.

Mitochondrial DNA differentiation

Population subdivision was evaluated based on mitochondrial DNA using spatial analysis of molecular variance (SAMOVA) at three levels: between groups within group-clusters (F_{SC}), between groups and clusters overall (F_{ST}), and among group-clusters (F_{CT}, Table 2). These analyses showed that genetic differentiation among group-clusters was significant ($0.301 < F_{CT} < 0.518$, $P < 0.01$; Table 2) for three, four, five and six clusters. However, genetic differentiation between groups within such clusters decreased only in the case of six clusters ($F_{SC} = -0.004$, $P < 0.001$), which indicated that homogeneity within clusters was achieved at this point (Dupanloup *et al.* 2002). With the organization in six population clusters, there was a tendency for the tundra/taiga wolf groups to form one cluster (BAA + BAB + BEA + BEB + BLA + BLB cluster; Table 2). In contrast, boreal coniferous forest groups clustered separately from tundra and taiga groups and also from each other (ABN, ABS, NTC, NTS groups). This result suggested greater genetic subdivision among boreal coniferous forest wolves. Finally, wolves sampled from the KAM group in the far northeastern tundra also clustered separately. These analyses provide only partial support for the tundra/taiga grouping suggested by microsatellite analysis and do not support a single grouping of boreal coniferous forest wolves.

For comparison to habitat groupings suggested by microsatellite data, we assessed the frequencies of mitochondrial DNA control region haplotypes in both tundra/taiga and boreal forest habitats. We found 16 mitochondrial haplotypes, 11 of which were located in the tundra/taiga and 10 in the boreal coniferous forest (Fig. S3, supplementary material). Haplotype distribution and frequency differed between the two habitats. For example, the frequency of haplotype lu32 was 71% (232 individuals) in wolves from the tundra/taiga, but only 22% (14 individuals) in wolves of the boreal coniferous forest. Similarly, haplotype lu39 was the second most frequent in the tundra/taiga wolves (17%, 56 individuals) but was not found in coniferous forest wolves. The binomial probability of missing a haplotype with this frequency in boreal coniferous forest wolves, given equal distributions in the two populations, is $P < 0.0001$. The exact test of population differentiation showed that the two populations were heterogeneous for frequencies of mitochondrial haplotypes ($P < 0.001$), as also found for autosomal microsatellites.

Levels of differentiation between the tundra/taiga and boreal coniferous forest wolves were an order of magnitude greater than those based on autosomal data ($F_{ST} = 0.282$; $P < 0.0001$). Similarly, although autosomal microsatellite diversity levels were comparable in different habitats, levels of mitochondrial gene diversity and nucleotide diversity were more than three times lower in tundra/taiga wolves than in their boreal coniferous forest conspecifics (0.153 ± 0.027 vs. 0.509 ± 0.079, and 0.0007 ± 0.001 vs. 0.002 ± 0.002, respectively). This difference in diversity reflects lower levels of sequence divergence among tundra/taiga haplotypes as well as a greater equitability in haplotype frequencies of boreal coniferous forest wolves. When analyses were conducted separately for wolves of different sexes, genetic differentiation between tundra/taiga and boreal coniferous forest wolves was approximately threefold higher for females ($F_{ST} = 0.353$; $P < 0.0001$) than males ($F_{ST} = 0.138$; $P < 0.0001$).

Differentiation in Y-chromosome haplotypes

We defined Y-chromosome haplotypes based on four microsatellite loci from 202 male wolves that were also typed for autosomal microsatellite and mitochondrial DNA markers. With procedures similar to those employed for mitochondrial DNA analyses, the distribution of genetic variation was evaluated for Y-chromosome haplotypes using SAMOVA (Table 2). These analyses, which tested different numbers of genetic clusters, showed that genetic differentiation

Table 2 Fixation indices corresponding to the clusters of groups inferred by SAMOVA algorithms for the 11 wolf populations typed for mitochondrial DNA and Y-chromosome polymorphism

Number of clusters	Cluster compositions	F_{SC}	F_{ST}	F_{CT}
Mitochondrial DNA				
Two clusters	1. ABN + ABS + BAA + BAB + BEA + BEB + BLA + BLB + KAM + NTC			
	2. NTS	0.105**	0.628**	0.585
Three clusters	1. ABN + ABS + BAA + BAB + BEA + BEB + BLA + BLB + KAM			
	2. NTC			
	3. NTS	0.095**	0.564**	0.518
Four clusters	1. ABS + BAA + BAB + BEA + BEB + BLA + BLB + KAM			
	2. ABN			
	4. NTC			
	3. NTS	0.097**	0.490**	0.435*
Five clusters	1. ABS + BAA + BEA + BEB + BLA + BLB + KAM			
	2. ABN			
	3. BAB			
	5. NTC			
	4. NTS	0.103**	0.420**	0.353*
Six clusters	1. BAA + BAB + BEA + BEB + BLA + BLB			
	2. ABN			
	3. ABS			
	4. KAM			
	6. NTC			
	5. NTS	–0.004**	0.298**	0.301*
Y-chromosome				
Two clusters	1. ABS + BAA + BAB + BEA + BEB + BLA + BLB + KAM + NTC + NTS			
	2. ABN	0.038**	0.178**	0.146
Three clusters	1. ABS + BAA + BEA + BEB + BLA + BLB + KAM + NTC + NTS			
	2. ABN			
	3. BAB	0.039**	0.138**	0.103
Four clusters	1. ABS + BAB + BLA + BLB + NTS			
	2. BAA + BEA + BEB + NTC			
	3. ABN			
	4. KAM	–0.015**	0.077**	0.090**
Five clusters	1. ABS + BLA + BLB			
	2. BAA + BEA + BEB + NTC			
	3. BAB + NTS			
	4. ABN			
	5. KAM	–0.018**	0.076**	0.093**
Six clusters	1. ABS + BAB + NTC + NTS			
	2. BAA + BEA + BEB			
	3. ABN			
	4. BLA			
	5. BLB			
	6. KAM	–0.022**	0.074**	0.094**

