"But how does evolution get passed on? Where does order come from? If you start with a lot of exploding firmament, *how* do you end up with butterflies? Were butterflies built in from the start? How? What bit of burning hydrogen carried the plans for people?"

Terry Pratchett, Ian Stewart, and Jack Cohen
The Science of Discworld III: Darwin's Watch

"'Well, I say it is the place of science only to observe,' he said. 'To seek cause where it may be found, but to realize that there are many things in the world for which no cause shall be found; not because it does not exist, but because we know too little to find it. It is not the place of science to insist on explanation, but only to observe, in hopes that the explanation will manifest itself.'"

Diana Gabaldon
Voyager

"Every so often, you have to unlearn what you thought you already knew and replace it by something more subtle"

Terry Pratchett, Ian Stewart, and Jack Cohen
The Science of Discworld

"A university is very much like a coral reef. It provides calm waters and food particles for delicate yet marvelously constructed organisms that could not possibly survive in the pounding surf of reality, where people ask questions like, 'Is what you do of any use?'"

Terry Pratchett, Ian Stewart, and Jack Cohen
The Science of Discworld

"Science is just working things out. And paying attention. And hoping there's someone around to dry you off."

Terry Pratchett, Ian Stewart, and Jack Cohen
The Science of Discworld II: The Globe

"The time to begin writing an article is when you have finished it to your satisfaction. By that time you begin to clearly and logically perceive what it is that you really want to say."

Mark Twain
Mark Twain's Notebook, 1902-1903

"Science is riddled with stories. In fact, if you can't tell a convincing story about your research, nobody will let you publish it. And even if they did, nobody else would understand it."

Terry Pratchett, Ian Stewart, and Jack Cohen
The Science of Discworld II: The Globe

University of Alberta

Ecological Genetics of Northern Wolves and Arctic Foxes

by

Lindsey Erin Carmichael ©

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ABSTRACT

Grey wolves (*Canis lupus*) and arctic foxes (*Alopex lagopus*) are the only canid species found throughout the mainland tundra and arctic islands of North America, but while they possess similar adaptations to life in northern ecosystems, their population genetics contrast in several ways. Arctic foxes were widely distributed throughout the Pleistocene, and current North American fox populations are connected by frequent long-distance movements. These migrations occur in response to periodic declines in local prey density, and result in high levels of gene flow between arctic foxes in different areas. In fact, though some fine-scale genetic structure exists within the Svalbard population, and despite the significant spatial separation of Svalbard from North America, most Svalbard foxes belong to the same genetic cluster as all North American arctic fox populations. Arctic foxes also display uniformly high levels of genetic variation that are greater than those observed in wolves. Like arctic foxes, wolves are characterized by high mobility, but are genetically structured according to habitat type – boreal forest, barren ground or arctic island. Among mainland populations, this isolation may be a function of natal habitat-biased dispersal. Non-random wolf movement may be cued by differences in vegetation cover (forest or tundra) or in the type and behavior of prey species encountered in different areas. At finer scales, behavior of prey also appears to influence: the extent of genetic differentiation among wolves within a habitat type; the amount of genetic

exchange between island and mainland wolves in the Western Arctic; and the substructuring of wolves within island populations (likely resulting from prey specialization on ungulate populations with divergent spatial ranges). Density and distribution of prey for both wolves and arctic foxes may be dramatically altered by continued warming of the arctic climate; these changes are expected to influence demographics of northern canids, and – by altering their movement patterns – gene flow among populations within each carnivore species. Island populations of wolves and foxes may also experience greater genetic isolation, accelerated loss of variation, and increased risk of inbreeding as migratory corridors over arctic sea ice are gradually lost.

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A journey of a thousand miles may begin with a single step, but uncounted more must follow before journey's end. Nor is the first step the hardest to take; the 101st, the 1001st, and the 10,001st are immeasurably more difficult. To all those who have seen me through this journey, who believed with unshakable certainty that I could do it, when I was unshakably convinced I couldn't... thank you. Thank you for seeing me safe to journey's end. And that you for understanding that sometimes you get the best light from a burning bridge.

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Chapter 1

General Introduction

Origins of Arctic Canids

Members of the family *Canidae* are found on all continents except Antarctica, thriving in habitats both unmodified and highly disturbed (Sillero-Zubiri & MacDonald 2004; Wandeler *et al.* 2003). Canids are at home in ecosystems as divergent as deserts and rain forests, but only two species are distributed throughout the arctic islands and tundra regions of North America. These are the grey wolf (*Canis lupus*) and the arctic fox (*Alopex lagopus*).

The antecedents of wolves (likely *Canis etruscus*) and arctic foxes (likely *Vulpes alopecoides*) evolved in the New World before invading Siberia via the Bering Land Bridge (Kurtén & Anderson 1980). Modern wolves and arctic foxes then arose in the Old World before recolonizing North America. Adaptation of these species to northern environments thus began with their earliest evolution, during repeated exposures to the Beringian Filter (Kurtén & Anderson 1980). Despite similar early origins, however, the later Pleistocene history of wolves and arctic foxes was quite different.

Morphological studies of modern wolf skulls indicate highest divergence at the periphery of the species' North American range; for example, in the Mexican wolf (*C. l. baileyi*) and the arctic island wolf (*C. l. arctos*). Such patterns have been termed "centrifugal evolution" and are thought to result from arrival of Old World colonists in waves, each subsequently isolated in a different glacial refugium (Nowak 2003). As many as five refugia have been proposed for wolves, three south of the Pleistocene ice sheets, one in Pearyland (Northern Greenland), and one in Beringia; current recognition of five wolf subspecies is based on this hypothesis, with original subspecies boundaries indicating the limits of post-glacial expansion for each previously isolated population (Brewster & Fritts 1995; Nowak 1995). However, recent surveys of mitochondrial DNA sequence diversity found little correspondence between haplotype distribution and geography (Vilà *et al.* 1999), and the contemporary northern wolf population possesses only a subset of the variation found in southern historical samples (Leonard *et al.* 2005). Current hypotheses may therefore be inconsistent with the true evolutionary history of this species.

Unlike wolves, arctic foxes were not restricted to isolated refugia during the Pleistocene glacial maxima. Instead, fossil (Kurtén & Anderson 1980), morphological (Bisaillon & Deroth 1980; Frafjord 1993) and molecular phylogeographical studies (Dalén *et al.* 2005) suggest this species expanded its range during cooling cycles, retreating to a compact but continuous polar distribution during warm interglacials. Gradual range contraction is consistent with the species' present occurrence: continuous throughout circumpolar tundra regions, with remnant populations on various islands in the polar oceans (Goltsman *et al.* 2005; Kurtén & Anderson 1980). The Canid Specialist Group of the International Union for the Conservation of Nature and Natural Resources (IUCN) recognizes four subspecies of arctic fox globally, with *A. l. lagopus* occupying most northern circumpolar regions and the remaining subspecies restricted to single small ice-free islands in the Bering Sea (Angerbjörn *et al.* 2004a).

Species and Life History Characteristics

The Grey Wolf

Grey wolves are the largest of the *Canidae*, with males weighing up to 62 kg and females slightly smaller (Mech & Boitani 2004); they are distinguished from red wolves (*Canis rufus*) by size, skull morphology, and distribution. Prior to European colonization of North America, grey wolves ranged from Mexico to the Canadian Arctic Archipelago – with red wolves found in the southeastern United States (USA) – but are currently restricted to Alaska, Canada, and a few small populations in the conterminous USA. Pelage colours in grey wolves are extremely variable and widely distributed; however, the frequency of pale wolves increases with increasing latitude and almost all individuals on the arctic islands (*C. l. arctos*) are white. This suggests pale colouration may be an adaptation for hunting in open tundra environments.

Wolves are opportunistic carnivores, and their diet is diverse and variable (e.g., Hayes *et al.* 2000; Kohira & Rexstad 1997; Kuyt 1972; Larter *et al.* 1994). However, stable wolf populations cannot be maintained in the absence of large ungulates (Mech 2005), and spatial and temporal variation in abundance of ungulate prey may have direct impacts on wolf social structure, breeding patterns, dispersal distances and territoriality.

Wolves are social carnivores, forming packs as large as 42 individuals that center around a dominant breeding pair (Mech & Boitani 2003). Subordinate females occasionally breed, but additional adult family members most frequently act as non-breeding helpers, protecting and provisioning the offspring of the dominant pair (Mech & Boitani 2003). These helpers may be mature relatives of the breeders, their offspring from previous years, or unrelated (Lehman *et al.* 1992; Mech & Boitani 2003). Regardless of composition, the size of a wolf pack is determined by the amount of prey available; competition increases within the pack during food shortages, and may be a primary impetus behind wolf dispersal (Mech & Boitani 2003).

Wolf home range sizes also vary with availability of prey, and wolves generally defend larger territories when ungulate density declines (Mech & Boitani 2003). In North American tundra regions, where migratory barren ground caribou (*Rangifer tarandus groenlandicus*) are the dominant prey species, wolves are territorial only during the breeding season. Adults make long distance foraging movements while denning (Frame *et al.* 2004), and once pups are old enough to travel with the pack, wolves abandon their home ranges to follow caribou herds over distances which may total thousands of kilometers, and dispersal may occur during these journeys (Walton *et al.* 2001). Even in regions with non-migratory prey, young wolves may disperse farther than 800 km (Fritts 1983), although shorter dispersals are more common when suitable vacant territories are available near natal ranges (Mech & Boitani 2003).

The Arctic Fox

Like wolves, arctic foxes display sexual size dimorphism, with males weighing up to 5 kg and females as much as 4 kg. They are the only canid species with fur covering their foot pads (*lagopus* meaning "hare foot") and the only canid to undergo a seasonal colour molt (Prestrud 1991). Summer fur is generally a grayish-brown, with winter fur either white or blue. The white morph is predominant throughout most of the species' range, but the frequency of blue foxes increases in coastal regions and on islands (Angerbjörn *et al.* 2004a). Since coastal and inland foxes also tend to use different food resources, this difference has historically prompted recognition of two ecotypes: "coastal" and "lemming" (Braestrup 1941).

Where lemmings (*Lemmus* and *Dicrostonyx* spp.) are present, they compose up to 90% of the arctic fox's diet (Macpherson 1969). In coastal regions, and in islands like Svalbard (Norway) that do not contain small mammals, foxes also eat nesting birds and their eggs, and will scavenge from both marine and terrestrial carrion (Eide *et al.* 2005; Roth 2002). When resources are abundant, foxes hunt in excess and cache prey for future use, and will also raid each other's caches when opportunity presents (Samelius & Alisauskas 2000). During periodic crashes of North American lemming populations, when caches are depleted and live prey unavailable, arctic foxes undertake vast foraging movements in search of alternative food and may wander hundreds or thousands of miles from their breeding ranges onto the sea ice or into the boreal forest (Wrigley & Hatch 1976). These seasonal migrations mean that North American arctic foxes, like tundra wolves, are territorial primarily during breeding season, with pair bonds perhaps dissolving in winter; reproductive foxes in Svalbard, which utilize more stable resources, may share and defend their home ranges year round (Prestrud 1992a).

Arctic foxes do not typically form social groups containing more than two adults (Angerbjörn *et al.* 2004a; Eide *et al.* 2004). Where additional adults occur, they may be littermates of the dominant pair or offspring from a previous season, but are less likely than wolves to care for or provision young (Strand *et al.* 2000; but see Goltsman *et al.* 2005 for exceptions). It is not clear whether additional adult foxes "help" by contributing to territorial defense, but they may increase success of the breeders indirectly by caching food within the shared home range (Eide *et al.* 2004). Indeed, supernumerary adults appear to be best tolerated when food resources are abundant, and may produce secondary litters under these circumstances (Goltsman *et al.* 2005). In addition to the potential for plural breeding, arctic foxes display a second reproductive adaptation to vagaries in their food supply: "lemming" foxes, with periodic access to superabundant food resources, have larger litters (6.3 ± 3.3, max 19) in all years than "coastal" foxes (4.2 ± 1.5, max 10), whose food resources are more predictable but less rich (Angerbjörn *et al.* 2004b).

Recent History and Current Status of Arctic Canids

The first wildlife legislation in North America was enacted in 1630 and offered a one-cent bounty for the killing of wolves (Kellert *et al.* 1996); their current restricted range is testament to the efficacy of this historical persecution. In Alaska and the Canadian North, wolf control (through poisoning or shooting) is also a contemporary phenomenon, enacted to reduce both predation pressure on ungulates used by humans and the loss of trapped arctic foxes to wolves (Boertje *et al.* 1996; Hayes & Harestad 2000; McEwen 1955; Usher 1965). While non-lethal methods of wolf control are in development (Boertje *et al.* 1995; Spence *et al.* 1999), wolves in Alaska and the Canadian Territories are also currently harvested for both private and commercial use. However, as this harvest represents a relatively small proportion of the estimated population, legal harvesting may not threaten northern wolf populations as a whole (Mech & Boitani 2004; Van Zyll de Jong & Carbyn 1999).

An external influence more likely to jeopardize wolf persistence, particularly in the Canadian Arctic Archipelago, is climate change. Severe winter weather producing deep or hard snow has been implicated in mortality of Peary caribou (*R. t. pearyi*) and muskoxen (*Ovibos moschatus*; Gunn *et al.* 1991; Larter & Nagy 2001a; Larter & Nagy 2001b). Since arctic foxes, arctic hares (*Lepus arcticus*), and small rodents are the only other mammalian prey in the Archipelago, islands without ungulates will not support wolf populations (Mech 2005). *C. l. arctos* may therefore be at increased risk of extinction, but its extent and current status are not well known (Miller 1995).

Since arctic foxes are naturally restricted to remote and largely inaccessible northern areas, human impact on the species throughout most of their range has been much more recent. Trapping for fur became common in the early 1900s, but current harvests are likely less than 20% of the total North American fox population, and harvests on Svalbard less than 1%. Given the species' high fecundity, these rates are likely sustainable over the long term (Geffen *et al.* 1996). However, climate change is also expected to have a negative impact on arctic foxes. Svalbard reindeer (*R. t. platyrhynchus*) are as susceptible to winter mortality as their North American cousins, and the effect of reindeer density declines on arctic foxes, who hunt reindeer calves and scavenge from carcasses (Prestrud 1992b), may mimic that of lemming population crashes (Fuglei *et al.* 2003). In North America, a greater threat to arctic fox persistence is the northward expansion of the red fox (*Vulpes vulpes*), which limits reproductive success of this species via both resource competition and direct aggression (Bailey 1992; Dalén *et al.* 2004; Tannerfeldt *et al.* 2002).

Thesis Objectives

From a genetic perspective, persistence of populations depends upon two factors: maintenance of adequate genetic variation and avoidance of inbreeding depression. Evolution cannot occur without pre-existing genetic variation, and genetically depauperate populations may therefore be unable to adapt to changing environmental conditions. Island populations, which are usually of smaller size, lose genetic variation quickly due to elevated rates of genetic drift (Frankham 2005). Drift may be countered by gene flow between populations, which may both increase genetic variation and reduce inbreeding (Vilà *et al.* 2003); however, island populations, by their very physical nature, are expected to experience less gene flow than contiguous mainland ones, and thus may also face higher risk of inbreeding depression.

The genetic variation and isolation of arctic island canids, which are capable of traversing annual sea ice, has not been previously examined. However, given the potentially dramatic effects of climatic change on arctic ecosystems, and the inherent demographic stochasticity arctic island canid populations may already face, genetic threats to their persistence are of particular concern. A major goal of this thesis is thus to determine the genetic nature and status of arctic island canid populations. In Chapter 3, microsatellite DNA fingerprinting is used to explore these issues in both wolves and arctic foxes. Chapters 2 and 4 examine island wolves in more detail, while fine-scale genetic structure of arctic foxes within a single island is examined in Chapter 5.

North American wolves have been the focus of numerous genetic studies undertaken at a range of scales and employing a variety of molecular markers (recently reviewed by Wayne & Vilà 2003); genetic analyses of arctic foxes are considerably less common, and with the exception of a single circumpolar phylogeographic investigation (Dalén *et al.* 2005), have never been conducted for either the contiguous North American or physically isolated Svalbard arctic fox populations. Furthermore, most population-level studies previously conducted have been devoted to historical, topographical or geological influences on genetic structuring of canids, while only recently have authors begun to consider the influences of habitat and prey on canid population genetics (Geffen *et al.* 2004; Sacks *et al.* 2004; Sacks *et al.* 2005). Since "organisms mostly form their own environments, and nearly all of the important context for organisms is other organisms," (p. 217, Pratchett *et al.* 2005) and since predator-prey relationships appear to influence social structure, reproduction, and movement of wolves and arctic foxes, it can thus be reasonably assumed that variation in type, distribution and abundance of prey may also impact the genetic structure of these predators. Therefore, the second major goal of this work is to identify the influence of ecology on the genetics of arctic canid species.

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Chapter Two

Prey Specialization May Influence Patterns of Gene Flow in Wolves of the Canadian Northwest[1]

Introduction

The grey wolf (*Canis lupus*) has one of the greatest natural ranges of any living mammal (Nowak 1983). Wolves are successful in a broad array of habitats with differing climates, topographies, and prey spectrums (Banfield 1974). The wolves of Northwestern Canada (British Columbia, Yukon and Northwest Territories, Fig. 2-1) typify this adaptability. Here, "wolf country" means everything from highland forest to barren-ground tundra (Seip 1992; Yukon Conservation Society 1995; Bergerud & Elliot 1998; Nagy & Larter 2000). It includes the Rocky Mountains, the Mackenzie Delta, and the islands of the Arctic Archipelago.

Topography is not the only inconstant in this region. Variation in the type and density of available prey is significant (Banfield 1974; Seip 1992; Yukon Conservation Society 1995; Bergerud & Elliot 1998; Nagy & Larter 2000). Indeed, this variation has necessitated the development of two distinct behavioral patterns in resident wolves. Throughout British Columbia and most of the Yukon, where moose are the dominant prey species, wolves are strictly territorial (Seip 1992; Yukon Conservation Society 1995). In contrast, wolves in barren-ground caribou ranges (Fig. 2-1a) are migratory; they associate with and follow the seasonal movements of the herds (Kuyt 1972; Heard & Williams 1992; Yukon Conservation Society 1995; Ballard *et al.* 1997; Walton *et al.* 2001).

Wolves, whether territorial or migratory, are highly mobile. Barren-ground wolf packs traverse thousands of kilometers every year (Kuyt 1972; Heard & Williams 1992; Walton *et al.* 2001), whereas individual wolves have been known to travel in excess of 800 km (Fritts 1983; Forbes & Boyd 1996). Furthermore, dispersal frequency is estimated to be near 25% (Lehman *et al.* 1992; Forbes & Boyd 1997). In a species with such high vagility, extensive gene flow might be expected to reduce genetic differentiation between even distant populations. Although geographical distance may still be of influence, we might expect other factors to have a greater impact on the genetic structure of wolf populations. One of these possibilities, topographic barriers to gene flow, has been investigated for smaller canids (Mercure *et al.* 1993), but such studies have not previously been conducted in wolves.

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Northwestern Canadian wolf habitat contains several candidate barriers to gene flow; we examined the Mackenzie River, and channels of the Arctic Ocean, as potential determinants of wolf population genetic structure. Our primary objective was to ascertain whether these topographic features act as barriers to wolf gene flow, and how their effect compares with that of linear distance. Second, we sought to establish whether patterns of gene flow observed among barren-ground wolves could be explained by the migration patterns of barren-ground caribou. Because high levels of gene flow are expected for wolves, we needed a molecular marker capable of detecting even small genetic differences between regions. The hypervariability of microsatellite loci (Tautz 1989; Weber & May, 1989) provided the required sensitivity.

Materials and Methods

Sampling Region

We sampled wolves from nine areas in Northwestern Canada (Fig. 2-1). Throughout the text, "Prince of Wales Strait" refers to the water separating Banks Island and Victoria Island, "Amundsen Gulf" to all water separating the islands from the mainland, and "Arctic Ocean" (or "ocean") to all of the above.

Sample Collection and Organization

Samples were contributed by biologists in Kluane National Park (Yukon Territory, n = 37), Ft. St. John (British Columbia, n = 41), and the Northwest and Yukon Territories (NT/YT, n = 413). Tissue samples were taken from hunted wolves throughout the NT/YT; Kluane National Park and Ft. St. John samples consisted of blood drawn from live-captured wolves.

Because sampling density of NT/YT wolves was highly irregular, it seemed most appropriate to partition individuals via breaks in the sampling distribution (Fig. 2-1b). NT/YT wolves were divided into seven geographic regions: Northern Richardson Mountains (Northern Richardsons); Southern Richardson Mountains (Southern Richardsons); Tuktoyaktuk/Inuvik (Tuk/Inuvik); Great Bear Lake; Paulatuk; Banks Island; Victoria Island (Fig. 2-1b). Location data were not available for wolves from Ft. St. John and Kluane National Park. However, because neither area is contiguous with any other, they were considered discrete geographic regions. Sample sizes are provided in Fig. 2-1b and Table 2-1.

Sample groupings used in this study are not meant to reflect the actual distribution of wolves, nor to imply the existence of discrete wolf populations in these areas. They were derived from our sampling distribution to allow comparison of animals in different geographic regions. However, most analysis methods employed (below) assume the existence of discrete populations in Hardy-Weinberg Equilibrium.

Laboratory Methods

DNA extraction was performed with DNeasy Tissue kits (QIAGEN) according to the manufacturer's instructions. Each wolf was genotyped using nine microsatellite loci isolated from domestic dog genomic libraries (Ostrander et al. 1993, 1995; Mellersh et al. 1997). One primer for each locus was labeled with 6-FAM (CXX140, CXX204, CXX250), TET (CXX377, CXX173) or HEX (CXX110, CXX251, CXX2079, CXX2328) (Applied Biosystems). CXX110, CXX140, CXX173, CXX250, CXX251 and locus CXX377 are dinucleotide repeats, while CXX2079 and CXX2328 are tetranucleotide repeats; however, single-base pair alleles were observed for several loci. Polymerase Chain Reaction (PCR) mixes and cycling conditions are available upon request.

Fragments were separated on 4.5% polyacrylamide sequencing gels under denaturing conditions, using the ABI 373 or ABI 377. ABI Prism Genescan Analysis (GENESCAN 2.02), and GENOTYPER 2.0 were used to assign genotypes. Readers interested in raw genotype data should contact the corresponding author.

Statistical Analysis

Disequilibrium, heterogeneity, and genetic diversity

Loci were tested for conformation to Hardy-Weinberg Equilibrium (HWE) using the Markov chain method (Guo & Thompson 1992) implemented in GENEPOP Version 3.1 (Raymond & Rousset 1995). GENEPOP was also used to screen for genotypic disequilibrium between each pair of loci. *G*-tests for heterogeneity (Sokal & Rohlf 1997) were performed to test for significant differences in allele frequencies between each pair of geographical regions. The Dunn-Sidák method (Sokal & Rohlf 1997) was applied to each set of tests to obtain corrected error rates of 0.05.

Allelic diversity (mean number of alleles across all nine loci) and unbiased expected heterozygosity (H_E, Nei & Roychoudhury 1974) were calculated for each region. We used Wilcoxon's signed ranks test (Sokal & Rohlf 1997) to screen for significant differences in H_E among regions (Kyle *et al.* 2001).

Genetic differentiation between regions

Nei's standard genetic distance (D_S, Nei 1972) and Wier & Cockerham's F_{ST} (1984) perform better than distance measures based on the stepwise mutation model, especially when imperfect microsatellites are included and low levels of differentiation expected (Takezaki & Nei 1996; Paetkau *et al.* 1997; Forbes & Hogg 1999). Pairwise F_{ST} was derived using GENEPOP Version 3.1, pairwise D_S

using programs available at http://www.biology.ualberta.ca/jbrzusto/GeneDist.php. We used PHYLIP Version 3.572 (Felsenstein 1995) to construct population gene trees based on both D_S and F_{ST}. Neighbor-joining (Saitou & Nei 1987) and Fitch-Margoliash (1967) tree topologies were compared. Bootstrap analysis (D_S, 1000 replications, neighbor-joining algorithm, majority-rule consensus) was performed to obtain a conservative estimate of support for the observed topology (Felsenstein 1985, Hillis & Bull 1993).

The assignment test (Paetkau *et al.* 1995; Rannala & Mountain 1997, Waser & Strobeck 1998) is a discriminant analysis technique that assigns each individual to the population or region in which its genotype is most likely to occur. The number of cross-assignments between two regions provides an indication of the relative genetic differentiation between them, and can be used to estimate relative migration rates (Waser & Strobeck 1998). All nine regions were simultaneously assigned using the program available at http://www.biology.ualberta.ca/jbrzusto/Doh.php, and the method of Titterington *et al.* (1981), which adjusts allele frequencies in every population to avoid zeros. This approach is independent of sample size, and replaces the $1/2N$ method discussed in Waser & Strobeck (1998).

Randomization tests were performed, using 1000 replicates, where new individuals were drawn from each region (each new individual was constructed based on the assumption of HWE and observed allele frequencies in that region, and each replicate sample included the same number of individuals as the actual sample). The null hypothesis under these conditions is that each population (or region) is distinct and in HWE. Results indicate the number of random replicates in which cross-assignment from population A to population B is equal to or greater than that observed in the real data. Significant results (less than 5% of the replicates) indicate more cross-assignments in the observed data than expected due to chance. Significance therefore suggests that some of these cross-assignments reflect true migration, rather than the absence (or low levels) of genetic differentiation between regions.

The Mantel test

The Mantel Test (1967) tests correlations between matrices of distance values and/or discrete, two-state characters. It was used to assess isolation-by-distance and the correlation between the presence of a physical barrier and increased genetic distance between regions.

For each region in the NT/YT, we calculated the average sampling location of all wolves included in that region. For Ft. St. John and Kluane National Park, where harvest site data were not available, we used the locations of the town site and the community of Kluane (eastern border of the Park), respectively. These coordinates (Fig. 2-1b) were entered into DISTGEO Version 3.01e (R PACKAGE version 3.0, Alain Vaudor) to produce a matrix of geographic distance between region pairs.

We constructed three additional matrices to test the correlation between each physical feature and increased genetic distance between wolves in different regions. Scoring was based on the assumption that the presence of a barrier increases genetic distance: a “one” was assigned to a pair of regions if the physical feature lay between them; if the feature did not separate the regions, they were given a “zero.” Matrices for tests involving the Mackenzie River did not include island populations: it was unclear how the islands should be scored. We tested two hypotheses with respect to the Arctic Ocean. First, ones were assigned to all regions separated by ocean, including Banks and Victoria Island (“ocean” tests in Table 2-4). Ones were then assigned to island-mainland pairs only (“Amundsen Gulf” tests in Table 2-4).

We used the R PACKAGE (Mantel Version 3.0) to test correlations between geographical distance, the Mackenzie River, the Arctic Ocean, and D_S between regions (1000 permutations of one matrix, Mantel 1967, normalized as in Sokal & Rohlf 1997). We also performed partial Mantel tests (Smouse *et al.* 1986), assuming that the impact of the barrier on genetic distance would be greater than that of geographic distance (Table 2-4). Parallel tests were conducted using F_{ST} as the genetic distance matrix.

Results

Tests for Disequilibrium and Heterogeneity

The following locus-region pairs deviated from HWE: CXX110, Kluane National Park; CXX204, Banks Island; CXX2328, Kluane National Park and Tuk/Inuvik. Null alleles were assumed absent as no locus deviated from HWE averaged over all regions. Furthermore, no pair of loci displayed genotypic disequilibrium across regions, suggesting all markers to be independent. All nine loci were retained for analysis.

Allele frequencies among geographical regions were compared using *G*-tests. All region pairs were significantly heterogeneous, allowing us to treat each region as a distinct entity. Since high gene flow occurs between some regions (below), the observation of significant heterogeneity might be attributed to local pack structure and relatedness. However, genotypic disequilibrium, a possible consequence of related individuals in a sample, was not observed in any region. Furthermore, no region violated HWE for all loci, suggesting the absence of significant intra-regional substructure (Wahlund 1928; Paetkau *et al.* 1997; Paetkau *et al.* 1998).

Genetic Diversity

Tuk/Inuvik and Victoria Island had the highest and lowest allelic diversities (and sample sizes), respectively (Table 2-1). Average allelic diversity of mainland populations was 6.21, of island populations 4.66. H_E ranged from 59.86% (Banks Island) to 74.42% (Great Bear Lake). Average H_E of mainland wolves was ≈ 8% higher than the average for the islands. Furthermore, H_E of the Banks Island population was significantly lower than that of Ft. St. John, Tuk/Inuvik, and Great Bear Lake ($P = 0.02$), the Northern Richardsons ($P = 0.05$), Kluane National Park and Paulatuk ($P = 0.10$). H_E of wolves on Victoria Island was not significantly lower than that of mainland wolves.

Genetic Differentiation and Gene Flow Between Regions

D_S ranged from 0.038 (Tuk/Inuvik - Paulatuk) to 0.554 (Banks Island - Ft. St. John), and F_{ST} from 0.009 (Tuk/Inuvik - Paulatuk) to 0.188 (Banks Island - Kluane National Park, Table 2-2). Previous studies have reported D_S values for wolves between 0.093 and 0.672 (Roy *et al.* 1994; Forbes & Boyd 1997), for wolverines, between 0.03-0.36 (Kyle & Strobeck 2001), and for arctic brown bears, between 0.13-0.54 (Paetkau *et al.* 1998, excluding Kodiak Island bears). F_{ST} was not calculated for brown bears (Paetkau *et al.* 1998), but ranged from 0.0017 to 0.2157 in wolverines (Kyle & Strobeck 2001) and averaged 0.168 for wolves (Roy *et al.* 1994).

All population trees constructed from these distances (and from D_{LR}, Paetkau *et al.* 1997) had the same topology as the neighbor-joining tree based on D_S (Fig. 2-2). Banks and Victoria Island paired in 99% of the bootstrap replicates. D_S and F_{ST} were highest between island and mainland regions (Table 2-2), suggesting genetic isolation of island from mainland wolves. Gene flow appeared to be more common across the Prince of Wales Straight, as Banks and Victoria Island wolves were genetically more similar to each other than to any mainland region (Table 2-2). The genetic isolation of the Banks Island population may be more pronounced; Victoria Island wolves appeared to be slightly closer to those in mainland regions (Table 2-2). However, these differences were small, and should be interpreted with caution.

D_S and F_{ST} results also implied structuring among mainland wolf populations. We observed a division across the Mackenzie River: wolves on the same side of the river were genetically closer than wolves on opposite sides of the river (Table 2-2). Furthermore, wolves on Banks and Victoria Island were genetically closer to mainland wolves east of the Mackenzie River than those west of the river. This east-west division occurred in 72% of the bootstrap replicates (Fig. 2-2).

Assignment test results provided additional support for trends observed with D_S and F_{ST}. Cross-assignment was more frequent between Banks and Victoria Island than between either island and the mainland (Table 2-3). Cross-assignment was also more common between regions on the same side of the Mackenzie River than regions separated by the River. For example, the Northern Richardsons are approximately equidistant from the Southern Richardsons and Tuk/Inuvik, but are separated from Tuk/Inuvik by the Mackenzie River (Fig. 2-1b). Frequency of cross-assignment between wolves in the Northern and Southern Richardsons was 21%, but only 4% between the Northern Richardsons and Tuk/Inuvik. Genetic differentiation also appears to increase as geographical distance between regions increases: the rate of cross-assignment between the Northern Richardsons and Ft. St. John (2.3%) was one-tenth that between the Northern and Southern Richardsons. Randomization suggested some of the cross-assignments observed are attributable to direct migration, as well as to the level of genetic differentiation between regions (Table 2-3).

Genetic differentiation between island and mainland wolves was generally quite high (Fig. 2-3). However, migration has occurred between the islands and the mainland (Figure 2-3, Table 2-3). Wolves TU9291 and IN8906, both sampled in the Tuk/Inuvik region, have genotypes $\approx 10^5$ times more likely to occur in an island population (1.10×10^{-12} vs. 2.93×10^{-7} for wolf TU9291 and 8.79×10^{-13} vs. 1.58×10^{-7} for wolf IN8906). Randomization tests confirm that these cross-assignments, unlike additional island-mainland cross-assignments shown in Table 2-3, would not be expected due to chance alone. Wolves TU9291 and IN8906 may therefore be actual migrants from Banks Island to the mainland.

Tests for Topographical Barriers to Gene Flow

Increases in genetic distance due to the presence of the Mackenzie River or Amundsen Gulf are clearly visible in Fig. 2-4. In contrast, Banks Island-Victoria Island (the star in Fig. 2-4) placed among regions separated by geographic distance alone, again suggesting that the Prince of Wales Straight is not a barrier to gene flow. We used the Mantel test to determine the statistical significance of these relationships.

Two sets of tests were performed, the first using D_S as the genetic distance, the second F_{ST}. Results were similar, with one exception. There was a significant positive correlation between geographical distance and D_S, but the correlation between geographical distance and F_{ST} was not significant at the 5% level (Table 2-4). A study of isolation-by-distance in bighorn sheep (Forbes & Hogg 1999) also reported stronger correlations for D_S than for F_{ST}.

The presence of the Mackenzie River between regions was associated with significantly increased genetic distance in both sets of tests (Table 2-4). A stronger correlation was observed between the ocean and genetic distance, the strongest between the Amundsen Gulf (island-mainland comparisons only) and genetic distance. These results confirmed that the Amundsen Gulf is a stronger barrier to gene flow than both the Mackenzie River and the Prince of Wales Straight.

Results suggested that physical barriers have a greater impact on genetic distance between regions than the geographic distance between them does; we used partial Mantel tests to examine this hypothesis (Table 2-4). The correlation between genetic distance and the Mackenzie River increased substantially when geographical distance was included, but remained lower than Mantel correlations for the ocean and Amundsen Gulf. Addition of geographical distance to ocean tests also increased the observed correlations, but to a lesser extent (Table 2-4).

We have used binary matrices in Mantel tests to assess relationships between topographic features and increased genetic distance; where appropriate, this technique could also be applied to anthropogenic barriers such as highways and high-density human populations.

Within and Among Island Populations

Local residents report two wolf phenotypes on Banks Island. Southern wolves range from white to pale grey, all with a reddish cast, while northern wolves lack this reddish tinge (J. Lucas Sr., pers. comm.). Because our Banks Island samples could be divided according to "northern" or "southern" harvest locations (North and South Banks respectively, division at 73°N, Fig. 2-1b), we were able to screen for substructure within the population. Because previous analysis suggested that island wolves were genetically far more similar to each other than to mainland wolves (Table 2-2, Table 2-3), we included the Victoria Island samples for comparison.

G-tests indicated significant differences in allele frequencies among the three sample sets. Closer examination of allele distributions on Banks Island revealed three alleles specific to North Banks, and seven specific to the south. Most of these alleles were quite rare; however, allele 144 of locus cxx.140, absent in northern wolves, had a frequency of 7% in the southern samples. Most were also observed in mainland regions and on Victoria Island (including allele 144, with a frequency of 11% on Victoria Island). Measures of genetic diversity for each region are shown in Table 2-1.

D_S /F_{ST} values between regions were as follows: North to South Banks Island, 0.020/0.005; South Banks to Victoria Island, 0.096/0.026; North Banks to Victoria Island, 0.117/0.040. Assignment test results were similar: cross-assignment was most common between North and South Banks, and least common between North Banks and Victoria Island (data not shown). Furthermore, cross-assignment between South Banks Island and Victoria Island was significantly greater than expected due to chance ($P = 0.02$ in randomization tests). The increased genetic similarity between Victoria Island and South Banks Island (compared to Victoria and North Banks Island) may support migration of wolves to South Banks Island from Victoria Island. However, observed differences in D_S and F_{ST} were small, and should be interpreted with caution.

Discussion

Mainland Wolves

Geographic distance between populations

Under the stepping-stone model of populations (Kimura 1953), probability of identity by descent decreases exponentially as the number of steps between populations is increased (Kimura & Weiss 1964). Therefore, the negative log of this probability will increase linearly with increasing geographic distance, and thus D_S is also expected to increase linearly with distance (Slatkin 1985; Fig. 2-4, Table 2-4). In contrast, F_{ST} is constrained by an upper bound, and cannot increase linearly with distance. F_{ST} may be approximately linear for some range of geographic distances, and some log transformation of F_{ST} may be linear for all distances, however, the behavior of this measure requires further investigation. Similarly, the behavior of D_{LR} under the stepping-stone model has yet to be examined. However, like D_S, D_{LR} is a logarithmic distance, and in this study, was strongly correlated to D_S ($r = 0.944$, $P = 0.001$, see also Paetkau *et al.* 1997). We might therefore predict D_{LR} to be linear with geographic distance as well. In contrast, the proportion of cross-assignments between two populations will not be linear with distance: as migration increases, cross-assignment will asymptote to 50%. However, unpublished simulations (P. Waser & C. Strobeck) suggest that cross-assignment may be approximately linear when migration rates are small.

Our results are consistent with the prediction of the stepping stone model; isolation-by-distance is one major determinant of population structure among mainland wolves in Northwestern Canada (Table 2-2, Fig. 2-4, Table 2-4). Previous microsatellite studies of isolation-by-distance in wolves are summarized by Forbes & Hogg (1999). Our "distance-only" comparisons (Fig. 2-4) correspond closely to their continent-wide wolf curve (Fig. 3c in Forbes & Hogg 1999) for distances > 600km. The drop below this curve for physically proximate populations (Fig. 2-4) may be due to a higher proportion of related individuals (thus lower genetic distance) in neighboring regions than in distant ones. Inclusion of these proximate populations may also have enhanced our ability to detect isolation-by-distance in highly vagile wolves. For example, Roy *et al.* (1994) did not find isolation-by-distance when wolves from widely-separated localities were examined, however, when data from closely situated populations was also considered, isolation-by-distance was significant (Forbes & Boyd 1997).

The Mackenzie River

Like geographical distance, the presence of the Mackenzie River was strongly correlated with increased genetic distance between regions (Table 2-4, Fig. 2-4). Gene flow among regions flanking the river was more common in the north-south, than in the east-west direction (Table 2-2, Fig. 2-2, Table 2-3). However, because the River is frozen 6-8 months of the year, it should not of itself pose a significant barrier to wolf movement. North-south migration of resident caribou herds (Fig. 2-1a) may be a greater determinant of the structure we observe because wolf movements are more likely to be driven by the movements of their prey. However, wolves that do attempt an east-west migration would face two additional challenges. A limited temporal restriction still exists as the River can only be crossed while frozen. Furthermore, the Mackenzie Delta has one of the highest levels of human activity in the NT, and avoidance of humans, or human-induced mortality of migrant wolves, might also reduce wolf gene flow across the River.

D_S, F_{ST}, and assignment test results suggest relatively low levels of genetic differentiation among mainland wolves on the same side of the Mackenzie River (Ft. St. John and Kluane National Park excluded, Table 2-2, Fig. 2-2, Table 2-3). The migratory behavior of wolves on the barren-grounds could contribute to this result in two ways. First, related wolves may be harvested in different regions at different times, as their packs follow migrating caribou herds. Secondly, these seasonal migrations may increase the probability of gene flow (for example, if a wolf from Tuk/Inuvik followed the caribou to Great Bear Lake, then mated with a wolf from this region). It is worth mentioning that, despite relatively high levels of gene flow in these areas, there remained significant genetic differences among wolves in different regions (*G*-tests, above).

Island Wolves

Genetic isolation of island wolves

D_S and F_{ST} values among mainland regions (0.038-0.329 and 0.009-0.097 respectively, Table 2-2) were within the ranges reported for continent-wide wolf populations (Roy *et al.* 1994; Forbes & Boyd 1997; Forbes & Hogg 1999). However, D_S values between Banks Island and the mainland (0.431-0.554, Table 2-2) exceeded most continent-wide distances, falling within the range of wolf-coyote comparisons (hybridizing and non-hybridizing populations, Roy *et al.* 1994). Similarly, F_{ST} values between Banks Island and mainland wolves (Table 2-2) included the continent-wide average (0.168) and fell between the averages for coyote-wolf populations (0.11/0.22, hybridizing/non-hybridizing populations respectively, Roy *et al.* 1994). Results for Victoria Island were similar, but less extreme (discussed further below).

The correlation between the presence of the Amundsen Gulf and increased genetic differentiation between regions was high and highly significant (Table 2-4, Fig. 2-4). Like the Mackenzie River, the Amundsen Gulf is frozen 6-8 months of the year, and could be crossed by wolves during this time. However, (with one exception, see below) prey is scarce on the pack ice, while high-density prey populations occupy both islands and the mainland. It is therefore reasonable to suggest that predator-prey relationships contribute to the barrier effect of the Amundsen Gulf, as well as that of the Mackenzie River.