*$P < 0.01$; **$P < 0.001$.

among clusters was significant ($0.090 < F_{CT} < 0.094$, $P < 0.001$; Table 2) for four, five and six clusters. Genetic differentiation between groups within clusters decreased for four clusters ($F_{SC} = -0.015$, $P < 0.001$), which indicated that homogeneity within clusters was achieved at this point (Dupanloup *et al.* 2002). With the organization in four population clusters, there was a tendency for some spatially contiguous wolf groups encompassing the tundra, taiga and boreal coniferous forest to form one cluster (BAA + BEA + BEB + NTC cluster; Table 2). In contrast, other populations clustered together although they were not contiguous and were not sampled in the same habitats (ABS + BAB + BLA + BLB + NTS groups). Finally, wolves sampled for the KAM group in the far northeastern tundra and those sampled from the ABN group in the boreal coniferous forest of our study area also clustered separately. These results suggested less spatially or habitat-determined genetic structure in Y-chromosome markers.

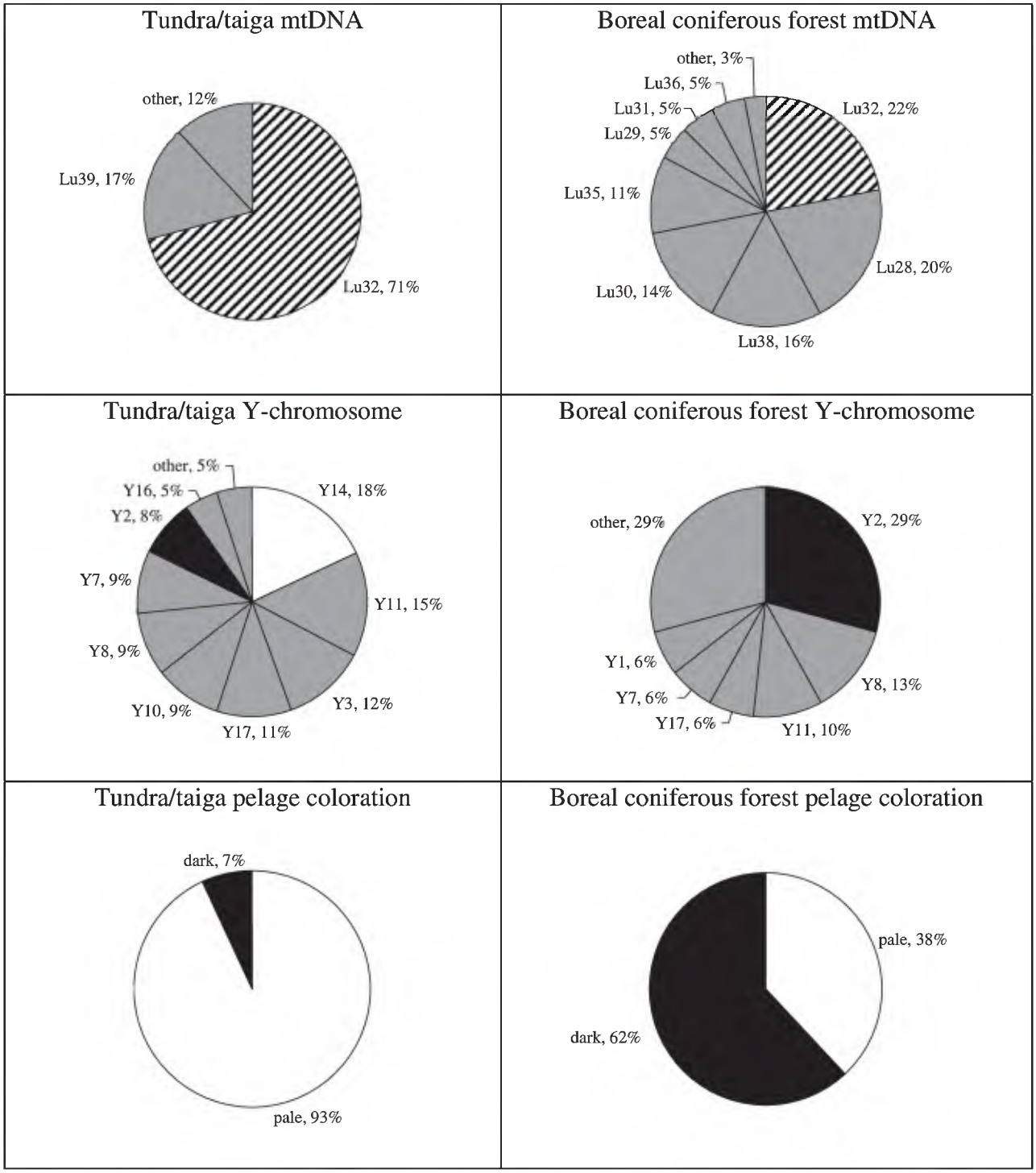

Fig. 5 Frequency of mitochondrial and Y-chromosome haplotypes (row 1 and 2, respectively) and pelage colouration (row 3) in tundra/taiga wolves (n = 337; left) and in boreal coniferous forest (n = 67; right). The most common colouration or Y-chromosome haplotype in the tundra/taiga and boreal coniferous forest are marked in white and black, respectively. The mitochondrial haplotype that was most common in both the tundra/taiga and boreal coniferous forest is displayed by diagonal lines.

As with mitochondrial data, we examined differentiation between tundra/taiga wolves ($n = 170$) and boreal coniferous forest wolves ($n = 32$) based on the frequencies of 19 Y-chromosome haplotypes for comparison to autosomal microsatellite markers. We found 13 Y-chromosome haplotypes in the tundra/taiga and 15 in the boreal coniferous forest (Fig. 5). Haplotype frequencies differed in the two habitats. For example, haplotype Y14, was the most frequent in tundra/taiga wolves and found in 31 individuals (18%), whereas it was found in only one boreal coniferous forest wolf (3%). The binomial probability of not finding by chance more Y14 haplotypes in boreal coniferous forest wolves if the two populations have equal distributions was $P < 0.0161$. The equitability of haplotype distribution was different in tundra/taiga and boreal coniferous forest wolves. In the latter, uncommon Y-chromosome haplotypes (those < 5% in occurrence) were present in 29% of the sample, whereas in tundra/taiga wolves, uncommon haplotypes were present only in 5% of the sample. However, Y-chromosome gene diversity values were similar in tundra/taiga wolves and in their boreal coniferous forest conspecifics (0.891 ± 0.692 and 0.897 ± 0.714, respectively).

The exact test of population differentiation showed that the two groupings were heterogeneous for frequencies of Y-chromosome haplotypes, with similar levels of statistical significance found for the other markers ($P < 0.001$). Differences between tundra/taiga and boreal coniferous forest wolves in Y-chromosome haplotype frequencies were also captured by *F*-statistics ($F_{ST} = 0.033$; $P < 0.0001$). If the haploid and paternal inheritance of Y-chromosome markers is taken into consideration, these values were lower than expected based upon a comparison with autosomal microsatellite loci. In fact, given equal contributions by sex, and an equal sex ratio as found in our study and elsewhere (Mech 1970; Appendix), Y-chromosome differentiation as represented by F_{ST} values should be four times larger than autosomal loci (Petit *et al.* 2002; Prugnolle & de Meeus 2002), rather than similar in magnitude as found in our analysis. These analyses do not account for the bias associated with differing levels of gene diversity between markers (Hedrick 1999). However, male-specific markers likely experience higher rates of gene flow when compared to biparental and female-inherited markers.

Association with distance and ecology

Analysis of 14 autosomal microsatellite loci typed in 404 wolves from 11 predefined groups suggested genetic differentiation between populations in different habitats (tundra/taiga vs. boreal coniferous forest). This conclusion is supported by neighbour-joining trees based on Nei's (1978) genetic distances as well as multiple-dimensional scaling analysis based on F_{ST} genetic distances (Fig. 6a, b). Furthermore, spatial autocorrelation analysis conducted on individual wolves found little evidence of differentiation with distance (Fig. S3, Supplementary material). Correlation values were not significantly different from zero for wolves sampled from all localities, or for wolves sampled in the tundra/taiga or in the boreal coniferous forest population separately. Consequently, geographical distance does not appear to strongly influence population or individual level differentiation calculated with autosomal microsatellite data.

Distance-based redundancy analysis conducted on autosomal microsatellite data and environmental variables seemed to contradict this result. In fact, when pairwise F_{ST} genetic distances among the 11 groups were compared to Euclidean distances using DISTLM's marginal test, a significant relationship between genetic differentiation and distance between localities was found ($P = 0.003$; 65% of variation explained; Table 3). Marginal tests were also significant for comparisons of genetic differentiation vs. habitat ($P = 0.001$; 67% of variation explained) or prey ($P = 0.004$; 67% of variation explained). However, when longitude was taken into account as a covariate in the multiple regression analysis (DISTLM's conditional test), latitudinal distance was correlated with F_{ST} distance ($P = 0.006$; 49% of variation explained), whereas when latitude was a covariate, longitudinal distance was not correlated with F_{ST} distance. These results suggest that differences in latitude are largely responsible for the geographical distance effect which in turn, is associated with the transition from boreal coniferous forest to taiga–tundra environments and from resident to migratory prey. Distance-based redundancy analysis conducted using Nei's distances were similar; however, prey type explained a larger proportion of genetic differentiation even when latitudinal and longitudinal distances were covariates ($P = 0.001$; 22% of remaining variation explained).