Whether by topographic barrier or prey specialization, our data suggests that Banks Island wolves are genetically isolated from the mainland (Table 2-2, Table 2-3, Fig. 2-4). It is recognized that genetic drift is accelerated in isolated populations, resulting in elevated genetic distances and decreased H_E. These symptoms of drift have been documented in other insular carnivore populations: Idaho wolverines (Kyle & Strobeck 2001), Kodiak Island brown bears, (Paetkau *et al.* 1998), and perhaps Vancouver Island wolves (Roy *et al.* 1994; Forbes & Boyd 1997), are a few examples (see also the review by Frankham 1997). Banks Island, and to a lesser extent Victoria Island, may also be insular populations, subject to increased genetic drift (Table 2-1, Table 2-2, Table 2-3, Fig. 2-4).

For Banks Island wolves, the effects of drift in isolation are likely accentuated by two confounding factors. Wolves were extirpated from Banks Island in the 1950's; natural recolonization began in the 1970's. The origin of the founders is unknown, but of the populations surveyed here, Victoria Island seems most likely (Table 2-2, Fig. 2-2, Fig. 2-4). Second, 30-50 Banks Island wolves, 15-25% of the total estimated population, are harvested annually. Harvesting bottlenecks, combined with the tendency to harvest multiple animals per pack, may also accelerate genetic drift on Banks Island.

Banks Island wolves were sampled during two time periods: 1991-93 (n = 44), and 1997-99 (n = 63). G-tests indicated significant differences in allele frequencies between these periods, and approximately two-thirds of the wolves assigned to their time of origin. D_S between the two time periods was small (0.044), but greater than distances between Tuk/Inuvik and Paulatuk (Table 2-2), and between North and South Banks Island (above). However, there was no significant decrease in genetic variation between 1991-93 and 1997-99 (data not shown).

Island-mainland wolf migration

Banks and Victoria Island wolves are genetically isolated from mainland wolves. However, genetic evidence (Table 2-3, Fig. 2-3), combined with recent sightings of white wolves, considered to be island wolves, in the Tuk/Inuvik region (B. Jacobson, pers. comm.), suggests that limited island-mainland migration does occur.

The Dolphin-Union caribou herd migrates annually from Victoria Island to the mainland and back (Fig. 2-1a, Miller 1989); it is therefore likely that the putative island wolves in our Tuk/Inuvik sample (Table 2-3, Figure 2-3) are indeed island wolves that followed this herd to the mainland. Thus, the Dolphin-Union caribou may serve as a corridor for wolf gene flow across the barrier of the Amundsen Gulf. The movements of the herd may also explain why Victoria Island wolves appear genetically closer to mainland animals than do the wolves of Banks Island (Table 2-2, assignment probabilities, data not shown). The Dolphin-Union migration occurs annually; Banks Island Peary caribou have not been observed on the mainland since 1952 (A. Carpenter & P. Esau, pers. comm.). Therefore, migration of wolves from Banks Island to the mainland (and vice versa) would be more likely to occur via Victoria Island than directly across the Amundsen Gulf.

Within and among island populations

Inter-island gene flow was much greater than island-mainland gene flow (Table 2-2, Fig. 2-2, Table 2-3). Indeed, the Prince of Wales Strait had no impact on genetic distance beyond that of simple geographical distance. Therefore, it is not a barrier to gene flow (Fig. 2-4, Table 2-4). This conclusion is supported by the observation of wolf tracks on the pack ice between Banks and Victoria Island.

Significant differences in allele frequencies (*G*-tests above) suggest limited substructure within the Banks Island wolf population. Wolves on North Banks Island appear slightly different, genetically, from wolves in the southern half of the island. Our division of the Banks Island sample set was based on observed colour dichotomy in these wolves. However, assortative mating by colour may not be likely; packs have been observed on both the island and the mainland that include wolves of multiple colour phenotypes. It seems more probable that any north-south differences reflect preferential occupation by wolves of areas with high prey density, for example, the large muskoxen populations of the Thomsen River (north) and the Egg and Masik River areas (south).

Conclusions and Implications

The presence of the Mackenzie River was associated with a significant increase in genetic distance between wolves in different regions. While the slight temporal restriction posed by the River itself, and high levels of human activity in the Mackenzie Delta likely contribute to this effect, migration patterns of barren-ground caribou may be the primary determinant of wolf population structure in this area. If further study supports this conclusion, caribou herd boundaries may suggest appropriate management units for barren-ground wolf populations.

The Amundsen Gulf presents a barrier to island-mainland gene flow that may also result from predator-prey interactions. Isolation of the Banks Island population is most pronounced, and may result from a combination of factors: drift in isolation, recent recolonization, and annual anthropogenic bottlenecks. Forbes & Hogg (1999) suggest that the combination of elevated genetic distance and reduced genetic diversity, observed here, may signify "a population of particular management concern." However, gene flow between Banks and Victoria Island is high, and the Dolphin-Union caribou herd may facilitate some island-mainland migration. Furthermore, although H_E on Banks Island is low compared to mainland populations surveyed here, diversity is not currently declining, and remains within or above the ranges reported for other, non-insular, wolf and carnivore populations (Roy *et al.* 1994; Forbes & Boyd 1997; Paetkau *et al.* 1997; Kyle *et al.* 2000, 2001; Kyle & Strobeck 2001).

The present study is the first to discover correlations between topographical features and population structure in wolves. Perhaps more exciting, much of the structure we observe could result from prey specialization by wolves in different regions. While our work is thus far limited to the wolves of Northwestern Canada, it will be interesting to determine whether similar relationships exist among other populations separated by physical barriers or prey specialization.

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Table 2-1 Genetic diversity in each geographical region. H_E denotes expected heterozygosity.

Geographic Region	Sample Size	Allelic Diversity	% H_E ± SE
Ft. St. John	41	5.67	72.78 ± 7.9
Kluane National Park	37	5.44	67.69 ± 10.0
Southern Richardsons	26	5.56	65.61 ± 11.7
Northern Richardsons	45	6.44	69.29 ± 8.8
Tuk/Inuvik	160	8.44	72.79 ± 10.4
Great Bear Lake	22	5.56	74.42 ± 8.1
Paulatuk	44	6.33	68.92 ± 12.9
Banks Island	107	5.33	59.86 ± 17.5
Victoria Island	9	4	64.42 ± 13.7
Island Populations			
South Banks Island	60	5	60.85 ± 16.8
North Banks Island	47	4.44	58.28 ± 18.5
Victoria Island	9	4	64.42 ± 13.7

Table 2-2 D_S and F_{ST} between regions: F_{ST} above diagonal; D_S below diagonal. Largest and smallest values are shown in bold.

	Ft. St. John	Kluane National Park	Southern Richardsons	Northern Richardsons	Tuk/ Inuvik	Great Bear Lake	Paulatuk	Banks Island	Victoria Island
Ft. St. John	0	0.055	0.073	0.063	0.073	0.059	0.097	0.185	0.140
Kluane National Park	0.173	0	0.049	0.053	0.054	0.075	0.079	**0.188**	0.131
Southern Richardsons	0.224	0.139	0	0.024	0.042	0.058	0.072	0.185	0.140
Northern Richardsons	0.205	0.155	0.080	0	0.043	0.050	0.080	0.184	0.150
Tuk/Inuvik	0.255	0.162	0.123	0.133	0	0.015	**0.009**	0.158	0.094
Great Bear Lake	0.238	0.257	0.191	0.179	0.079	0	0.025	0.156	0.110
Paulatuk	0.329	0.230	0.205	0.240	**0.038**	0.108	0	0.180	0.098
Banks Island	**0.554**	0.512	0.480	0.511	0.460	0.431	0.492	0	0.031
Victoria Island	0.521	0.413	0.425	0.511	0.325	0.418	0.319	0.095	0

Table 2-3 Summary of cross-assignment between regions. Indicates the number of animals sampled in each region (row) that assigned to each region (column). Outlined values indicate the number of animals that assigned to the population where they were sampled. Cross-assignments significant at the 5% level are indicated with asterisks.

Sampling Region	**Assigned Region**									**Sample Size**
	Ft. St. John	Kluane National Park	Southern Richardsons	Northern Richardsons	Tuk/Inuvik	Great Bear Lake	Paulatuk	Banks Island	Victoria Island	
Ft. St. John	37	1	0	1	0	2*	0	0	0	41
Kluane National Park	4	29	0	0	2	1	1	0	0	37
Southern Richardsons	0	1	12	9*	2	1	1	0	0	26
Northern Richardsons	1	2	6	30	3	2	1	0	0	45
Tuk/Inuvik	4	0	11*	6	89	12	35*	2*	1	160
Great Bear Lake	0	0	1	1	4	12	4	0	0	22
Paulatuk	0	0	0	0	8	5	31	0	0	44
Banks Island	0	0	0	0	0	0	1	93	13*	107
Victoria Island	0	0	0	0	0	0	0	4	5	9

Table 2-4 Results of the Mantel test. Genetic: Nei's D_S or F_{ST}. Geographical: geographical distance between regions. River: presence/absence of the Mackenzie River. Ocean: Amundsen Gulf and Prince of Wales Straight. Amundsen Gulf: water between mainland and islands only.

	D_S		F_{ST}	
Mantel Tests	**r value**	**probability (r)**	**r value**	**probability (r)**
Genetic vs geographical	0.491	0.030	0.432	0.096
Genetic vs river	0.562	0.032	0.540	0.032
Genetic vs ocean	0.813	0.032	0.800	0.032
Genetic vs Amundsen Gulf	0.892	0.032	0.873	0.032
Partial Mantel Tests				
Genetic/river – geographical	0.781	0.011	0.705	0.004
Genetic/ocean - geographical	0.854	0.001	0.821	0.002
Genetic/Amundsen Gulf - geographical	0.919	0.001	0.880	0.002

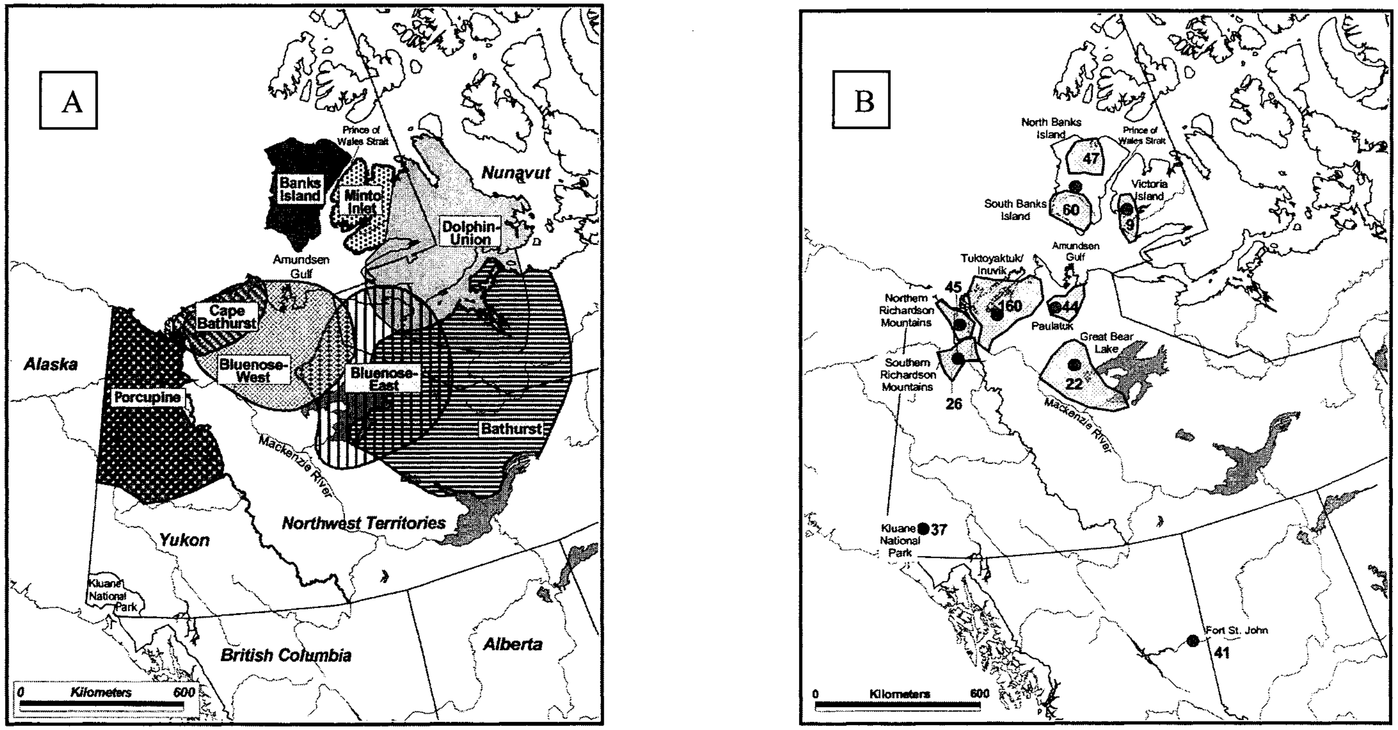

Figure 2-1 **A)** Topographic features and home ranges of barren-ground caribou herds within the study area. **B)** Boundaries of wolf sampling locations for NT/YT regions. Locations were not available for Ft. St. John and Kluane National Park wolves (see Methods). Sample size is indicated for each region, and geographic coordinates used in Mantel tests (see Methods) are marked with circles.

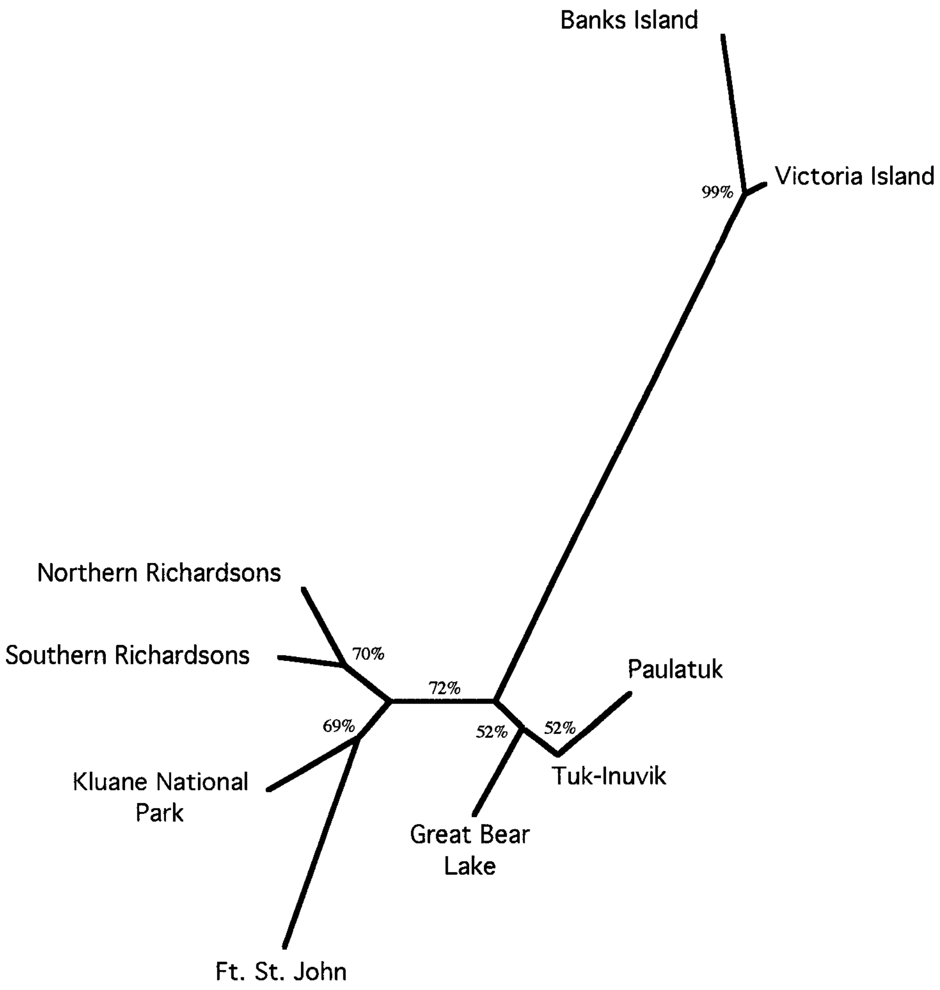

Figure 2-2 Neighbor-joining tree based on D_S values between each pair of regions. Bootstrap support is indicated for each grouping (1000 replications).

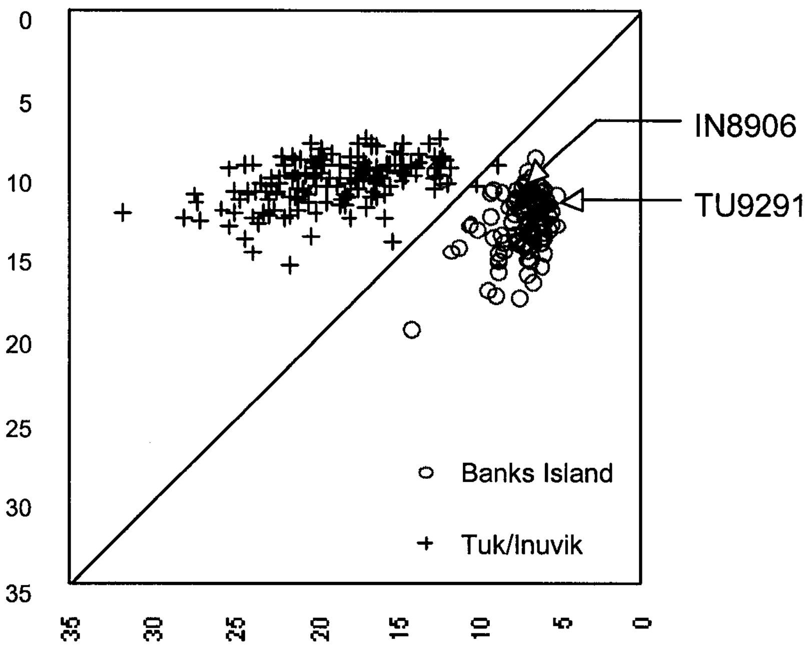

Figure 2-3 Assignment test results between Banks Island and Tuk/Inuvik. Individuals are plotted according to the negative log likelihood that their genotype would appear in each region. Individuals appearing on the same side of the diagonal as the axis for their region have genotypes more likely to occur in the region from which they are sampled, and vice versa. Cross assignment of wolf IN8906 and wolf TU9219 was significant in randomization tests (see Table 2-2, 2-3 and Results).

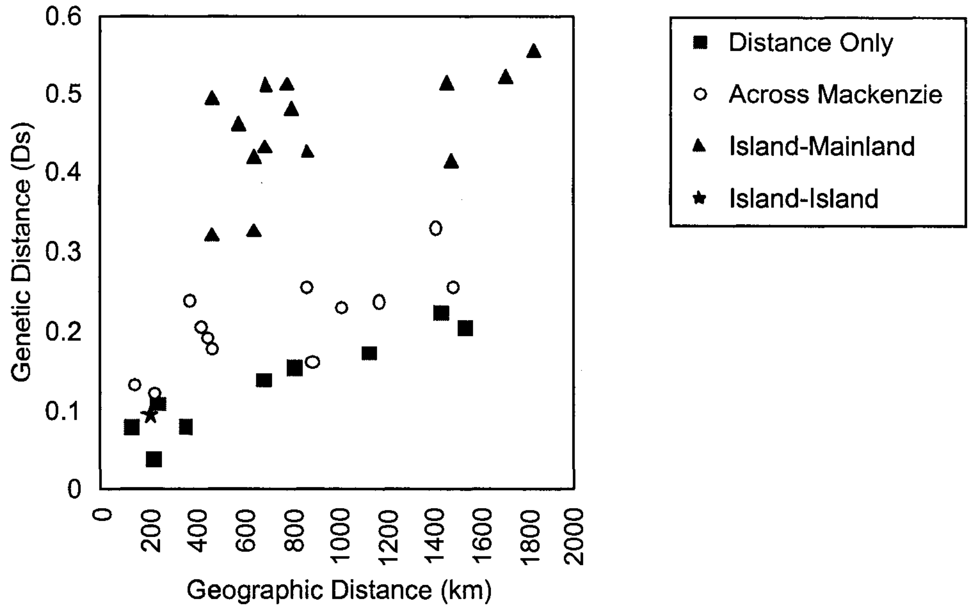

Figure 2-4 Plots D_S against geographic distance between regions. Region pairs are subdivided according to the presence or absence of physical barriers between them (for example, "Across Mackenzie" refers to regions that are separated by the Mackenzie River).

Chapter 3

Historical and Ecological Determinants of Genetic Structure in Arctic Canids

Introduction

Canid species inhabit forests and jungles, prairies and savannas, mountains, deserts and coastlines; they are able to thrive in undisturbed habitats and in human cities (IUCN/SSC 2004; Wandeler *et al.* 2003). However, only two species, the arctic fox (*Alopex lagopus*) and the grey wolf (*Canis lupus*), are found throughout the mainland tundra and arctic archipelago of North America (Angerbjörn *et al.* 2004; Mech & Boitani 2004). Arctic foxes are also found on islands in the northern oceans, including Svalbard (Norway). While different in many ways, wolves and arctic foxes have developed similar strategies for survival in harsh northern environments.

Fossil evidence suggests modern wolves and arctic foxes reached North America via the Bering Land Bridge during later phases of the Pleistocene (Kurtén & Anderson 1980). During the Wisconsinan glaciation, grey wolves may have persisted in up to five North American refugia: three south of the ice sheets, one in Pearyland (Greenland), and one in Beringia (Brewster & Fritts 1995). These distinct groups expanded throughout North America at the onset of the current interglacial (Nowak 2003); the present reduced range of this species is a consequence of recent persecution (Leonard *et al.* 2005). Unlike wolves, arctic foxes were found throughout North America during the last glaciation, their current range reflecting the progressive contraction of species-suitable habitat towards the pole and the northward expansion of their primary competitor, the red fox (*Vulpes vulpes*, Dalén *et al.* 2004; Dalén *et al.* 2005; Kurtén & Anderson 1980; Tannerfeldt *et al.* 2002).

Both wolves and arctic foxes are well-adapted to persistence in harsh northern climates. The arctic fox is the only canid with fur-covered foot pads, and can maintain homeostasis at external temperatures as low at -40°C without increasing its basal metabolic rate (Prestrud 1991). Pelage colour in wolves varies throughout their range, but the frequency of pale and white pelts increases dramatically above treeline, a likely adaptation for hunting in tundra ecosystems (Musiani 2003).

In addition to climatic extremes, arctic ecosystems are characterized by variation in the location and density of prey species, and wolves and foxes have developed flexible behaviors to cope with this instability. Two fox ecotypes are generally recognized: "coastal" foxes, feeding on birds, eggs, and carrion from the marine ecosystem (e.g. polar bear kills); and "lemming" foxes, which subsist primarily on small mammals of cyclical abundance (Braestrup 1941). The stable resource base of coastal foxes results in smaller home ranges which may be occupied and defended year round (Anthony 1997; Audet *et al.* 2002; Eide *et al.* 2004). However, lemming foxes are territorial primarily during the breeding season, and in winter, many arctic foxes migrate distances up to 2300 km in search of food (Eberhardt *et al.* 1983). Long-range foraging movements have also been documented through regions which do not support breeding populations, such as sea ice (640 km) and the southern boreal forest (1000 km, Wrigley & Hatch 1976). "Normal" dispersal distances of 40-60 km are typical of European alpine foxes, but may not be applicable to large, continuous populations in North America (Landa *et al.* 1998; Strand *et al.* 2000). In any case, long-distance dispersal occurs in all fox populations, and is thought to be an adaptation to large-scale synchrony of lemming population dynamics (Audet *et al.* 2002; Dalén 2005).

The behavior of tundra wolves more closely resembles that of sympatric arctic foxes than that of boreal forest wolves. Forest wolves feed primarily on resident ungulates like moose, elk, and deer, and defend their territories in all seasons (e.g. Hayes *et al.* 2000; Huggard 1993; Mech & Boitani 2003). Mainland tundra and Baffin Island wolves rely on migratory barren ground caribou and are territorial only while denning; during the fall and winter, wolves follow the movements of the caribou from their calving areas on the tundra to wintering grounds below treeline which may be thousands of kilometers away (Heard & Williams 1992; Kuyt 1972; Walton *et al.* 2001). Dispersal distances of forest wolves vary with availability of vacant territories, and can be as great as 886 km (Fritts 1983; Mech & Boitani 2003). Studies distinguishing dispersal distances from migratory movements of tundra wolves have not been conducted, but dispersal during migration was recently documented (Walton *et al.* 2001). Gene flow among tundra wolves could therefore be much greater than that of wolves in the boreal forest or on arctic islands without migratory caribou populations.

Whether territorial or migratory, wolves form packs which generally center around a dominant breeding pair (Mech & Boitani 2003). Groups average 6-8 individuals, and may include offspring of the breeders and additional non-breeding helpers; by comparison, arctic foxes form smaller groups most often consisting of a mated pair and their offspring (Audet *et al.* 2002), which may not persist after denning season. In both species, increased resource richness leads to larger social groups and later dispersals; reproductive output of arctic foxes is also tied to the productivity of their environment, as foxes may wean as many as 19 cubs in a peak lemming year (Angerbjörn *et al.* 2004).

Genetic studies have been conducted on wolf populations worldwide, employing a range of molecular markers and investigating social structure, population structure, and phylogeographic structure in various regions (e.g. Blanco *et al.* 2005; Flagstad *et al.* 2003; Kyle *et al.* 2006; Roy *et al.* 1994; Vilà *et al.* 1999). In Chapter 2 (Carmichael *et al.* 2001), microsatellites were used to explore the genetics of northern wolves, but this study was restricted to a small portion of the Canadian northwest. Relative to wolves, the population genetics of arctic foxes have received far less attention (but see Dalén *et al.* 2005; Dalén *et al.* 2002; Meinke *et al.* 2001), and have not been investigated in North America or the Svalbard Archipelago. The present study explores population-level genetics of wolves and arctic foxes throughout these northern ecosystems. By comparing results for each arctic canid, the historical, physical, and/or ecological factors with greatest influence on the contemporary genetics of these species may be identified. Such data could be particularly useful for conservation of carnivores in a changing arctic environment.

Materials and Methods

Sample Collection, Laboratory Analysis and Dataset Validation

Contemporary samples of 1,700 arctic foxes, distributed throughout the Svalbard Archipelago and their North American range, were collected (Fig. 3-1a). The sampling area for wolves extended across the North American Arctic and included boreal forest wolves for comparison to tundra populations (Fig. 3-1b). Over 2,000 wolves were analyzed, including 491 individuals previously examined in Chapter 2.

Tissue and blood samples were stored frozen while dry material such as pelt or hair was kept at room temperature. DNeasy tissue kits were used to extract genomic DNA from all samples (QIAGEN, Germany). Microsatellite loci were PCR-amplified using fluorescently-labeled primers from domestic dogs. Fifteen loci were amplified in wolves: CPH5 and CPH16 (Fredholm & Wintero 1995); CXX110, CXX140, CXX173, CXX250, CXX251, CXX377 (Ostrander *et al.* 1993); CXX618, CXX671, CXX733, CXX745, CXX758, CXX781, CXX2079 (Mellersh *et al.* 1997). We used 13 loci for arctic foxes: CPH5, CPH8, CPH9, CPH15 (Fredholm & Wintero 1995); CXX140, CXX147, CXX173, CXX250 (Ostrander *et al.* 1993); CXX671, CXX733, CXX745, CXX758, CXX771 (Mellersh *et al.* 1997). Eight loci were common between the species; six of the wolf markers were also used in Chapter 2.

For arctic foxes, single-locus amplifications of CPH5, CPH8, CPH9, CXX140, CXX147, CXX250, or CXX745 contained 0.16 μmol each primer, 0.12 mmol dNTPs, 2.5 mmol $MgCl_2$, 1 x PCR buffer (50 mmol KCl, 10 mmol Tris-HCl, pH 8.8, 0.1% Triton X100), 1 U Taq polymerase, and approximately 40 ng template in 15 μl total. For multiplex reactions of CXX173/CXX671, CPH15/CXX758, or CXX733/CXX771 dNTP concentration was increased to

0.16 mmol and $MgCl_2$ to 2.7 mmol. Wolf loci were amplified in the following multiplexes: CPH5/CXX2079; CXX671/CXX173/CXX377; CXX745/CPH16; CXX140/CXX250/CXX251; CXX618/CXX758/CXX110; and CXX733/CXX781. Reactions contained 0.16 mmol dNTPs, 1.7-2.5 mmol $MgCl_2$, and 0.5-2.5U Taq, with primer concentrations in each reaction scaled for optimal product balance. Finally, the pseudoautosomal loci DBX and DBY were amplified from all samples as a molecular sex test (Seddon 2005) using: 0.15 μmol each DBX primer, 0.1 μmol each DBY primer, 0.2 mmol dNTPs, 2.25 mmol $MgCl_2$, and 2.4 U Taq polymerase.

All PCR amplifications were conducted in Eppendorf Mastercycler ep thermocyclers (Eppendorf AG, Hamburg, Germany) with: 2 min at 94°C; 3 cycles of 45 sec at 94°, 30 sec at 50°, 10 sec at 72°; 30 cycles of 35 sec at 94°, 35 sec at 50°, 5 sec at 72°; and 30 min at 72°. Reaction products were separated on an ABI 377 Sequencer (Applied Biosystems) and genotypes were assigned using GENESCAN 3.1 and GENOTYPER 2.0 software (Applied Biosystems). All genotypes were checked twice by eye and all ambiguous results repeated.

The Microsatellite Excel Toolkit (Park 2001) was used to check the dataset for typographical errors and for samples with identical genotypes. Most matching pairs consisted of a fur house sample and one collected directly from the hunter; the sample with the least reliable biological data was excluded. One pair of identical wolves appeared to represent monozygotic twins (Chapter 8), and therefore both individuals were retained. After elimination of matching individuals, 1,924 wolves and 1,514 arctic foxes remained for analysis.

Population Delineation and Preliminary Analysis

Capture locations of all samples were mapped using ARCGIS 9.1 (Environmental Systems Research Institute 1999-2004). Arctic fox samples were grouped based on gaps in the sampling distribution (Fig. 3-1a). Wolves were divided into geographic regions based on three hierarchical criteria: 1) gaps in the sampling distribution, 2) ranges of associated barren ground caribou herds (Chapter 2), and 3) political boundaries of Canadian provinces in southern regions (Fig. 3-1b). Regions considered to be occupied by migratory barren ground wolves, and regions considered to be occupied by sedentary forest wolves, are shown in Fig. 3-1b. This categorization was based on the distribution limit of migratory barren ground caribou (e.g. Musiani 2003).

Regions for each species were tested for genic differentiation, linkage disequilibrium, and Hardy-Weinberg equilibrium using the Markov Chain method of GENEPOP 3.4 (Raymond & Rousset 1995) with dememorization of 10,000, 1000 batches, and 10,000 iterations per batch. Genic differentiation results were combined across loci using Fisher's method (Sokal & Rohlf 1995), and Bonferroni corrections used to obtain P values of 0.05 for all tests. Some geographic regions were subsequently pooled to facilitate interspecific comparisons between analogous populations of wolves and foxes (Fig. 3-1). All other analyses of wolves were conducted using genetically defined clusters as the unit of comparison (Fig. 3-1c).

Throughout the chapter, "region" refers to a geographically defined group of samples, "cluster" refers to a genetically defined group of samples, and "population" is used inclusively.

Comparisons Between Species

Allele sizes for the eight common microsatellites were calibrated for consistency between arctic foxes and wolves; genetic variation was then assessed for 11 comparable geographic regions in each species (Table 3-1, Fig. 3-1). Expected heterozygosity H_E (Nei & Roychoudhury 1974) was calculated in the Microsatellite Excel Toolkit, and significant differences in H_E identified using Wilcoxon's signed-ranks tests (Sokal & Rohlf 1995). The rarefaction method implemented in CONTRIB 1.01 (Petit *et al.* 1998) was used to calculate allelic richness after correction for variation in sample size, with a rarefaction size of 20 allele copies (Table 1). Nei's standard genetic distance (D_S) was calculated within and between species using PHYLIP version 3.65 (Table 3-2, Felsenstein 1995; Nei 1972).

Genetic Clustering of Each Species

Bayesian clustering of genotypes was performed in STRUCTURE 2.1, including all loci and without any prior spatial information (Pritchard *et al.* 2000). Initial runs for arctic foxes consisted of 100,000 burn-in cycles followed by 1,000,000 iterations of the Markov Chain. The admixture model was selected and a unique α (percent admixture) estimated for each cluster; λ, describing the allele frequency distribution of each locus, was also inferred. Setting the number of clusters, K, to vary between 1 and 4, indicated that an appropriate value for λ was 0.5 and that α was unequal between clusters and often small; ALPHAPROPSD was therefore set to 0.1. These final parameters were used to conduct two replicates each of K = 1-7. A similar exploration indicated that $\lambda = 0.4$ was most appropriate for wolves; all other parameters were identical to those for arctic foxes. As greater variation was observed between wolf runs, three replicates each of K = 1-13 were performed to examine convergence of the Markov Chain. The number of clusters in each species was determined based on peaking of lnProb(D) (Pritchard *et al.* 2000), level of admixture in each cluster, and the partitioning of individuals between clusters.

STRUCTURE results for wolves were confirmed using GENELAND, a Bayesian clustering program that incorporates spatial coordinates of individuals into the analysis via Voronoi tessellation; GENELAND therefore assigns greater probability to genetic clusters that are continuous within the spatial landscape (Guillot *et al.* 2005). STRUCTURE results suggested that $K = 7$ was most appropriate for wolves (Fig. 3-2), and the following settings in GENELAND were thus employed: delta.coord 0.15 (to "de-noise" the spatial coordinates); 1,000,000 iterations; burn-in 100,000 iterations; thinning 1000; the Dirichlet allele frequency model (shown to perform best by Guillot *et al.* 2005); and 7 populations. Arctic foxes were not analyzed in the GENELAND framework as STRUCTURE suggested K was most likely 1 (see Results).

Outputs from STRUCTURE and GENELAND were combined to devise wolf genetic clusters which were used for all further analysis (Appendix); since foxes formed a single cluster, fox geographic regions were used instead. Fig. 3-1c indicates ecotype (migratory barren ground or sedentary forest) for each wolf genetic cluster.

Genetic Distance, Assignment, and Sex-Biased Dispersal

One thousand bootstrap pseudoreplicates of wolf clusters and fox regions were created in PHYLIP 3.65. Nei's D_S was calculated for each replicate, and a neighbor-joining majority-rule consensus tree constructed (Felsenstein 1985; Saitou & Nei 1987). Euclidean distance was calculated among populations within species using average latitude and longitude and the "Geographic Distances" subroutine of MANTEL 4.0 (Casgrain & Legendre 2001). A Mantel test (Mantel 1967) was then performed with 9999 permutations to assess isolation by distance in each species.

Paetkau *et al.*'s assignment test (1995) was conducted with allele frequencies adjusted to avoid zeros (Titterington *et al.* 1981). To identify levels of cross-assignment greater than those expected due to correlation of allele frequencies between clusters, 10,000 replicates were performed, creating new individuals and assuming Hardy-Weinberg Equilibrium (Chapter 2). In addition to providing estimates of the relative number of migrants between two populations, assignment indices can be used as an indicator of relative differentiation, and were employed to explore contrasts between wolves in different habitat types. The variance of corrected assignment indices (vAIc) method, implemented in FSTAT, was also used to test for sex-biased dispersal in both species (Goudet 1995; Goudet *et al.* 2002; Prugnolle & de Meeus 2002).

Determinants of Genetic Structure in Wolves

In Chapter 2, partial Mantel tests were used to estimate correlations between physical barriers and genetic distance between populations while controlling for the influence of physical distance (Smouse *et al.* 1986). The inability to simultaneously assess more than two predictor variables, and recent concerns regarding the validity of associated significance estimates (Raufaste & Rousset 2001), are limitations of this technique. An alternative recently applied to population genetic data in wolves is distance-based redundancy analysis (dbRDA, Geffen *et al.* 2004; McArdle & Anderson 2001). dbRDA allows the user to test up to N-1 predictor variables (N = number of populations) either individually, or fitted in sequence to produce a combined model. Significance estimates in dbRDA have also been proven adequate (McArdle & Anderson 2001). This approach was used to test correlations between Nei's D_S among all wolf clusters and a suite of 22 potential determinants of genetic structure. The eight factors most related to D_S in preliminary tests were retained for full analysis and are described below.

Results in Chapter 2 suggested wolf genetic structure may result from specialization on particular prey types. To test this idea directly, a predictor was designed which indicated the dominant prey species within the range of each wolf cluster, based upon distribution of large ungulate species and available wolf diet studies (Hayes *et al.* 1997; Hayes *et al.* 2000; Kohira & Rexstad 1997; Larter *et al.* 1994; Mahoney & Virgl 2003; Olsen *et al.* 2001; R Popko pers. comm.; Schaefer *et al.* 1999; Spaulding *et al.* 1998; Stenhouse *et al.* 1995; Urton & Hobson 2005). However, wolf diet is complex and variable over space and time, and a number of assumptions had to be made during construction of this predictor. To simplify and to focus on an aspect of prey behavior that influences movement patterns of associated wolves (Ballard *et al.* 1997; Walton *et al.* 2001), an indicator denoting the behavior, sedentary or migratory, of the dominant prey species in the range of each wolf cluster was then defined (migratory barren-ground caribou = 0, all other species = 1). These predictors were tested singly and as a set called "prey."

Water barriers between populations – the Mackenzie River, channels of the Arctic ocean and the straights between the Coastal Islands and the mainland (Fig. 3-1b) – were coded in a similar fashion. Annual minimum temperature and annual rainfall in each area were obtained from Environment Canada (2000) and the National Climatic Data Center's online databases (2000), and vegetation was categorized using the World Wildlife Fund's Terrestrial Ecosystems (Environmental Systems Research Institute 2006). These habitat descriptors were tested separately and as a set called "habitat." Finally, average latitude and longitude for each cluster were tested individually, as a set called "spatial", and in combination with other variable sets.

The program PCO was used to perform principle coordinate analysis (PCA) on our genetic distance matrix (Anderson 2003b); dbRDA was then conducted on all variables using DISTLM*forward* (Anderson 2003a). Marginal tests of each predictor or set of predictors were made, followed by sequential tests using a forward selection procedure to produce a combined model of genetic differentiation in wolves.

Results

Equilibrium and Differentiation in Each Species

All wolf geographic regions (Fig. 3-1b) possessed significantly different allele frequencies. Allele frequencies in arctic fox regions (Fig. 3-1a) were generally homogeneous: the Svalbard population was one consistent exception. Ten locus pairs also deviated from linkage equilibrium in the Svalbard fox population, suggesting population substructuring in addition to slight differentiation from North America (below).

CPH5 and CXX110 showed significant association in eight out of 21 wolf regions, indicating potential physical linkage (all other Bonferroni-corrected significant results occurred in a single population). Since CXX110 was less variable and more difficult to type, it was excluded from further analysis. In arctic foxes, CPH8 suffered a significant deficiency of heterozygotes in 12 of 17 regions. CPH8 also accounted for over 50% of the missing data in our fox samples, and was excluded for likely possession of null alleles. We therefore proceeded with 14 microsatellite loci in wolves and 12 loci in arctic foxes, eight of which were common between species.

Variation and Differentiation Between Species

Average H_E for wolves was 62%, and island populations were significantly less variable than mainland wolves ($P = 0.05$, Table 1). In arctic foxes, H_E averaged 80% in all types of populations; however, arctic foxes were significantly more variable ($P = 0.05$) than island wolves alone. Allelic richness results for both species duplicated these trends. Equivalent results were also obtained considering all loci and all populations for each species.

Allele size ranges in wolves and foxes overlapped only partially, and in 6 of the 8 common loci, arctic foxes had larger average allele size. Species allele frequencies were relatively divergent; Nei's D_S averaged 2.19 between species (range 1.37-2.99), while wolf D_S averaged 0.25 (range 0.03-0.66), and fox D_S 0.08 (0.02-0.19). Genetic distances between wolf populations were consistently larger than those for foxes (Table 3-2).

Genetic Clustering of Each Species

STRUCTURE analysis was conducted using all loci. lnProb(D) for arctic foxes increased slightly as K was increased (Fig. 3-2a). However, for $K = 2$, an average of 97% of the individuals in each geographic region assigned to a single cluster, and this trend persisted as K was increased. While some genuine complexity seemed to exist within the Svalbard group, this population also consistently assigned to the single cluster containing the vast majority of North American arctic foxes (Fig. 3-3). The increase in probability with larger K thus appeared to result from over-parameterization of the model, and STRUCTURE seemed to be segregating rare alleles, rather than partitioning individuals according to true genetic discontinuities. A single panmictic unit including North America and Svalbard seems most likely for arctic foxes.

In contrast, given the plateau in lnProb(D) and cohesion of the clusters (Fig. 3-2a and 3-2b), $K = 7$ was the most appropriate choice for wolves. In general, STRUCTURE recovered an Atlantic group, a western and eastern boreal forest group (Western Woods and Forest) and a western and eastern barren ground group (Western Barrens and Eastern Barrens), shown in Fig. 3-1c. Assignment of mainland clusters was nearly identical in GENELAND as in STRUCTURE, however, the methods differed with regards to island populations. GENELAND separated Coastal Island wolves and grouped all arctic island wolves into a single cluster; STRUCTURE divided the arctic islands into a Western grouping (Banks and Victoria Island) and an Eastern grouping (North and South Baffin Island), and did not delineate Coastal Island wolves until $K = 9$ (data not shown). This difference is most likely due to spatial concentration of the Coastal samples, which would receive high weighting in the GENELAND framework. Both methods described the High Arctic region as a mixture of Western Island wolves, Eastern Island wolves, and mainland types, possibly due to small sample size in this region ($n = 11$).

Results from STRUCTURE and GENELAND were combined to devise genetic clusters of wolves (Fig. 3-1c) in all regions. North and South Baffin Island were pooled, but all other island populations remained distinct for three reasons: 1) the conflict between the clustering methods; 2) the obvious physical boundaries of islands in the landscape; 3) and to retain the ability to perform detailed examinations of island wolf genetics (Chapter 4). Ten clusters of wolves were therefore used for all analysis detailed below. Since arctic foxes formed a single cluster, we performed parallel analyses on ungrouped arctic fox regions (Fig. 3-1a).

Relationships Among Canid Populations

A bootstrap consensus tree of D_S between wolf clusters is shown in

Fig. 3-4a. Moderate to high levels of support (48-93%) were observed for all nodes except that for the Atlantic population, and clusters were grouped roughly according to their physical locations (Fig. 3-1c). Despite the visual correspondence between tree topology and geography, however, no significant association between physical and genetic distance among clusters was observed (Mantel test, $r = 0.43$, $P = 0.08$).

In contrast to results for wolf clusters, there was no association, visual or statistical, between geography and D_S in arctic foxes (Fig. 3-5, $r = 0.15$, $P = 0.24$). Indeed, subpopulations located on the same island appear on opposite sides of the tree (Fig 3-4b). These observations confirm that arctic foxes form a single genetic unit.

Classical assignment tests for wolf clusters and fox regions were next performed (Paetkau *et al.* 1995). Unsurprisingly, island wolves were most distinct in both genetic distance (not shown) and assignment analyses (Table 3-3). It was interesting to note, however, that divergence in assignment indices for wolves suggested higher differentiation among boreal forest populations than barren ground ones (Fig. 3-6). Assignment across habitat types was more complex. Differentiation between the Western Woods and the Western Barrens was similar to that among forest populations (Fig. 3-6a, 3-7a), while differentiation between the Eastern Barrens and the Forest was similar to that observed in the barren grounds (Fig. 3-6b, 3-7b), despite comparable physical separation in these cases (Fig. 3-7). In contrast, arctic fox populations displayed overlapping assignment indices (data not shown) and self-assignment rates below 14% in North America (42% in Svalbard). The vAIc test for sex-biased dispersal was used to determine whether low differentiation in foxes reflected high male-mediated gene flow. No signal was detected in foxes, but male wolves were the more dispersing sex in our study area (female vAIc = 20.56, male vAIc = 25.27, $P = 0.05$).

Determinants of Population Structure in Wolves

Model testing was not pursued for arctic foxes as the level of structure seemed too low to provide any useful signal. However, despite the small number of clusters, patterns in wolves were strong enough to produce several significant results.

We began by assessing complexity in our genetic distance matrix (D_S) using PCA. Several vectors with large and negative Eigenvalues were obtained, suggesting our wolf D_S was highly non-metric (Laub & Muller 2004 Table 3-4a). Studies of pattern-recognition have shown correspondences between negative Eigenvalues and hidden aspects of data variation: for example, context versus frequency of words in different texts, or shape versus stroke weight of numerals

(Laub & Muller 2004). The aspect of D_S quantified by our negative Eigenvectors is not clear, but exclusion of such vectors biases significance calculations in dbRDA (McArdle & Anderson 2001). They were therefore included despite resultant oddities such as negative F statistics (with associated P values above 0.95) for some predictor variables, and sequential tests that explained more than 100% of the variation in D_S (Table 3-5). This complexity does not invalidate the dbRDA procedure (MJ Anderson, pers. comm.).

The suite of predictor variables included minimum annual temperature, rainfall, vegetation, isolation by a water barrier, behavior and species of primary prey for each cluster, and average longitude and latitude. Consistent with Geffen *et al.*, (2004) minimum temperature explained 98% of the variation in D_S ($P =$ 0.0001) when the eight predictors were tested individually; addition of longitude to temperature in a sequential test explained 113% of the variation in D_S. Significant positive associations were also obtained between latitude or rainfall and D_S, while behavior of prey (migratory or non-migratory) was significantly negatively associated with genetic distance (Table 3-4b). This negative association signified correlation to the "imaginary" dimensions of D_S identified by negative Eigenvalues in the PCA (MJ Anderson, pers. comm.).

When variables were grouped into sets, the spatial coordinates displayed the strongest relationship to D_S, explaining 98.14% of the genetic distance ($P =$ 0.0005, Table 3-4b). However, tests for correlations between predictors indicated that each spatial variable was strongly correlated, positively or negatively, to most of the other predictors in our matrix, implying that the high explanatory power of the spatial variables is more complex than a simple causal increase in D_S with geographic distance.

Discussion

Genetic variation within and between species

Since all markers used in this study were originally developed for domestic dogs (*Canis familiaris*), potential ascertainment bias might be predicted to inflate variation observed in wolves, relative to the more distantly related arctic foxes (Bardeleben *et al.* 2005; Ellegren *et al.* 1997). However, larger allele sizes and greater genetic variation were observed in arctic foxes. Furthermore, a reciprocal study of 24 ovine and 448 bovine microsatellites found no evidence of ascertainment bias (Crawford *et al.* 1998). We therefore suggest trends reported here result primarily from divergent species and life history characteristics, rather than any significant methodological constraints.

Arctic foxes averaged 80% heterozygosity regardless of the type of population; heterozygosity in mainland wolves averaged 71%, while island wolves were significantly less diverse (Table 1). All wolf populations contained less variation than all fox populations, but the difference was significant between foxes and island wolves only. Similar trends were observed in the average number of alleles per population, and the pattern likely results from both historical and contemporary events.

Wolves persisted in small populations in a number of distinct refugia during the last glaciation (Brewster & Fritts 1995), while arctic foxes were widely distributed, and would not have experienced the bottlenecks undergone by wolves (Dalén *et al.* 2005; Kurtén & Anderson 1980). In addition, arctic foxes occur at higher density than wolves, (Angerbjörn *et al.* 2004; Mech & Boitani 2004), and likely possess a higher effective population size. Whereas only two wolves normally breed in a pack of 6-8 individuals (but see Mech & Boitani 2003), foxes form smaller social groups, and a higher proportion of adults thus breeds each generation (Macpherson 1969). Given their respective species and life histories, it is unsurprising that arctic foxes in general possess more genetic variation than wolves.

Since arctic foxes can travel long distances over sea ice, it is also unsurprising that island and mainland populations are equally variable (Table 3-1). More interesting is the fact fox populations surveyed here are more variable than populations in Greenland (H_E = 0.54-0.73 Meinke *et al.* 2001) and Scandinavia (H_E = 0.58-0.77), while equal to the large Russian population (H_E = 0.83 Dalén 2005). Russian foxes, like North American ones, have likely persisted at high density since the Pleistocene, while Scandinavian foxes have endured recent, severe, and prolonged bottlenecks (Dalén 2005). The lower variation in Greenland foxes is more difficult to explain, but portions of the Greenland coast are ice-free year round, perhaps impeding gene flow; drift-in-isolation may therefore be higher in this population (Dalén *et al.* 2005).

Reduced variation might be expected among wolves on southern ice-free islands (Roy *et al.* 1994; Weckworth *et al.* 2005), where immigration from larger mainland populations is physically hindered, but wolves can cross sea ice as arctic foxes do, and evidence for restricted gene flow between arctic island and mainland wolves does exist (Chapter 2, 4). However, due to differences in energetics and resulting home range sizes, island wolf populations are likely to be smaller than sympatric arctic foxes, resulting in elevated genetic drift. While both species are harvested, wolves, with longer generation times and smaller litter sizes, may be more susceptible to harvesting bottlenecks (Macpherson 1969; Mech & Boitani 2003). If wolves were isolated in a Pearyland refugium during the Pleistocene, lower variation in contemporary island populations may also be a relic of historical demographic bottlenecks (Nowak 1995).

Methodology of Cluster Identification

Two technical aspects of the STRUCTURE analysis performed here merit comment. Default settings for the admixture model assume a uniform allele frequency distribution ($\lambda = 1.0$) and that all clusters are equally admixed (Pritchard & Wen 2004). Under these assumptions, $K = 18$ was most probable for our wolves (data not shown). Fixing λ equal to the inferred value of 0.4 (representing skewed allele frequencies) while allowing a unique level of admixture in each cluster produced the far more reasonable result $K = 7$ discussed above. This discrepancy suggests STRUCTURE's default settings may be inappropriate for other microsatellite datasets, and for other systems including genetic barriers of unequal permeability. A further advantage of optimizing admixture separately for each cluster is the ability to assess cohesion of the inferred groups, which can be used as an additional indicator of appropriate K (Fig. 3-2b).

The behavior of STRUCTURE in the absence of genetic discontinuity is also of interest. Increasing K for arctic foxes produced small increases in probability, while clusters were created without any real content: improvement through sequestering of rare alleles, rather than divergent groups of individuals. Taken together, these results recommend greater caution in application of STRUCTURE than has been common (e.g. Cegelski *et al.* 2006; McRae *et al.* 2005; Weckworth *et al.* 2005). Confirmation of results using GENELAND (Guillot *et al.* 2005) or the recently released STRUCTURAMA (Huelsenbeck & Andolfatto submitted) may also be prudent.

Absence of Genetic Structure in Arctic Foxes

Potential complexity within the Svalbard population notwithstanding (Fig. 3-3), North American and Svalbard foxes formed a single genetic cluster. Dalén *et al.* (2005) attributed this pattern in mtDNA phylogeography to the inverse response of polar-adapted species to climatic cycles: expanding during ice ages and contracting into a single circumpolar populations during interglacials. With the exception of foxes in alpine habitats and on sea ice-free islands like Iceland, world-wide arctic fox populations have likely been physically continuous since the Pleistocene (Dalén *et al.* 2005). Spatial synchrony of lemming population cycles has selected for development of long-distance migrations in foxes, allowing them to escape areas devoid of adequate prey (Audet *et al.* 2002; Dalén 2005); high levels of gene flow resulting from these movements would maintain homogeneous allele frequencies across large geographic areas. It is therefore likely that no population in our sampling range has experienced significant genetic isolation since initial colonization. Large population sizes, in combination with this physical and temporal continuity, would effectively suppress genetic differentiation in these regions.

No fox populations were separated by F_{ST} above 0.02, and our pairwise values averaged 0.002 (data not shown). In contrast, pairwise F_{ST} ranged from 0.06-0.2 in Scandinavian foxes (Dalén 2005), while Meinke *et al.* observed values from 0.07 to 0.262 among coastal Greenland populations (2001). Higher differentiation, like low variation, is expected among alpine foxes in Scandinavia; Greenland foxes are tundra foxes, but restricted to coastal regions (Meinke *et al.* 2001). If fox movement occurs only around Greenland's circumference, gene flow between populations may thus be restricted; greater resource stability may also reduce the number of long distance movements made by Greenland foxes relative to North American populations.

Ecologically-Defined Genetic Structure of Grey Wolves

Unlike panmictic foxes, wolves form genetically distinct subpopulations whose boundaries correlate with habitat type – forest, tundra, or island – and vary longitudinally within each type. As in arctic foxes, these patterns could have been initiated during the last glaciation. Indeed, the five subspecies of North American wolves are thought to have descended from populations in distinct glacial refugia, possibly representing individual waves of invasion by Old World wolves (Nowak 1995; Nowak 2003). While such a history could contribute to the observed genetics of wolves, the population boundaries derived here do not correspond to proposed subspecific ranges (Nowak 1995). The discrepancy could result from non-genetic influences on wolf skull morphology, from which subspecies were defined, or from more recent self-organizing dynamics superimposed upon remnant post-glacial signal. Preliminary mtDNA sequencing of a subset of these samples has been conducted (unpublished data), and haplotype frequency differentiation consistent with subspecies level divisions in these areas has not been identified. Analysis of further samples, and perhaps of Y chromosome DNA, could help resolve this issue.

Since historical forces do not sufficiently explain observed genetic differentiation of wolves, contemporary influences must be considered. D_S between wolf populations was higher than among foxes in almost all cases (Table 3-2). Similarly, with the exception of Svalbard at 42%, self-assignment rates for arctic foxes were below 14%; the smallest self-assignment rate for wolves was 59% (Table 3-3). Therefore, with the possible exception of tundra animals, wolves likely disperse shorter distances, or disperse long-distances less often than foxes do. More interesting is the observation that the amount of wolf gene flow varies with habitat type, and gene flow occurs in non-random directions. Differentiation was lower among barren ground populations than territorial forest populations (Fig. 3-6a, b), consistent with the extensive annual movements which facilitate long-distance dispersal of tundra wolves (Walton *et al.* 2001), and with the high potential for gene flow when wolves follow distinct caribou herds into common wintering grounds. Despite separation by half the distance, differentiation between Western Barrens (tundra) and Western Woods (forest) wolves was equivalent to that among forest wolves, suggesting the transition between wooded and tundra habitats discourages gene flow between wolf populations (Fig. 3-7a). Indeed, boundaries of Bayesian-derived genetic clusters correspond to boundaries of habitat types (Fig. 3-1c).

Previous studies have also suggested a connection between habitat and wolf dispersal. Correlations exist between water barriers and genetic structure of wolves in the Canadian Northwest; however, as these barriers should be passable while frozen, Carmichael *et al.* (2001, Chapter 2) suggested that behavior of prey species flanking each barrier supported wolf movements parallel to these barriers, while human avoidance or increased hunting pressure further reduced cross-barrier dispersal. More recently, Geffen *et al.* (2004) demonstrated a link between climatic variation and structure of wolf populations. dbRDA was therefore used to quantify the influence of habitat on genetic structure of wolves in our study area.

Water barriers were highly correlated to D_S in partial Mantel tests (data not shown), but were not significant predictors of genetic distance between wolf populations using dbRDA (Table 3-5). Barriers did, however, display some correspondence to the negative Eigenvectors within D_S. Taken together, these results may support the suggestion (Chapter 2) that the relationship between barriers and D_S is not a straightforward causative one. Consistent with previous results (Geffen *et al.* 2004), the single greatest predictor of genetic differentiation among wolf populations was climate (minimum annual temperature, Table 3-5). Like the correspondence with barriers, and as suggested by Geffen *et al.* (2004), it is not clear that this result represents a causal link between climate and gene flow; indeed, it is difficult to imagine how temperature could directly influence the amount or direction of genetic exchange between wolf populations. However, two correlates of temperature (Table 3-4b), vegetation type (0.7332) and prey species (-0.4712) could direct the dispersal choices of individual wolves (Geffen *et al.* 2004). Description of these complex factors required simplifying assumptions that may have hampered our ability to detect correlations directly, but it is interesting to note that the behavior of the dominant prey species in each area (resident or migratory) is significantly correlated to the imaginary vectors within wolf Ds (Table 3-5).

Geffen *et al.* (2004) used latitude and longitude of each population to signify geographic distance between groups. We have also used these coordinates to describe the spatial relationship between populations (Table 3-4b), but are uncertain they describe a parameter as directly relevant to the dispersal of wolves as the distance in kilometers between regions. In fact, while distance was not significantly correlated with wolf D_S, the combined spatial variables explained more variation in D_S than minimum temperature alone (Table 3-5). The high explanatory power of this predictor set may therefore also reflect a more complex, underlying causal process. This idea is supported by the positive correlation between latitude and the barrier descriptor (0.5156), dominant prey species (0.7424), and vegetation (0.5310), and by negative correlations between latitude and prey behavior (-0.0544), temperature (-0.8524), and rainfall (-0.5771). Similarly, longitude is positively correlated to prey behavior (0.2056) and vegetation (0.2656), while negatively correlated to the barrier variable (-0.2068), prey species (-0.1747), temperature (-0.2934), and rainfall (-0.2625). In other words, these spatial descriptors provide a strong summary of all variables describing the habitat and ecology of wolves in each region (Table 3-4b).

Considered together, the outcomes of Bayesian clustering, classical assignment, and dbRDA analysis support the hypothesis that natal habitat-biased dispersal drives genetic differentiation in wolves (Davis & Stamps 2004; Geffen *et al.* 2004; Sacks *et al.* 2004). For northern wolves, a familiar level of vegetation cover – forest or tundra – could signify a suitable habitat, encouraging dispersing wolves to remain within their natal habitat type. Dispersers that settle in familiar areas may also increase their reproductive success via cultural mechanisms, as they will have learned to hunt resident prey while with their natal pack (Sacks *et al.* 2005). In our study area, learned behavior is most likely to isolate forest from tundra wolves, which have adapted their denning and territorial behavior to cope with the large scale seasonal movements of barren ground caribou (Heard & Williams 1992; Walton *et al.* 2001). Prey specialization as a barrier to gene flow has been suggested by other authors (Chapter 2; Geffen *et al.* 2004; Musiani 2003), and has been used to explain differences in skull morphology between wolf populations in other regions (Brewster & Fritts 1995).

Wolf differentiation along an east-west axis may result from habitat variation along this gradient (Geffen *et al.* 2004), for example, in the western Arctic, where forest and tundra wolves occur at the same latitude. In this area, however, wolves which cross habitat types must also cross the human-populated Mackenzie Delta region, and increased mortality of these dispersers, overlaid upon the change in habitat type, could create a barrier more intractable to wolves than either influence alone (Blanco *et al.* 2005; Chapter 2). This study also demonstrates habitat-correlated isolation of wolves in the north-south direction, between the Eastern Barrens and the southern Forest population (Fig. 3-1c). Assortative mating via pelage colour could contribute to this pattern, as a significant increase in frequency of pale and white wolves is observed with increasing latitude (Musiani 2003). However, wolves from the Eastern Barrens invade the range of Forest wolves following the southern winter migration of barren ground caribou, and since this period includes wolf breeding season (Mech 2002), a high potential for admixture exists. While dominant wolves are most likely to mate with dominant partners within their own pack, gene flow between forest and tundra wolves may occur via interbreeding of subordinate individuals, as occurs in other canids (e.g. Baker *et al.* 2004). The frequency of such events in wolves is not well known, but gene flow may be overestimated by our assignment data for two reasons (Table 3-3, Fig. 3-7b). Samples contributed by hunters and trappers are most often collected during winter, and may thus represent admixture of Eastern Barrens and Forest individuals without admixture of tundra and forest genes (mingling without gene flow). Furthermore, hunters from Northern Saskatchewan often harvest wolves in the Northwest Territories, but return home before selling the resultant pelts (D Bewick, pers. comm.). Samples identified by fur houses as originating in Saskatchewan (Forest cluster) might therefore truly originate in the Eastern Barrens. Significant cross-assignment from the Forest into the Eastern Barrens cluster (Table 3-3) is, however, supportive of some genetic exchange between forest and tundra wolves in this area.

Conclusions

Population structure in both wolves and arctic foxes appears to be strongly influenced by ecological factors such as distribution of habitat types, and adaptation of carnivores to variability in their prey base. Arctic foxes of the "coastal" ecotype in Greenland show levels of differentiation comparable to the isolated and depleted Scandinavian subpopulations; arctic foxes inhabiting the large, continuous landscape of North America and Svalbard show no differentiation whatsoever. Panmixia is likely maintained through long distance movements in response to spatiotemporal changes in availability of lemming prey. In contrast, wolves exhibit natal habitat-biased dispersal – likely resulting both from physical aspects of their environment and learned hunting behaviors – which may in some areas be reinforced by assortative mating or anthropogenic influences. Whatever the underlying mechanisms, dispersal of wolves is decidedly non-random, and genetic discontinuities correspond closely to changes in habitat type.

Differential responses to historical climate change are also potential contributors to the contrasting genetic structure in these arctic canid species. While wolves are thought to have been isolated in multiple, widely spaced refugia during the Pleistocene, arctic foxes enjoyed an extensive range expansion. During the current interglacial, wolf populations have expanded and intermingled, while foxes have retreated, following arctic temperatures toward the pole and avoiding intra-guild competition with temperate-adapted red foxes (Dalén *et al.* 2004; Tannerfeldt *et al.* 2002). As the arctic climate continues to warm and sea ice becomes scarcer, arctic foxes may persist only in those isolated high arctic islands red foxes cannot reach. The fox populations surveyed here will then begin to resemble currently isolated populations, with higher differentiation and lower genetic variation. A greater future threat to the persistence of the species than increased risk of inbreeding may be the inability of foxes to escape crashes in lemming population density; however, as long as sea birds nest on the arctic islands (Bety *et al.* 2001; Samelius & Alisauskas 2000) arctic foxes are likely to persist.

Predictions for wolves are more difficult to make, but as climate change provokes shifts in the distribution of vegetation and prey species (Brotton & Wall 1997; Grace *et al.* 2002; Mech 2005), we may observe further intermingling of wolf types and a loss of regional differentiation, at least in mainland regions. It is likely that the forthcoming climatic changes will have influences as dramatic as those of the Pleistocene on the distribution and genetics of arctic canids, and indeed, of all arctic species.

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Appendix

Wolf samples were divided into genetic clusters using results of STRUCTURE and GENELAND analysis, and according to the following protocol:

1) Geographic regions formerly designated Banks Island, Victoria Island, and the High Arctic (Fig. 3-1b) were treated as distinct clusters for three reasons:
    a) conflict between clustering methods
    b) inherent physical boundaries
    c) to allow fine-scale analysis of island wolf genetics

2) Geographic regions North and South Baffin were pooled into a single cluster based on agreement between clustering methods and physical position on the same island (Fig. 3-1b).

3) The Coastal Islands region was designated a cluster due to partitioning in GENELAND at $K = 7$, identical partitioning in STRUCTURE at $K = 9$ (data not shown), and physical coherence of the sampling locations (Fig. 3-1b). A single additional sample was added to this group based on clustering results (Pacific region, below).

4) Mainland clusters were established in the following manner:
    a) Samples were sorted according to GENELAND class, then STRUCTURE cluster. As STRUCTURE analysis is aspatial, it is more sensitive to admixture; as GENELAND analysis is inherently spatial, it is most sensitive to population substructure. Division of samples into units of analysis requires emphasis on differentiation, rather than admixture, and GENELAND results therefore took precedence when clustering outcomes conflicted.
    b) Spatial sorting, with longitude or latitude dominant, was used to assess distribution of samples within each cluster.
    c) When multiple wolves were sampled at a single location, and >1 class/cluster was inferred, all wolves were assigned to the dominant cluster for that location.
    d) Gaps in the distribution of spatial coordinates for wolf samples were used to fine-tune boundaries between genetic clusters. To be used as a demarcation, these gaps were required to correspond to shifts in the dominance of class/cluster category. This rule was employed most often in establishing the Forest cluster, where sampling location data for some individuals may have been compromised (see Discussion).

Data used to perform cluster partitioning is shown in Table 3-A1 below.

Table 3-A1 Individual, sampling location, geographic region, final genetic cluster, and Bayesian cluster assignments are shown for all wolf samples included in this study. Cluster abbreviations follow those in **Table 3-3**. Region abbreviations are as follows: Alaska (AK), Alberta (AB), Atlantic (AT), Banks Island (BI), Bathurst (BA), Bluenose W (BW), British Columbia (BC), Cape Bathurst (CB), Coastal Island (CI), High Arctic (HA), Mackenzie (MA), Manitoba (MB), Maritime (MR), NE Main (NE), North Baffin (NB), Pacific (PA), Porcupine (PO), Qamanirjuaq (QA), Saskatchewan (SK), South Baffin (SB), Southampton (SH), Victoria Island (VI), Yukon (YK). Maritime, Pacific, and Southampton samples were not included in regional analysis due to extremely low sample size, but were pooled into the genetic clusters shown below.

Individual	**Latitude**	**Longitude**	**Region**	**Cluster**	**STRUCTURE**	**GENELAND**
CFX-456	54.070	-124.550	BC	WW	B	Class 2
CXI-972	54.230	-125.750	BC	WW	A	Class 2
CXG-169	54.230	-125.750	BC	WW	B	Class 2
CXI-971	54.230	-125.750	BC	WW	B	Class 2
CXI-973	54.230	-125.750	BC	WW	B	Class 2
CXI-974	54.230	-125.750	BC	WW	B	Class 2
QAE-863	54.230	-125.750	BC	WW	F	Class 2
CXI-340	54.430	-124.250	BC	WW	A	Class 2
CXF-782	54.430	-124.250	BC	WW	B	Class 2
CXI-336	54.430	-124.250	BC	WW	B	Class 2
CXI-337	54.430	-124.250	BC	WW	B	Class 2
CXI-338	54.430	-124.250	BC	WW	B	Class 2
CXI-339	54.430	-124.250	BC	WW	B	Class 2
CXK-566	54.520	-128.600	BC	WW	B	Class 1
CYH-729	54.770	-127.170	BC	WW	B	Class 2
CXD-826	55.250	-127.670	BC	WW	B	Class 2
CXH-480	55.750	-120.530	BC	WW	A	Class 2
CXH-481	55.760	-120.530	BC	WW	B	Class 2
CXL-488	56.200	-120.680	BC	WW	B	Class 2
Y31	56.230	-120.920	BC	WW	A	Class 2
Y39	56.230	-120.920	BC	WW	A	Class 2
Y45	56.230	-120.920	BC	WW	A	Class 2
Y30	56.230	-120.920	BC	WW	B	Class 2
Y32	56.230	-120.920	BC	WW	B	Class 2
Y33	56.230	-120.920	BC	WW	B	Class 2
Y34	56.230	-120.920	BC	WW	B	Class 2
Y35	56.230	-120.920	BC	WW	B	Class 2
Y36	56.230	-120.920	BC	WW	B	Class 2
Y37	56.230	-120.920	BC	WW	B	Class 2
Y38	56.230	-120.920	BC	WW	B	Class 2
Y40	56.230	-120.920	BC	WW	B	Class 2

Y41	56.230	-120.920	BC	WW	B	Class 2
Y42	56.230	-120.920	BC	WW	B	Class 2
Y43	56.230	-120.920	BC	WW	B	Class 2
Y44	56.230	-120.920	BC	WW	B	Class 2
Y46	56.230	-120.920	BC	WW	B	Class 2
Y47	56.230	-120.920	BC	WW	B	Class 2
Y48	56.230	-120.920	BC	WW	B	Class 2
Y49	56.230	-120.920	BC	WW	B	Class 2
Y50	56.230	-120.920	BC	WW	B	Class 2
Y51	56.230	-120.920	BC	WW	B	Class 2
Y52	56.230	-120.920	BC	WW	B	Class 2
Y53	56.230	-120.920	BC	WW	B	Class 2
Y54	56.230	-120.920	BC	WW	B	Class 2
Y55	56.230	-120.920	BC	WW	B	Class 2
Y56	56.230	-120.920	BC	WW	B	Class 2
Y57	56.230	-120.920	BC	WW	B	Class 2
Y58	56.230	-120.920	BC	WW	B	Class 2
Y59	56.230	-120.920	BC	WW	B	Class 2
Y60	56.230	-120.920	BC	WW	B	Class 2
Y61	56.230	-120.920	BC	WW	B	Class 2
Y62	56.230	-120.920	BC	WW	B	Class 2
Y63	56.230	-120.920	BC	WW	B	Class 2
Y64	56.230	-120.920	BC	WW	B	Class 2
Y65	56.230	-120.920	BC	WW	B	Class 2
Y66	56.230	-120.920	BC	WW	B	Class 2
Y67	56.230	-120.920	BC	WW	B	Class 2
Y68	56.230	-120.920	BC	WW	B	Class 2
Y69	56.230	-120.920	BC	WW	B	Class 2
Y70	56.230	-120.920	BC	WW	B	Class 2
CXL-670	56.250	-120.850	BC	WW	B	Class 2
CWC-233	56.280	-120.950	BC	WW	A	Class 2
CXI-955	56.280	-120.950	BC	WW	A	Class 2
SDU-697	57.430	-125.630	BC	WW	B	Class 2
UAM10338	60.000	-160.000	AK	WW	B	Class 2
KNP12	60.050	-137.500	YK	WW	B	Class 2
KNP17	60.050	-137.500	YK	WW	B	Class 2
PMY09	60.080	-128.220	YK	WW	B	Class 2
27607	60.100	-137.380	YK	WW	B	Class 2
41778	60.100	-137.380	YK	WW	B	Class 2
PMY41140	60.100	-137.380	YK	WW	B	Class 2
PMY41141	60.100	-137.380	YK	WW	B	Class 2
PMY41142	60.100	-137.380	YK	WW	B	Class 2
PMY41143	60.100	-137.380	YK	WW	B	Class 2
PMY41208	60.100	-137.380	YK	WW	B	Class 2
YT04	60.100	-137.380	YK	WW	B	Class 2
PMY41155	60.220	-132.130	YK	WW	B	Class 2
UAM15611	60.400	-150.330	AK	WW	B	Class 2
UAM15610	60.450	-150.530	AK	WW	B	Class 2
KNP09	60.480	-137.170	YK	WW	B	Class 2
KNP11	60.480	-137.170	YK	WW	B	Class 2
KNP18	60.480	-137.170	YK	WW	B	Class 2

KNP01	60.500	-137.620	YK	WW	B	Class 2
KNP02	60.500	-137.620	YK	WW	B	Class 2
KNP10	60.500	-137.620	YK	WW	B	Class 2
KNP15	60.500	-137.620	YK	WW	B	Class 2
KNP16	60.500	-137.620	YK	WW	B	Class 2
KNP23	60.650	-138.870	YK	WW	B	Class 2
KNP24	60.750	-139.500	YK	WW	B	Class 2
KNP25	60.750	-139.500	YK	WW	B	Class 2
KNP26	60.750	-139.500	YK	WW	B	Class 2
KNP41007	60.750	-139.500	YK	WW	B	Class 2
KNP41008	60.750	-139.500	YK	WW	B	Class 2
KNP41009	60.750	-139.500	YK	WW	B	Class 2
KNP41015	60.750	-139.500	YK	WW	B	Class 2
KNP41023	60.750	-139.500	YK	WW	B	Class 2
KNP41024	60.750	-139.500	YK	WW	B	Class 2
KNP41034	60.750	-139.500	YK	WW	B	Class 2
KNP41035	60.750	-139.500	YK	WW	B	Class 2
KNP41036	60.750	-139.500	YK	WW	B	Class 2
KNP41050	60.750	-139.500	YK	WW	B	Class 2
KNP41055	60.750	-139.500	YK	WW	B	Class 2
KNP41056	60.750	-139.500	YK	WW	E	Class 2
KNP41054	60.750	-137.500	YK	WW	B	Class 2
KNP41060	60.750	-137.500	YK	WW	B	Class 2
KNP41070	60.750	-137.500	YK	WW	B	Class 2
KNP41071	60.750	-137.500	YK	WW	B	Class 2
UAM15613	60.770	-150.500	AK	WW	B	Class 2
PMY41207	60.820	-137.430	YK	WW	B	Class 2
PMY41209	60.820	-137.430	YK	WW	B	Class 2
KNP19	60.830	-139.750	YK	WW	B	Class 2
KNP20	60.830	-139.750	YK	WW	B	Class 2
PMY41200	60.830	-139.750	YK	WW	B	Class 2
PMY41201	60.830	-139.750	YK	WW	B	Class 2
PMY41202	60.830	-139.750	YK	WW	B	Class 2
PMY41206	60.830	-139.750	YK	WW	B	Class 2
YT41100	60.830	-137.080	YK	WW	A	Class 2
KNP41025	60.830	-137.080	YK	WW	B	Class 2
KNP41026	60.830	-137.080	YK	WW	B	Class 2
KNP41027	60.830	-137.080	YK	WW	B	Class 2
KNP41057	60.830	-137.080	YK	WW	B	Class 2
KNP41058	60.830	-137.080	YK	WW	B	Class 2
KNP41059	60.830	-137.080	YK	WW	B	Class 2
KNP41061	60.830	-137.080	YK	WW	B	Class 2
KNP41069	60.830	-137.080	YK	WW	B	Class 2
KNP41072	60.830	-137.080	YK	WW	B	Class 2
YT41101	60.830	-137.080	YK	WW	B	Class 2
YT41102	60.830	-137.080	YK	WW	B	Class 2
YT41103	60.830	-137.080	YK	WW	B	Class 2
YT41105	60.830	-137.080	YK	WW	B	Class 2
YT41106	60.830	-137.080	YK	WW	B	Class 2
YT41107	60.830	-137.080	YK	WW	B	Class 2
PMY04	60.900	-135.200	YK	WW	B	Class 2

KNP07	60.950	-137.850	YK	WW	B	Class 2
KNP08	60.950	-137.850	YK	WW	B	Class 2
KNP41033	60.950	-137.850	YK	WW	B	Class 2
KNP41045	60.950	-137.850	YK	WW	B	Class 2
KNP41046	60.950	-137.850	YK	WW	B	Class 2
KNP41068	60.950	-137.850	YK	WW	B	Class 2
AF33503	61.067	-136.833	YK	WW	B	Class 2
AF33504	61.067	-136.833	YK	WW	B	Class 2
AF33505	61.067	-136.833	YK	WW	B	Class 2
AF33508	61.067	-136.833	YK	WW	B	Class 2
PMY41203	61.120	-136.580	YK	WW	B	Class 2
PMY41205	61.120	-136.580	YK	WW	B	Class 2
PMY41210	61.120	-136.580	YK	WW	B	Class 2
PMY41211	61.120	-136.580	YK	WW	B	Class 2
PMY41212	61.120	-136.580	YK	WW	B	Class 2
KNP41001	61.120	-136.370	YK	WW	B	Class 2
KNP41002	61.120	-136.370	YK	WW	B	Class 2
PMY41145	61.220	-136.950	YK	WW	B	Class 2
PMY41151	61.220	-136.950	YK	WW	B	Class 2
YT01	61.270	-136.930	YK	WW	B	Class 2
YT02	61.270	-136.930	YK	WW	B	Class 2
KNP41013	61.300	-140.100	YK	WW	B	Class 2
KNP41014	61.300	-140.100	YK	WW	B	Class 2
KNP41062	61.320	-138.670	YK	WW	B	Class 2
KNP41063	61.320	-138.670	YK	WW	B	Class 2
KNP21	61.420	-139.570	YK	WW	B	Class 2
KNP22	61.420	-139.570	YK	WW	B	Class 2
KNP03	61.430	-139.100	YK	WW	B	Class 2
PMY41153	61.430	-137.550	YK	WW	B	Class 2
PMY41154	61.430	-137.550	YK	WW	B	Class 2
KNP41003	61.450	-137.180	YK	WW	B	Class 2
KNP41004	61.450	-137.180	YK	WW	B	Class 2
KNP41005	61.450	-137.180	YK	WW	B	Class 2
KNP41006	61.450	-137.180	YK	WW	B	Class 2
KNP41064	61.470	-139.020	YK	WW	B	Class 2
KNP41065	61.470	-139.020	YK	WW	B	Class 2
KNP41028	61.550	-137.530	YK	WW	A	Class 2
KNP41040	61.550	-137.530	YK	WW	B	Class 2
KNP41041	61.550	-137.530	YK	WW	B	Class 2
KNP41016	61.570	-136.970	YK	WW	B	Class 2
KNP41017	61.570	-136.970	YK	WW	B	Class 2
PMY02	61.580	-130.120	YK	WW	B	Class 2
PMY41150	61.720	-137.500	YK	WW	B	Class 2
KNP41010	61.770	-139.230	YK	WW	B	Class 2
KNP41011	61.770	-139.230	YK	WW	B	Class 2
KNP41012	61.770	-139.230	YK	WW	B	Class 2
KNP41051	61.780	-138.930	YK	WW	B	Class 2
KNP41052	61.780	-138.930	YK	WW	B	Class 2
KNP41053	61.780	-138.930	YK	WW	B	Class 2
KNP41018	61.900	-137.780	YK	WW	B	Class 2
KNP41019	61.900	-137.780	YK	WW	B	Class 2

KNP41020	61.900	-137.780	YK	WW	B	Class 2
KNP41021	61.900	-137.780	YK	WW	B	Class 2
KNP41022	61.900	-137.780	YK	WW	B	Class 2
KNP41037	61.970	-137.180	YK	WW	B	Class 2
KNP41038	61.970	-137.180	YK	WW	B	Class 2
KNP41039	61.970	-137.180	YK	WW	B	Class 2
PMY01	61.970	-132.420	YK	WW	B	Class 2
KNP41067	62.080	-138.480	YK	WW	B	Class 2
PMY05	62.080	-136.150	YK	WW	B	Class 2
PMY06	62.080	-136.150	YK	WW	B	Class 2
PMY07	62.080	-136.150	YK	WW	B	Class 2
PMY08	62.080	-136.150	YK	WW	B	Class 2
PMY13	62.080	-136.150	YK	WW	B	Class 2
PMY10	62.300	-133.100	YK	WW	B	Class 2
PMY12	62.300	-133.100	YK	WW	B	Class 2
UAM10336	62.330	-145.150	AK	WW	B	Class 2
KNP41066	62.480	-139.470	YK	WW	B	Class 2
ARF01	62.830	-143.670	AK	WW	B	Class 2
ARF02	62.830	-143.670	AK	WW	B	Class 2
ARF03	62.830	-143.670	AK	WW	B	Class 2
PMY03	63.580	-135.830	YK	WW	B	Class 2
UAM28891	63.844	-148.580	AK	WW	B	Class 2
UAM46953	63.924	-147.829	AK	WW	B	Class 2
NW21	64.000	-128.000	MA	WW	B	Class 2
NW22	64.000	-128.000	MA	WW	B	Class 2
NW24	64.000	-128.000	MA	WW	B	Class 2
NW25	64.000	-128.000	MA	WW	B	Class 2
NW26	64.000	-128.000	MA	WW	B	Class 2
NW33	64.000	-128.000	MA	WW	B	Class 2
NW34	64.000	-128.000	MA	WW	B	Class 2
GQQ-362	64.050	-139.420	YK	WW	B	Class 2
UAM46959	64.115	-147.894	AK	WW	B	Class 2
UAM46949	64.132	-146.113	AK	WW	B	Class 2
UAM46979	64.221	-147.678	AK	WW	B	Class 2
UAM63629	64.250	-147.350	AK	WW	B	Class 2
UAM63747	64.250	-147.350	AK	WW	E	Class 2
UAM47431	64.333	-147.983	AK	WW	B	Class 2
UAM46969	64.371	-147.445	AK	WW	B	Class 2
ARF11	64.500	-158.000	AK	WW	B	Class 2
ARF12	64.500	-158.000	AK	WW	B	Class 2
ARF13	64.500	-158.000	AK	WW	E	Class 2
UAM63756	64.500	-149.000	AK	WW	B	Class 2
ARF10	64.670	-151.830	AK	WW	B	Class 2
UAM63628	64.700	-147.700	AK	WW	B	Class 2
NW01	64.900	-125.570	MA	WW	B	Class 2
NW09	64.900	-125.570	MA	WW	B	Class 2
ARF17	65.000	-152.000	AK	WW	B	Class 2
ARF09	65.000	-151.000	AK	WW	B	Class 2
KNP04	65.120	-140.520	YK	WW	A	Class 2
NW03	65.270	-126.820	MA	WW	B	Class 2
NW04	65.270	-126.820	MA	WW	B	Class 2