Distance-based redundancy analysis conducted on mitochondrial DNA data also indicated association with habitat and prey type and less association with distance. Marginal tests were significant for habitat ($P = 0.027$; 60% of variation explained; Table 3) or prey ($P = 0.004$; 80% of variation explained). Such relationships were significant also when both latitudinal and longitudinal distances were covariates ($P = 0.025$; 25% $P = 0.001$; 57% of remaining variation explained by habitat and prey, respectively). By contrast, no significant relationships were found when genetic distances based on Y-chromosome data were used (Table 3). For all tests, no difference in results was found in using log-transformed or untransformed distances and consequently, only results for untransformed distances are presented. In conclusion, with the exception of Y-chromosome data, these results support our characterization of tundra/taiga wolves and boreal coniferous forest wolves as separate populations, with a boundary coincident to the southern limit of the caribou winter migration (Fig. 4).

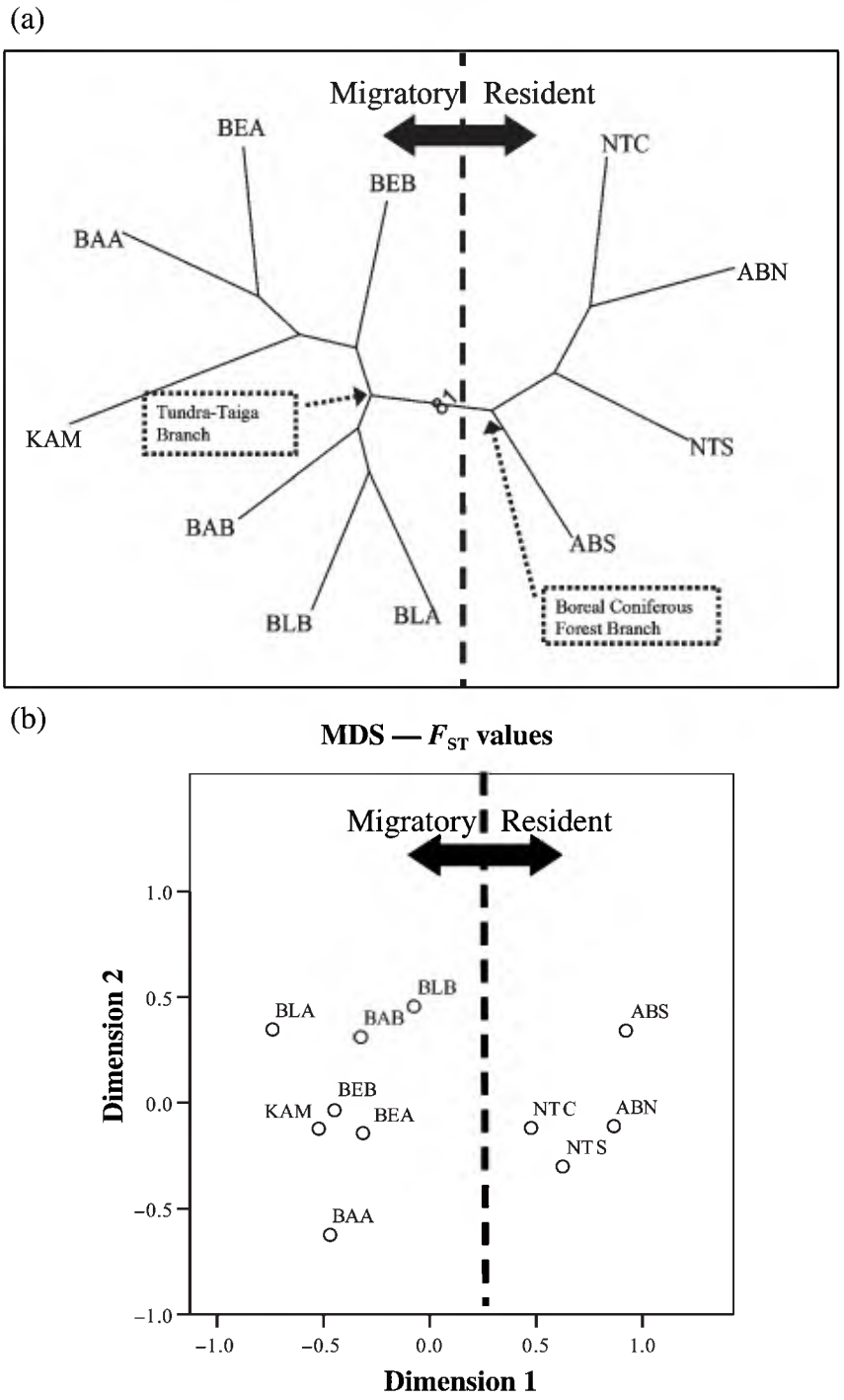

Fig. 6 Neighbour-joining tree (a) and MDS plot (b) based on Nei 's (1978) unbiased genetic distance between the 11 predefined wolf populations for microsatellite loci. Bootstrap support in 1000 replicates is indicated on the branches if >50%. Normalized raw stress for MDS was 0.050. Tundra and northern Canadian Shield taiga (taiga) samples were from migratory wolves and boreal coniferous forest samples were from resident wolves.

Differentiation in coat colour

We examined 337 and 67 pelts from tundra/taiga and boreal coniferous forest wolves, respectively. When the fur grading and pelt guide from Obbard (1987) was used to standardize descriptions of colour morphs, wolves fell discretely into light (white to near white) and dark (grey through black) categories. Other minor coat colour variations, resulting from various mixtures of black, grey, brown, red and white hairs, were also observed. These colour categories and pelage variants were similar to those described for wolf populations elsewhere (McBride 1980; Mech 1988; Gipson *et al.* 2002). We found a distinct difference in colour frequency between the two inferred populations (Fig. 5). Tundra and taiga wolves were paler in colour than wolves of the neighbouring boreal coniferous forest as 93%

Table 3 Tests for the relationships of F_{ST} and Nei's genetic distance among wolf groups at different sites (obtained using autosomal microsatellites, mitochondrial DNA or Y-chromosome data) with individual sets of predictor variables, using the dbRDA multivariate *F*-statistic. On the left are the marginal tests of individual sets, on the right are the partial (conditional) tests, where the variables of latitude or longitude or both have been included as covariates in each analysis. *P* values less than 0.05 are highlighted in bold. The column headed 'var' indicates the percentage of the multivariate genetic variation explained by the particular predictor variables. Under conditional tests, the effects of latitude and longitude as covariates with habitat, prey and each other were explicitly assessed for microsatellite data because geographical distance was found to be significant in the marginal tests

Marginal tests				Conditional tests			
Variable set	*F*	*P*	var	Variable set	*F*	*P*	var
Autosomal microsatellites							
F_{ST}							
Euclidean distance	7.299	**0.003**	65%	Lat and long covariates			
Habitat	18.388	**0.001**	67%	Habitat	4.570	0.083	14%
Prey movements	18.092	**0.004**	67%	Prey movements	4.555	0.063	14%
				Latitudinal distance	10.995	**0.006**	49%
				Longitudinal distance	5.100	0.057	23%
Nei's Distance							
Euclidean distance	11.734	**0.002**	75%	Lat and long covariates			
Habitat	17.718	**0.001**	66%	Habitat	3.391	0.084	8%
Prey movements	20.549	**0.003**	70%	Prey movements	45.592	**0.001**	22%
				Latitudinal distance	20.415	**0.006**	65%
				Longitudinal distance	4.433	0.071	14%
Mitochondrial DNA (F_{ST})							
Euclidean distance	4.055	0.125	50%	Lat and long covariates			
Habitat	13.359	**0.027**	60%	Habitat	7.018	**0.025**	25%
Prey movements	49.442	**0.004**	85%	Prey movements	53.130	**0.001**	57%
Y-Chromosome haplotypes (F_{ST})							
Euclidean distance	0.888	0.505	18%	Lat and long covariates			
Habitat	0.470	0.682	5%	Habitat	0.016	0.972	0%
Prey movements	1.056	0.585	11%	Prey movements	1.728	0.244	16%

of wolves from tundra/taiga areas exhibited light pelage colouration whereas only 38% of boreal coniferous forest wolves did (Yates' $\chi^2 = 64.52$, $P < 0.0001$). Frequency distribution analysis of colour morphs was therefore consistent with a significant subdivision corresponding to tundra/taiga and boreal coniferous forest wolves.

Discussion

We documented a distinct genetic partition between grey wolf populations coinciding with the ecological boundary between boreal coniferous forest and tundra/taiga habitats and spanning over a thousand kilometers in length in northern North America. This conclusion is supported by GENELAND analysis of autosomal microsatellite data with low rates of misassignment (< 10%) and is consistent with the analysis of mitochondrial data. The subgroups defined by mitochondrial DNA analysis indicate a further partition within the boreal coniferous forest populations, but only at $K = 6$ does a single tundra population (KAM) appear as a distinct cluster from the tundra/taiga group. The boreal coniferous forest subdivisions may represent additional habitat associations not well sampled by this study (Carmichael *et al.* in press), but these are not supported by the Y-chromosome analysis (Table 2). However, sample sizes are smaller for the Y-chromosome haplotypes analysis as only males are used. Consequently, there may be low statistical power for uncovering population structure. Finally, coat colour differences were substantial between wolves in the two environments, with light colours predominating in taiga and tundra habitats.

Limited evidence of isolation by distance suggests that ecology rather than spatial separation is more important in restricting gene flow for wolves in the large expanse of northern North America. Barren-ground caribou are the most important prey for tundra wolves (Kuyt 1972; Parker 1973; Heard & Williams 1992; Walton *et al.* 2001a), and boreal coniferous forest and tundra/taiga populations differ primarily with respect to their migratory behaviour and that of their prey. Consequently, our results show that significant genetic differentiation results from prey-based specialization in the absence of topological barriers to dispersal. This result implies that ecological specialization, such as changes in coat colour and hunting behaviour, can occur even in parapatric populations of highly mobile vertebrates (see below).