NW05	65.270	-126.820	MA	WW	B	Class 2
NW06	65.270	-126.820	MA	WW	B	Class 2
NW10	65.270	-126.820	MA	WW	B	Class 2
NW18	65.270	-126.820	MA	WW	B	Class 2
NW16	65.270	-126.820	MA	WW	D	Class 2
NW19	65.270	-126.820	MA	WW	D	Class 2
NW32	65.270	-126.820	MA	WW	D	Class 2
ARF19	66.000	-156.000	AK	WW	B	Class 2
ARF14	66.000	-149.000	AK	WW	B	Class 2
ARF15	66.000	-149.000	AK	WW	B	Class 2
ARF04	66.000	-143.000	AK	WW	B	Class 2
ARF06	66.500	-160.000	AK	WW	A	Class 2
ARF05	66.500	-160.000	AK	WW	B	Class 2
MP9214	66.500	-136.500	PO	WW	B	Class 2
MP9213	66.733	-136.283	PO	WW	B	Class 2
ARF08	66.830	-161.000	AK	WW	B	Class 2
MP9221	66.833	-136.300	PO	WW	B	Class 2
ARF16	67.000	-160.000	AK	WW	B	Class 2
ARF07	67.000	-158.000	AK	WW	B	Class 2
IN9202	67.050	-136.500	PO	WW	B	Class 2
MP9218	67.050	-136.267	PO	WW	B	Class 2
MP9219	67.050	-136.267	PO	WW	B	Class 2
MP9217	67.050	-136.250	PO	WW	B	Class 2
MP9220	67.050	-136.250	PO	WW	B	Class 2
MP9224	67.050	-136.250	PO	WW	B	Class 2
MP9216	67.050	-136.250	PO	WW	E	Class 2
MP9211	67.067	-136.150	PO	WW	B	Class 2
MP9207	67.083	-136.133	PO	WW	B	Class 2
MP9215	67.100	-136.117	PO	WW	B	Class 2
MP9201	67.117	-136.117	PO	WW	B	Class 2
MP9202	67.117	-136.117	PO	WW	B	Class 2
MP9222	67.117	-136.000	PO	WW	B	Class 2
MP9223	67.117	-134.750	PO	WW	B	Class 2
MP9204	67.133	-136.100	PO	WW	A	Class 2
MP9203	67.133	-136.083	PO	WW	B	Class 2
MP9206	67.150	-137.117	PO	WW	B	Class 2
MP9208	67.150	-136.333	PO	WW	B	Class 2
MP9205	67.150	-136.117	PO	WW	B	Class 2
MP9209	67.200	-136.050	PO	WW	B	Class 2
MP9210	67.217	-136.050	PO	WW	B	Class 2
MP9212	67.450	-134.917	PO	WW	E	Class 2
MP9301	67.667	-134.833	PO	WW	A	Class 3
AK9230	67.950	-135.750	PO	WW	B	Class 2
AK9232	67.950	-135.750	PO	WW	B	Class 2
AK9233	67.950	-135.750	PO	WW	B	Class 2
AK8909	67.950	-135.533	PO	WW	B	Class 2
AK9305	67.950	-135.533	PO	WW	B	Class 2
AK9306	67.950	-135.533	PO	WW	B	Class 2
AK9228	68.133	-135.883	PO	WW	A	Class 2
AK8902	68.133	-135.883	PO	WW	B	Class 2
AK8904	68.133	-135.883	PO	WW	B	Class 2

AK9202	68.133	-135.883	PO	WW	B	Class 2
AK9210	68.133	-135.883	PO	WW	B	Class 2
AK9211	68.133	-135.883	PO	WW	B	Class 2
AK9221	68.133	-135.883	PO	WW	B	Class 2
AK9222	68.133	-135.883	PO	WW	B	Class 2
AK9223	68.133	-135.883	PO	WW	B	Class 2
AK9224	68.133	-135.883	PO	WW	B	Class 2
AK9302	68.133	-135.883	PO	WW	B	Class 2
AK9304	68.167	-135.883	PO	WW	B	Class 2
AK8905	68.200	-135.167	PO	WW	B	Class 2
AK8901	68.217	-135.883	PO	WW	B	Class 2
AK8906	68.217	-135.883	PO	WW	B	Class 2
AK9201	68.217	-135.883	PO	WW	B	Class 2
AK9207	68.217	-135.883	PO	WW	B	Class 2
AK9208	68.217	-135.883	PO	WW	B	Class 2
AK9209	68.217	-135.883	PO	WW	B	Class 2
AK9218	68.217	-135.883	PO	WW	B	Class 2
AK9219	68.217	-135.883	PO	WW	B	Class 2
AK9220	68.217	-135.883	PO	WW	B	Class 2
AK9229	68.217	-135.883	PO	WW	B	Class 2
AK9235	68.217	-135.883	PO	WW	B	Class 2
AK93JM	68.217	-135.883	PO	WW	B	Class 2
AK8903	68.300	-135.800	PO	WW	B	Class 2
AK9212	68.300	-135.800	PO	WW	B	Class 2
AK9213	68.300	-135.800	PO	WW	B	Class 2
AK9214	68.300	-135.800	PO	WW	B	Class 2
AK9215	68.300	-135.800	PO	WW	B	Class 2
AK9225	68.300	-135.800	PO	WW	B	Class 2
AK9231	68.300	-135.800	PO	WW	B	Class 2
AK9301	68.350	-135.367	PO	WW	B	Class 2
AK9303	68.417	-136.000	PO	WW	B	Class 2
AK9204	68.917	-137.333	PO	WW	B	Class 2
AK9206	68.917	-137.333	PO	WW	B	Class 2
AK9217	68.917	-137.333	PO	WW	B	Class 2
AK9203	68.917	-137.333	PO	WW	E	Class 2
AK9205	68.917	-137.333	PO	WW	E	Class 2
PBQ-943	54.130	-108.430	SK	FO	A	Class 1
CVV-658	54.150	-115.680	AB	FO	A	Class 1
CWE-317	54.150	-115.680	AB	FO	A	Class 1
CWE-348	54.150	-115.680	AB	FO	A	Class 1
CVZ-118	54.150	-113.870	AB	FO	A	Class 1
CWF-159	54.150	-113.870	AB	FO	B	Class 1
CVU-850	54.270	-110.730	AB	FO	A	Class 1
CVU-851	54.270	-110.730	AB	FO	A	Class 1
CVV-208	54.270	-110.730	AB	FO	A	Class 1
CVX-108	54.270	-110.730	AB	FO	A	Class 1
CVX-109	54.270	-110.730	AB	FO	A	Class 1
CVZ-098	54.270	-110.730	AB	FO	A	Class 1
GJS-017	54.270	-110.730	AB	FO	A	Class 1
CUX-352	54.330	-110.480	AB	FO	A	Class 1
CVX-351	54.330	-110.480	AB	FO	A	Class 1

CVX-353	54.330	-110.480	AB	FO	A	Class 1
GJT-330	54.330	-110.480	AB	FO	E	Class 1
BSM-158	54.330	-109.770	SK	FO	A	Class 1
BSM-159	54.330	-109.770	SK	FO	A	Class 1
GQT-672	54.330	-109.770	SK	FO	A	Class 1
GWK-247	54.330	-109.770	SK	FO	A	Class 1
PBQ-864	54.330	-109.770	SK	FO	A	Class 1
PBT-197	54.330	-109.770	SK	FO	A	Class 1
RGH-655	54.330	-109.770	SK	FO	A	Class 1
CWC-082	54.450	-110.920	AB	FO	A	Class 1
GPX-042	54.550	-94.470	MB	FO	A	Class 1
PBO-563	54.580	-101.370	MB	FO	A	Class 1
PBO-564	54.580	-101.370	MB	FO	A	Class 1
PBO-778	54.580	-101.370	MB	FO	A	Class 1
GQQ-553	54.580	-101.370	MB	FO	E	Class 1
BMT-928	54.620	-97.770	MB	FO	A	Class 1
BMT-927	54.620	-97.770	MB	FO	D	Class 1
CWA-676	54.680	-112.220	AB	FO	A	Class 1
CWF-200	54.720	-115.400	AB	FO	A	Class 1
CWF-201	54.720	-115.400	AB	FO	A	Class 1
CWF-202	54.720	-115.400	AB	FO	A	Class 1
CWF-203	54.720	-115.400	AB	FO	A	Class 1
CVV-814	54.720	-113.280	AB	FO	A	Class 1
CVZ-588	54.720	-113.280	AB	FO	A	Class 1
CWD-016	54.720	-113.280	AB	FO	A	Class 1
CVV-813	54.720	-113.280	AB	FO	B	Class 1
CVY-194	54.770	-111.970	AB	FO	A	Class 1
BMD-395	54.770	-101.850	MB	FO	A	Class 1
BMP-291	54.770	-101.850	MB	FO	A	Class 1
PBC-794	54.770	-101.850	MB	FO	A	Class 1
PBC-795	54.770	-101.850	MB	FO	A	Class 1
BMO-688	54.770	-101.850	MB	FO	D	Class 1
CVZ-649	54.820	-112.550	AB	FO	A	Class 1
CWB-560	54.850	-112.320	AB	FO	A	Class 1
GJX-713	54.850	-112.320	AB	FO	A	Class 1
GJZ-078	54.850	-112.320	AB	FO	A	Class 1
BM8-008	54.900	-98.620	MB	FO	A	Class 1
PSI-792	54.900	-98.620	MB	FO	F	Class 1
WMB03-23	54.930	-95.250	MB	FO	A	Class 1
WMB03-26	54.930	-95.250	MB	FO	A	Class 1
WMB03-25	54.930	-95.250	MB	FO	E	Class 1
CWB-685	55.070	-114.030	AB	FO	A	Class 1
CWB-717	55.070	-114.030	AB	FO	A	Class 1
BRZ-851	55.100	-105.280	SK	FO	A	Class 6
BRZ-852	55.100	-105.280	SK	FO	A	Class 6
BSE-448	55.100	-105.280	SK	FO	A	Class 6
BSK-931	55.100	-105.280	SK	FO	A	Class 6
BSK-933	55.100	-105.280	SK	FO	A	Class 6
BSK-937	55.100	-105.280	SK	FO	A	Class 6
BSK-938	55.100	-105.280	SK	FO	A	Class 6
BSK-939	55.100	-105.280	SK	FO	A	Class 6

DGW-700	55.100	-105.280	SK	FO	A	Class 6
DGW-739	55.100	-105.280	SK	FO	A	Class 6
DGW-802	55.100	-105.280	SK	FO	A	Class 6
DGW-887	55.100	-105.280	SK	FO	A	Class 6
GWC-809	55.100	-105.280	SK	FO	A	Class 6
GWC-814	55.100	-105.280	SK	FO	A	Class 6
GWC-833	55.100	-105.280	SK	FO	A	Class 6
GWC-836	55.100	-105.280	SK	FO	A	Class 6
GWX-929	55.100	-105.280	SK	FO	A	Class 6
BSK-935	55.100	-105.280	SK	FO	B	Class 6
GWC-803	55.100	-105.280	SK	FO	B	Class 6
BSB-933	55.100	-105.280	SK	FO	D	Class 6
BSK-932	55.100	-105.280	SK	FO	D	Class 6
DGW-692	55.100	-105.280	SK	FO	D	Class 6
DGW-699	55.100	-105.280	SK	FO	D	Class 6
GWC-799	55.100	-105.280	SK	FO	D	Class 6
GWC-852	55.100	-105.280	SK	FO	D	Class 6
GWC-861	55.100	-105.280	SK	FO	D	Class 6
GWK-708	55.100	-105.280	SK	FO	D	Class 6
GWM-807	55.100	-105.280	SK	FO	D	Class 6
GWM-811	55.100	-105.280	SK	FO	D	Class 6
RDX-895	55.100	-105.280	SK	FO	D	Class 6
BRZ-850	55.100	-105.280	SK	FO	E	Class 6
BRZ-853	55.100	-105.280	SK	FO	E	Class 6
BSK-940	55.100	-105.280	SK	FO	E	Class 6
DGW-688	55.100	-105.280	SK	FO	E	Class 6
DGW-694	55.100	-105.280	SK	FO	E	Class 6
DGW-701	55.100	-105.280	SK	FO	E	Class 6
DGW-786	55.100	-105.280	SK	FO	E	Class 6
DGW-787	55.100	-105.280	SK	FO	E	Class 6
DGW-835	55.100	-105.280	SK	FO	E	Class 6
DGW-837	55.100	-105.280	SK	FO	E	Class 6
DGW-883	55.100	-105.280	SK	FO	E	Class 6
GWC-780	55.100	-105.280	SK	FO	E	Class 6
GWC-785	55.100	-105.280	SK	FO	E	Class 6
GWC-787	55.100	-105.280	SK	FO	E	Class 6
GWC-788	55.100	-105.280	SK	FO	E	Class 6
GWC-790	55.100	-105.280	SK	FO	E	Class 6
GWC-791	55.100	-105.280	SK	FO	E	Class 6
GWC-796	55.100	-105.280	SK	FO	E	Class 6
GWC-797	55.100	-105.280	SK	FO	E	Class 6
GWC-802	55.100	-105.280	SK	FO	E	Class 6
GWC-805	55.100	-105.280	SK	FO	E	Class 6
GWC-810	55.100	-105.280	SK	FO	E	Class 6
GWC-818	55.100	-105.280	SK	FO	E	Class 6
GWC-821	55.100	-105.280	SK	FO	E	Class 6
GWC-843	55.100	-105.280	SK	FO	E	Class 6
GWC-845	55.100	-105.280	SK	FO	E	Class 6
GWC-848	55.100	-105.280	SK	FO	E	Class 6
GWC-857	55.100	-105.280	SK	FO	E	Class 6
GWC-862	55.100	-105.280	SK	FO	E	Class 6

GWD-057	55.100	-105.280	SK	FO	E	Class 6
GWD-059	55.100	-105.280	SK	FO	E	Class 6
GWD-066	55.100	-105.280	SK	FO	E	Class 6
GWD-068	55.100	-105.280	SK	FO	E	Class 6
GWD-073	55.100	-105.280	SK	FO	E	Class 6
GWD-074	55.100	-105.280	SK	FO	E	Class 6
GWK-478	55.100	-105.280	SK	FO	E	Class 6
GWK-710	55.100	-105.280	SK	FO	E	Class 6
GWK-711	55.100	-105.280	SK	FO	E	Class 6
GWK-715	55.100	-105.280	SK	FO	E	Class 6
GWK-716	55.100	-105.280	SK	FO	E	Class 6
GWK-719	55.100	-105.280	SK	FO	E	Class 6
GWK-720	55.100	-105.280	SK	FO	E	Class 6
GWK-721	55.100	-105.280	SK	FO	E	Class 6
GWK-723	55.100	-105.280	SK	FO	E	Class 6
GWK-724	55.100	-105.280	SK	FO	E	Class 6
GWK-728	55.100	-105.280	SK	FO	E	Class 6
GWK-735	55.100	-105.280	SK	FO	E	Class 6
GWK-736	55.100	-105.280	SK	FO	E	Class 6
GWK-737	55.100	-105.280	SK	FO	E	Class 6
GWK-743	55.100	-105.280	SK	FO	E	Class 6
GWK-745	55.100	-105.280	SK	FO	E	Class 6
GWM-778	55.100	-105.280	SK	FO	E	Class 6
GWM-781	55.100	-105.280	SK	FO	E	Class 6
GWM-783	55.100	-105.280	SK	FO	E	Class 6
GWM-784	55.100	-105.280	SK	FO	E	Class 6
GWM-785	55.100	-105.280	SK	FO	E	Class 6
GWM-800	55.100	-105.280	SK	FO	E	Class 6
GWM-805	55.100	-105.280	SK	FO	E	Class 6
GWM-806	55.100	-105.280	SK	FO	E	Class 6
GWM-813	55.100	-105.280	SK	FO	E	Class 6
GWM-824	55.100	-105.280	SK	FO	E	Class 6
GWM-826	55.100	-105.280	SK	FO	E	Class 6
RDX-896	55.100	-105.280	SK	FO	E	Class 6
RDX-897	55.100	-105.280	SK	FO	E	Class 6
RGD-855	55.100	-105.280	SK	FO	E	Class 6
BSK-936	55.100	-105.280	SK	FO	F	Class 6
AXZ-514	55.120	-116.870	AB	FO	A	Class 1
AXZ-515	55.120	-116.870	AB	FO	A	Class 1
SDU-275	55.120	-116.870	AB	FO	A	Class 1
AXZ-516	55.120	-116.870	AB	FO	B	Class 1
GJV-259	55.120	-116.870	AB	FO	B	Class 1
SDR-504	55.170	-118.800	AB	FO	A	Class 1
SDS-277	55.170	-118.800	AB	FO	A	Class 1
GOP-360	55.170	-108.150	SK	FO	E	Class 1
GWI-032	55.220	-106.400	SK	FO	A	Class 1
CWE-303	55.280	-114.770	AB	FO	A	Class 1
CVV-574	55.320	-115.630	AB	FO	A	Class 1
CWF-004	55.320	-115.630	AB	FO	A	Class 1
CWE-093	55.320	-115.630	AB	FO	B	Class 1
BRW-382	55.420	-104.550	SK	FO	A	Class 1

BRW-383	55.420	-104.550	SK	FO	A	Class 1
BSE-168	55.420	-104.550	SK	FO	A	Class 1
BSM-305	55.420	-104.550	SK	FO	A	Class 1
BMA-405	55.520	-106.570	SK	FO	A	Class 1
WMB03-17	55.530	-103.280	SK	FO	A	Class 1
BMK-399	55.580	-97.150	MB	FO	A	Class 1
BMK-400	55.580	-97.150	MB	FO	A	Class 1
BMP-505	55.580	-97.150	MB	FO	A	Class 1
BMP-506	55.580	-97.150	MB	FO	A	Class 1
GPN-107	55.580	-97.150	MB	FO	A	Class 1
BMK-397	55.580	-97.150	MB	FO	D	Class 1
BMK-398	55.580	-97.150	MB	FO	F	Class 1
PAZ-024	55.580	-97.150	MB	FO	F	Class 1
GQM-277	55.730	-97.150	MB	FO	A	Class 1
PBL-756	55.730	-97.150	MB	FO	A	Class 1
PBL-758	55.730	-97.150	MB	FO	A	Class 1
PBL-759	55.730	-97.150	MB	FO	A	Class 1
PBL-761	55.730	-97.150	MB	FO	A	Class 1
PBL-762	55.730	-97.150	MB	FO	A	Class 1
PBL-763	55.730	-97.150	MB	FO	A	Class 1
PBO-254	55.730	-97.150	MB	FO	A	Class 1
PBL-757	55.730	-97.150	MB	FO	F	Class 1
PBL-760	55.730	-97.150	MB	FO	F	Class 1
PBQ-456	55.730	-97.150	MB	FO	F	Class 1
GRR-734	55.730	-97.150	MB	FO	G	Class 1
WMB03-08	55.750	-101.180	MB	FO	A	Class 1
SDT-680	55.780	-118.830	AB	FO	A	Class 1
WMB03-05	55.780	-98.880	MB	FO	A	Class 1
WMB03-10	55.780	-98.880	MB	FO	A	Class 1
WMB03-14	55.780	-98.880	MB	FO	A	Class 1
WMB03-15	55.780	-98.880	MB	FO	A	Class 1
WMB03-22	55.780	-98.880	MB	FO	A	Class 1
BSJ-430	55.850	-108.480	SK	FO	A	Class 1
GQV-446	55.850	-108.480	SK	FO	A	Class 1
PBS-483	55.850	-108.480	SK	FO	A	Class 1
PBS-484	55.850	-108.480	SK	FO	A	Class 1
PBS-485	55.850	-108.480	SK	FO	A	Class 1
PBS-487	55.850	-108.480	SK	FO	A	Class 1
PBS-488	55.850	-108.480	SK	FO	A	Class 1
PBS-489	55.850	-108.480	SK	FO	A	Class 1
PBR-282	55.850	-108.480	SK	FO	B	Class 1
CWE-920	55.950	-113.770	AB	FO	A	Class 1
GJG-214	55.950	-113.770	AB	FO	A	Class 1
K34997	55.950	-113.770	AB	FO	A	Class 1
AXI-897	55.980	-87.630	MB	FO	A	Class 1
AXI-898	55.980	-87.630	MB	FO	A	Class 1
WMB03-09	56.010	-95.820	MB	FO	A	Class 1
WMB03-07	56.020	-95.820	MB	FO	A	Class 1
WMB03-12	56.020	-95.820	MB	FO	A	Class 1
WMB03-18	56.020	-95.820	MB	FO	A	Class 1
WMB03-20	56.170	-102.250	SK	FO	A	Class 1

WMB03-21	56.170	-102.250	SK	FO	A	Class 1
SDR-652	56.250	-118.600	AB	FO	A	Class 1
SDR-653	56.250	-118.600	AB	FO	A	Class 1
SDR-654	56.250	-118.600	AB	FO	A	Class 1
SDR-655	56.250	-118.600	AB	FO	A	Class 1
SDR-656	56.250	-118.600	AB	FO	A	Class 1
SDR-657	56.250	-118.600	AB	FO	A	Class 1
SDR-658	56.250	-118.600	AB	FO	A	Class 1
SDR-659	56.250	-118.600	AB	FO	A	Class 1
PBG-652	56.450	-94.200	MB	FO	A	Class 1
WMB03-19	56.450	-94.200	MB	FO	A	Class 1
PBG-651	56.450	-94.200	MB	FO	F	Class 1
BMH-590	56.470	-99.750	MB	FO	A	Class 1
BMH-591	56.470	-99.750	MB	FO	A	Class 1
BMH-592	56.470	-99.750	MB	FO	A	Class 1
BMJ-462	56.470	-99.750	MB	FO	A	Class 1
BMJ-463	56.470	-99.750	MB	FO	A	Class 1
PBN-658	56.470	-99.750	MB	FO	A	Class 1
BMJ-461	56.470	-99.750	MB	FO	E	Class 1
PBQ-285	56.480	-109.430	SK	FO	A	Class 1
PBQ-286	56.480	-109.430	SK	FO	A	Class 1
AXS-674	56.530	-117.670	AB	FO	B	Class 1
SDV-409	56.730	-111.380	AB	FO	A	Class 1
BLH-495	56.770	-98.920	MB	FO	A	Class 1
BLH-496	56.770	-98.920	MB	FO	A	Class 1
PSM-580	56.770	-98.920	MB	FO	A	Class 1
PBD-195	56.820	-101.070	MB	FO	A	Class 1
PBN-869	56.820	-101.070	MB	FO	A	Class 1
PBN-870	56.820	-101.070	MB	FO	A	Class 1
PBN-871	56.820	-101.070	MB	FO	A	Class 1
PBN-872	56.820	-101.070	MB	FO	A	Class 1
GDE-773	56.820	-101.070	MB	FO	E	Class 1
WMB03-13	57.080	-102.020	SK	FO	E	Class 6
SDU-369	58.050	-116.350	AB	FO	A	Class 1
SDU-370	58.050	-116.350	AB	FO	B	Class 1
UYQ-264G	58.180	-116.400	AB	FO	B	Class 1
W98	60.020	-111.540	AB	FO	A	Class 1
W99	60.250	-113.000	AB	FO	A	Class 1
W97	61.104	-116.498	AB	FO	A	Class 1
NW29	66.250	-128.630	BW	WB	B	Class 3
NW30	66.250	-128.630	BW	WB	B	Class 3
NW27	66.250	-128.630	BW	WB	D	Class 3
NW28	66.250	-128.630	BW	WB	D	Class 3
NW31	66.250	-128.630	BW	WB	D	Class 3
FG8905	66.250	-128.617	BW	WB	A	Class 3
FG8904	66.283	-128.617	BW	WB	D	Class 3
FG8902	66.283	-128.533	BW	WB	D	Class 3
FG9301	66.350	-126.583	BW	WB	A	Class 3
CO9204	66.883	-126.250	BW	WB	A	Class 3
CO9205	66.883	-126.250	BW	WB	D	Class 3
CO9206	66.883	-126.250	BW	WB	D	Class 3

FG8901	66.983	-126.400	BW	WB	B	Class 3
NW08	67.030	-126.120	BW	WB	A	Class 3
NW07	67.030	-126.120	BW	WB	D	Class 3
NW23	67.030	-126.120	BW	WB	E	Class 3
CO9304	67.050	-126.033	BW	WB	A	Class 3
CO9301	67.050	-126.033	BW	WB	D	Class 3
CO9302	67.050	-126.033	BW	WB	D	Class 3
CO9303	67.050	-126.033	BW	WB	D	Class 3
CO9305	67.050	-126.033	BW	WB	D	Class 3
FG9201	67.167	-126.000	BW	WB	D	Class 3
FG9202	67.167	-126.000	BW	WB	D	Class 3
CO9201	67.167	-125.167	BW	WB	B	Class 3
CO9202	67.167	-125.167	BW	WB	E	Class 3
CO9203	67.167	-125.167	BW	WB	E	Class 3
IN9315	67.567	-133.667	CB	WB	A	Class 3
IN9308	67.967	-133.167	CB	WB	D	Class 3
IN9314	68.000	-132.917	CB	WB	A	Class 3
IN9317	68.000	-132.917	CB	WB	B	Class 3
IN9316	68.000	-132.917	CB	WB	D	Class 3
IN9319	68.000	-132.917	CB	WB	D	Class 3
IN9313	68.000	-132.917	CB	WB	F	Class 3
IN9312	68.117	-132.667	CB	WB	D	Class 3
IN9201	68.167	-132.833	CB	WB	B	Class 3
IN8903	68.200	-131.500	CB	WB	D	Class 3
IN8904	68.200	-131.500	CB	WB	D	Class 3
PA9301	68.267	-125.500	BW	WB	D	Class 3
PA9302	68.267	-125.500	BW	WB	D	Class 3
PA9303	68.267	-125.500	BW	WB	D	Class 3
PA9304	68.267	-125.500	BW	WB	D	Class 3
PA9306	68.267	-125.500	BW	WB	D	Class 3
PA9201	68.267	-125.500	BW	WB	E	Class 3
PA9202	68.267	-125.500	BW	WB	E	Class 3
PA9203	68.267	-125.500	BW	WB	E	Class 3
PA9204	68.267	-125.500	BW	WB	E	Class 3
PA9305	68.267	-125.500	BW	WB	E	Class 3
IN9213	68.283	-127.250	BW	WB	D	Class 3
IN9214	68.283	-127.250	BW	WB	D	Class 3
IN9215	68.283	-127.250	BW	WB	D	Class 3
IN9217	68.283	-127.250	BW	WB	D	Class 3
IN9218	68.283	-127.250	BW	WB	D	Class 3
IN9219	68.283	-127.250	BW	WB	D	Class 3
IN9220	68.283	-127.250	BW	WB	D	Class 3
IN9222	68.283	-127.250	BW	WB	D	Class 3
IN9216	68.283	-127.250	BW	WB	E	Class 3
IN9221	68.283	-127.250	BW	WB	E	Class 3
IN9305	68.500	-132.667	CB	WB	D	Class 3
IN9318	68.517	-133.633	CB	WB	D	Class 3
IN8906	68.583	-133.583	CB	WB	E	Class 3
IN9303	68.583	-133.167	CB	WB	B	Class 3
IN9304	68.583	-133.167	CB	WB	D	Class 3
PA0189	68.633	-125.167	BW	WB	A	Class 3

PA0389	68.633	-125.167	BW	WB	A	Class 3
PA0289	68.633	-125.167	BW	WB	D	Class 3
PA0489	68.633	-125.167	BW	WB	D	Class 3
PA0889	68.633	-125.167	BW	WB	D	Class 3
PA0589	68.633	-125.167	BW	WB	E	Class 3
PA0789	68.633	-125.167	BW	WB	E	Class 3
PA0989	68.633	-125.167	BW	WB	E	Class 3
PA1189	68.633	-125.167	BW	WB	E	Class 3
IN9301	68.667	-133.783	CB	WB	D	Class 3
TU9372	68.667	-132.833	CB	WB	E	Class 3
IN9306	68.700	-134.167	CB	WB	A	Class 3
CHA35	68.717	-134.117	CB	WB	D	Class 3
TU9331	68.717	-133.250	CB	WB	D	Class 3
TU9366	68.717	-132.833	CB	WB	B	Class 3
TU9368	68.717	-132.833	CB	WB	B	Class 3
TU9367	68.717	-132.833	CB	WB	D	Class 3
TU8901	68.733	-129.550	CB	WB	D	Class 3
TU9230	68.733	-129.550	CB	WB	D	Class 3
TU9228	68.733	-129.533	CB	WB	A	Class 3
IN9307	68.750	-133.333	CB	WB	B	Class 3
PA1389	68.750	-124.917	BW	WB	D	Class 3
PA1489	68.750	-124.917	BW	WB	D	Class 3
PA1589	68.750	-124.917	BW	WB	D	Class 3
PA1689	68.750	-124.917	BW	WB	D	Class 3
PA1789	68.750	-124.917	BW	WB	D	Class 3
PA2189	68.750	-124.917	BW	WB	D	Class 3
PA2289	68.750	-124.917	BW	WB	D	Class 3
PA2489	68.750	-124.917	BW	WB	D	Class 3
PA1989	68.750	-124.917	BW	WB	E	Class 3
PA2389	68.750	-124.917	BW	WB	E	Class 3
TU9231	68.817	-132.500	CB	WB	A	Class 3
TU9290	68.833	-133.000	CB	WB	A	Class 3
TU9291	68.833	-133.000	CB	WB	C	Class 3
TU9289	68.833	-133.000	CB	WB	D	Class 3
TU9348	68.833	-133.000	CB	WB	D	Class 3
IN9309	68.833	-128.500	CB	WB	D	Class 3
TU9370	68.867	-133.467	CB	WB	D	Class 3
TU9282	68.867	-133.450	CB	WB	A	Class 3
TU9359	68.867	-133.000	CB	WB	D	Class 3
TU9240	68.867	-127.000	BW	WB	D	Class 3
BJ-004	68.880	-126.950	BW	WB	C	Class 3
BJ-005	68.880	-126.950	BW	WB	E	Class 3
BJ-006	68.880	-126.950	BW	WB	E	Class 3
IN9302	68.883	-134.167	CB	WB	A	Class 3
TU9360	68.883	-132.583	CB	WB	D	Class 3
TU9324	68.900	-133.417	CB	WB	D	Class 3
TU9326	68.900	-133.417	CB	WB	D	Class 3
TU9273	68.900	-132.417	CB	WB	D	Class 3
TU9274	68.900	-132.417	CB	WB	D	Class 3
TU9275	68.900	-132.333	CB	WB	D	Class 3
TU9288	68.917	-132.667	CB	WB	B	Class 3

TU9271	68.917	-132.667	CB	WB	F	Class 3
TU9285	68.917	-131.967	CB	WB	D	Class 3
TU9213	68.933	-132.750	CB	WB	D	Class 3
TU9214	68.933	-132.750	CB	WB	D	Class 3
TU9209	68.933	-132.083	CB	WB	D	Class 3
TU9210	68.933	-132.083	CB	WB	D	Class 3
TU9321	68.950	-134.133	CB	WB	A	Class 3
TU9322	68.950	-134.133	CB	WB	A	Class 3
TU9320	68.950	-134.133	CB	WB	B	Class 3
TU9266	68.950	-133.667	CB	WB	D	Class 3
TU9242	68.950	-132.167	CB	WB	D	Class 3
TU9243	68.950	-132.167	CB	WB	D	Class 3
TU9276	68.950	-132.167	CB	WB	D	Class 3
TU9277	68.950	-132.167	CB	WB	D	Class 3
TU9283	68.950	-132.167	CB	WB	D	Class 3
TU9284	68.950	-132.167	CB	WB	D	Class 3
TU9262	68.950	-132.117	CB	WB	D	Class 3
TU9344	68.967	-132.533	CB	WB	D	Class 3
TU9345	68.967	-132.533	CB	WB	D	Class 3
TU9369	68.967	-132.533	CB	WB	F	Class 3
TU9212	69.000	-134.000	CB	WB	D	Class 3
TU9330	69.000	-133.617	CB	WB	D	Class 3
TU9341	69.000	-133.383	CB	WB	D	Class 3
TU9340	69.000	-133.383	CB	WB	E	Class 3
TU9347	69.000	-132.500	CB	WB	D	Class 3
TU9272	69.000	-132.417	CB	WB	D	Class 3
TU9227	69.000	-128.417	CB	WB	B	Class 3
TU9225	69.000	-128.417	CB	WB	D	Class 3
TU9226	69.000	-128.417	CB	WB	D	Class 3
TU9280	69.033	-132.250	CB	WB	D	Class 3
TU9281	69.033	-132.250	CB	WB	D	Class 3
TU9278	69.033	-131.950	CB	WB	B	Class 3
TU9224	69.067	-132.000	CB	WB	A	Class 3
TU9333	69.083	-133.167	CB	WB	A	Class 3
TU9334	69.083	-133.167	CB	WB	A	Class 3
TU9335	69.083	-133.167	CB	WB	D	Class 3
TU9319	69.083	-133.150	CB	WB	B	Class 3
TU9223	69.083	-132.583	CB	WB	D	Class 3
TU9346	69.100	-133.533	CB	WB	E	Class 3
TU9287	69.100	-132.000	CB	WB	A	Class 3
TU9286	69.100	-132.000	CB	WB	D	Class 3
TU9361	69.100	-131.250	CB	WB	D	Class 3
TU9362	69.100	-131.250	CB	WB	D	Class 3
TU9343	69.133	-134.333	CB	WB	A	Class 3
TU9342	69.133	-133.800	CB	WB	D	Class 3
TU9303	69.133	-133.350	CB	WB	D	Class 3
TU9311	69.133	-133.350	CB	WB	D	Class 3
TU9312	69.133	-133.350	CB	WB	D	Class 3
TU9302	69.133	-133.350	CB	WB	E	Class 3
TU9327	69.133	-131.250	CB	WB	B	Class 3
TU9329	69.133	-131.250	CB	WB	D	Class 3

TU9307	69.150	-133.650	CB	WB	B	Class 3
TU9309	69.150	-133.650	CB	WB	B	Class 3
TU9310	69.150	-133.650	CB	WB	B	Class 3
TU9314	69.150	-133.650	CB	WB	B	Class 3
TU9304	69.150	-133.650	CB	WB	D	Class 3
TU9305	69.150	-133.650	CB	WB	D	Class 3
TU9306	69.150	-133.650	CB	WB	D	Class 3
TU9308	69.150	-133.650	CB	WB	D	Class 3
TU9313	69.150	-133.650	CB	WB	D	Class 3
TU9315	69.150	-133.650	CB	WB	D	Class 3
TU9318	69.150	-133.650	CB	WB	D	Class 3
TU9316	69.150	-133.650	CB	WB	E	Class 3
TU9317	69.150	-133.650	CB	WB	E	Class 3
PA9206	69.150	-124.100	BW	WB	A	Class 3
PA9208	69.150	-124.100	BW	WB	D	Class 3
PA9207	69.150	-124.100	BW	WB	E	Class 3
TU9265	69.167	-132.500	CB	WB	D	Class 3
PA9205	69.167	-124.150	BW	WB	D	Class 3
TU9323	69.200	-132.000	CB	WB	D	Class 3
TU9336	69.217	-132.500	CB	WB	D	Class 3
TU9263	69.217	-131.333	CB	WB	D	Class 3
TU9264	69.217	-131.333	CB	WB	D	Class 3
TU9207	69.250	-131.333	CB	WB	D	Class 3
TU9208	69.250	-131.333	CB	WB	D	Class 3
TU9353	69.267	-132.700	CB	WB	D	Class 3
TU9354	69.267	-132.700	CB	WB	D	Class 3
TU9355	69.267	-132.700	CB	WB	E	Class 3
TU9332	69.283	-132.583	CB	WB	D	Class 3
TU9349	69.300	-134.283	CB	WB	F	Class 2
TU9268	69.300	-133.583	CB	WB	A	Class 3
TU9269	69.300	-133.583	CB	WB	A	Class 3
TU9270	69.300	-133.583	CB	WB	D	Class 3
IN9310	69.333	-133.167	CB	WB	A	Class 3
TU9220	69.333	-133.000	CB	WB	B	Class 3
TU9221	69.333	-133.000	CB	WB	E	Class 3
TU9337	69.333	-132.800	CB	WB	D	Class 3
TU9339	69.333	-132.800	CB	WB	D	Class 3
TU9338	69.333	-132.800	CB	WB	E	Class 3
TU9239	69.333	-131.500	CB	WB	B	Class 3
TU9371	69.333	-129.000	CB	WB	E	Class 3
TU9301	69.367	-134.167	CB	WB	B	Class 2
TU9211	69.367	-130.850	CB	WB	B	Class 3
TU9350	69.450	-134.550	CB	WB	B	Class 2
TU9201	69.500	-133.667	CB	WB	B	Class 2
TU9203	69.500	-133.667	CB	WB	B	Class 2
TU9204	69.500	-133.667	CB	WB	B	Class 2
TU9205	69.500	-133.667	CB	WB	B	Class 2
TU9206	69.500	-133.667	CB	WB	B	Class 2
TU9236	69.500	-130.800	CB	WB	D	Class 3
TU9237	69.500	-130.800	CB	WB	D	Class 3
TU9356	69.533	-129.750	CB	WB	A	Class 3

TU9217	69.550	-131.000	CB	WB	A	Class 3
TU9215	69.633	-131.417	CB	WB	D	Class 3
TU9216	69.633	-131.417	CB	WB	D	Class 3
TU9218	69.633	-131.417	CB	WB	D	Class 3
TU9219	69.633	-131.417	CB	WB	D	Class 3
TU9232	69.633	-131.250	CB	WB	D	Class 3
TU9233	69.633	-131.250	CB	WB	D	Class 3
TU9234	69.633	-131.250	CB	WB	D	Class 3
TU9235	69.633	-131.250	CB	WB	D	Class 3
TU9351	69.700	-131.500	CB	WB	B	Class 2
TU9352	69.700	-131.500	CB	WB	F	Class 2
TU8908	69.700	-129.000	CB	WB	D	Class 3
BJ-003	69.700	-128.970	CB	WB	B	Class 3
BJ-001	69.700	-128.970	CB	WB	C	Class 3
BJ-002	69.700	-128.970	CB	WB	C	Class 3
BJ-007	69.700	-128.970	CB	WB	C	Class 3
TU9222	69.717	-131.583	CB	WB	B	Class 2
TU9357	69.750	-128.833	CB	WB	D	Class 5
TU9358	69.767	-128.833	CB	WB	D	Class 5
IN9211	69.833	-134.000	CB	WB	B	Class 2
IN9212	69.833	-134.000	CB	WB	D	Class 2
PBD-885	58.620	-101.480	MB	EB	A	Class 6
PBO-887	58.620	-101.480	MB	EB	A	Class 6
WMB03-01	58.620	-101.480	MB	EB	A	Class 6
PBD-889	58.620	-101.480	MB	EB	B	Class 6
BMF-001	58.620	-101.480	MB	EB	E	Class 6
PBD-886	58.620	-101.480	MB	EB	E	Class 6
WMB03-03	58.620	-101.480	MB	EB	E	Class 6
WMB03-06	58.620	-101.480	MB	EB	E	Class 6
WMB03-16	58.620	-101.480	MB	EB	E	Class 6
PBO-888	58.620	-101.480	MB	EB	F	Class 6
WMB03-02	58.620	-101.480	MB	EB	F	Class 6
PBJ-981	58.720	-94.120	MB	EB	A	Class 6
PBK-539	58.720	-94.120	MB	EB	A	Class 6
BMR-614	58.720	-94.120	MB	EB	E	Class 6
PBJ-982	58.720	-94.120	MB	EB	E	Class 6
PBJ-980	58.720	-94.120	MB	EB	F	Class 6
BSB-325	59.320	-107.200	SK	EB	A	Class 6
BSJ-006	59.320	-107.200	SK	EB	A	Class 6
BSM-448	59.320	-107.200	SK	EB	A	Class 6
BSJ-003	59.320	-107.200	SK	EB	B	Class 6
BSJ-005	59.320	-107.200	SK	EB	B	Class 6
BSM-453	59.320	-107.200	SK	EB	D	Class 6
BSB-326	59.320	-107.200	SK	EB	E	Class 6
BSJ-004	59.320	-107.200	SK	EB	E	Class 6
BSJ-008	59.320	-107.200	SK	EB	E	Class 6
BSJ-009	59.320	-107.200	SK	EB	E	Class 6
BSJ-010	59.320	-107.200	SK	EB	E	Class 6
BSJ-011	59.320	-107.200	SK	EB	E	Class 6
BSM-447	59.320	-107.200	SK	EB	E	Class 6
BSM-450	59.320	-107.200	SK	EB	E	Class 6