The migratory system of wolves and barren-ground caribou

We show for the first time that tundra grey wolves migrate with barren-ground caribou and maintain close proximity throughout their migration. Our findings suggest that tundra/taiga wolves are associated with these caribou. Our spatial resolution did not allow testing for location–habitat association at a fine scale. However, our quantitative findings are consistent with the wolf-barren ground caribou attraction premise that was hypothesized based on observations of tundra wolf predation on caribou (Kelsall 1968; Kuyt 1972; Parker 1973; Walton *et al.* 2001a). Our identification of a migratory population of grey wolves and caribou in the taiga–tundra is analogous to the coincident pattern of migration of ungulates and spotted hyenas in East Africa (Hofer & East 1995; Trinkel *et al.* 2004). However, in contrast, grey wolves south of the treeline, which live in boreal coniferous forest environments, are generally territorial and relatively sedentary, and specialize on resident prey such as moose, elk, deer and non-migratory woodland caribou (Young & Goldman 1944; Mech 1970). The annual spring migration of tundra/taiga wolves and barren-ground caribou exceeds 1000 km and results in grey wolves giving birth in tundra summering areas, which are far from their winter range in the taiga. Large postcalving aggregations of caribou typically start returning towards the treeline in July and August (Urquhart 1981; Gunn *et al.* 2002) at a time when wolf pups can move only near the den (Mech 1970). The simultaneous migration of wolves with caribou during fall, when the whole pack can move together, through the spring of the following year has been previously inferred (Kelsall 1968; Heard & Williams 1992; Walton *et al.* 2001a), and is shown directly for the first time in our telemetry study (Figs 2 and 3). Consequently, young wolves spend a critical period of their development migrating with and learning to prey on migratory barren-ground caribou (Pruitt 1959; Calef 1981). This distinctive developmental history suggests a unique, ecologically specialized form of wolf may be evolving in the Canadian tundra.

Mating in tundra/taiga wolves occurs in February and March when they have returned to the taiga and potentially can mingle with boreal coniferous forest wolves. Nonmigratory wolves must defend and maintain fixed territorial boundaries as a prerequisite to successful production and rearing of young (see Mech 1970). Foreign wolves that enter a territory would normally be repulsed or killed (Mech 1977; Peterson & Page 1988). In our taiga sample, the abundance of wolves that were genetically similar to tundra wolves may reflect interbreeding between resident and migratory tundra wolves or, alternatively, that a surge in predation pressure by immigrant tundra wolves depresses resident taiga prey density such that only a small resident population can be sustained year round.

Prey-mediated differentiation of tundra/taiga and boreal coniferous forest wolves

We found that ecological factors such as prey and habitat are the dominant variables explaining genetic variation among populations. Distance-based redundancy analysis conducted on autosomal microsatellite and mitochondrial DNA data showed a significant association between genetic differentiation and variation in habitat type (tundra/taiga or boreal coniferous forest) and in prey type (migratory caribou or resident prey). For autosomal microsatellite data, an association with latitude but not longitude was also detected (Table 3) which reflects the south to north habitat transition from boreal coniferous forest to taiga–tundra environments. These results support our characterization of tundra/taiga wolves and boreal coniferous forest wolves as separate populations, with a boundary coincident with the southward extent of caribou migration (Fig. 4). However, no associations were detected among environmental variables and genetic distances based on Y-chromosome haplotype data possibly because of high rates of gene flow for male-specific markers (see below).

The potential importance of migratory behaviour and ecological factors were also supported by previous genetic studies. For example, Carmichael *et al.* (2001) assessed variation in nine microsatellite loci in populations east and west of the Mackenzie River and on the high Arctic Islands in North America. They found that the Mackenzie River was a significant cause of differentiation as were marine water barriers separating Banks and Victoria Islands from the mainland. They hypothesized that the genetic-isolating effects of rivers and islands are not primarily due to the presence of water, because rivers freeze and sea ice forms several months each year. Rather, they suggested that wolves follow different migratory caribou herds east and west of the Mackenzie River. Similarly, insular differentiation of wolves is due to the presence of resident island caribou herds. Consequently, Carmichael *et al.* suggested movements of prey restrict dispersal of grey wolves. The importance of prey and ecological factors for wolf dispersal was also suggested by the analysis of Geffen *et al.* (2004) who found that environmental factors, such as temperature and climate, explained more than twice the genetic variation in mtDNA and microsatellite loci than geographical distance did. These authors suggested that the natal environment of North American wolves predetermined where adults would disperse. For example, individuals reared in a forested environment with high elk density will disperse potentially long distances to find similar prey and habitat. Finally, Pilot *et al.* (2006) found that genetic differentiation in European wolves was correlated with climate, habitat types, and diet composition and also suggested that natal-habitat biased dispersal was the underlying mechanism

linking population ecology with genetic structure. Apparently, in large canids, ecotypic divergence may be the primary mode of differentiation (Carmichael *et al.* 2001; Musiani 2003; Geffen *et al.* 2004; Sacks *et al.* 2004, 2005; Pilot *et al.* 2006; Carmichael *et al.* in press) presenting an alternative to topographically induced population structure (Avise 2000).

The potential effect of the developmental environment on food preferences is well established in many mammals including humans (reviewed by Birch 1999), and hunting skills may be habitat dependent (Partridge & Green 1985; Magurran 1986; Smith & Skúlason 1996), although such skills could also be learned in carnivores (Estes *et al.* 2003). Experimental and empirical studies on a diverse array of animals support natal habitat-based differentiation (Stamps 2001; Davis & Stamps 2004), and some observations support natal habitat-based dispersal, differentiation and fragmentation also in coyotes, a close relative of the grey wolf (Sacks *et al.* 2005). Whereas random dispersal between different types of habitat will produce gene flow that limits local adaptation (Lenormand 2002), the combination of reduced gene flow and local adaptation facilitated by natal habitat-based dispersal might lead eventually to speciation (Sorenson *et al.* 2003). The results of our study support a critical role for foraging ecology (a factor potentially linked to natal habitat-based dispersal) in explaining genetic and phenotypic patterns in North American wolves that may be similar to patterns in raptors, hyenas and killer whales where resident and migratory prey populations are found (Hofer & East 1993; Hoelzel 1994, 1998; Lank *et al.* 2003).