BSM-451	59.320	-107.200	SK	EB	E	Class 6
BSM-452	59.320	-107.200	SK	EB	E	Class 6
BSJ-007	59.320	-107.200	SK	EB	F	Class 6
BSM-449	59.320	-107.200	SK	EB	F	Class 6
W1	60.680	-102.930	QA	EB	A	Class 1
W2	60.680	-102.930	QA	EB	A	Class 1
W3	60.680	-102.930	QA	EB	A	Class 1
W4	60.680	-102.930	QA	EB	A	Class 1
W5	60.680	-102.930	QA	EB	A	Class 1
W6	60.680	-102.930	QA	EB	A	Class 1
W16	60.720	-104.170	QA	EB	A	Class 6
W18	60.720	-104.170	QA	EB	A	Class 6
W23	60.720	-104.170	QA	EB	A	Class 6
W27	60.720	-104.170	QA	EB	A	Class 6
W31	60.720	-104.170	QA	EB	A	Class 6
W32	60.720	-104.170	QA	EB	A	Class 6
W36	60.720	-104.170	QA	EB	A	Class 6
W38	60.720	-104.170	QA	EB	A	Class 6
W40	60.720	-104.170	QA	EB	A	Class 6
W44	60.720	-104.170	QA	EB	A	Class 6
W49	60.720	-104.170	QA	EB	A	Class 6
W53	60.720	-104.170	QA	EB	A	Class 6
W54	60.720	-104.170	QA	EB	A	Class 6
W60	60.720	-104.170	QA	EB	A	Class 6
W63	60.720	-104.170	QA	EB	A	Class 6
W65	60.720	-104.170	QA	EB	A	Class 6
W66	60.720	-104.170	QA	EB	A	Class 6
W68	60.720	-104.170	QA	EB	A	Class 6
W69	60.720	-104.170	QA	EB	A	Class 6
W71	60.720	-104.170	QA	EB	A	Class 6
W72	60.720	-104.170	QA	EB	A	Class 6
W74	60.720	-104.170	QA	EB	A	Class 6
W75	60.720	-104.170	QA	EB	A	Class 6
W84	60.720	-104.170	QA	EB	A	Class 6
W85	60.720	-104.170	QA	EB	A	Class 6
W93	60.720	-104.170	QA	EB	A	Class 6
W94	60.720	-104.170	QA	EB	A	Class 6
W26	60.720	-104.170	QA	EB	B	Class 6
W50	60.720	-104.170	QA	EB	B	Class 6
W81	60.720	-104.170	QA	EB	C	Class 6
W25	60.720	-104.170	QA	EB	D	Class 6
W46	60.720	-104.170	QA	EB	D	Class 6
W52	60.720	-104.170	QA	EB	D	Class 6
W61	60.720	-104.170	QA	EB	D	Class 6
W67	60.720	-104.170	QA	EB	D	Class 6
W90	60.720	-104.170	QA	EB	D	Class 6
W92	60.720	-104.170	QA	EB	D	Class 6
W15	60.720	-104.170	QA	EB	E	Class 6
W17	60.720	-104.170	QA	EB	E	Class 6
W19	60.720	-104.170	QA	EB	E	Class 6
W20	60.720	-104.170	QA	EB	E	Class 6

W21	60.720	-104.170	QA	EB	E	Class 6
W22	60.720	-104.170	QA	EB	E	Class 6
W24	60.720	-104.170	QA	EB	E	Class 6
W28	60.720	-104.170	QA	EB	E	Class 6
W29	60.720	-104.170	QA	EB	E	Class 6
W30	60.720	-104.170	QA	EB	E	Class 6
W33	60.720	-104.170	QA	EB	E	Class 6
W34	60.720	-104.170	QA	EB	E	Class 6
W35	60.720	-104.170	QA	EB	E	Class 6
W37	60.720	-104.170	QA	EB	E	Class 6
W39	60.720	-104.170	QA	EB	E	Class 6
W42	60.720	-104.170	QA	EB	E	Class 6
W43	60.720	-104.170	QA	EB	E	Class 6
W47	60.720	-104.170	QA	EB	E	Class 6
W48	60.720	-104.170	QA	EB	E	Class 6
W51	60.720	-104.170	QA	EB	E	Class 6
W55	60.720	-104.170	QA	EB	E	Class 6
W56	60.720	-104.170	QA	EB	E	Class 6
W57	60.720	-104.170	QA	EB	E	Class 6
W58	60.720	-104.170	QA	EB	E	Class 6
W59	60.720	-104.170	QA	EB	E	Class 6
W62	60.720	-104.170	QA	EB	E	Class 6
W64	60.720	-104.170	QA	EB	E	Class 6
W70	60.720	-104.170	QA	EB	E	Class 6
W73	60.720	-104.170	QA	EB	E	Class 6
W76	60.720	-104.170	QA	EB	E	Class 6
W77	60.720	-104.170	QA	EB	E	Class 6
W78	60.720	-104.170	QA	EB	E	Class 6
W80	60.720	-104.170	QA	EB	E	Class 6
W82	60.720	-104.170	QA	EB	E	Class 6
W83	60.720	-104.170	QA	EB	E	Class 6
W87	60.720	-104.170	QA	EB	E	Class 6
W89	60.720	-104.170	QA	EB	E	Class 6
W91	60.720	-104.170	QA	EB	E	Class 6
W95	60.720	-104.170	QA	EB	E	Class 6
W96	60.720	-104.170	QA	EB	E	Class 6
W14	60.720	-104.170	QA	EB	F	Class 6
W79	60.720	-104.170	QA	EB	F	Class 6
W86	60.720	-104.170	QA	EB	F	Class 6
W88	60.720	-104.170	QA	EB	F	Class 6
AR185	61.100	-94.050	QA	EB	A	Class 6
BKW-365	61.100	-94.050	QA	EB	A	Class 6
BKW-366	61.100	-94.050	QA	EB	A	Class 6
BKW-369	61.100	-94.050	QA	EB	A	Class 6
BKW-375	61.100	-94.050	QA	EB	A	Class 6
BKW-376	61.100	-94.050	QA	EB	A	Class 6
BKW-381	61.100	-94.050	QA	EB	A	Class 6
BKW-382	61.100	-94.050	QA	EB	A	Class 6
BKW-383	61.100	-94.050	QA	EB	A	Class 6
BLB-195	61.100	-94.050	QA	EB	A	Class 6
BLB-199	61.100	-94.050	QA	EB	A	Class 6

BLB-210	61.100	-94.050	QA	EB	A	Class 6
BLB-214	61.100	-94.050	QA	EB	A	Class 6
BLB-222	61.100	-94.050	QA	EB	A	Class 6
BLB-230	61.100	-94.050	QA	EB	A	Class 6
BUB-220	61.100	-94.050	QA	EB	A	Class 6
K34843	61.100	-94.050	QA	EB	A	Class 6
BLB-205	61.100	-94.050	QA	EB	B	Class 6
BLB-211	61.100	-94.050	QA	EB	B	Class 6
BLB-191	61.100	-94.050	QA	EB	C	Class 6
BLB-201	61.100	-94.050	QA	EB	C	Class 6
AR172	61.100	-94.050	QA	EB	D	Class 6
AR187	61.100	-94.050	QA	EB	D	Class 6
BKW-367	61.100	-94.050	QA	EB	D	Class 6
BKW-379	61.100	-94.050	QA	EB	D	Class 6
BKW-389	61.100	-94.050	QA	EB	D	Class 6
BLB-204	61.100	-94.050	QA	EB	D	Class 6
AR166	61.100	-94.050	QA	EB	E	Class 6
AR167	61.100	-94.050	QA	EB	E	Class 6
AR168	61.100	-94.050	QA	EB	E	Class 6
AR169	61.100	-94.050	QA	EB	E	Class 6
AR170	61.100	-94.050	QA	EB	E	Class 6
AR171	61.100	-94.050	QA	EB	E	Class 6
AR181	61.100	-94.050	QA	EB	E	Class 6
AR182	61.100	-94.050	QA	EB	E	Class 6
AR183	61.100	-94.050	QA	EB	E	Class 6
AR184	61.100	-94.050	QA	EB	E	Class 6
AR188	61.100	-94.050	QA	EB	E	Class 6
AR189	61.100	-94.050	QA	EB	E	Class 6
AR190	61.100	-94.050	QA	EB	E	Class 6
BKW-364	61.100	-94.050	QA	EB	E	Class 6
BKW-368	61.100	-94.050	QA	EB	E	Class 6
BKW-371	61.100	-94.050	QA	EB	E	Class 6
BKW-372	61.100	-94.050	QA	EB	E	Class 6
BKW-373	61.100	-94.050	QA	EB	E	Class 6
BKW-374	61.100	-94.050	QA	EB	E	Class 6
BKW-377	61.100	-94.050	QA	EB	E	Class 6
BKW-378	61.100	-94.050	QA	EB	E	Class 6
BKW-380	61.100	-94.050	QA	EB	E	Class 6
BKW-384	61.100	-94.050	QA	EB	E	Class 6
BKW-385	61.100	-94.050	QA	EB	E	Class 6
BKW-386	61.100	-94.050	QA	EB	E	Class 6
BKW-387	61.100	-94.050	QA	EB	E	Class 6
BKW-388	61.100	-94.050	QA	EB	E	Class 6
BKW-390	61.100	-94.050	QA	EB	E	Class 6
BKW-391	61.100	-94.050	QA	EB	E	Class 6
BKW-392	61.100	-94.050	QA	EB	E	Class 6
BKW-393	61.100	-94.050	QA	EB	E	Class 6
BKW-394	61.100	-94.050	QA	EB	E	Class 6
BKW-395	61.100	-94.050	QA	EB	E	Class 6
BLB-192	61.100	-94.050	QA	EB	E	Class 6
BLB-193	61.100	-94.050	QA	EB	E	Class 6

BLB-194	61.100	-94.050	QA	EB	E	Class 6
BLB-196	61.100	-94.050	QA	EB	E	Class 6
BLB-198	61.100	-94.050	QA	EB	E	Class 6
BLB-202	61.100	-94.050	QA	EB	E	Class 6
BLB-203	61.100	-94.050	QA	EB	E	Class 6
BLB-206	61.100	-94.050	QA	EB	E	Class 6
BLB-207	61.100	-94.050	QA	EB	E	Class 6
BLB-208	61.100	-94.050	QA	EB	E	Class 6
BLB-209	61.100	-94.050	QA	EB	E	Class 6
BLB-212	61.100	-94.050	QA	EB	E	Class 6
BLB-213	61.100	-94.050	QA	EB	E	Class 6
BLB-215	61.100	-94.050	QA	EB	E	Class 6
BLB-216	61.100	-94.050	QA	EB	E	Class 6
BLB-217	61.100	-94.050	QA	EB	E	Class 6
BLB-218	61.100	-94.050	QA	EB	E	Class 6
BLB-219	61.100	-94.050	QA	EB	E	Class 6
BLB-221	61.100	-94.050	QA	EB	E	Class 6
BLB-223	61.100	-94.050	QA	EB	E	Class 6
BLB-224	61.100	-94.050	QA	EB	E	Class 6
BLB-225	61.100	-94.050	QA	EB	E	Class 6
BLB-226	61.100	-94.050	QA	EB	E	Class 6
BLB-227	61.100	-94.050	QA	EB	E	Class 6
BLB-229	61.100	-94.050	QA	EB	E	Class 6
BLB-231	61.100	-94.050	QA	EB	E	Class 6
BUB-197	61.100	-94.050	QA	EB	E	Class 6
PKW-370	61.100	-94.050	QA	EB	E	Class 6
PSI-641	61.100	-94.050	QA	EB	E	Class 6
PSI-642	61.100	-94.050	QA	EB	E	Class 6
AR186	61.100	-94.050	QA	EB	F	Class 6
AR05	61.170	-100.260	QA	EB	B	Class 6
AR06	61.170	-100.260	QA	EB	E	Class 6
AR01	61.200	-100.190	QA	EB	D	Class 6
AR02	61.240	-100.240	QA	EB	E	Class 6
AR03	61.240	-100.240	QA	EB	E	Class 6
AR04	61.240	-100.240	QA	EB	E	Class 6
AR07	61.240	-100.240	QA	EB	E	Class 6
AR08	61.240	-100.240	QA	EB	E	Class 6
3383b	61.530	-105.580	QA	EB	A	Class 6
3396	61.530	-105.580	QA	EB	A	Class 6
3397a	61.530	-105.580	QA	EB	A	Class 6
3397b	61.530	-105.580	QA	EB	A	Class 6
3398a	61.530	-105.580	QA	EB	A	Class 6
3409b	61.530	-105.580	QA	EB	A	Class 6
3414	61.530	-105.580	QA	EB	A	Class 6
W103	61.530	-105.580	QA	EB	A	Class 6
W110	61.530	-105.580	QA	EB	A	Class 6
W111	61.530	-105.580	QA	EB	A	Class 6
W118	61.530	-105.580	QA	EB	A	Class 6
W126	61.530	-105.580	QA	EB	A	Class 6
W129	61.530	-105.580	QA	EB	A	Class 6
W132	61.530	-105.580	QA	EB	A	Class 6

W133	61.530	-105.580	QA	EB	A	Class 6
W135	61.530	-105.580	QA	EB	A	Class 6
W138	61.530	-105.580	QA	EB	A	Class 6
W140	61.530	-105.580	QA	EB	A	Class 6
W142	61.530	-105.580	QA	EB	A	Class 6
W143	61.530	-105.580	QA	EB	A	Class 6
W155	61.530	-105.580	QA	EB	A	Class 6
W165	61.530	-105.580	QA	EB	A	Class 6
W166	61.530	-105.580	QA	EB	A	Class 6
W168	61.530	-105.580	QA	EB	A	Class 6
W176	61.530	-105.580	QA	EB	A	Class 6
W183	61.530	-105.580	QA	EB	A	Class 6
W189	61.530	-105.580	QA	EB	A	Class 6
W193	61.530	-105.580	QA	EB	A	Class 6
W200	61.530	-105.580	QA	EB	A	Class 6
W202	61.530	-105.580	QA	EB	A	Class 6
W206	61.530	-105.580	QA	EB	A	Class 6
W207	61.530	-105.580	QA	EB	A	Class 6
W208	61.530	-105.580	QA	EB	A	Class 6
W213	61.530	-105.580	QA	EB	A	Class 6
W218	61.530	-105.580	QA	EB	A	Class 6
W219	61.530	-105.580	QA	EB	A	Class 6
W225	61.530	-105.580	QA	EB	A	Class 6
W226	61.530	-105.580	QA	EB	A	Class 6
W112	61.530	-105.580	QA	EB	B	Class 6
W153	61.530	-105.580	QA	EB	B	Class 6
3362b	61.530	-105.580	QA	EB	D	Class 6
3364b	61.530	-105.580	QA	EB	D	Class 6
3371	61.530	-105.580	QA	EB	D	Class 6
3374b	61.530	-105.580	QA	EB	D	Class 6
3376b	61.530	-105.580	QA	EB	D	Class 6
3402	61.530	-105.580	QA	EB	D	Class 6
3412a	61.530	-105.580	QA	EB	D	Class 6
W109	61.530	-105.580	QA	EB	D	Class 6
W117	61.530	-105.580	QA	EB	D	Class 6
W127	61.530	-105.580	QA	EB	D	Class 6
W149	61.530	-105.580	QA	EB	D	Class 6
W151	61.530	-105.580	QA	EB	D	Class 6
W154	61.530	-105.580	QA	EB	D	Class 6
W164	61.530	-105.580	QA	EB	D	Class 6
W174	61.530	-105.580	QA	EB	D	Class 6
W190	61.530	-105.580	QA	EB	D	Class 6
W216	61.530	-105.580	QA	EB	D	Class 6
3360	61.530	-105.580	QA	EB	E	Class 6
3361a	61.530	-105.580	QA	EB	E	Class 6
3361b	61.530	-105.580	QA	EB	E	Class 6
3362a	61.530	-105.580	QA	EB	E	Class 6
3362c	61.530	-105.580	QA	EB	E	Class 6
3363a	61.530	-105.580	QA	EB	E	Class 6
3363b	61.530	-105.580	QA	EB	E	Class 6
3364a	61.530	-105.580	QA	EB	E	Class 6

3365a	61.530	-105.580	QA	EB	E	Class 6
3365b	61.530	-105.580	QA	EB	E	Class 6
3366a	61.530	-105.580	QA	EB	E	Class 6
3366b	61.530	-105.580	QA	EB	E	Class 6
3368a	61.530	-105.580	QA	EB	E	Class 6
3368c	61.530	-105.580	QA	EB	E	Class 6
3369a	61.530	-105.580	QA	EB	E	Class 6
3370a	61.530	-105.580	QA	EB	E	Class 6
3370b	61.530	-105.580	QA	EB	E	Class 6
3372	61.530	-105.580	QA	EB	E	Class 6
3373a	61.530	-105.580	QA	EB	E	Class 6
3373b	61.530	-105.580	QA	EB	E	Class 6
3374a	61.530	-105.580	QA	EB	E	Class 6
3374c	61.530	-105.580	QA	EB	E	Class 6
3375a	61.530	-105.580	QA	EB	E	Class 6
3375b	61.530	-105.580	QA	EB	E	Class 6
3376a	61.530	-105.580	QA	EB	E	Class 6
3376c	61.530	-105.580	QA	EB	E	Class 6
3377	61.530	-105.580	QA	EB	E	Class 6
3378	61.530	-105.580	QA	EB	E	Class 6
3379a	61.530	-105.580	QA	EB	E	Class 6
3379b	61.530	-105.580	QA	EB	E	Class 6
3379c	61.530	-105.580	QA	EB	E	Class 6
3380a	61.530	-105.580	QA	EB	E	Class 6
3380b	61.530	-105.580	QA	EB	E	Class 6
3380c	61.530	-105.580	QA	EB	E	Class 6
3380d	61.530	-105.580	QA	EB	E	Class 6
3381a	61.530	-105.580	QA	EB	E	Class 6
3381b	61.530	-105.580	QA	EB	E	Class 6
3382a	61.530	-105.580	QA	EB	E	Class 6
3382b	61.530	-105.580	QA	EB	E	Class 6
3383a	61.530	-105.580	QA	EB	E	Class 6
3383c	61.530	-105.580	QA	EB	E	Class 6
3384a	61.530	-105.580	QA	EB	E	Class 6
3384b	61.530	-105.580	QA	EB	E	Class 6
3385a	61.530	-105.580	QA	EB	E	Class 6
3385b	61.530	-105.580	QA	EB	E	Class 6
3385c	61.530	-105.580	QA	EB	E	Class 6
3386a	61.530	-105.580	QA	EB	E	Class 6
3386b	61.530	-105.580	QA	EB	E	Class 6
3387a	61.530	-105.580	QA	EB	E	Class 6
3387b	61.530	-105.580	QA	EB	E	Class 6
3388a	61.530	-105.580	QA	EB	E	Class 6
3388b	61.530	-105.580	QA	EB	E	Class 6
3389a	61.530	-105.580	QA	EB	E	Class 6
3389b	61.530	-105.580	QA	EB	E	Class 6
3389c	61.530	-105.580	QA	EB	E	Class 6
3389d	61.530	-105.580	QA	EB	E	Class 6
3390a	61.530	-105.580	QA	EB	E	Class 6
3390b	61.530	-105.580	QA	EB	E	Class 6
3390c	61.530	-105.580	QA	EB	E	Class 6

3391a	61.530	-105.580	QA	EB	E	Class 6
3391b	61.530	-105.580	QA	EB	E	Class 6
3392a	61.530	-105.580	QA	EB	E	Class 6
3392b	61.530	-105.580	QA	EB	E	Class 6
3392c	61.530	-105.580	QA	EB	E	Class 6
3393a	61.530	-105.580	QA	EB	E	Class 6
3393b	61.530	-105.580	QA	EB	E	Class 6
3393c	61.530	-105.580	QA	EB	E	Class 6
3393d	61.530	-105.580	QA	EB	E	Class 6
3394b	61.530	-105.580	QA	EB	E	Class 6
3394c	61.530	-105.580	QA	EB	E	Class 6
3395a	61.530	-105.580	QA	EB	E	Class 6
3395b	61.530	-105.580	QA	EB	E	Class 6
3397c	61.530	-105.580	QA	EB	E	Class 6
3398b	61.530	-105.580	QA	EB	E	Class 6
3398c	61.530	-105.580	QA	EB	E	Class 6
3398d	61.530	-105.580	QA	EB	E	Class 6
3399a	61.530	-105.580	QA	EB	E	Class 6
3399b	61.530	-105.580	QA	EB	E	Class 6
3400a	61.530	-105.580	QA	EB	E	Class 6
3400b	61.530	-105.580	QA	EB	E	Class 6
3400c	61.530	-105.580	QA	EB	E	Class 6
3401a	61.530	-105.580	QA	EB	E	Class 6
3401b	61.530	-105.580	QA	EB	E	Class 6
3403	61.530	-105.580	QA	EB	E	Class 6
3404a	61.530	-105.580	QA	EB	E	Class 6
3405a	61.530	-105.580	QA	EB	E	Class 6
3405b	61.530	-105.580	QA	EB	E	Class 6
3406a	61.530	-105.580	QA	EB	E	Class 6
3406b	61.530	-105.580	QA	EB	E	Class 6
3406c	61.530	-105.580	QA	EB	E	Class 6
3407a	61.530	-105.580	QA	EB	E	Class 6
3407b	61.530	-105.580	QA	EB	E	Class 6
3407c	61.530	-105.580	QA	EB	E	Class 6
3408a	61.530	-105.580	QA	EB	E	Class 6
3408b	61.530	-105.580	QA	EB	E	Class 6
3408c	61.530	-105.580	QA	EB	E	Class 6
3409a	61.530	-105.580	QA	EB	E	Class 6
3410	61.530	-105.580	QA	EB	E	Class 6
3411	61.530	-105.580	QA	EB	E	Class 6
3412b	61.530	-105.580	QA	EB	E	Class 6
3413	61.530	-105.580	QA	EB	E	Class 6
3415a	61.530	-105.580	QA	EB	E	Class 6
3415b	61.530	-105.580	QA	EB	E	Class 6
3416a	61.530	-105.580	QA	EB	E	Class 6
3416b	61.530	-105.580	QA	EB	E	Class 6
3416c	61.530	-105.580	QA	EB	E	Class 6
3417	61.530	-105.580	QA	EB	E	Class 6
3418	61.530	-105.580	QA	EB	E	Class 6
3419	61.530	-105.580	QA	EB	E	Class 6
3420a	61.530	-105.580	QA	EB	E	Class 6

3420b	61.530	-105.580	QA	EB	E	Class 6
3421	61.530	-105.580	QA	EB	E	Class 6
3423	61.530	-105.580	QA	EB	E	Class 6
3424	61.530	-105.580	QA	EB	E	Class 6
3425	61.530	-105.580	QA	EB	E	Class 6
3426	61.530	-105.580	QA	EB	E	Class 6
3427	61.530	-105.580	QA	EB	E	Class 6
3428a	61.530	-105.580	QA	EB	E	Class 6
3429	61.530	-105.580	QA	EB	E	Class 6
3432	61.530	-105.580	QA	EB	E	Class 6
3433	61.530	-105.580	QA	EB	E	Class 6
3434a	61.530	-105.580	QA	EB	E	Class 6
3434b	61.530	-105.580	QA	EB	E	Class 6
W101	61.530	-105.580	QA	EB	E	Class 6
W104	61.530	-105.580	QA	EB	E	Class 6
W105	61.530	-105.580	QA	EB	E	Class 6
W114	61.530	-105.580	QA	EB	E	Class 6
W120	61.530	-105.580	QA	EB	E	Class 6
W123	61.530	-105.580	QA	EB	E	Class 6
W125	61.530	-105.580	QA	EB	E	Class 6
W130	61.530	-105.580	QA	EB	E	Class 6
W131	61.530	-105.580	QA	EB	E	Class 6
W136	61.530	-105.580	QA	EB	E	Class 6
W137	61.530	-105.580	QA	EB	E	Class 6
W139	61.530	-105.580	QA	EB	E	Class 6
W141	61.530	-105.580	QA	EB	E	Class 6
W146	61.530	-105.580	QA	EB	E	Class 6
W148	61.530	-105.580	QA	EB	E	Class 6
W150	61.530	-105.580	QA	EB	E	Class 6
W157	61.530	-105.580	QA	EB	E	Class 6
W160	61.530	-105.580	QA	EB	E	Class 6
W163	61.530	-105.580	QA	EB	E	Class 6
W170	61.530	-105.580	QA	EB	E	Class 6
W172	61.530	-105.580	QA	EB	E	Class 6
W173	61.530	-105.580	QA	EB	E	Class 6
W175	61.530	-105.580	QA	EB	E	Class 6
W177	61.530	-105.580	QA	EB	E	Class 6
W179	61.530	-105.580	QA	EB	E	Class 6
W181	61.530	-105.580	QA	EB	E	Class 6
W186	61.530	-105.580	QA	EB	E	Class 6
W187	61.530	-105.580	QA	EB	E	Class 6
W188	61.530	-105.580	QA	EB	E	Class 6
W191	61.530	-105.580	QA	EB	E	Class 6
W197	61.530	-105.580	QA	EB	E	Class 6
W203	61.530	-105.580	QA	EB	E	Class 6
W204	61.530	-105.580	QA	EB	E	Class 6
W209	61.530	-105.580	QA	EB	E	Class 6
W210	61.530	-105.580	QA	EB	E	Class 6
W212	61.530	-105.580	QA	EB	E	Class 6
W214	61.530	-105.580	QA	EB	E	Class 6
W215	61.530	-105.580	QA	EB	E	Class 6

3394a	61.530	-105.580	QA	EB	F	Class 6
3430	61.530	-105.580	QA	EB	F	Class 6
W100	61.530	-105.580	QA	EB	F	Class 6
W102	61.530	-105.580	QA	EB	F	Class 6
W106	61.530	-105.580	QA	EB	F	Class 6
W107	61.530	-105.580	QA	EB	F	Class 6
W108	61.530	-105.580	QA	EB	F	Class 6
W115	61.530	-105.580	QA	EB	F	Class 6
W116	61.530	-105.580	QA	EB	F	Class 6
W119	61.530	-105.580	QA	EB	F	Class 6
W121	61.530	-105.580	QA	EB	F	Class 6
W122	61.530	-105.580	QA	EB	F	Class 6
W128	61.530	-105.580	QA	EB	F	Class 6
W144	61.530	-105.580	QA	EB	F	Class 6
W145	61.530	-105.580	QA	EB	F	Class 6
W156	61.530	-105.580	QA	EB	F	Class 6
W158	61.530	-105.580	QA	EB	F	Class 6
W159	61.530	-105.580	QA	EB	F	Class 6
W161	61.530	-105.580	QA	EB	F	Class 6
W162	61.530	-105.580	QA	EB	F	Class 6
W167	61.530	-105.580	QA	EB	F	Class 6
W169	61.530	-105.580	QA	EB	F	Class 6
W171	61.530	-105.580	QA	EB	F	Class 6
W178	61.530	-105.580	QA	EB	F	Class 6
W180	61.530	-105.580	QA	EB	F	Class 6
W182	61.530	-105.580	QA	EB	F	Class 6
W185	61.530	-105.580	QA	EB	F	Class 6
W192	61.530	-105.580	QA	EB	F	Class 6
W194	61.530	-105.580	QA	EB	F	Class 6
W196	61.530	-105.580	QA	EB	F	Class 6
W198	61.530	-105.580	QA	EB	F	Class 6
W199	61.530	-105.580	QA	EB	F	Class 6
W205	61.530	-105.580	QA	EB	F	Class 6
W217	61.530	-105.580	QA	EB	F	Class 6
W220	61.530	-105.580	QA	EB	F	Class 6
W221	61.530	-105.580	QA	EB	F	Class 6
W223	61.530	-105.580	QA	EB	F	Class 6
W224	61.530	-105.580	QA	EB	F	Class 6
3422	61.530	-105.580	QA	EB	G	Class 6
3303b	61.620	-105.750	QA	EB	A	Class 6
3319a	61.620	-105.750	QA	EB	A	Class 6
3330	61.620	-105.750	QA	EB	A	Class 6
3342b	61.620	-105.750	QA	EB	A	Class 6
3343	61.620	-105.750	QA	EB	A	Class 6
3349	61.620	-105.750	QA	EB	A	Class 6
3354a	61.620	-105.750	QA	EB	A	Class 6
3318d	61.620	-105.750	QA	EB	B	Class 6
3329b	61.620	-105.750	QA	EB	B	Class 6
3336a	61.620	-105.750	QA	EB	B	Class 6
3315b	61.620	-105.750	QA	EB	D	Class 6
3318a	61.620	-105.750	QA	EB	D	Class 6

3318c	61.620	-105.750	QA	EB	D	Class 6
3325b	61.620	-105.750	QA	EB	D	Class 6
3339c	61.620	-105.750	QA	EB	D	Class 6
3345a	61.620	-105.750	QA	EB	D	Class 6
3348	61.620	-105.750	QA	EB	D	Class 6
3351	61.620	-105.750	QA	EB	D	Class 6
3354b	61.620	-105.750	QA	EB	D	Class 6
3357	61.620	-105.750	QA	EB	D	Class 6
3300a	61.620	-105.750	QA	EB	E	Class 6
3301a	61.620	-105.750	QA	EB	E	Class 6
3301b	61.620	-105.750	QA	EB	E	Class 6
3301c	61.620	-105.750	QA	EB	E	Class 6
3302a	61.620	-105.750	QA	EB	E	Class 6
3302b	61.620	-105.750	QA	EB	E	Class 6
3303c	61.620	-105.750	QA	EB	E	Class 6
3304a	61.620	-105.750	QA	EB	E	Class 6
3304b	61.620	-105.750	QA	EB	E	Class 6
3304c	61.620	-105.750	QA	EB	E	Class 6
3304d	61.620	-105.750	QA	EB	E	Class 6
3305	61.620	-105.750	QA	EB	E	Class 6
3306a	61.620	-105.750	QA	EB	E	Class 6
3306b	61.620	-105.750	QA	EB	E	Class 6
3306c	61.620	-105.750	QA	EB	E	Class 6
3307a	61.620	-105.750	QA	EB	E	Class 6
3307b	61.620	-105.750	QA	EB	E	Class 6
3307c	61.620	-105.750	QA	EB	E	Class 6
3308a	61.620	-105.750	QA	EB	E	Class 6
3308b	61.620	-105.750	QA	EB	E	Class 6
3308c	61.620	-105.750	QA	EB	E	Class 6
3308d	61.620	-105.750	QA	EB	E	Class 6
3309a	61.620	-105.750	QA	EB	E	Class 6
3309b	61.620	-105.750	QA	EB	E	Class 6
3310a	61.620	-105.750	QA	EB	E	Class 6
3310b	61.620	-105.750	QA	EB	E	Class 6
3310c	61.620	-105.750	QA	EB	E	Class 6
3310d	61.620	-105.750	QA	EB	E	Class 6
3311a	61.620	-105.750	QA	EB	E	Class 6
3311b	61.620	-105.750	QA	EB	E	Class 6
3311c	61.620	-105.750	QA	EB	E	Class 6
3312	61.620	-105.750	QA	EB	E	Class 6
3313a	61.620	-105.750	QA	EB	E	Class 6
3313b	61.620	-105.750	QA	EB	E	Class 6
3313c	61.620	-105.750	QA	EB	E	Class 6
3313d	61.620	-105.750	QA	EB	E	Class 6
3314a	61.620	-105.750	QA	EB	E	Class 6
3314b	61.620	-105.750	QA	EB	E	Class 6
3314c	61.620	-105.750	QA	EB	E	Class 6
3314d	61.620	-105.750	QA	EB	E	Class 6
3315a	61.620	-105.750	QA	EB	E	Class 6
3316a	61.620	-105.750	QA	EB	E	Class 6
3316b	61.620	-105.750	QA	EB	E	Class 6

3317b	61.620	-105.750	QA	EB	E	Class 6
3318b	61.620	-105.750	QA	EB	E	Class 6
3319b	61.620	-105.750	QA	EB	E	Class 6
3320	61.620	-105.750	QA	EB	E	Class 6
3321a	61.620	-105.750	QA	EB	E	Class 6
3321b	61.620	-105.750	QA	EB	E	Class 6
3321c	61.620	-105.750	QA	EB	E	Class 6
3322a	61.620	-105.750	QA	EB	E	Class 6
3322b	61.620	-105.750	QA	EB	E	Class 6
3323a	61.620	-105.750	QA	EB	E	Class 6
3323b	61.620	-105.750	QA	EB	E	Class 6
3324a	61.620	-105.750	QA	EB	E	Class 6
3325a	61.620	-105.750	QA	EB	E	Class 6
3326	61.620	-105.750	QA	EB	E	Class 6
3327a	61.620	-105.750	QA	EB	E	Class 6
3327b	61.620	-105.750	QA	EB	E	Class 6
3327c	61.620	-105.750	QA	EB	E	Class 6
3328a	61.620	-105.750	QA	EB	E	Class 6
3328b	61.620	-105.750	QA	EB	E	Class 6
3329a	61.620	-105.750	QA	EB	E	Class 6
3331	61.620	-105.750	QA	EB	E	Class 6
3332	61.620	-105.750	QA	EB	E	Class 6
3333a	61.620	-105.750	QA	EB	E	Class 6
3334a	61.620	-105.750	QA	EB	E	Class 6
3334b	61.620	-105.750	QA	EB	E	Class 6
3334c	61.620	-105.750	QA	EB	E	Class 6
3335a	61.620	-105.750	QA	EB	E	Class 6
3335b	61.620	-105.750	QA	EB	E	Class 6
3336b	61.620	-105.750	QA	EB	E	Class 6
3337a	61.620	-105.750	QA	EB	E	Class 6
3337b	61.620	-105.750	QA	EB	E	Class 6
3337c	61.620	-105.750	QA	EB	E	Class 6
3338a	61.620	-105.750	QA	EB	E	Class 6
3338b	61.620	-105.750	QA	EB	E	Class 6
3338c	61.620	-105.750	QA	EB	E	Class 6
3338d	61.620	-105.750	QA	EB	E	Class 6
3339a	61.620	-105.750	QA	EB	E	Class 6
3339b	61.620	-105.750	QA	EB	E	Class 6
3339d	61.620	-105.750	QA	EB	E	Class 6
3339e	61.620	-105.750	QA	EB	E	Class 6
3340a	61.620	-105.750	QA	EB	E	Class 6
3340b	61.620	-105.750	QA	EB	E	Class 6
3340c	61.620	-105.750	QA	EB	E	Class 6
3341a	61.620	-105.750	QA	EB	E	Class 6
3341b	61.620	-105.750	QA	EB	E	Class 6
3341c	61.620	-105.750	QA	EB	E	Class 6
3342a	61.620	-105.750	QA	EB	E	Class 6
3344	61.620	-105.750	QA	EB	E	Class 6
3345b	61.620	-105.750	QA	EB	E	Class 6
3346a	61.620	-105.750	QA	EB	E	Class 6
3346b	61.620	-105.750	QA	EB	E	Class 6

3347a	61.620	-105.750	QA	EB	E	Class 6
3347b	61.620	-105.750	QA	EB	E	Class 6
3347c	61.620	-105.750	QA	EB	E	Class 6
3350	61.620	-105.750	QA	EB	E	Class 6
3352	61.620	-105.750	QA	EB	E	Class 6
3353a	61.620	-105.750	QA	EB	E	Class 6
3353b	61.620	-105.750	QA	EB	E	Class 6
3355	61.620	-105.750	QA	EB	E	Class 6
3356	61.620	-105.750	QA	EB	E	Class 6
3359a	61.620	-105.750	QA	EB	E	Class 6
3359b	61.620	-105.750	QA	EB	E	Class 6
3333b	61.620	-105.750	QA	EB	F	Class 6
W13	61.650	-105.580	QA	EB	D	Class 6
W10	61.650	-105.580	QA	EB	E	Class 6
W11	61.650	-105.580	QA	EB	E	Class 6
W12	61.650	-105.580	QA	EB	E	Class 6
W7	61.650	-105.580	QA	EB	E	Class 6
W8	61.650	-105.580	QA	EB	E	Class 6
W9	61.650	-105.580	QA	EB	E	Class 6
RI87	62.820	-92.080	QA	EB	E	Class 6
RI89	63.130	-92.800	QA	EB	E	Class 6
RI88	63.580	-92.250	QA	EB	C	Class 6
CI193	63.580	-92.250	QA	EB	E	Class 6
CI195	63.580	-92.250	QA	EB	E	Class 6
CI194	63.830	-91.000	QA	EB	E	Class 6
CI192	64.120	-90.750	QA	EB	E	Class 6
CH24	64.150	-84.450	SH	EB	B	Class 6
CH20	64.160	-84.460	SH	EB	E	Class 6
CH23	64.180	-84.460	SH	EB	E	Class 6
CH22	64.200	-84.500	SH	EB	E	Class 6
CH21	64.200	-84.450	SH	EB	E	Class 6
RI75	64.430	-93.100	QA	EB	E	Class 6
RI76	64.430	-93.100	QA	EB	E	Class 6
RI77	64.430	-93.100	QA	EB	E	Class 6
RI78	64.430	-93.100	QA	EB	E	Class 6
RI79	64.430	-93.100	QA	EB	E	Class 6
RI80	64.430	-93.100	QA	EB	E	Class 6
RI81	64.430	-93.100	QA	EB	E	Class 6
RI83	64.430	-93.100	QA	EB	E	Class 6
RI84	64.430	-93.100	QA	EB	E	Class 6
RI85	64.430	-93.100	QA	EB	E	Class 6
RI86	64.430	-93.100	QA	EB	E	Class 6
RI82	64.430	-93.100	QA	EB	G	Class 6
CI191	64.480	-91.070	QA	EB	E	Class 6
BL40	64.500	-99.000	QA	EB	E	Class 6
BL41	64.500	-99.000	QA	EB	E	Class 6
FF9203	65.033	-122.267	BW	EB	A	Class 6
FF9201	65.083	-123.500	BW	EB	A	Class 6
FF9202	65.083	-123.500	BW	EB	A	Class 6
NW15	65.180	-123.420	BW	EB	A	Class 6
NW11	65.180	-123.420	BW	EB	D	Class 6

NW12	65.180	-123.420	BW	EB	D	Class 6
NW20	65.180	-123.420	BW	EB	D	Class 6
NW02	65.180	-123.420	BW	EB	E	Class 6
NW17	65.180	-123.420	BW	EB	E	Class 6
NW13	65.180	-123.420	BW	EB	F	Class 6
NW14	65.180	-123.420	BW	EB	G	Class 6
FF9394	65.517	-123.950	BW	EB	B	Class 6
BLK-490	66.530	-86.250	NE	EB	E	Class 6
BLK-491	66.530	-86.250	NE	EB	E	Class 6
BLK-492	66.530	-86.250	NE	EB	E	Class 6
BLK-493	66.530	-86.250	NE	EB	E	Class 6
GOS-886	66.530	-86.250	NE	EB	E	Class 6
HB19	66.530	-86.250	NE	EB	E	Class 6
KU151	66.570	-116.430	BA	EB	A	Class 6
KU146	66.570	-116.430	BA	EB	E	Class 6
CB173	66.770	-102.600	BA	EB	E	Class 6
CB174	66.770	-102.600	BA	EB	E	Class 6
CB175	66.770	-102.600	BA	EB	E	Class 6
CB176	66.770	-102.600	BA	EB	E	Class 6
CB177	66.770	-102.600	BA	EB	E	Class 6
CB178	66.770	-102.600	BA	EB	E	Class 6
CB179	66.770	-102.600	BA	EB	E	Class 6
CB180	66.770	-102.600	BA	EB	E	Class 6
KU157	67.030	-115.280	BA	EB	E	Class 6
KU159	67.120	-116.120	BA	EB	E	Class 6
KU145	67.390	-114.380	BA	EB	E	Class 6
KU147	67.390	-114.380	BA	EB	E	Class 6
KU148	67.390	-114.380	BA	EB	E	Class 6
KU150	67.390	-114.380	BA	EB	E	Class 6
KU149	67.390	-114.380	BA	EB	F	Class 6
KIT198	67.680	-107.930	BA	EB	E	Class 6
KIT201	67.680	-107.930	BA	EB	E	Class 6
KIT202	67.680	-107.930	BA	EB	E	Class 6
KU158	67.820	-115.080	BA	EB	A	Class 6
KIT203	67.820	-115.080	BA	EB	D	Class 6
KIT199	67.820	-115.080	BA	EB	E	Class 6
KIT204	67.820	-115.080	BA	EB	E	Class 6
CB220	68.450	-105.200	BA	EB	E	Class 6
CB206	68.500	-107.000	BA	EB	C	Class 5
CB213	68.500	-107.000	BA	EB	C	Class 5
KIT200	68.500	-107.000	BA	EB	E	Class 5
CB205	68.500	-104.750	BA	EB	D	Class 6
CB218	68.500	-104.750	BA	EB	D	Class 6
HB104	68.780	-81.230	NE	EB	E	Class 6
HB16	68.780	-81.230	NE	EB	G	Class 6
PB38	68.880	-90.080	NE	EB	D	Class 6
PB34	68.880	-90.080	NE	EB	E	Class 6
PB35	68.880	-90.080	NE	EB	E	Class 6
PB36	68.880	-90.080	NE	EB	E	Class 6
PB37	68.880	-90.080	NE	EB	E	Class 6
PB39	68.880	-90.080	NE	EB	E	Class 6

TA154	69.130	-92.500	NE	EB	A	Class 6
TA156	69.130	-92.500	NE	EB	D	Class 6
CVR-188	69.380	-81.800	NE	EB	E	Class 5
CVR-194	69.380	-81.800	NE	EB	E	Class 5
CVR-189	69.380	-81.800	NE	EB	G	Class 5
CVR-190	69.380	-81.800	NE	EB	G	Class 5
CVR-191	69.380	-81.800	NE	EB	G	Class 5
CYH-002	69.380	-81.800	NE	EB	G	Class 5
CVR-193	69.380	-81.800	NE	EB	G	Class 5
TA153	69.620	-93.300	NE	EB	E	Class 6
TA155	69.620	-93.300	NE	EB	E	Class 6
BGK-072	45.100	-64.300	MR	AT	F	Class 7
BGR-524	46.170	-64.570	MR	AT	F	Class 7
BTR-035	47.220	-67.980	MR	AT	F	Class 7
BTR-036	47.220	-67.980	MR	AT	F	Class 7
BTR-037	47.220	-67.980	MR	AT	F	Class 7
RFI-955	49.780	-56.630	AT	AT	F	Class 7
BAO-873	51.730	-56.420	AT	AT	F	Class 7
2003004	52.680	-61.400	AT	AT	F	Class 7
CYE-405	52.900	-66.890	AT	AT	F	Class 7
BAI-329	52.950	-66.920	AT	AT	F	Class 7
FCN-987	52.950	-66.920	AT	AT	F	Class 7
VQ2-276	52.950	-66.920	AT	AT	F	Class 7
K26514	53.400	-60.170	AT	AT	F	Class 7
PXY-414	53.550	-64.020	AT	AT	F	Class 7
PXY-787	53.550	-64.020	AT	AT	F	Class 7
PXY-788	53.550	-64.020	AT	AT	F	Class 7
QAP-504	53.550	-64.020	AT	AT	F	Class 7
QAP-505	53.550	-64.020	AT	AT	F	Class 7
2003002	53.580	-60.470	AT	AT	F	Class 7
2003001	53.580	-60.450	AT	AT	F	Class 7
CVK-168	54.180	-58.430	AT	AT	F	Class 7
BAF-117	54.900	-59.780	AT	AT	F	Class 7
BAF-118	54.900	-59.780	AT	AT	F	Class 7
BAF-122	54.900	-59.780	AT	AT	F	Class 7
BAI-443	54.900	-59.780	AT	AT	F	Class 7
UAM18418	53.720	-166.770	PA	CI	B	Class 4
ARF18	55.000	-131.000	CI	CI	B	Class 4
UAM18015	55.220	-132.080	CI	CI	F	Class 4
UAM18016	55.252	-132.255	CI	CI	B	Class 4
UAM17282	55.317	-131.000	CI	CI	F	Class 4
UAM24105	55.333	-131.500	CI	CI	B	Class 4
UAM17134	55.570	-132.530	CI	CI	B	Class 4
UAM17136	55.570	-132.530	CI	CI	B	Class 4
UAM17137	55.570	-132.530	CI	CI	B	Class 4
UAM17279	55.933	-131.383	CI	CI	F	Class 4
UAM18012	56.069	-133.080	CI	CI	B	Class 4
UAM17933	56.070	-133.070	CI	CI	B	Class 4
UAM18152	56.070	-133.070	CI	CI	B	Class 4
UAM18440	56.450	-133.200	CI	CI	B	Class 4
UAM18421	56.500	-133.100	CI	CI	B	Class 4

UAM18422	56.500	-133.100	CI	CI	B	Class 4
UAM18427	56.500	-133.100	CI	CI	B	Class 4
UAM18432	56.550	-133.000	CI	CI	B	Class 4
UAM18438	56.550	-133.000	CI	CI	B	Class 4
UAM18436	56.580	-132.800	CI	CI	B	Class 4
UAM18435	56.600	-133.130	CI	CI	B	Class 4
UAM18419	56.630	-133.250	CI	CI	B	Class 4
UAM18420	56.630	-133.250	CI	CI	B	Class 4
UAM44525	56.630	-133.100	CI	CI	B	Class 4
UAM18181	56.700	-133.670	CI	CI	B	Class 4
UAM18175	56.700	-133.670	CI	CI	B	Class 4
UAM18178	56.700	-133.670	CI	CI	B	Class 4
UAM18184	56.700	-133.670	CI	CI	B	Class 4
UAM18186	56.700	-133.670	CI	CI	B	Class 4
UAM18188	56.700	-133.670	CI	CI	B	Class 4
UAM18424	56.700	-133.670	CI	CI	B	Class 4
UAM18430	56.700	-133.670	CI	CI	B	Class 4
UAM18439	56.700	-133.670	CI	CI	B	Class 4
UAM18425	56.770	-133.200	CI	CI	B	Class 4
UAM18426	56.770	-133.200	CI	CI	B	Class 4
UAM18434	56.830	-132.970	CI	CI	B	Class 4
S07	71.220	-122.470	BI	BI	C	Class 5
S08	71.220	-122.470	BI	BI	C	Class 5
SW35	71.220	-122.470	BI	BI	C	Class 5
SW37	71.220	-122.470	BI	BI	C	Class 5
SW38	71.220	-122.470	BI	BI	C	Class 5
SHS423	71.350	-122.750	BI	BI	C	Class 5
SHS424	71.350	-122.750	BI	BI	C	Class 5
SHS425	71.350	-122.750	BI	BI	C	Class 5
SHS421	71.400	-122.800	BI	BI	C	Class 5
SHS9329	71.717	-123.367	BI	BI	C	Class 5
SHS9330	71.717	-123.367	BI	BI	C	Class 5
SHS9331	71.717	-123.367	BI	BI	C	Class 5
SHS9332	71.717	-123.367	BI	BI	C	Class 5
SHS9333	71.717	-123.367	BI	BI	C	Class 5
SHS9334	71.717	-123.367	BI	BI	C	Class 5
SHS9335	71.717	-123.367	BI	BI	C	Class 5
SHS9336	71.717	-123.367	BI	BI	C	Class 5
SHS9337	71.717	-123.367	BI	BI	C	Class 5
HW41	71.820	-124.550	BI	BI	E	Class 5
SHS442	71.833	-124.533	BI	BI	C	Class 5
SHS9302	71.875	-122.500	BI	BI	C	Class 5
SHS9303	71.875	-122.500	BI	BI	C	Class 5
SHS9304	71.875	-122.500	BI	BI	C	Class 5
SHS9305	71.875	-122.500	BI	BI	C	Class 5
SHS9306	71.875	-122.500	BI	BI	C	Class 5
SHS9105	71.900	-124.867	BI	BI	C	Class 5
SHS978-05	71.958	-124.750	BI	BI	C	Class 5
SH023	71.970	-126.000	BI	BI	C	Class 5
SHS9201	71.978	-125.049	BI	BI	C	Class 5
SHS9204	71.978	-125.049	BI	BI	C	Class 5

SHS9301	71.978	-125.049	BI	BI	C	Class 5
SHS9340	71.978	-125.049	BI	BI	C	Class 5
SHS9203	71.980	-125.000	BI	BI	C	Class 5
SHS426	71.980	-124.833	BI	BI	C	Class 5
SHS9106	71.983	-125.250	BI	BI	C	Class 5
SHS978-07	71.983	-125.250	BI	BI	C	Class 5
SHS978-14	71.983	-125.250	BI	BI	C	Class 5
SHS978-36	71.983	-125.250	BI	BI	C	Class 5
SHS978-33	71.983	-125.250	BI	BI	E	Class 5
SHS9202	71.992	-124.867	BI	BI	C	Class 5
SHS978-08	72.000	-125.100	BI	BI	C	Class 5
SHS978-09	72.000	-124.600	BI	BI	C	Class 5
SHS978-10	72.000	-124.600	BI	BI	C	Class 5
SHS416	72.000	-124.530	BI	BI	C	Class 5
SHS417	72.000	-124.530	BI	BI	C	Class 5
SHS978-35	72.000	-123.000	BI	BI	C	Class 5
SHS431	72.033	-125.217	BI	BI	C	Class 5
SHS9103	72.033	-124.583	BI	BI	C	Class 5
SHS9108	72.033	-124.583	BI	BI	C	Class 5
SHS978-03	72.033	-124.583	BI	BI	C	Class 5
SHS427	72.050	-124.867	BI	BI	C	Class 5
SHS428	72.050	-124.867	BI	BI	C	Class 5
SHS429	72.050	-124.867	BI	BI	C	Class 5
SHS430	72.050	-124.867	BI	BI	C	Class 5
SHS978-04	72.248	-124.000	BI	BI	C	Class 5
SHS978-12	72.263	-123.985	BI	BI	C	Class 5
SH038	72.270	-123.980	BI	BI	C	Class 5
S04	72.280	-124.480	BI	BI	C	Class 5
S05	72.280	-124.480	BI	BI	C	Class 5
S06	72.280	-124.480	BI	BI	C	Class 5
SW13	72.280	-124.480	BI	BI	C	Class 5
SW14	72.280	-124.480	BI	BI	C	Class 5
SW15	72.280	-124.480	BI	BI	C	Class 5
SW16	72.280	-124.480	BI	BI	C	Class 5
SW17	72.280	-124.480	BI	BI	C	Class 5
SW18	72.280	-124.480	BI	BI	C	Class 5
SW19	72.280	-124.480	BI	BI	C	Class 5
SW36	72.280	-124.480	BI	BI	C	Class 5
SHS9339	72.333	-124.167	BI	BI	C	Class 5
SHS456	72.359	-123.719	BI	BI	C	Class 5
SHS457	72.359	-123.719	BI	BI	C	Class 5
SHS432	72.433	-125.033	BI	BI	C	Class 5
SHS433	72.433	-125.033	BI	BI	C	Class 5
SHS434	72.433	-125.033	BI	BI	C	Class 5
SHS418	72.483	-122.833	BI	BI	C	Class 5
SHS419	72.483	-122.833	BI	BI	C	Class 5
SHS420	72.483	-122.833	BI	BI	C	Class 5
SHS422	72.483	-122.833	BI	BI	C	Class 5
SW30	72.930	-124.480	BI	BI	C	Class 5
SW31	72.930	-124.480	BI	BI	C	Class 5
SW32	72.930	-124.480	BI	BI	C	Class 5

SHN455	73.229	-119.556	BI	BI	C	Class 5
SHN978-15	73.400	-122.000	BI	BI	C	Class 5
SHN9311	73.417	-121.950	BI	BI	C	Class 5
SHN9312	73.417	-121.950	BI	BI	C	Class 5
SHN9313	73.417	-121.950	BI	BI	C	Class 5
SHN9314	73.417	-121.950	BI	BI	C	Class 5
SHN9315	73.417	-121.950	BI	BI	C	Class 5
SHN9316	73.417	-121.950	BI	BI	C	Class 5
SHN9317	73.417	-121.950	BI	BI	C	Class 5
SHN9318	73.417	-121.950	BI	BI	C	Class 5
SHN9319	73.417	-121.950	BI	BI	C	Class 5
SHN9320	73.417	-121.950	BI	BI	C	Class 5
SHN9321	73.417	-121.950	BI	BI	C	Class 5
SHN9322	73.417	-121.950	BI	BI	C	Class 5
SHN9323	73.417	-121.950	BI	BI	C	Class 5
SHN9324	73.417	-121.950	BI	BI	C	Class 5
SHN9325	73.417	-121.950	BI	BI	C	Class 5
SHN978-16	73.425	-121.980	BI	BI	C	Class 5
SHN978-17	73.440	-121.925	BI	BI	C	Class 5
SHN453	73.444	-119.950	BI	BI	C	Class 5
SHN454	73.444	-119.950	BI	BI	C	Class 5
SH027	73.470	-122.950	BI	BI	C	Class 5
SH028	73.470	-122.950	BI	BI	C	Class 5
SH029	73.470	-122.950	BI	BI	C	Class 5
SH030	73.470	-122.950	BI	BI	C	Class 5
SH031	73.470	-122.950	BI	BI	C	Class 5
SH033	73.470	-122.950	BI	BI	C	Class 5
SH036	73.470	-122.950	BI	BI	C	Class 5
SW20	73.470	-122.950	BI	BI	C	Class 5
SW21	73.470	-122.950	BI	BI	C	Class 5
SW22	73.470	-122.950	BI	BI	C	Class 5
SW53	73.470	-122.950	BI	BI	C	Class 5
SW54	73.470	-122.950	BI	BI	C	Class 5
SW55	73.470	-122.950	BI	BI	C	Class 5
SW56	73.470	-122.950	BI	BI	C	Class 5
S01	73.570	-124.080	BI	BI	C	Class 5
S02	73.570	-124.080	BI	BI	C	Class 5
S03	73.570	-124.080	BI	BI	C	Class 5
SH051	73.570	-124.080	BI	BI	C	Class 5
SH052	73.570	-124.080	BI	BI	C	Class 5
SW10	73.570	-124.080	BI	BI	C	Class 5
SW11	73.570	-124.080	BI	BI	C	Class 5
SW12	73.570	-124.080	BI	BI	C	Class 5
SW25	73.570	-124.080	BI	BI	C	Class 5
SW27	73.570	-124.080	BI	BI	C	Class 5
SW28	73.570	-124.080	BI	BI	C	Class 5
SW29	73.570	-124.080	BI	BI	C	Class 5
SW33	73.570	-124.080	BI	BI	C	Class 5
SHN452	73.622	-119.988	BI	BI	C	Class 5
HW57	73.820	-119.920	BI	BI	C	Class 5
SH024	73.820	-119.920	BI	BI	C	Class 5

SH025	73.880	-116.330	BI	BI	C	Class 5
SH026	73.880	-116.330	BI	BI	C	Class 5
SH035	73.880	-116.330	BI	BI	C	Class 5
SW23	73.880	-116.330	BI	BI	C	Class 5
SHN9328	73.967	-119.750	BI	BI	C	Class 5
SHN978-18	73.971	-120.150	BI	BI	C	Class 5
SHN978-19	73.971	-120.150	BI	BI	C	Class 5
SHN978-20	73.971	-120.150	BI	BI	C	Class 5
SHN978-21	73.971	-120.150	BI	BI	C	Class 5
SHN978-22	73.971	-120.150	BI	BI	C	Class 5
SHN978-23	73.971	-120.150	BI	BI	C	Class 5
SHN9326	74.000	-119.833	BI	BI	C	Class 5
SHN449	74.016	-120.068	BI	BI	C	Class 5
SHN450	74.016	-120.068	BI	BI	C	Class 5
SHN451	74.016	-120.068	BI	BI	C	Class 5
SHN9327	74.025	-119.867	BI	BI	C	Class 5
SHN978-24	74.050	-119.570	BI	BI	C	Class 5
SHN978-25	74.050	-119.570	BI	BI	C	Class 5
SHN978-26	74.050	-119.570	BI	BI	C	Class 5
SHN978-27	74.050	-119.570	BI	BI	C	Class 5
SHN978-28	74.050	-119.570	BI	BI	C	Class 5
SHN978-29	74.050	-119.570	BI	BI	C	Class 5
SHN978-30	74.050	-119.570	BI	BI	C	Class 5
SHN978-31	74.050	-119.570	BI	BI	C	Class 5
SHN444	74.128	-119.825	BI	BI	C	Class 5
SHN445	74.128	-119.825	BI	BI	C	Class 5
SHN446	74.128	-119.825	BI	BI	C	Class 5
SHN447	74.128	-119.825	BI	BI	C	Class 5
SHN448	74.128	-119.825	BI	BI	C	Class 5
SW24	74.130	-119.750	BI	BI	C	Class 5
SW26	74.130	-119.750	BI	BI	C	Class 5
CB215	68.920	-104.370	VI	VI	E	Class 6
CB219	68.920	-104.370	VI	VI	E	Class 6
CB209	69.100	-105.050	VI	VI	C	Class 5
CB207	69.180	-104.700	VI	VI	C	Class 5
HW47	70.420	-115.000	VI	VI	C	Class 5
HW59	70.420	-115.000	VI	VI	C	Class 5
HW76	70.420	-115.000	VI	VI	C	Class 5
HW61	70.420	-115.000	VI	VI	C	Class 5
HW62	70.420	-115.000	VI	VI	C	Class 5
HW72	70.420	-115.000	VI	VI	C	Class 5
HW74	70.420	-115.000	VI	VI	C	Class 5
HW58	70.420	-115.000	VI	VI	D	Class 5
HW44	70.420	-115.000	VI	VI	E	Class 5
HW45	70.420	-115.000	VI	VI	E	Class 5
HW46	70.420	-115.000	VI	VI	E	Class 5
HW73	70.420	-115.000	VI	VI	E	Class 5
HW82	70.730	-117.750	VI	VI	C	Class 5
HW89	71.250	-117.420	VI	VI	C	Class 3
HW77	71.250	-116.800	VI	VI	C	Class 5
HW78	71.250	-116.800	VI	VI	C	Class 5

HW79	71.250	-116.800	VI	VI	C	Class 5
HW80	71.250	-116.800	VI	VI	C	Class 5
HW83	71.250	-116.800	VI	VI	C	Class 5
HW81	71.250	-116.800	VI	VI	E	Class 5
HW48	71.330	-117.000	VI	VI	C	Class 5
HW52	71.330	-117.000	VI	VI	C	Class 5
HW60	71.330	-117.000	VI	VI	C	Class 5
HW63	71.330	-117.000	VI	VI	C	Class 5
HW64	71.330	-117.000	VI	VI	C	Class 5
HW49	71.330	-117.000	VI	VI	E	Class 5
HW87	71.350	-117.420	VI	VI	E	Class 3
HW90	71.360	-117.430	VI	VI	E	Class 5
HW91	71.360	-117.430	VI	VI	E	Class 5
HW85	71.420	-113.420	VI	VI	C	Class 5
HW84	71.420	-113.420	VI	VI	C	Class 5
HW67	71.430	-117.470	VI	VI	C	Class 5
HW68	71.430	-117.470	VI	VI	E	Class 5
HW06	71.533	-117.767	VI	VI	C	Class 5
HW69	71.580	-118.870	VI	VI	C	Class 5
HW05	71.720	-117.490	VI	VI	C	Class 5
HW08	71.720	-117.490	VI	VI	C	Class 5
HW09	71.720	-117.490	VI	VI	C	Class 5
HW03	71.720	-117.490	VI	VI	C	Class 5
HW04	71.720	-117.490	VI	VI	C	Class 5
HW07	71.720	-117.490	VI	VI	C	Class 5
HW01	71.900	-117.300	VI	VI	C	Class 5
HW86	71.900	-111.580	VI	VI	C	Class 5
HW65	72.770	-111.020	VI	VI	C	Class 5
HW66	72.770	-111.020	VI	VI	C	Class 5
HW70	72.770	-111.020	VI	VI	C	Class 5
HW71	72.770	-111.020	VI	VI	C	Class 5
HW75	72.770	-111.020	VI	VI	E	Class 5
GF210	75.530	-82.500	HA	HA	C	Class 5
GF217	76.420	-82.880	HA	HA	E	Class 5
GF214	77.100	-84.320	HA	HA	C	Class 5
GF135	77.120	-83.330	HA	HA	C	Class 5
GF136	77.190	-84.260	HA	HA	C	Class 5
GF44	77.190	-84.260	HA	HA	G	Class 5
GF45	77.190	-84.260	HA	HA	G	Class 5
GF211	77.220	-85.420	HA	HA	C	Class 5
GF212	77.220	-85.420	HA	HA	E	Class 5
GF208	77.220	-85.420	HA	HA	G	Class 5
GF216	77.220	-85.420	HA	HA	G	Class 5
KI112	62.500	-70.250	SB	BAF	E	Class 5
KI109	62.500	-70.250	SB	BAF	G	Class 5
KI110	62.500	-70.250	SB	BAF	G	Class 5
KI111	62.500	-70.250	SB	BAF	G	Class 5
KI113	62.500	-70.250	SB	BAF	G	Class 5
KI115	62.500	-70.250	SB	BAF	G	Class 5
KI107	62.500	-70.250	SB	BAF	G	Class 5
KI108	62.500	-70.250	SB	BAF	G	Class 5

KI116	62.500	-70.250	SB	BAF	G	Class 5
KI09	62.600	-69.500	SB	BAF	G	Class 5
KI106	62.830	-69.870	SB	BAF	G	Class 5
KI47	62.830	-69.870	SB	BAF	G	Class 5
KI114	62.830	-69.870	SB	BAF	G	Class 5
IQ43	62.830	-66.580	SB	BAF	G	Class 5
KI53	62.900	-69.850	SB	BAF	G	Class 5
KI52	62.930	-69.800	SB	BAF	G	Class 5
KI51	63.120	-69.730	SB	BAF	G	Class 5
IQ98	63.600	-68.820	SB	BAF	G	Class 5
IQ101	63.730	-68.570	SB	BAF	G	Class 5
IQ92	63.730	-68.570	SB	BAF	G	Class 5
IQ93	63.730	-68.570	SB	BAF	G	Class 5
IQ97	63.730	-68.570	SB	BAF	G	Class 5
IQ91	63.730	-68.570	SB	BAF	G	Class 5
IQ100	63.750	-68.520	SB	BAF	G	Class 5
IQ102	63.750	-68.520	SB	BAF	G	Class 5
IQ103	63.750	-68.520	SB	BAF	G	Class 5
IQ99	63.750	-68.520	SB	BAF	G	Class 5
KI105	63.750	-68.520	SB	BAF	G	Class 5
IQ33	63.900	-68.320	SB	BAF	G	Class 5
CD130	64.160	-76.580	SB	BAF	E	Class 6
CD127	64.160	-76.580	SB	BAF	G	Class 6
IQ61	64.170	-69.420	SB	BAF	G	Class 5
IQ62	64.170	-69.420	SB	BAF	G	Class 5
CD94	64.230	-76.530	SB	BAF	G	Class 5
CD95	64.230	-76.530	SB	BAF	G	Class 5
CD129	64.250	-75.350	SB	BAF	G	Class 5
CD138	64.280	-75.490	SB	BAF	G	Class 5
CD137	64.400	-73.580	SB	BAF	E	Class 6
CD139	64.400	-73.580	SB	BAF	E	Class 6
CD96	64.430	-74.800	SB	BAF	G	Class 5
CD131	64.450	-75.600	SB	BAF	E	Class 5
CD128	64.450	-75.600	SB	BAF	G	Class 5
CD140	64.450	-75.600	SB	BAF	G	Class 5
PG63	65.170	-65.500	SB	BAF	G	Class 5
PG64	65.170	-65.500	SB	BAF	G	Class 5
PG65	65.170	-65.500	SB	BAF	G	Class 5
PG67	65.980	-71.200	SB	BAF	G	Class 5
PG69	66.050	-68.330	SB	BAF	G	Class 5
PG70	66.050	-68.330	SB	BAF	G	Class 5
PG90	66.050	-68.330	SB	BAF	G	Class 5
PG66	66.120	-65.620	SB	BAF	G	Class 5
PG08	66.130	-65.720	SB	BAF	G	Class 5
PG72	66.130	-65.720	SB	BAF	G	Class 5
PG73	66.130	-65.720	SB	BAF	G	Class 5
PG74	66.130	-65.720	SB	BAF	G	Class 5
PG01	66.480	-70.330	SB	BAF	G	Class 5
PG02	66.480	-70.330	SB	BAF	G	Class 5
PG05	66.480	-70.330	SB	BAF	G	Class 5
PG06	66.480	-70.330	SB	BAF	G	Class 5

PG07	66.480	-70.330	SB	BAF	G	Class 5
ANP01	66.550	-66.920	SB	BAF	G	Class 5
PG68	66.550	-66.920	SB	BAF	G	Class 5
PG03	66.570	-67.450	SB	BAF	G	Class 5
PG04	66.570	-67.450	SB	BAF	G	Class 5
CR26	68.500	-71.330	SB	BAF	G	Class 5
CR28	68.500	-71.330	SB	BAF	G	Class 5
CR29	68.500	-71.330	SB	BAF	G	Class 5
CR27	68.500	-71.330	SB	BAF	G	Class 5
CR30	69.620	-67.550	SB	BAF	G	Class 5
II14	69.650	-80.070	NB	BAF	G	Class 5
HB25	69.780	-77.250	NB	BAF	G	Class 5
II12	69.830	-83.000	NB	BAF	G	Class 6
II160	69.930	-81.720	NB	BAF	E	Class 5
II15	70.080	-84.830	NB	BAF	E	Class 6
KI48	70.100	-63.800	SB	BAF	G	Class 5
KI49	70.100	-63.800	SB	BAF	G	Class 5
KI50	70.100	-63.800	SB	BAF	G	Class 5
KI54	70.100	-63.800	SB	BAF	G	Class 5
II162	70.170	-82.500	NB	BAF	G	Class 5
HB17	70.170	-82.500	NB	BAF	G	Class 5
HB18	70.170	-82.500	NB	BAF	G	Class 5
II10	70.200	-81.480	NB	BAF	G	Class 5
II11	70.200	-81.480	NB	BAF	G	Class 5
PI32	70.250	-81.700	NB	BAF	E	Class 5
II13	70.250	-81.700	NB	BAF	G	Class 5
PI31	70.250	-81.700	NB	BAF	G	Class 5
II161	70.250	-78.580	NB	BAF	G	Class 5
II164	70.250	-78.580	NB	BAF	G	Class 5
PI144	70.620	-80.680	NB	BAF	G	Class 5
AB124	71.190	-85.510	NB	BAF	D	Class 6
AB126	71.190	-85.510	NB	BAF	E	Class 6
AB125	71.190	-85.510	NB	BAF	G	Class 6
AB121	71.190	-85.510	NB	BAF	G	Class 6
AB122	71.190	-85.510	NB	BAF	G	Class 6
AB120	71.230	-85.100	NB	BAF	E	Class 6
AB123	71.230	-85.100	NB	BAF	E	Class 6
AB118	71.560	-84.270	NB	BAF	E	Class 6
AB196	72.100	-84.500	NB	BAF	G	Class 6
AB197	72.100	-84.500	NB	BAF	G	Class 6
PI141	72.100	-79.000	NB	BAF	G	Class 5
PI60	72.100	-79.000	NB	BAF	G	Class 5
AB119	72.250	-80.340	NB	BAF	G	Class 6
AB117	72.250	-80.340	NB	BAF	G	Class 6
AB132	72.350	-84.430	NB	BAF	D	Class 6
AB133	72.550	-84.170	NB	BAF	E	Class 6
AB134	72.560	-84.100	NB	BAF	C	Class 6
PI143	72.700	-77.980	NB	BAF	F	Class 5
PI142	72.700	-77.980	NB	BAF	G	Class 5
PI58	72.700	-77.980	NB	BAF	G	Class 5
PI56	72.700	-77.980	NB	BAF	G	Class 5

PI57	72.700	-77.980	NB	BAF	G	Class 5
PI59	72.700	-77.980	NB	BAF	G	Class 5
AB152	72.980	-85.100	NB	BAF	G	Class 5
II163	73.030	-85.170	NB	BAF	G	Class 5
CVK-163	73.030	-85.170	NB	BAF	G	Class 5
II165	73.030	-85.170	NB	BAF	G	Class 5

Table 3-1 Genetic variation in analogous populations of wolves and arctic foxes, averaged over eight common microsatellite loci.

Arctic Foxes					**Grey Wolves**				
Region[*]	**N**[†]	**H_E**[§]	**H_E SD**	**A^R (20)**[¥]	**Region**[*]	**N**[†]	**H_E**[§]	**H_E SD**	**A^R (20)**[¥]
Mackenzie	20	0.78	0.04	6.54	Mackenzie	337	0.72	0.03	5.55
Karrak	48	0.80	0.03	6.78	Bathurst	29	0.71	0.05	5.66
Kivalliq	303	0.81	0.04	7.13	Qamanirjuaq	597	0.72	0.04	5.86
Manitoba	45	0.81	0.03	6.86	Manitoba	84	0.76	0.04	5.97
NE Main	99	0.81	0.04	7.05	NE Main	25	0.65	0.06	5.21
Atlantic	25	0.81	0.04	7.14	Atlantic	19	0.69	0.07	5.27
Mainland		**0.80**		**6.92**	**Mainland**		**0.71**		**5.58**
Banks	10	0.81	0.04	7.25	Banks	163	0.61	0.04	3.66
Victoria	95	0.80	0.04	6.96	Victoria	52	0.66	0.04	4.39
High Arctic	17	0.77	0.07	6.85	High Arctic	11	0.56	0.07	3.20
N. Baffin	68	0.81	0.03	7.06	N. Baffin	43	0.61	0.09	4.35
S. Baffin	27	0.81	0.04	6.95	S. Baffin	73	0.57	0.07	3.99
Island		**0.80**		**7.01**	**Island**		**0.60**		**3.92**

[*] Analogous geographic regions appear in the same row, and averages for population type are given in bold. Regions are shown in Figure 3-1a and b.

[†] number of individuals sampled in each region

[§] expected heterozygosity, with standard deviation indicated by SD

[¥] allelic richness, with rarefaction size (in allele copies) given in brackets

Table 3-2 Nei's standard genetic distance (D_S) between regions. Fox populations are given below the diagonal, with wolf populations above. The square matrix shows fox-wolf comparisons.

	MA	KA	KI	MB	NE	AT	BI	VI	HA	NB	SB
Mackenzie (MA)		0.108	0.056	0.066	0.113	0.324	0.232	0.166	0.279	0.152	0.350
Karrak/Bathurst (KA)	0.107		0.030	0.096	0.061	0.275	0.275	0.193	0.228	0.097	0.266
Kivalliq/Qaminirjuaq (KI)	0.090	0.032		0.055	0.057	0.253	0.234	0.143	0.221	0.086	0.272
Manitoba (MB)	0.127	0.045	0.021		0.125	0.270	0.265	0.170	0.295	0.185	0.382
NE Main (NE)	0.094	0.046	0.016	0.034		0.306	0.367	0.274	0.267	0.073	0.175
Atlantic (AT)	0.121	0.067	0.040	0.062	0.047		0.517	0.568	0.592	0.283	0.420
Banks (BI)	0.132	0.157	0.117	0.149	0.099	0.140		0.111	0.282	0.390	0.660
Victoria (VI)	0.100	0.041	0.019	0.036	0.030	0.078	0.114		0.260	0.288	0.525
High Arctic (HA)	0.192	0.113	0.077	0.107	0.092	0.135	0.186	0.082		0.223	0.368
N. Baffin (NB)	0.067	0.048	0.024	0.043	0.029	0.057	0.112	0.039	0.116		0.099
S. Baffin (SB)	0.108	0.070	0.042	0.053	0.050	0.072	0.168	0.053	0.103	0.066	
Mackenzie (MA)	2.364	2.037	2.050	2.093	2.079	2.137	2.256	2.160	2.171	2.177	2.127
Karrak/Bathurst (KA)	2.457	2.004	2.103	2.171	2.092	2.229	2.264	2.169	2.311	2.247	2.231
Kivalliq/Qaminirjuaq (KI)	2.355	1.968	2.011	2.085	2.019	2.120	2.174	2.087	2.145	2.152	2.090
Manitoba (MB)	2.106	1.815	1.819	1.880	1.825	1.881	1.955	1.869	1.965	1.957	1.927
NE Main (NE)	2.464	2.046	2.083	2.144	2.063	2.154	2.211	2.201	2.275	2.201	2.128
Atlantic (AT)	1.629	1.427	1.424	1.493	1.368	1.528	1.602	1.399	1.409	1.550	1.506
Banks (BI)	2.633	2.322	2.254	2.356	2.380	2.568	2.344	2.350	2.492	2.455	2.714
Victoria (VI)	2.524	2.298	2.278	2.307	2.403	2.514	2.534	2.417	2.552	2.492	2.677
High Arctic (HA)	2.702	2.615	2.590	2.809	2.763	2.823	2.486	2.695	2.993	2.647	2.579
N. Baffin (NB)	2.727	2.253	2.334	2.444	2.268	2.405	2.401	2.479	2.368	2.444	2.308
S. Baffin (SB)	2.331	2.004	1.969	1.988	1.854	2.117	1.994	2.095	1.987	2.049	1.916

Table 3-3 Assignment among wolf clusters. The proportion of individuals sampled in each cluster, which assign to each cluster, is indicated by each row. Self-assignment proportions are outlined, and bold values represent significantly more cross-assignment than predicted given each sample's allele frequencies.

Sampling Cluster	**Assigned Cluster**									
	WW	FO	WB	EB	AT	CI	BI	VI	HA	BAF
Western Woods (WW)	0.904	**0.047**	0.037	0.012	0.000	0.000	0.000	0.000	0.000	0.000
Forest (FO)	0.050	0.589	0.074	**0.275**	0.000	0.000	0.000	0.004	0.000	**0.008**
Western Barrens (WB)	**0.084**	**0.110**	0.679	0.089	0.004	0.000	**0.008**	**0.025**	0.000	0.000
Eastern Barrens (EB)	**0.024**	**0.192**	0.080	0.635	**0.036**	0.000	0.001	0.013	0.000	**0.020**
Atlantic (AT)	0.000	0.040	0.000	0.000	0.960	0.000	0.000	0.000	0.000	0.000
Coastal Islands (CI)	**0.056**	0.000	**0.028**	0.000	0.000	0.917	0.000	0.000	0.000	0.000
Banks Island (BI)	0.000	0.000	0.000	0.000	0.000	0.000	0.939	**0.061**	0.000	0.000
Victoria Island (VI)	0.000	0.000	**0.038**	0.038	0.000	0.000	**0.231**	0.692	0.000	0.000
High Arctic (HA)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Baffin Island (BAF)	0.000	0.000	0.000	0.060	0.000	0.000	0.000	**0.009**	0.000	0.931

	% Variation Explained	
Axis	Individual	Cumulative
1	112.99	112.99
2	14.18	127.18
3	9.21	136.39
4	2.35	138.74
5	0.01	138.75
6	0.00	138.75
7	-0.29	138.45
8	-1.43	137.02
9	-2.42	134.60
10	-34.60	100.00

Table 3-4a Principle coordinate analysis of Nei's D_S among wolf clusters. The large negative Eigenvalue of axis 10 indicates non-metricity and implies complexity within the genetic distance.

Table 3-4b Correlation among predictor variables used in distance-based redundancy analysis of Nei's D_S among wolf clusters. Variable sets are indicated in bold.

		Spatial		**Prey**		**Habitat**		
	Barrier	Latitude	Longitude	Behavior	Species	Temperature	Rain	Vegetation
Barrier	1							
Latitude	0.5156	1						
Longitude	-0.2068	-0.097	1					
Behavior	0.6124	-0.0544	0.2056	1				
Species	0.5278	0.7424	-0.1747	0.068	1			
Temperature	-0.2059	-0.8524	-0.2934	0.1393	-0.4712	1		
Rain	0.1137	-0.5771	-0.2625	0.214	-0.0516	0.8482	1	
Vegetation	0.7013	0.531	0.2656	0.6247	0.7332	-0.3735	-0.0262	1

Table 3-5 Distance-based redundancy analysis of Nei's D_S among wolf clusters. We analyzed individual variables (single predictors) alone, then sequentially to obtain a combined model. Analysis was then repeated while treating variables as predictor sets (grouped as in Table 3-4b). Significant *P* values in marginal tests are shown in bold. The column headed % Var indicates the amount of variation in D_S explained by a particular variable, with Cumulative indicating the total variation explained by all fitted variables in sequential tests. Explanatory power of greater than 100% results from non-metricity in the D_S matrix.

Test		**Single Predictors**			
Marginal	Variable	F	P	% Var	
	Barrier	-0.65	0.9273	-8.80	
	Latitude	11.42	**0.0115**	58.80	
	Longitude	3.83	0.1188	32.37	
Prey <	Behavior	-0.56	**0.9779**	-7.49	
	Species	0.24	0.6685	2.97	
	Temperature	392.34	**0.0001**	98.00	
	Rain	23.09	**0.0017**	74.27	
	Vegetation	0.21	0.6477	2.54	
Sequential	Variable	F	P	% Var	Cumulative
	Temperature	392.34	0.0001	98.00	98.00
	Longitude	-8.06	0.7760	15.17	113.18
		Predictor Sets			
Marginal	Variable	F	P	% Var	
	Barrier	-0.65	0.9287	-8.80	
	Spatial	185.06	**0.0005**	98.14	
	Prey	-0.12	0.8796	-3.46	
	Habitat	5.15	0.0623	72.03	
Sequential	Variable	F	P	% Var	Cumulative
	Spatial	185.06	0.0005	98.14	98.14

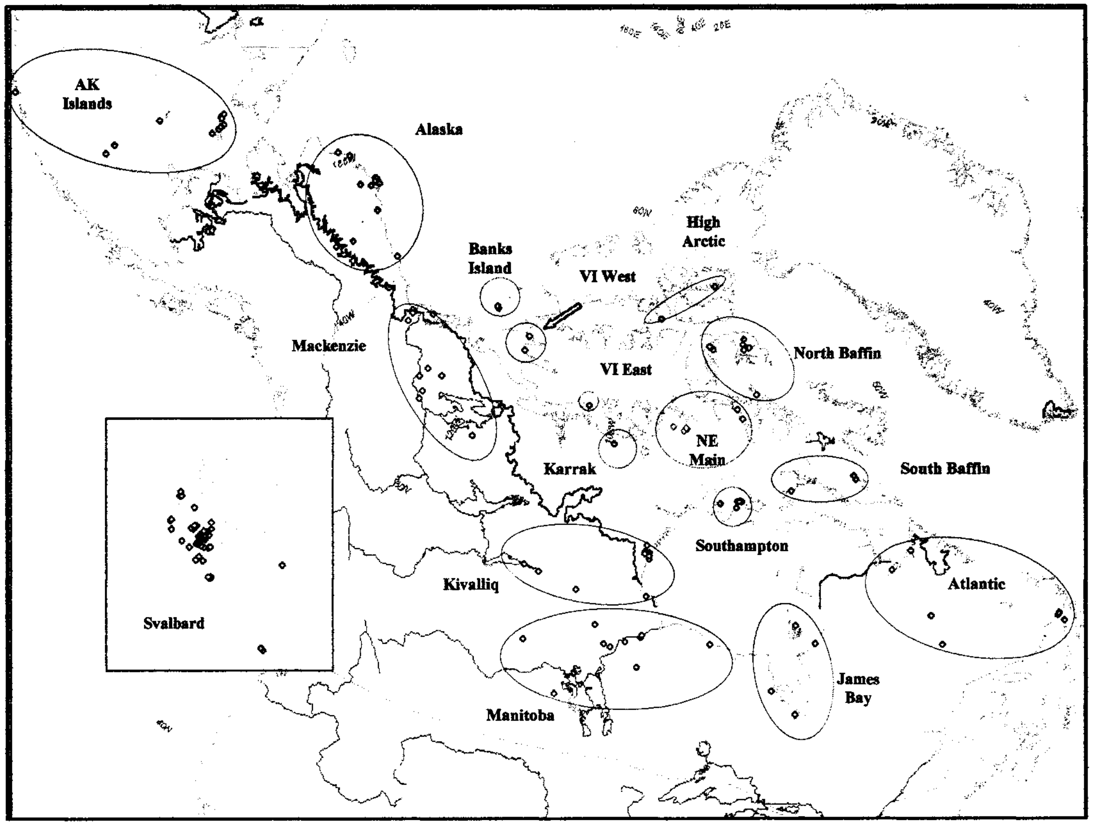

Figure 3-1a Sampling range of arctic foxes, with treeline indicated by a black line. Fox samples are shown grouped into geographic regions. In some analyses, Victoria East and Victoria West were pooled into a single population, "Victoria," to facilitate direct comparisons to wolves.