Habitat adaptation and specialization on migratory barren-ground caribou or on nonmigratory prey in our study area likely occurred relatively recently, beginning in the Holocene. Wolves and their primary prey re-occupied previously glaciated areas in northern Canada, which were snow-covered for most of the year, beginning about 13 000 years ago (Kurtén & Anderson 1980; Guthrie 1990). Northern climates fluctuate and so northern species typically experience great fluctuations in numbers (Post & Stenseth 1999; Weladji *et al.* 2002) and 10-fold population size reductions have been observed in caribou (see Kelsall 1968; Klein 1991; Caughley & Gunn 1993; Morneau & Payette 2000). Consequently, the lower mtDNA diversity values in tundra–taiga wolves may be due to past decreases in numbers linked to harsh climate, prey (i.e. caribou) shortages, or hunting by local people (Musiani & Paquet 2004). Because of the haploid and nonrecombinant nature of mitochondrial DNA inheritance, mtDNA is expected to be more sensitive to population size changes. These characteristics are common to Y-chromosome markers as well, but we find no significant difference between levels of variation of tundra/taiga and boreal coniferous forest wolves. This difference between haploid marker systems may reflect a higher rate of gene flow in Y-chromosome markers, which would tend to restore diversity, although these conclusions should be taken with caution since homoplasy could be large for these markers (Sundqvist *et al.* 2006).

Sex bias in variation and dispersal

Mitochondrial DNA variation was lower in the tundra/taiga population relative to the boreal coniferous forest, whereas levels of variation of biparentally inherited microsatellite loci and Y-chromosome markers were comparable. Similarly, the level of differentiation between tundra/taiga and boreal coniferous forest populations, based on mitochondrial DNA sequence variation ($F_{ST} = 0.28$) was much higher than that revealed by analysis of nuclear microsatellite loci ($F_{ST} \approx 0.03$ for autosomal microsatellite loci and Y-chromosome haplotypes). Given equal reproduction of the sexes, mitochondrial and Y-chromosome diversity and divergence should be about four times larger than that of autosomal loci (Petit *et al.* 2002; Prugnolle & de Meeus 2002). These considerations do not account for homoplasy and higher polymorphism in autosomal microsatellites or for differences in error associated with F_{ST} estimates from different markers, and assume values are due to equilibrium gene flow only. However, the differentiation in mitochondrial DNA is larger than expected whereas the differentiation in Y-chromosome microsatellite loci is less than expected.

In wolves, a single-mated pair reproduces within a pack (with exceptions, Murie 1944; Haber 1977; Harrington *et al.* 1982; Mech *et al.* 1998) and sex ratios approach one (Mech 1970; this study's sex ratio, Appendix). Consequently, sex-biased gene flow could contribute to the disparity in variation and differentiation between mitochondrial and nuclear markers (e.g. Lehman *et al.* 1991), where females from the boreal coniferous forest population rarely mate and den with migratory males from the tundra and vice-versa. Such a bias in gene flow would reduce mitochondrial DNA variation in the tundra/taiga population and lead to greater differentiation by drift.

We suggest that the behavioural mechanism underlying this asymmetry could involve the constraint of natal habitat-based dispersal in wolves. In wolves, mating occurs during the winter when both migratory and nonmigratory wolves may be in close proximity in the taiga. Here, interpopulation matings may occur; however, matings between nonmigratory males and migratory females would not involve a habitat shift for either partner as nonmigratory males could return to their natal pack or habitat after an extrapack mating. Similarly, migratory females could return to their natal habitat in the tundra with a fetus having only a nuclear DNA contribution from the boreal coniferous forest male. In contrast, in order to transfer boreal coniferous forest mtDNA haplotypes to tundra/

taiga populations, breeding nonmigratory females would have to disperse from their natal pack and accompany migratory males to the tundra, a habitat distinct from their natal environment. Therefore, the natal habitat-based dispersal hypothesis predicts boreal coniferous forest females would rarely abandon their natal forest habitat to accompany migratory males to the tundra to den, and vice-versa. Consequently, the observed bias in variability and gene flow of mtDNA is consistent with a strong natal habitat association of adults. Our telemetry results supported this hypothesis as all the satellite-collared wolves returned to their tundra denning locations and no dispersal events were detected to different habitat types.

In general, even if interpopulation matings occur in the taiga, they only would result in successful gene flow if the mated pair established a new pack or an impregnated female returned to her natal pack to give birth. The former would result in a habitat shift for one member of the mated pair and be dependent on the availability of vacant territories. The latter would likely result in a second litter within the female's natal pack and be less likely to survive (Cluff *et al.* 2003). Therefore, these alternatives may contribute to gene flow between the two populations only to a minor extent.