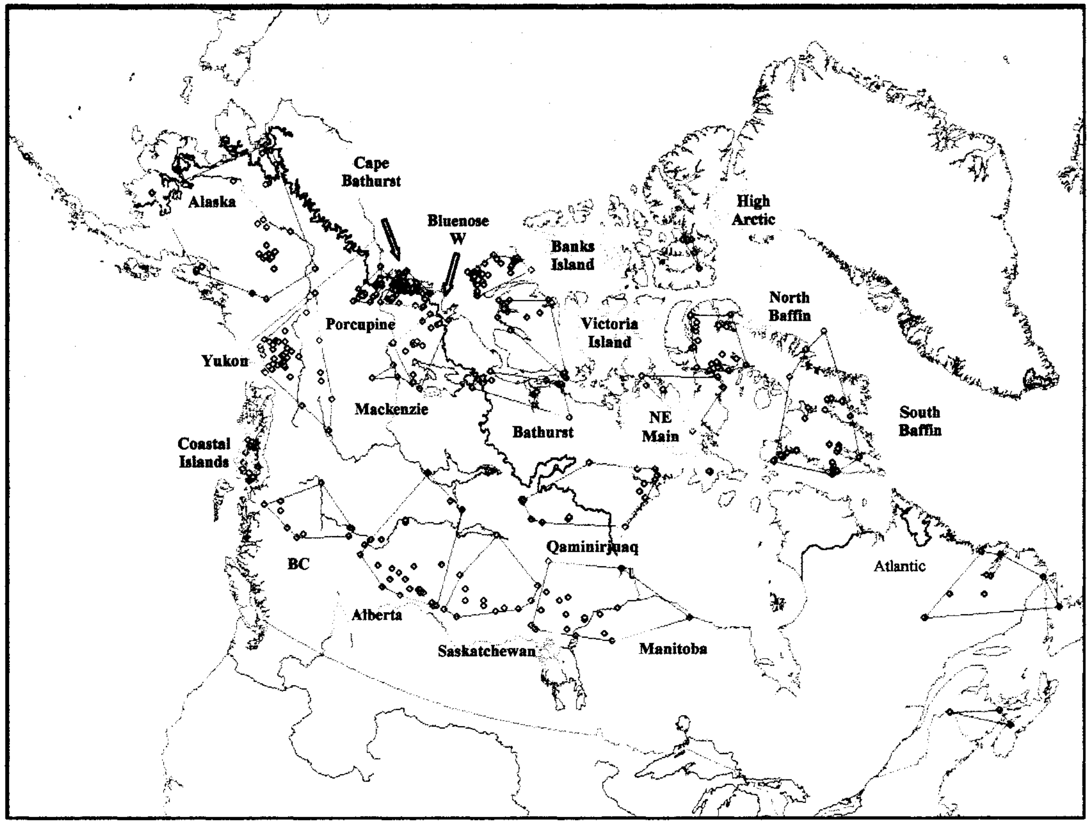

Figure 3-1b Sampling range of grey wolves, with treeline indicated by a black line. Wolf samples are shown grouped into geographic regions. Cape Bathurst, Bluenose W, Bathurst, NE Main and Qaminurjuaq are composed primarily of migratory wolves; all other populations are non-migratory. In some analyses, Porcupine, Mackenzie, Cape Bathurst and Bluenose West were pooled into a single population, "Mackenzie," to facilitate direct comparisons to arctic foxes.

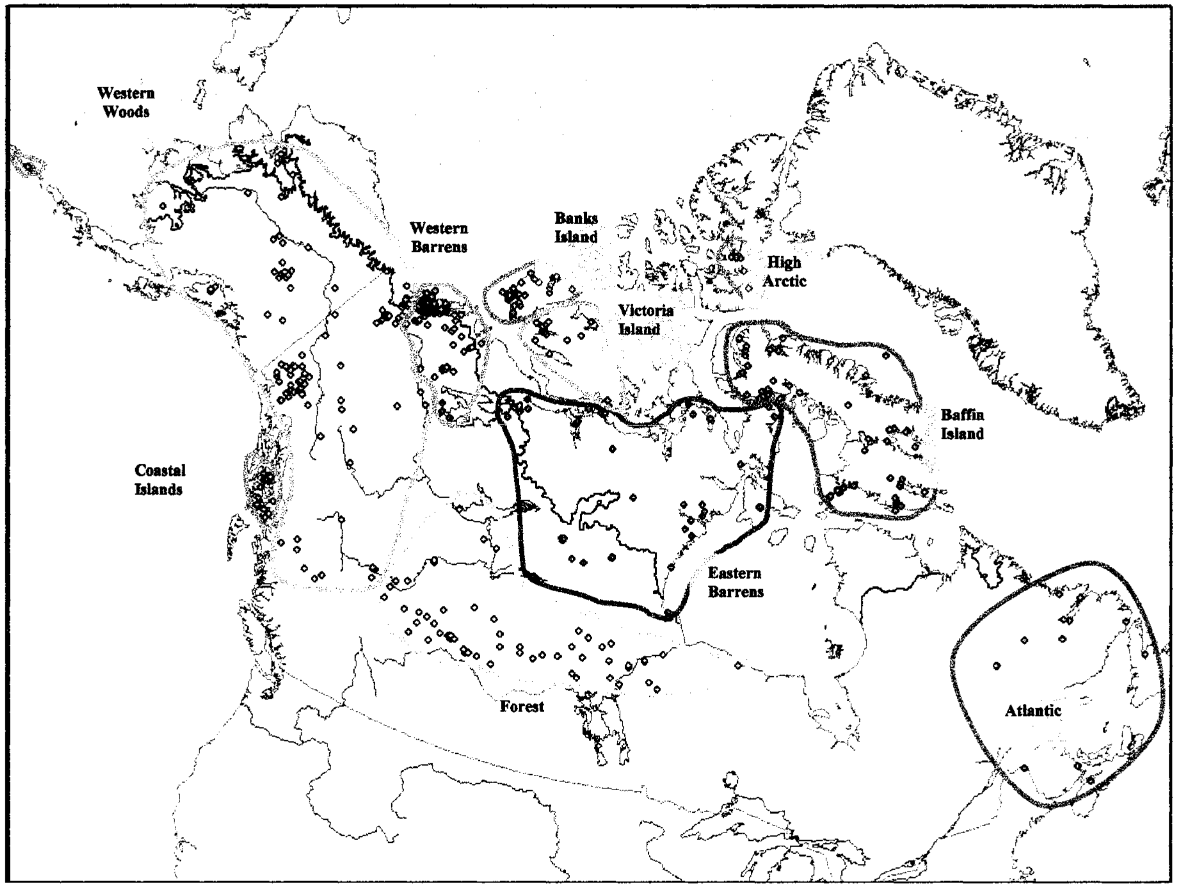

Figure 3-1c Grey wolf samples grouped into genetic clusters based on results of analyses in STRUCTURE and GENELAND. Western Barrens and Eastern Barrens represent migratory wolves, with all other populations considered sedentary.

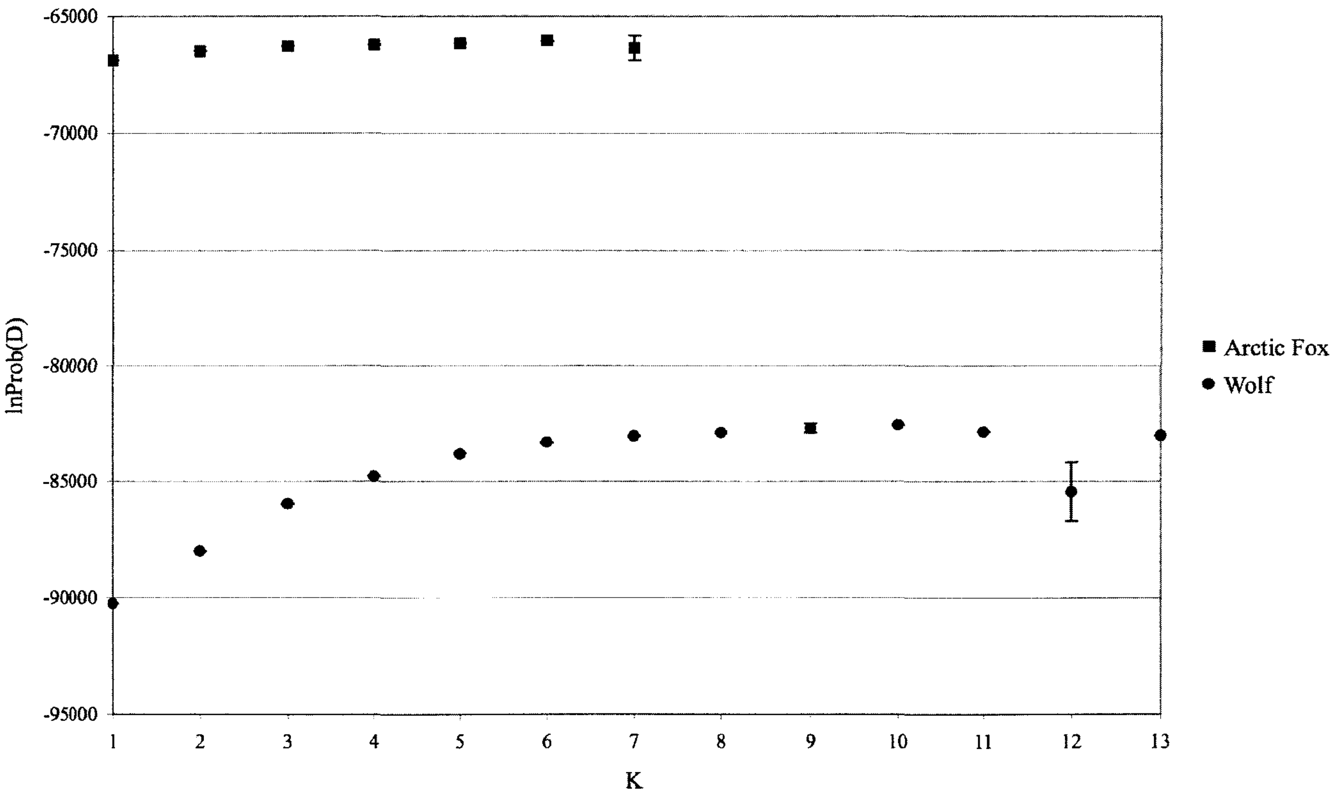

Figure 3-2a Average lnProb(D) as number of clusters is increased during STRUCTURE analysis of arctic foxes and grey wolves. Probability of wolf data began to peak around K=7. All values of K were similarly likely for arctic foxes.

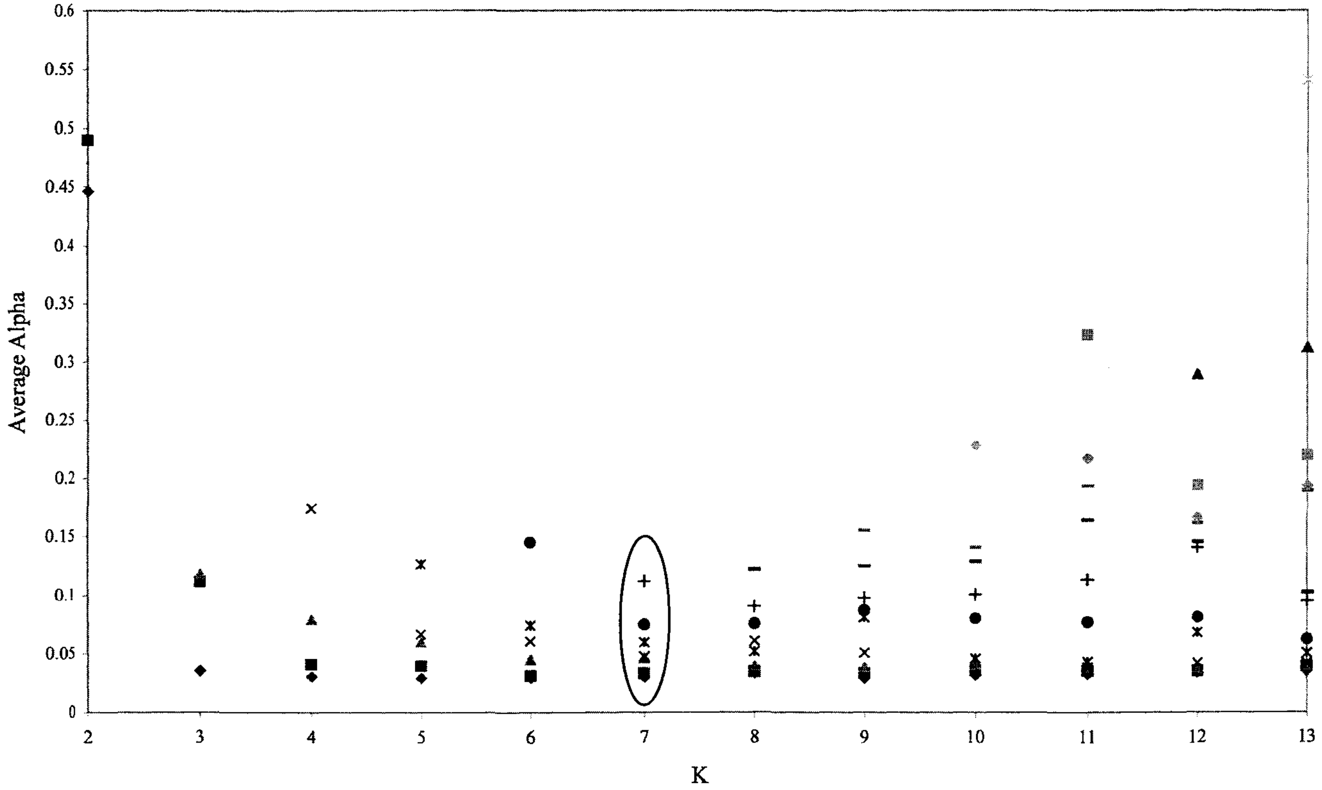

Figure 3-2b Average admixture of each wolf cluster as K is increased. Data from equivalent clusters at each value of K was pooled across three replicates. Lowest levels of admixture were obtained with K=7, suggesting highest group cohesion under this model.

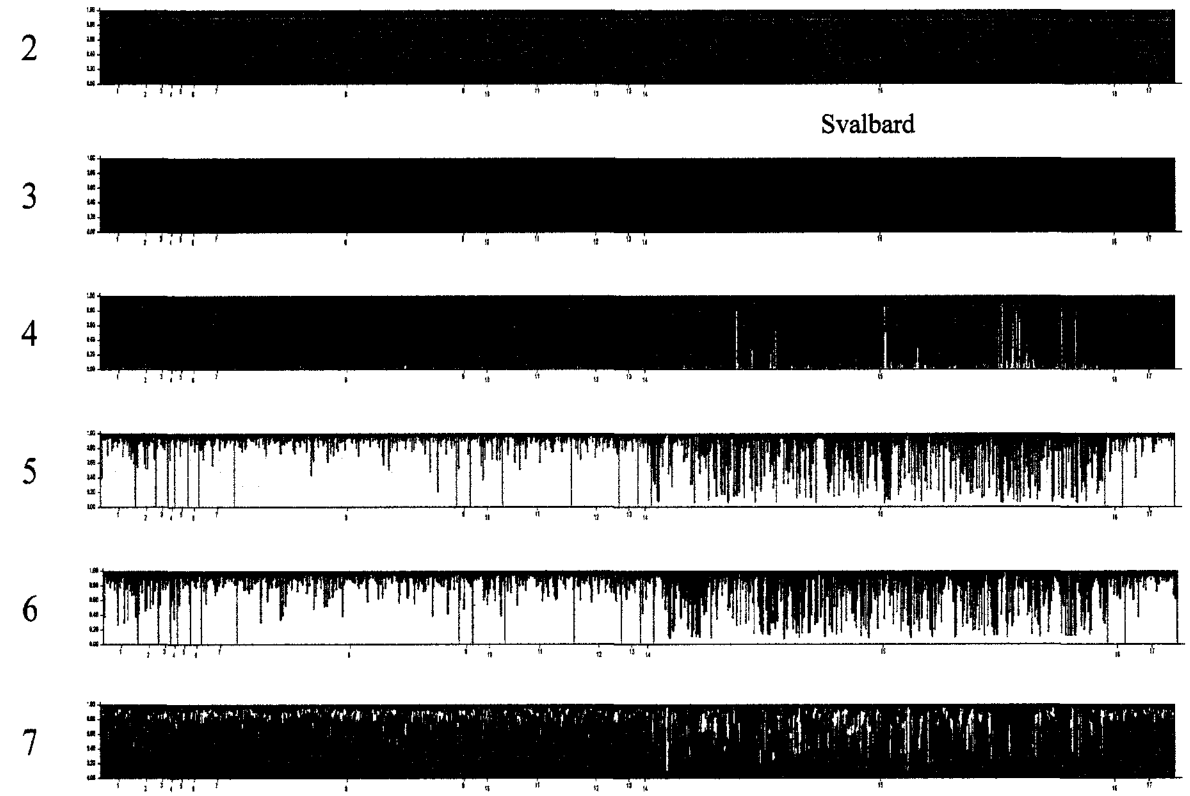

Figure 3-3 Individual assignment of arctic foxes after clustering in STRUCTURE. Vertical bars represent individual foxes. Number of clusters (K) is shown at right. Region breaks were not used during clustering, but are shown for reference, with regions arranged in alphabetical order. Svalbard was the only geographical region that appeared to contain true, if low-level, genetic differentiation; partitioning of all other foxes seemed to reflect segregation of rare alleles rather than of divergent individuals.

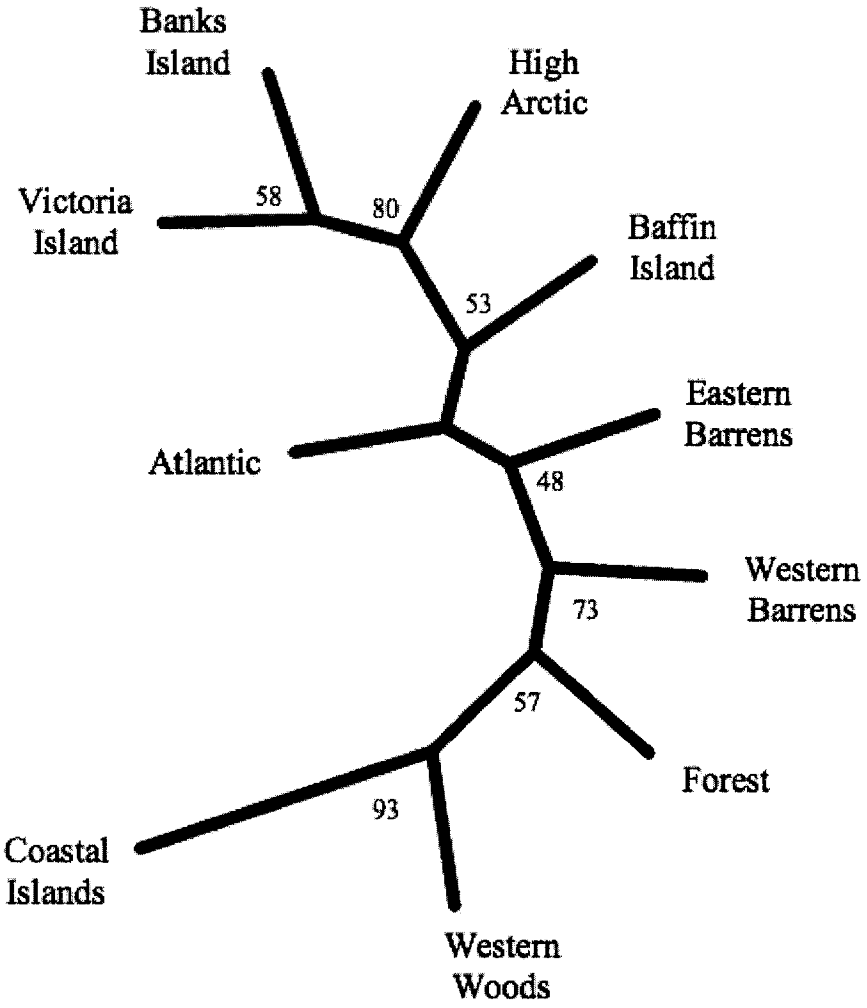

Figure 3-4a Majority-rule consensus tree of wolf clusters based on Nei's D_S. Bootstrap support values for each node are indicated.

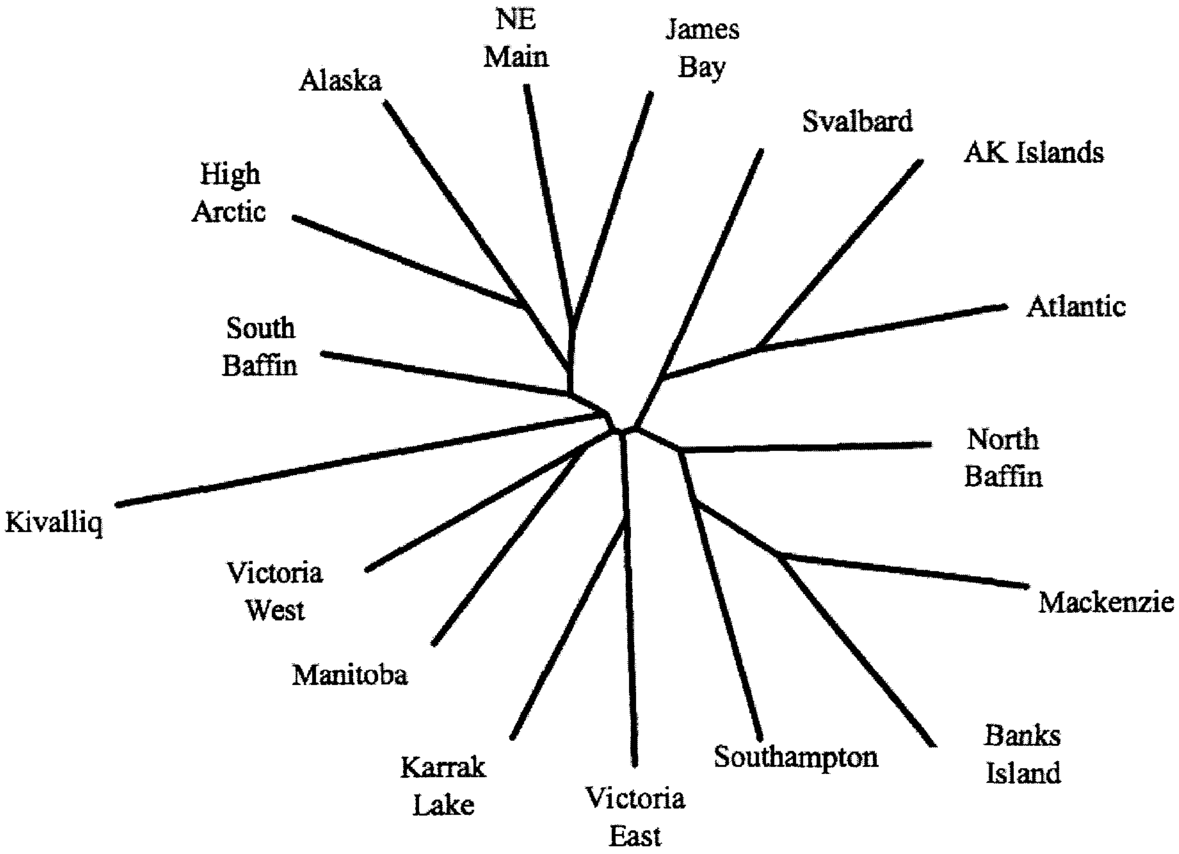

Figure 3-4b Majority rule consensus tree of arctic fox regions, based on Nei's D_S. Bootstrap support is not indicated, as no grouping occurred in more than 40% of trees.

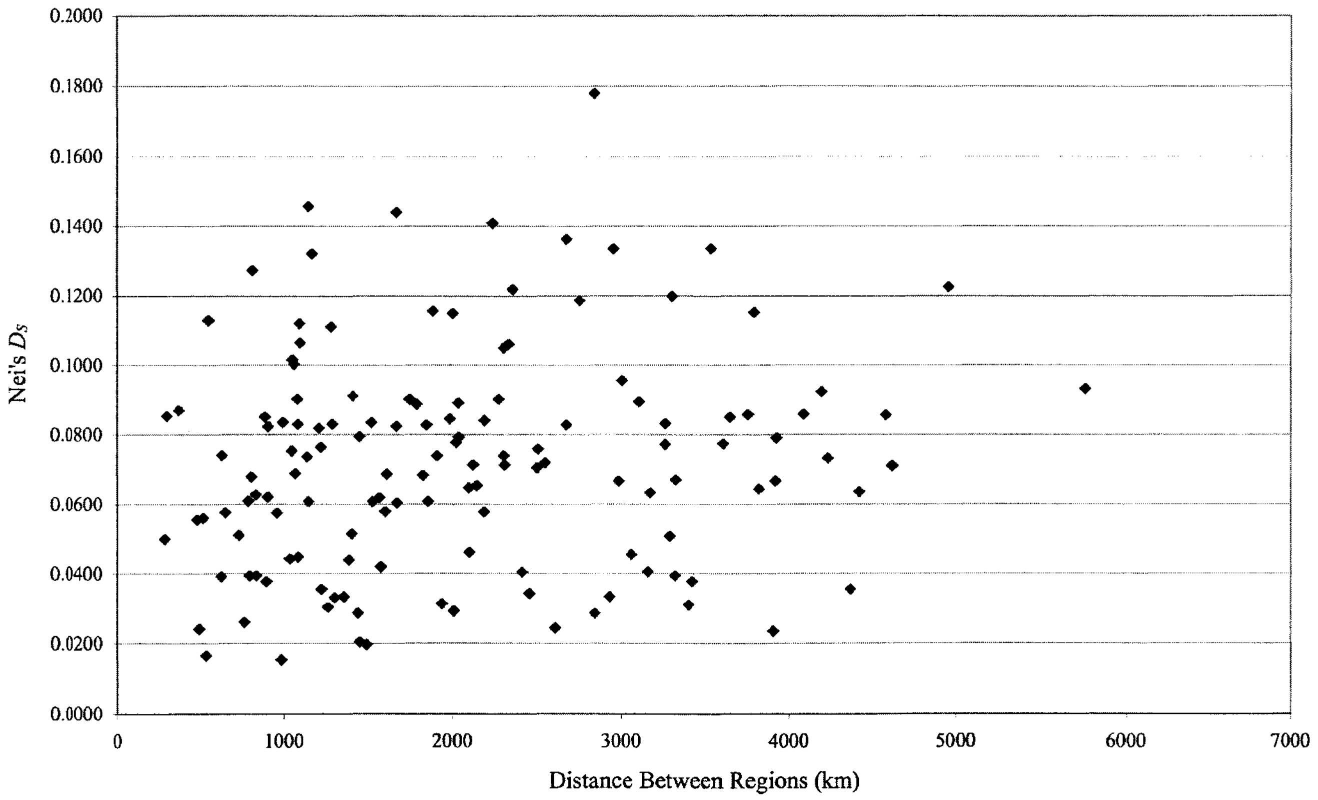

Figure 3-5 Nei's D_S between pairs of arctic fox regions, relative to the geographic distance between them. Mantel tests confirmed that isolation-by-distance does not occur in this species.

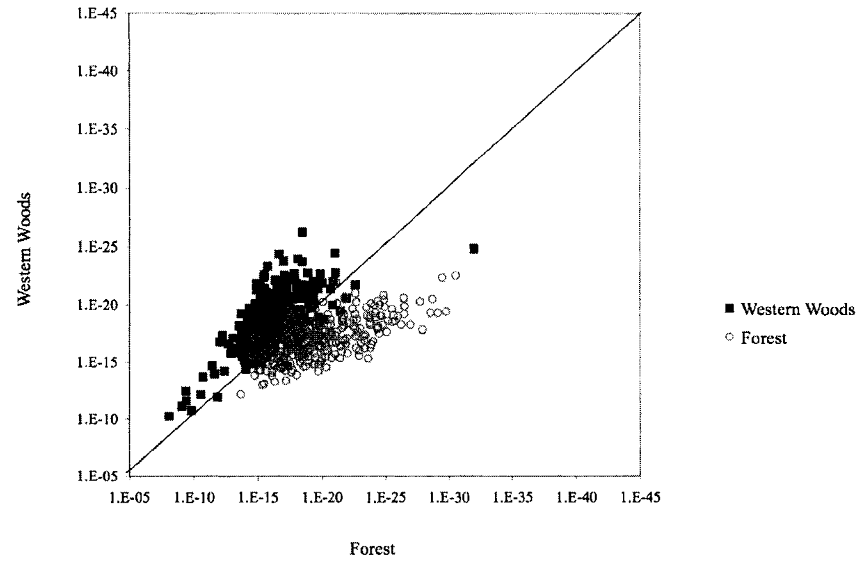

Figure 3-6a Assignment among wolf clusters within the boreal forest habitat type. Symbols indicate the sampling cluster of each wolf. Individuals are plotted according to the probability that their genotype would arise in each cluster; the diagonal line represents genotypes equally likely in both. The Western Woods and Forest clusters are 1816 km apart. The low level of overlap in assignment indices is suggestive of moderate genetic differentiation.

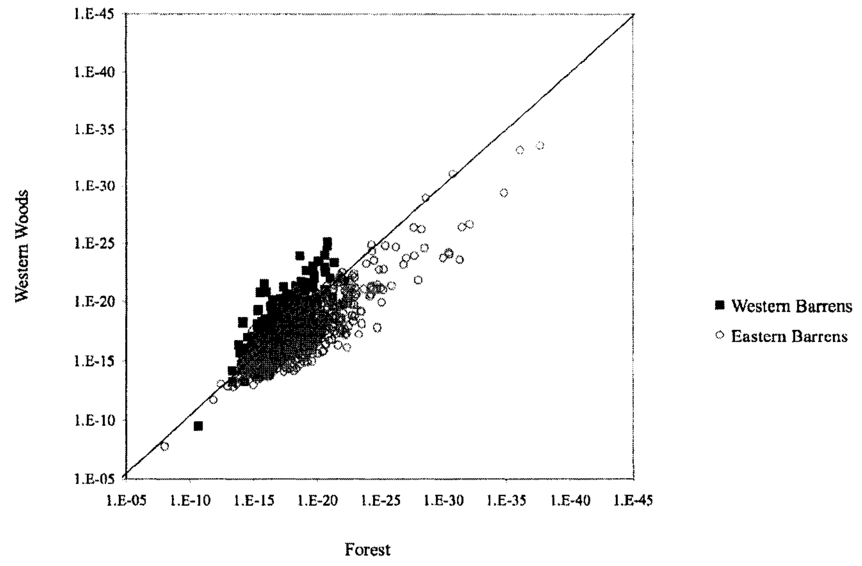

Figure 3-6b Assignment among wolves within the barren ground habitat type. Western Barrens and Eastern Barrens are separated by 1462 km. Increased overlap in assignment indices relative to the boreal forest may be partially due to decreased geographic distance, but also signifies lower genetic differentiation within the barren ground habitat type.

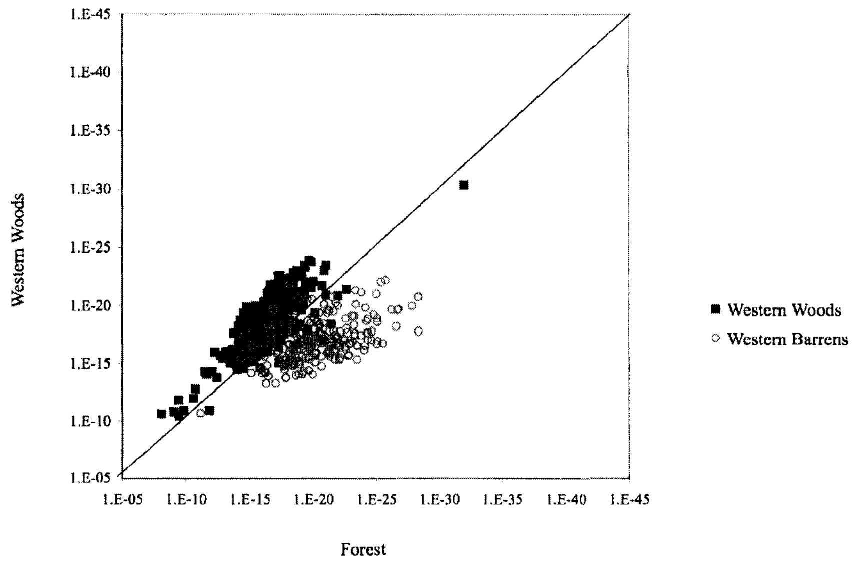

Figure 3-7a Assignment among wolves occupying different habitat types. Despite a physical separation roughly half that represented in **6b** (766 km), differentiation is equivalent to that within the boreal forest habitat type.

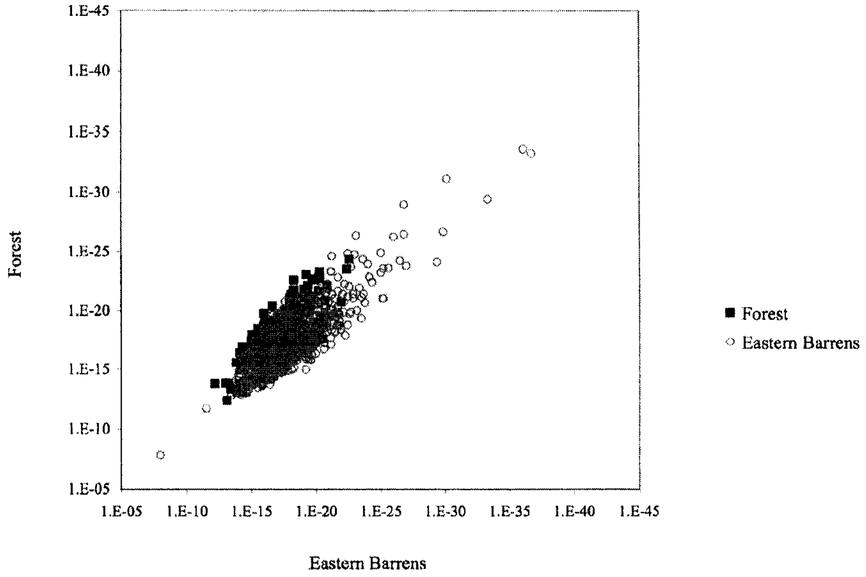

Figure 3-7b Assignment among wolves occupying different habitat types. In this case, genetic differentiation appears equivalent to that observed within the barren-ground habitat type.

Chapter 4

Northwest Passages: Genetics of Arctic Island Wolves Over Space and Time

Introduction

Grey wolves (*Canis lupus*) have inhabited the Canadian arctic islands since the Pleistocene (Kurtén & Anderson 1980). Indeed, contemporary wolves in this region may have descended from a refugial population in northeast Greenland that expanded across the Arctic Archipelago after the last glacial maximum (Nowak 2003). Supporting this idea are the unusually large carnassial teeth found in contemporary wolves on nearly all arctic islands, which have prompted the subspecific designation *C. l. arctos*: only Baffin Island wolves are thought to belong to the mainland subspecies *C. l. occidentalis* (Nowak 1995). The extended tenure of wolves on the arctic islands does not imply populations have remained stable or static over time, however. Banks Island wolves collected in 1914-16 were classified as *C. l. bernardi* based on their long, narrow, flat skulls, but samples dating from 1953-55 were short, broad, high, and thus morphologically consistent with *C. l. arctos* (Manning & Macpherson 1958). Manning & MacPherson (1958) attributed this change to extinction of the original population, followed by replacement with immigrants from neighboring Melville or Victoria Islands. Over the last century, significant changes in skull morphology have been documented in other arctic island populations as well (Clutton-Brock *et al.* 1994).

Wolves in the Canadian Arctic Archipelago have experienced recent fluctuations in population size as well as morphology. On Banks Island in the 1950s, wolf scavenging on trapped arctic foxes prompted a poisoning program which severely reduced or extirpated this population (McEwen 1955; Usher 1965); wolves began to reappear in the late 1970s and 1980s, and have increased to approximately 200 individuals (Chapter 2, Carmichael *et al.* 2001; Gunn *et al.* 1991; Larter & Nagy 2000; Miller 1995; Miller & Reintjes 1995). Similarly, wolves had continually inhabited the Fosheim Peninsula of Ellesmere Island for decades, were not observed by field workers in 2001-02, and have since returned to the area (Mech 2005). It is not clear whether changes in population size on individual islands result only from human harvesting and local interplay between predator and prey density, or whether expansion of wolf populations on a given island is prompted by over-ice recolonization from elsewhere in the Archipelago (Manning & Macpherson 1958; Usher 1965).

The arctic islands may be the harshest habitat in the range of the grey wolf. Average daily minimum temperature is below -20°C, and lows of less than

-50°C have been documented (Environment Canada 2000). Climatic variation resulting in deep winter snow, or ice buildup following freezing rain, may have negative impacts on survival of caribou (*Rangifer tarandus*) and muskoxen (*Ovibos moschatus*; Gunn *et al.* 1991; Larter & Nagy 2001a; Larter & Nagy 2001b). Small prey species like arctic hares (*Lepus arcticus*), arctic foxes (*Alopex lagopus*), sea birds and microtine rodents (e.g. *Lemmus* sp.) are insufficient to meet the nutritional requirements of large carnivores in years of low ungulate density, and wolf populations decline with reductions in ungulate prey (Mech 2005). In addition to these climatic and energetic constraints, wolves on the arctic islands may face anthropogenic threats to persistence. While absence of infrastructure means arctic island wolves do not suffer road or railway mortalities as those in southern populations do, they are currently considered big game species throughout the Canadian Territories and are harvested for both commercial and subsistence use (Van Zyll de Jong & Carbyn 1999). On some islands, this harvest may be as high as 25% of the total population annually (Chapter 2).

Considering these significant external impediments to continued lupine inhabitance of the Canadian arctic islands, genetic threats to persistence may be of particular concern. If island populations are demographically isolated, mating between close relatives may increase, and fitness may be lost through inbreeding depression (Frankham 2005). Since demographic isolation also results in genetic isolation, loss of genetic variation via accelerated drift may hamper the population's ability to adapt to a changing environment (Frankham 2005). Arctic island wolves may have additional significance for conservation: since these populations might have originated from a glacial refugium spatially distinct from southern wolf refugia (Nowak 1995), they may retain unique adaptations and genetic characteristics not found in mainland wolves, thus representing a disproportionate fraction of total wolf genetic variation (Leonard *et al.* 2005).

The purpose of this study was to use nuclear microsatellite loci to determine: 1) the relationship of arctic island wolves to mainland wolves, and the rate of migration between them, 2) the degree of gene flow and differentiation among island populations, and 3) the genetic status of each island population, encompassing relative variation, level of inbreeding, and stability over time. An improved understanding of the nature and characteristics of wolves on the arctic islands may facilitate assignment of an appropriate conservation priority and permit management decisions suited to wolf populations inhabiting the changing arctic environment.

Materials and Methods

Sample Collection, Laboratory Analysis and Dataset Validation

Contemporary samples of 1,924 wolves from across the North American Arctic (Fig. 4-1) were examined. The dataset contained only one set of duplicate genotypes: a pair of wolves believed to be identical twins (Chapter 8). Samples discussed here were also analyzed in Chapter 3, and include wolves from the following Canadian arctic islands: Banks Island (n = 163); Victoria Island (n = 52); the High Arctic (Devon Island and Ellesmere Island, pooled due to low sample size, n = 11); and Baffin Island (North and South Baffin, n = 116). Coastal Island wolves from southeastern Alaska were included for comparison to northern populations (n = 35).

DNA extraction, PCR amplification of microsatellites and molecular sexing loci, and gel electrophoresis conditions are described in Chapter 3.

Genetic Delineation of Wolf Populations

Bayesian clustering of genotypes was conducted in STRUCTURE 2.1 (Pritchard *et al.* 2000) without any prior spatial information and using 100,000 burn-in cycles followed by 1,000,000 iterations of the Markov Chain. A unique level of admixture (α) was assumed for each cluster, the shape of the allele frequency distribution of each locus (λ) was inferred, and the number of clusters (K) was allowed to vary between 1 and 6. Three replicates each of K = 1-13 were then performed, setting λ to 0.4 and ALPHAPROPSD to 0.1 (Chapter 3). Peaking of lnProb(D) (Pritchard *et al.* 2000) and level of admixture in each cluster indicated K = 7 was most appropriate for this data. STRUCTURE results were confirmed using the spatial model implemented in GENELAND 1.0.5 (Chapter 3; Guillot *et al.* 2005). Delineation of mainland clusters was nearly identical between GENELAND and STRUCTURE, but the methods differed with regards to island populations (see Results). Ten wolf clusters were therefore used as the basis of all further analysis (Fig. 4-1).