Conservation implications

The migratory wolves of the tundra/taiga represent a unique ecotype adapted to existence in the Arctic and for predation on barren-ground caribou. They are genetically distinct in autosomal, Y-chromosome and mitochondrial DNA markers, are much lighter coloured than their boreal coniferous forest counterparts and live in a unique habitat. Consequently, since they are genetically and ecologically distinct, they would be considered an evolutionary significant unit (ESU) under synthetic versions of the concept (Crandall *et al.* 2000; Fraser & Bernatchez 2001; Delaney & Wayne 2005) and should be a priority in the conservation of North American wolves. They are the only grey wolf population known to undertake long-distance migration. A potential qualifying concern is how rapidly such behavioural and phenotypic differences can evolve and how dependent evolution is on standing variation (Long *et al.* 2000; Barton & Keightley 2002). For example, coat colour is clearly a segregating trait in most North American wolf populations (Young & Goldman 1944; McBride 1980; Mech 1988; Gipson *et al.* 2002). However, light colour is likely recessive (T. Anderson, unpublished data), and therefore even with strong selection, hundreds of generations may be needed to restore the light allele to high frequency (Hartl & Clark 1989). The tundra migratory system has likely taken thousands of years to evolve and consequently, tundra wolves may not be readily replaced should they go extinct.

A second concern is the effect global warming may have on the extent and continuity of tundra habitats. Global warming will likely reduce the available habitat for migration and may even cause an end to migratory systems because of climatic fluctuations and demographic crises for northern ungulates (Post & Stenseth 1999; Weladji *et al.* 2002). Similarly, Mech (2004) showed how climate change might be affecting wolves in the high Arctic. Minimally, tundra habitat will be lost and become increasingly fragmented (Kittel *et al.* 2000; Hansen *et al.* 2001; Theurillat & Guisan 2001). As a result, population size for tundra migratory wolves may decrease and isolation of some population segments may increase. In other areas, as migratory systems are lost, tundra wolves must become nonmigratory and interact genetically more with resident wolves or go extinct. Consequently, unique adaptations for tundra life may be lost. These potential scenarios need more careful modelling to identify populations under the greatest threats. Additionally, behavioural and ecological flexibility of wolves needs to be better assessed to determine how populations will respond to climate changes. For example, how well can migratory wolves succeed as resident territorial wolves should migratory prey be lost?

Conclusions

This study shows that tundra/taiga and boreal coniferous forest wolves are genetically, phenotypically and behaviourally distinct ecotypes and demonstrate the potential importance of ecological factors in explaining differentiation in highly mobile species. Our results parallel those on killer whales (*Orcinus orca*), where resident and migratory populations are reproductively isolated and feed on different prey (Hoelzel 1994, 1998). Selection for light colouration and specialization on migratory prey in snow-covered habitats likely provided the important prerequisites for divergence of tundra/taiga wolves and explains the sex bias in variation and genetic divergence. Finally, conservation of the tundra/taiga phenotypes should be a priority in future wolf-management plans, especially given the likely effects of global warming.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Histogram of simulated values representing the number of populations in the Markov chain Monte Carlo (MCMC) run with variable number of populations. A clear mode at npop=2 suggests that this value is the best estimate of the number of populations present in the data set.

Figure S2. Network for mitochondrial DNA haplotypes observed in wolves. The network was estimated under the 95% statistical limits of parsimony using the algorithm in Clement *et al.* (2000). Bigger circles represent haplotypes that were found in our sample with circle-size proportional to haplotype occurrence. Haplotype relative frequency in tundra/taiga wolves is shown in white and in boreal forest wolves in black. Smaller squares represent hypothetical haplotypes.

Figure S3. Correlograms showing the combined spatial correlation *r* as a function of distance (in class sizes of 100 km), 95% CI about the null hypothesis of a random distribution of wolves, and 95% confidence error bars about *r* as determined by bootstrapping. Wolf samples from all localities are included in the upper panel, whereas the central and lower panels include wolves sampled in the tundra/taiga and coniferous forest population, respectively.

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Appendix

Number of individuals sampled, central latitude (Lat) and longitude (Long), dominant habitat and prey in the diet, percent of dark individuals and of females in each of 11 predefined Northern Canadian wolf populations

Group	*N*	Central Lat.	Long.	Habitat*	Dominant prey	Dark/ Total	Females/ Total
Kaminuriak (KAM)	49	64.770	–96.839	Tundra	Kaminuriak migratory caribou	2%	45%
Bluenose-a (BLA)	15	67.147	–117.481	Tundra	Bluenose migratory caribou	33%	47%
Bathurst-a (BAA)	33	64.680	–109.885	Tundra	Bathurst migratory caribou	0%	55%
Beverly a (BEA)	60	62.890	–108.460	Tundra	Beverly migratory caribou	8%	52%
Beverly b (BEB)	150	61.334	–104.962	Taiga	Beverly migratory caribou (winter)	3%	47%
Bluenose-b (BLB)	20	65.328	–123.158	Taiga	Bluenose migratory caribou (winter)	10%	55%
Bathurst-b (BAB)	9	62.545	–114.802	Taiga	Bathurst migratory caribou (winter)	33%	78%
Northwest Territories-central (NTC)	8	63.686	–124.242	Boreal coniferous forest	Resident ungulates†	33%	50%
Northwest Territories-south (NTS)	14	61.311	–117.499	Boreal coniferous forest	Resident ungulates†	64%	50%
Alberta-north (ABN)	8	59.571	–111.883	Boreal coniferous forest	Resident ungulates†	57%	25%
Alberta-south (ABS)	38	56.539	–112.572	Boreal coniferous forest	Resident ungulates†	53%	66%

*Tundra, Northern Canadian Shield Taiga (Taiga) or boreal coniferous forest (coniferous forest); †Deer (*Odocoileus* spp.), elk (*Cervus elaphus*), moose (*Alces alces*) and woodland caribou (*Rangifer tarandus caribou*).