Variation within Wolf Clusters

Expected heterozygosity H_E (Nei & Roychoudhury 1974) within each genetic cluster was estimated in the Microsatellite Excel Toolkit (Park 2001), and significant differences in H_E were tested using Wilcoxon's signed-ranks tests with $P = 0.05$ (Sokal & Rohlf 1995). The rarefaction method implemented in CONTRIB 1.01 (Petit *et al.* 1998) was then used to calculate allelic richness after correction for variation in sample size. The High Arctic sample was smallest, and rarefaction size was therefore set to 22 allele copies (Table 4-1).

BOTTLENECK 1.2.02 was used to test for recent bottlenecks in all island populations (Piry *et al.* 1999; Spencer *et al.* 2000). Tests were conducted under the Infinite Allele Model (IAM, Kimura & Crow 1964), the Stepwise Mutation Model for microsatellites (SMM, Ohta & Kimura 1973), and the Two-Phase Mutation Model (TPM, di Rienzo *et al.* 1994). The following combinations of variance and probability were used under the TPM: 4% and 90%; 12% and 70%; 12% and 90%; and 12% and 95%. All tests were assessed for significance with a one-tailed Wilcoxon's test for excess heterozygosity. Consistent evidence of population declines was found for Banks Island and the High Arctic; the M-ratio test was therefore performed to confirm results for these populations, using a microsatellite mutation rate of 5×10^{-4}, a 90% chance of single-step mutations, an average multi-step mutation size of 3.5 repeats, and 10,000 permutations to assess significance (Garza & Williamson 2001). Pre-bottleneck effective population size (*Ne*) was estimated separately for each island. The maximum theoretical carrying capacity for wolves on the Queen Elizabeth Islands (including Ellesmere and Devon Island) is approximately 205 individuals (Miller 1995); *Ne*'s of 205, 100, 50, and 20 were tested to account for dispersion of wolves across the entire island chain, and for complexity in wolf social structure (i.e., variable pack size and number of breeders within each pack; Mech & Boitani 2003). A total of 54 wolves were observed on Banks Island in 1955 prior to initiation of wolf control (McEwen 1955); 43 wolves were poisoned before the program was discontinued in 1959 due to absence of wolves on the island (Usher 1965). Pre-bottleneck *Ne* was therefore set to 54 and 43. Assuming that each of the eight groups of two or more wolves observed prior to poisoning in 1955 represented a different pack (McEwen 1955), each with two breeding adults, *Ne* = 16 was also tested. Finally, a pre-bottleneck *Ne* of 200, the current estimated total population of wolves on Banks Island, was assumed (J Nagy, unpublished data).

Banks Island has been continuously occupied by wolves since the 1970s, but the present population sample was collected in three intervals: 1991-93 (n = 43), 1997-99 (n = 63), and 2002-04 (n = 57). The total sample (pooled for all other analyses) was therefore subdivided and changes in allele frequencies in the re-established population used to estimate current *Ne*. Since genetic exchange occurs between Banks Island and other wolf populations (Chapter 2 and Results), maximum likelihood estimates of *Ne* were computed using the program M Ne 1.01, which accounts for the influence of both genetic drift and migration on allele frequencies over time (Wang & Whitlock 2003). For this analysis, each sampling interval was assumed to be separated by two wolf generations (Mech & Seal 1987), and for simplicity, generations were considered discrete. The following populations were tested as sources of migrants to Banks Island: Victoria and the High Arctic Islands; the Western Barrens; and island and mainland sources combined.

Relationships Among Wolf Clusters

PHYLIP 3.65 was used to calculate Nei's standard genetic distance (D_S) among all genetically defined clusters, and between sampling periods for Banks Island (Felsenstein 1995; Nei 1972). Mainland wolf clusters derived in STRUCTURE encompassed broad geographical areas; to identify gene flow corridors between island and mainland wolves at a finer physical scale, 1000 bootstrap pseudoreplicates were generated based upon the smaller geographic regions defined in Chapter 3. D_S was calculated for each replicate, and a neighbor-joining majority-rule consensus tree constructed (Felsenstein 1985; Saitou & Nei 1987). For reference, membership of each geographic region in each STRUCTURE cluster (Fig. 4-1) is shown in Fig. 4-2.

Paetkau *et al.*'s classical assignment test (1995) was conducted among clusters with allele frequencies adjusted to avoid zeros (Titterington *et al.* 1981), and 10,000 randomizations – creating new individuals from each population's allele frequencies – to discriminate between cross-assignments signaling true migration and those resulting from correlated allele frequencies (Chapter 2). Further assignment was performed using the Bayesian model implemented in BAYESASS, which also provides estimates of inbreeding within each population and the asymmetrical rates of migration between them (Wilson & Rannala 2003). Each run consisted of 3,000,000 iterations, with a burn-in of 999,999 cycles and a thinning interval of 2000. After determining appropriate update values for allele frequencies (0.05), rates of migration (0.025), and inbreeding in each population (0.08), two replicates were performed with unique random number seeds and convergence of the outcomes graphically assessed. Results of all four assignment methods (STRUCTURE, GENELAND, BAYESASS and classical) were compared to identify island-mainland migrants.

Results

Delineation of Wolf Populations

Results from STRUCTURE and GENELAND were combined to devise ten wolf clusters for analysis (Fig. 4-1, Chapter 3). On the mainland, both methods recovered an Atlantic group, a western and eastern boreal forest group (Western Woods and Forest) and western and eastern barren ground groups (Western Barrens and Eastern Barrens). Under the optimal model K = 7 (Chapter 3), STRUCTURE pooled the Coastal Islands with the Western Woods and split the arctic islands into a Western cluster (Banks and Victoria Island) and an Eastern cluster (North and South Baffin Island). GENELAND, in contrast, grouped all arctic islands into one population and segregated the Coastal Islands, likely because of the high spatial concentration of these samples (Guillot *et al.* 2005). While

STRUCTURE recognized the Western Islands at K = 3, and the Eastern Islands at K = 4, the Coastal Islands were not segregated until K = 9 (data not shown).

Because of this inconsistency, and to retain the ability to explore the genetics of island wolves in detail, the Coastal Islands, Victoria Island, and Baffin Island (Northern and Southern regions combined, Chapter 3) were considered distinct populations. Clustering analysis of Banks Island alone did not recover discrete sampling intervals, and the total sample (163 individuals) was therefore treated as a single genetic cluster. In contrast, STRUCTURE described the High Arctic region as a mixture of Western Island wolves, Eastern Island wolves, and mainland types. However, these samples were analyzed as a discrete population to allow further genetic characterization of this physically isolated region.

Low Variation, Population Bottlenecks, and Island Inbreeding

On average, island wolves had 14% less H_E than mainland wolves; all island populations were significantly less variable than those on the mainland (Table 4-1). Victoria Island was also significantly more diverse than the High Arctic (Table 4-1). In contrast to the relatively consistent H_E among islands, allelic richness (A^r) values suggested two general categories of island wolves: the Coastal Islands, Victoria Island, and Baffin Island had more than 4 alleles per locus, while Banks Island and the High Arctic had fewer than 4 alleles per locus (Table 4-1). These estimates were corrected for sample size, and thus suggest a genuine loss of allelic diversity in the latter two populations.

Similar contrasts between H_E and A^r have also been observed in the recently bottlenecked Phillip Island red fox population (Lade *et al.* 1996). Since Banks Island and the High Arctic are also known to have suffered demographic bottlenecks in the last 50 years (Mech 2005; Usher 1965), we tested for significant genetic signatures of population declines. Results from BOTTLENECK were highly dependent on the mutational model assumed: under the IMM, all 10 populations (including mainland wolves) showed significant ($P < 0.05$) or nearly significant ($P < 0.1$) evidence of bottlenecks, while under the SMM, no population was significant. All parameter sets under the TPM produced intermediate results. Despite this, Banks Island showed evidence of population decline under all models but the SMM (e.g., $P = 0.01$, TPM with variance = 12% and probability = 70%), and the High Arctic population was always marginally significant (e.g., $P = 0.08$, TPM 12% and 70%). M ratios were calculated to confirm these results assuming a range of pre-bottleneck effective population sizes (*Ne*). Average M was 0.64 for the High Arctic and 0.70 for Banks Island under all conditions, and all ratios were significantly different from equilibrium expectations ($P \leq 0.01$).

Despite evidence of bottlenecks in these two populations, inbreeding did not appear to be significant (Table 4-1). Banks Island's inbreeding coefficient was 0.003; although F_{IS} was 0.63 in the High Arctic, the genetic complexity of this population (identified by STRUCTURE analysis) suggests a Wahlund effect rather than non-random mating. Similarly, an F_{IS} of 0.43 in Victoria Island likely reflects the presence of multiple migrants within the population (below). However, the F_{IS} of 0.18 in the Coastal Islands (Table 4-1) may represent true inbreeding (below).

Since the total Banks Island sample consisted of wolves hunted between 1991-93, 1997-99, and 2002-04, changes in allele frequencies over time were used to estimate the effective size of the current population. *Ne* ranged from 51 (mainland and island migration sources combined) to 53 (island sources of migration only), or 25% of the estimated total population (Chapter 2).

H_E of Banks Island wolves declined from 63.24% in 1991-93, to 62.68% in 1997-99, to 61.14% in 2002-04, but these changes were not significant. In contrast, A^r was initially 3.86, and increased to 4.36 in later sampling periods. Alleles were shared between 1997-99 and 2002-04 that were not observed in the 1991-93 sample, and all sampling periods contained alleles unique to that time.

Differentiation of Island and Mainland Wolves

Largest D_S was found between the Coastal Islands and all other populations (Table 4-2), suggesting greatest genetic isolation. Among the arctic islands, High Arctic was most distinct, perhaps reflecting the population's physical separation or its small sample size. D_S between islands, and between islands and the mainland, was greater than between any pair of mainland populations with three exceptions: Victoria Island and Eastern Barrens (0.160); Baffin Island and Eastern Barrens (0.159); and Banks and Victoria Island (0.089). Isolation by distance was not observed (Chapter 3).

Drift over time in the Banks Island population was quantified by calculating D_S between our three sampling intervals (Chapter 2). Genetic distance was 0.032 between 1991-93 and 1997-99, and 0.031 between 1997-99 and 2002-04. These values are equal to or greater than those among several mainland clusters (Table 4-2), however, as noted above, STRUCTURE analysis of Banks Island alone did not recover sampling intervals as distinct clusters.

Mainland wolf clusters derived in STRUCTURE spanned large geographical areas. To identify mainland wolves most similar to the island populations at a finer scale, and thus to identify specific locations where island-mainland gene flow might occur, these large genetic clusters were subdivided into the geographic regions defined in Chapter 3. D_S was calculated among all regions and the resultant neighbor-joining tree is shown in Fig. 4-2. The Western Islands and the High Arctic plotted proximate to the Bathurst region within the Eastern Barrens cluster, while North and South Baffin Island were nearest the NE Mainland region of the Eastern Barrens; these mainland groups are those physically proximate to each island set (Chapter 3). It is also worth noting that D_S between North Baffin Island and the mainland was smaller than that between South Baffin Island and the mainland with one exception: the genetic distances to the Atlantic population (0.398 vs. 0.078 respectively).

Migration and Gene Flow Among Island and Mainland Wolves

In classical assignment tests (Paetkau *et al.* 1995), self-assignment rates for mainland wolf populations averaged 75.34% (range 58.9-96%); the average for island wolves was 89.58%, ranging from 69.2% in Victoria Island to 100% in the High Arctic (data not shown). In contrast to the relatively low rate for Victoria, Banks Island – the other Western Island population – had a self-assignment rate of 93.9%. This difference resulted from lower genetic differentiation and thus higher cross-assignment between Victoria Island and the mainland (Fig. 4-3a, b), and from apparent high gene flow from Banks Island to Victoria (below). In the Eastern Arctic, Baffin Island's relationship with mainland wolves was comparable to that of Victoria Island, rather than that of Banks Island (Fig. 4-3c).

Two independent Bayesian estimations of migration rates were performed among our populations; since both runs reached stationarity and appeared well converged (data not shown), results are presented from the replicate with the highest likelihood (Table 4-3). Total migration between Baffin Island and the mainland was low but approximately equal in each orientation, averaging 0.0157 ± 0.0020. In contrast, total migration between the Western Islands and the mainland was directionally biased: 0.0013 from mainland to Banks Island and 0.0161 from Banks Island to the mainland; 0.0523 from mainland to Victoria Island and 0.0065 from Victoria to the mainland. The difference in frequency of island-mainland movements for each Western Island was supported by agreement between all four assignment tests (STRUCTURE, GENELAND, BAYESASS and classical): 4 of 52 individuals sampled in Victoria Island likely originated in barren ground populations; 4 of 941 barren ground wolves originated in Victoria Island; 3 of 941 barren ground wolves migrated from Banks Island; no wolf sampled on Banks Island had migrated from the mainland.

In addition to differences in relationships of the Western Islands to the mainland, BAYESASS indicated highly asymmetric migration between them: 26% of Victoria Island samples had migrated from Banks Island, with no movement in the opposite direction (Table 4-3). If all cross-assigned wolves in classical tests are assumed to represent migrants (as suggested by significance of these counts), 23% of individuals on Victoria Island originated in Banks Island, while 6% of the Banks Island wolves had migrated from Victoria (data not shown). The conflict between methods arises from BAYESASS's lower bound of 0.67 on self-migration rates, which could affect estimates involving the Victoria Island sample, where non-migration is near this limit (G. A. Wilson, pers. comm.; Wilson & Rannala 2003). Regardless of the exact rate, it can be concluded that migration between the Western Islands occurs primarily in an eastern orientation, from Banks Island into Victoria Island.

Discussion

Coastal Island Wolves

The genetics of Coastal Island wolves have been discussed in detail by Weckworth *et al.* (2005); they were included here for comparison to arctic island populations. In this study, the Coastal Island population had the only positive F_{IS} value potentially attributable to inbreeding. Although inbreeding has not been previously detected in this population, it is consistent with isolation of these wolves from mainland populations via intervening mountain ranges (Weckworth *et al.* 2005), which may also have contributed to D_S values between the Coastal Islands and all other populations greater than any other observed. However, STRUCTURE partitioned the arctic island populations as early as K = 3, while Coastal samples were not segregated until K = 9. Such conflicts between assignment tests and genetic distances likely reflect respective sensitivities of each method to events at different time scales (Castric & Bernatchez 2004). One possible interpretation of these results under this assumption is that Coastal Island wolves have been isolated for a longer period of time, while recent drift in arctic island populations – resulting from isolation, demographic bottlenecks, or a combination thereof – has been rapid and severe.

Banks Island Wolves

Poisoning of the Banks Island wolf population began in the mid 1950s and concluded when residents no longer observed wolves on the island. However, it is not clear whether the contemporary population descended from individuals persisting in the remote, uninhabited north-east corner of the island, or from wolves immigrating from other arctic islands (Usher 1965), and while relatively high H_E suggests rapid recovery of this population, we cannot distinguish between severe decline of resident wolves and a founder effect dating from establishment of a novel population. However, significant changes in skull morphology between Banks Island wolves collected in 1914-16 and in 1953-55 (prior to wolf control) suggest extinction and recolonization occurred early in the century (Manning & Macpherson 1958), and such events may thus have produced the contemporary population as well. D_S between Banks and Victoria Island is less than half the next smallest island-to-island genetic distance (Table 4-2); therefore, of populations surveyed here, Victoria Island is the most likely origin of post-poisoning Banks Island wolves.

Since 1974, the Banks Island muskoxen population has increased from 3800 to 65,000 individuals, and is currently estimated at 45,000 (Gunn *et al.* 1991; Larter & Nagy 2001a). Peary caribou (*R. t. pearyi*) populations declined during the same period (Larter & Nagy 2000), but there is no doubt island wolves have access to ample prey. Therefore, resource competition is not a likely explanation for the high rate of wolf migration from Banks to Victoria Island (Table 4-3). However, with an estimated 200 individuals, the Banks Island wolf population is larger than at any time in the past 50 years, and wolves may be leaving the island in search of unoccupied territories (Mech & Boitani 2003). It is interesting to speculate that the current direction of migration arises from a reversal of events which led to colonization of Banks Island by wolves from Victoria Island earlier this century.

If, on average, two wolves breed in a pack of 6-8 individuals (Mech & Boitani 2003), the calculated effective population size of 51-53 is higher than expected for a total estimated population size of 200 wolves. This result may indicate that the total wolf population is larger than previously thought, or could reflect a perturbation of wolf social structure resulting from annual harvesting of approximately 25%. Mainland wolf populations that are harvested or controlled display small pack sizes, with more and younger breeding pairs, resulting from high turn-over in individual packs and territories more frequently vacated (Hayes & Harestad 2000). Wolf harvesting as a key influence is supported by observation of measurable changes in allele composition and frequencies over time; indeed, D_S between sampling intervals is on par with some distances between distinct mainland clusters (Table 4-2). In addition to a relatively high rate of harvest, genetic drift in this population may be accelerated by hunters' tendency to harvest several individuals from the same pack (Chapter 2).

Drift over time may be of less concern than declining variation in Banks Island wolves. Already a "less variable" island population (Table 4-1), Banks Island may also be losing heterozygosity over time. However, allelic richness has increased since 1991-93 – due to introduction of novel alleles, probably via migration – and inbreeding is not currently observed on the island. Continued monitoring of drift and variation in this population may be warranted, especially if ingress is reduced or the ungulate prey base begins to decline.

Victoria Island Wolves

Relative to Banks Island, Victoria Island wolves are less genetically differentiated from mainland populations (Table 4-2, Fig. 4-3), and the rate of migration between Victoria and the mainland is substantially higher (Table 4-3). D_S between the islands is consistent with separation by linear distance only, and wolf tracks have been observed on the sea ice between the islands, suggesting no barrier to gene flow exists (Chapter 2). Therefore, Victoria Island likely acts as the prime contact point between the Western Arctic Islands and the mainland, with gene flow occurring between Banks Island and Victoria, and between Victoria Island and the mainland. It is interesting to note that, historically, Banks Island Inuit would not travel directly south to the mainland, as sea ice conditions in the Amundsen Gulf were considered unstable and treacherous; as we suggest for wolves, the Inuit traveled first from Banks Island to Victoria Island, despite the increased distance involved (Usher 1965).

While sea ice conditions may also influence the success of wolf migration, we support the hypothesis that annual over-ice migrations of the Dolphin-Union caribou herd – which calve on Victoria Island, but winter on the mainland (Fig. 4-4) – facilitate island-mainland wolf gene flow, and that wolves migrate incidentally while in pursuit of their prey (Chapter 2). In other words, wolf movement from Banks to Victoria Island may stem from density-dependant effects arising via abundant prey, while movement of wolves between Victoria Island and the mainland results from spatial variation in the availability of said prey. It is not clear, however, why migration occurs primarily from the mainland to the island, instead of equally in both directions (Table 4-3). One possibility is that Western Island wolves have dense muskoxen populations as an alternative to migrating Dolphin-Union caribou, while mainland wolves are largely dependent on caribou (Kuyt 1972), and must either follow the Dolphin-Union herd or switch to an alternate barren-ground population until their return (Walton *et al.* 2001). The difference may also result from timing of wolf dispersal relative to the position of their prey, but available data cannot be used to test this hypothesis. Regardless of the underlying mechanism, it seems clear that mainland wolves arrive in the Western Islands via Victoria Island: in addition to identifiable migrants (Fig. 4-3b) and a high Wahlund-induced F_{IS} (Table 4-1), Victoria Island contains the highest genetic diversity of any island surveyed here (Table 4-1).

High Arctic Island Wolves

Samples were collected from Ellesmere and Devon Islands between 1999-2002. Since this period corresponded to the collapse of the High Arctic prey system (Mech 2005), only 11 individuals could be obtained, and results presented here should be considered preliminary. Despite this limitation, the data suggests several interesting genetic features of the High Arctic island wolf population.

STRUCTURE identified this sample as a mixture of Western Island, Eastern Island, and mainland wolves. However, when the results of all four assignment tests were combined, the High Arctic population consisted of two resident wolves, five migrants from the Western Islands, two likely hybrids of residents and Western Island migrants, and a Baffin Island immigrant (data not shown). It is interesting to note that the only two resident wolves, GF44 and GF45, were harvested in November of 1999 prior to the population decline. The putative hybrids were sampled in 2002, after the first migrants had been harvested on the island. While these results are tentative, our sample appears to contain pre-bottleneck residents and post-bottleneck colonists of the High Arctic Islands. This hypothesis suggests that high observed F_{IS} reflects a Wahlund effect, although inbreeding could also have occurred in such a small population. This hypothesis would also explain why significant evidence of genetic bottlenecks was detected in M-ratio tests, but not tests for relative excess heterozygosity conducted in BOTTLENECK (Piry *et al.* 1999): the relative decline in heterozygosity produced by a Wahlund effect would have reduced the power of this test.

Baffin Island Wolves

Morphological studies suggest Baffin Island wolves are more closely related to the mainland that to other arctic island populations (Nowak 1995). While the magnitude of D_S between Baffin and other clusters supports this idea, clustering analysis using STRUCTURE and GENELAND, and levels of differentiation within classical assignment tests (data not shown), suggest a greater current affinity to island wolves over mainland ones. These observations may be analogous to those for Coastal Island wolves, reflecting on the one hand post-glacial colonization of Baffin Island, and on the other, divergent behaviors with opposing influences on the genetics of island and mainland wolves (island wolves, with a more spatially stable prey base, are likely more territorial than mainland barren-ground wolves in general).

Functionally, Baffin is most similar to Victoria Island: it is the contact point for migration between Eastern Island wolves and those on the mainland (Fig. 4-3). Lower migration relative to Victoria (Table 4-3) may be due to estimation using the STRUCTURE cluster composed of the total Baffin Island population; fine-scale analysis with Baffin Island divided into two geographically defined subpopulations (Chapter 3) revealed greater differentiation between South Baffin and the mainland than North Baffin and the mainland. Migration rates between regions within the Eastern Barrens cluster and the Baffin Island subpopulations reflected this result (data not shown). Baffin caribou herds use distinct calving grounds in the northern and southern parts of the island (Ferguson 1989), and the differentiation of Baffin wolf subpopulations from each other and from the mainland may result from prey specialization on distinct groups of caribou (Clark 1971).

D_S between the South Baffin region and the NE Mainland region (Eastern Barrens cluster) was almost twice the distance between South Baffin and the

Atlantic population (0.149 vs. 0.078). It is interesting to speculate that wolf migration through the South Baffin region connects the arctic islands to southern tundra populations in Quebec and Labrador, while North Baffin connects the Eastern Islands to mainland arctic tundra wolves. Samples from Northern Quebec would be required to test this hypothesis.

Origin of Arctic Island Wolves and Metapopulation Status

Morphological data has been used to suggest Baffin Island was colonized – along with mainland tundra populations – by wolves from southern glacial refugia, while northern arctic island populations arose from wolves previously isolated in Pearyland (North Greenland, Nowak 1995). The current study is only partially supportive of this hypothesis. While arctic island wolves are genetically distinct from mainland populations, D_S values were greatest for the southern Coastal Islands, implying arctic island wolves were more recently isolated. Baffin Island wolves currently show a greater affinity to other arctic island wolves than mainland wolves, and it is not clear at what time this affinity developed. Furthermore, although Banks and Victoria Island shared an allele found in no other population (total frequency 1%, data not shown), private alleles were not found within any island populations. However, these results are based solely upon microsatellites, which may not be sensitive to events on the time scale represented by post-glacial expansion (Paetkau *et al.* 1997).

Showing low correlation between distribution and geography, mitochondrial DNA haplotypes are generally unsupportive of subspecific differentiation in North American wolves (Leonard *et al.* 2005; Vilà *et al.* 1999), and recent results suggest most or all mainland North American wolves may have expanded from refugia in the southern United States (Leonard *et al.* 2005). However, studies to date have not included wolves from the arctic islands, and therefore could not speculate on the origins of wolves in these regions. Our own preliminary mitochondrial DNA sequencing found lower haplotype diversity in, and no haplotypes unique to the arctic islands, but whether this result reflects low sample size, recent admixture with mainland populations, or colonization of the arctic islands by wolves from southern refugia is currently unclear (data not shown). This early data, in combination with those discussed above, does not support colonization from a Pearyland refugium, but further mitochondrial or Y chromosome sequencing, including samples from Greenland, will be required to resolve this issue. Sequencing of Banks Island samples from 1914-16, 1953-55, and contemporary populations would also help determine whether past changes in skull morphology represent sequential invasion by different groups of wolves, classification error (Manning & Macpherson 1958) or changing environmental influences on phenotype.

Issues of taxonomy aside, arctic island wolves appear to act as a metapopulation (Elmhagen & Angerbjorn 2001), and, given the history of the Banks Island population, may have done so for some time. Classical metapopulations exist in discrete habitat patches inhabited by breeding subpopulations, here represented by populations on individual arctic islands. Vulnerability of High Arctic prey systems suggests all island subpopulations face the risk of extinction; however, dynamics of prey populations are asynchronous between islands and wolves are therefore unlikely to be extirpated from all islands simultaneously. Connection of arctic islands by winter sea ice permits movement between them, and genetic data suggest such movement does in fact occur (Table 4-3). Management decisions should therefore consider the status of arctic island wolves as a whole.

Conservation of Arctic Island Wolves in a Changing Climate

While prey population dynamics are influenced by a number of factors, there is some evidence that severe winter conditions associated with climate warming (e.g. deep snow and freezing rain) periodically reduce survival of caribou and muskoxen on the arctic islands (Larter & Nagy 2001b; Mech 2005). Peary caribou populations have been in decline throughout the region for some time, and the combination of climate, human harvesting, and wolf predation is likely integral to this process (Gunn *et al.* 2006; Larter & Nagy 2000). Indeed, wolf density on many islands has increased with increasing muskoxen density, resulting in higher predation on caribou that are easier to catch. Similar dynamics have been observed in southern wolf ecosystems (Boertje *et al.* 1996; Seip 1992), and are also implicated in the decline of Channel Island foxes (Roemer *et al.* 2001).

The challenge for conservation is that Peary caribou are currently endangered (COSEWIC 2004), while arctic island wolves are one of the most distinct populations in North America, and may yet represent a distinct wolf subspecies. How then, are we to conserve both unique island wolves, and the unique caribou subspecies upon which they prey? Should climatic conditions inimical to ungulates occur more frequently, the question may become academic as both species are ultimately driven to extinction. The risk of extinction for wolves will doubtless be greatest on smaller high arctic islands, where demographic stochasticity may have a more immediate effect.

Results presented here suggest arctic island wolves may soon face genetic, as well as ecological threats to persistence. Island populations already display reduced genetic variation (Table 4-1) that may restrict their ability to adapt to a changing environment. At present, inbreeding depression is not a significant risk, probably due to intermittent arrival of divergent migrant wolves from the large, continuous mainland population. However, as global warming continues, the number of ice-free weeks in the Northwest Passage will likely increase, especially in the Western Arctic (Johnston 2002). As winter ice cover becomes thinner, and ice is absent for longer periods, the international shipping community will call for increased traffic through the Passage (Charron 2005; Johnston 2002). Facilitation of this traffic with icebreakers will further reduce the opportunity for mainland wolves to reach the arctic islands. If the ice free period overlaps the migratory periods of the Dolphin-Union caribou herd, wolf gene flow from the mainland to the Western Islands could be eliminated entirely.

This situation represents a second conservation challenge. Current introgression of mainland genotypes into the island metapopulation may be considered undesirable, as it "dilutes" the unique island stock (Kyle *et al.* 2006). Since conservation efforts are facilitated by recognition of populations as unique subspecies, and given potential conflict with conservation efforts for Peary caribou, future declines in arctic island wolf populations may receive little attention. However, this very gene flow may be required for persistence of arctic island wolves, as it counters the genetic drift and inbreeding depression that may otherwise occur (Vilà *et al.* 2003). Whether or not island wolves are considered taxonomically distinct, they are the only large terrestrial carnivore present in the Arctic Archipelago, and therefore fill a unique niche in the ecosystem. As observed in a wolf-free Yellowstone (Ripple & Larsen 2000), "ecosystems are dynamic, and loss of a species in one place may cause unexpected trouble elsewhere" (p. 311, Pratchett *et al.* 2002). It is unclear whether combined climatic, demographic, ecological, genetic, and anthropogenic factors will result in wolf-free arctic islands, but if island wolves are to persist, anthropogenic intervention may yet be key.

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Table 4-1 Genetic variation in island and mainland wolves.

		Variation						Significant[£]		
Cluster[*]	**N**[†]	H_E[§]	H_E **SD**	A^R **(22)**[¥]	F_{IS}[¶]	**CI**	**BI**	**VI**	**HA**	**BAF**
Western Woods	322	0.73	0.02	5.67	0.009	*	*	*	*	*
Forest	258	0.74	0.03	5.92	0.033	*	*	*	*	*
Western Barrens	237	0.74	0.02	5.92	0.024	*	*	*	*	*
Eastern Barrens	704	0.74	0.03	6.04	0.017	*	*	*	*	*
Atlantic	25	0.75	0.03	6.06	0.070	*	*	*	*	*
Mainland		**0.74**		**5.92**						
Coastal Islands (CI)	36	0.61	0.05	4.19	0.181					
Banks Island (BI)	163	0.63	0.03	3.65	0.003					
Victoria Island (VI)	52	0.65	0.03	4.30	0.427				*	
High Arctic (HA)	11	0.49	0.06	3.07	0.629					
Baffin Island (BAF)	116	0.60	0.04	4.20	0.031					
Island		**0.60**		**3.88**						

[*] Genetically defined clusters of wolves (Fig. 4-1). Averages for population type are given in bold.

[†] sample size

[§] expected heterozygosity, with standard deviation indicated by SD

[¥] allelic richness, with rarefaction size (in alleles) given in brackets

[¶] population inbreeding estimated in BAYESASS

[£] stars indicate significant differences in heterozygosity between pairs of populations

Table 4-2 Nei's standard genetic distance (D_S) between clusters. Mainland-mainland comparisons appear in the upper left triangle, island-island comparisons in the lower right triangle, with island-mainland comparisons in the square. Largest and smallest genetic distances are shown in bold.

	WW	FO	WB	EB	AT	CI	BI	VI	HA	BAF
Western Woods (WW)	0									
Forest (FO)	0.112	0								
Western Barrens (WB)	0.105	0.045	0							
Eastern Barrens (EB)	0.165	**0.039**	**0.039**	0						
Atlantic (AT)	0.351	0.262	0.267	0.218	0					
Coastal Islands (CI)	0.363	0.438	0.454	0.509	0.662	0				
Banks Island (BI)	0.302	0.270	0.240	0.225	0.381	0.887	0			
Victoria Island (VI)	0.325	0.216	0.185	0.160	0.424	0.871	0.089	0		
High Arctic (HA)	0.493	0.442	0.355	0.332	0.500	**1.229**	0.260	0.245	0	
Baffin Island (BAF)	0.360	0.255	0.222	0.159	0.345	0.729	0.424	0.343	0.344	0

Table 4-3 Bayesian estimates of migration rates among wolf populations. Direction of migration is from populations in the left column to those right of the vertical line. "Self-migration" rates (analogous to self-assignment rates) are outlined, rates greater than 2% shown in bold, and populations are abbreviated as in Table 2. Standard deviation of migration rates averaged 0.005 and did not exceed 0.0382 (HA-HA).

	To									
From	WW	FO	WB	EB	AT	CI	BI	VI	HA	BAF
Western Woods	0.9865	0.0040	**0.0541**	0.0013	0.0028	0.0020	0.0003	0.0049	0.0158	0.0009
Forest	0.0076	0.8405	0.0086	**0.0805**	0.0030	0.0018	0.0003	0.0050	0.0162	0.0009
Western Barrens	0.0021	0.0021	0.8987	0.0026	0.0033	0.0016	0.0003	**0.0204**	0.0163	0.0015
Eastern Barrens	0.0015	**0.1478**	**0.0237**	0.8901	0.0038	0.0019	0.0003	0.0172	**0.0238**	0.0130
Atlantic	0.0003	0.0006	0.0016	0.0145	0.9690	0.0015	0.0003	0.0049	0.0156	0.0009
Coastal	0.0003	0.0007	0.0010	0.0004	0.0029	0.9855	0.0003	0.0045	0.0155	0.0009
Banks Island	0.0004	0.0011	0.0087	0.0022	0.0037	0.0015	0.9975	**0.2590**	**0.0997**	0.0010
Victoria Island	0.0006	0.0006	0.0015	0.0007	0.0025	0.0014	0.0003	0.6746	0.0159	0.0009
High Arctic	0.0003	0.0005	0.0009	0.0009	0.0051	0.0014	0.0003	0.0047	0.7439	0.0009
Baffin Island	0.0004	0.0021	0.0012	0.0068	0.0038	0.0015	0.0003	0.0048	**0.0373**	0.9791

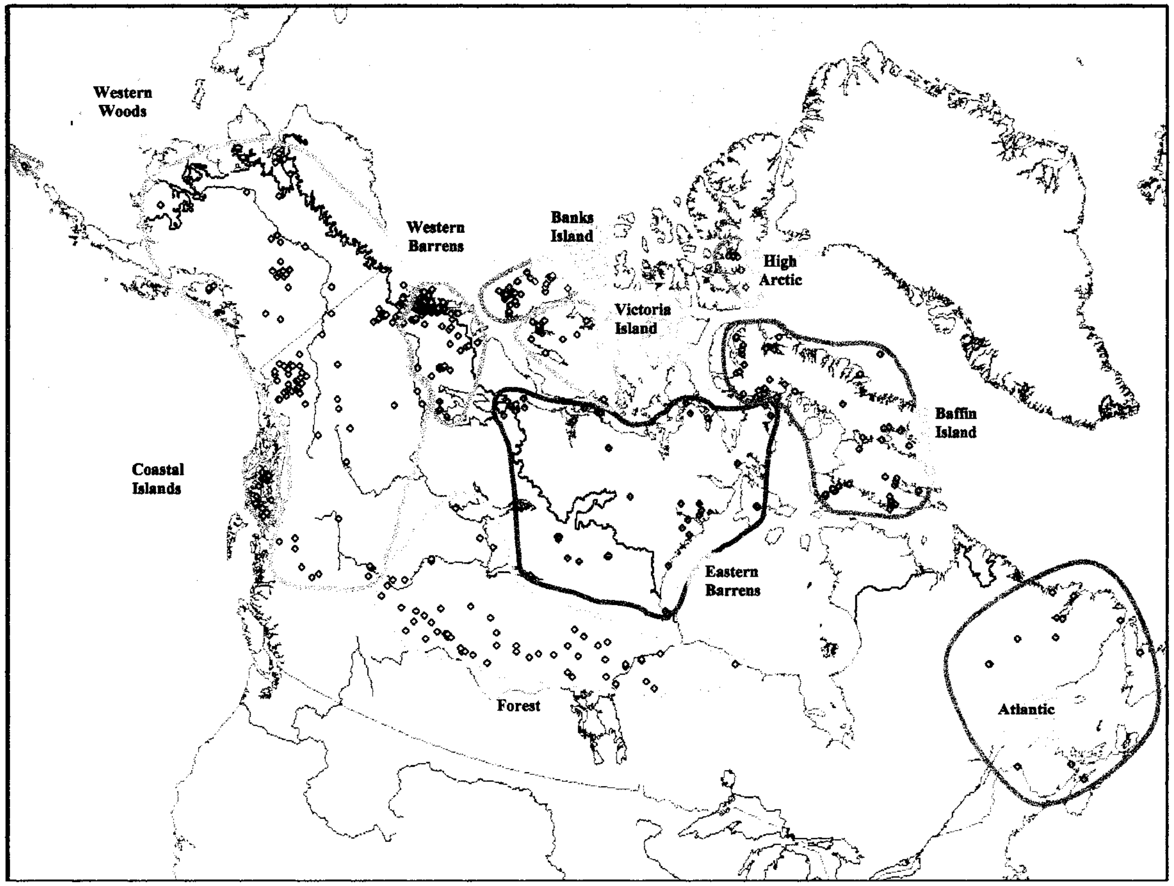

Figure 4-1 Genetic clusters of grey wolf samples based on STRUCTURE and GENELAND analysis. Figure adapted from Chapter 3.

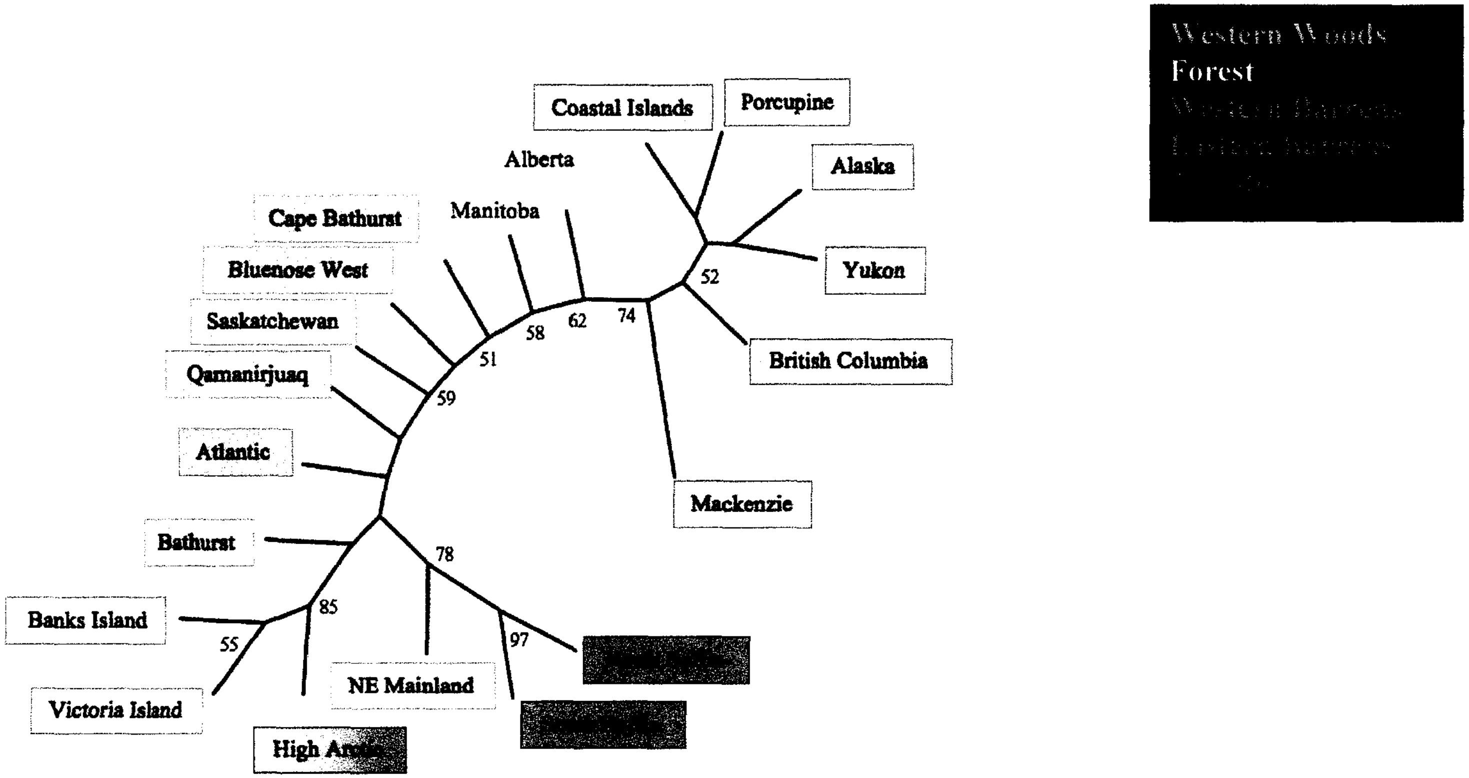

Figure 4-2 Neighbor-joining consensus tree of Nei's standard genetic distance between wolves in physical regions (delimited in Chapter 3). Bootstrap support values are indicated for nodes appearing in more than 50% of the pseudoreplicates. Coloured boxes indicate membership of each region in its corresponding STRUCTURE cluster (K = 7); colour gradients represent regions that were split between two clusters. While STRUCTURE grouped the Coastal Islands with the Western Woods, and created a Western (light blue) and Eastern (dark blue) Island group, all island populations were considered distinct in our analyses (Fig. 4-1).

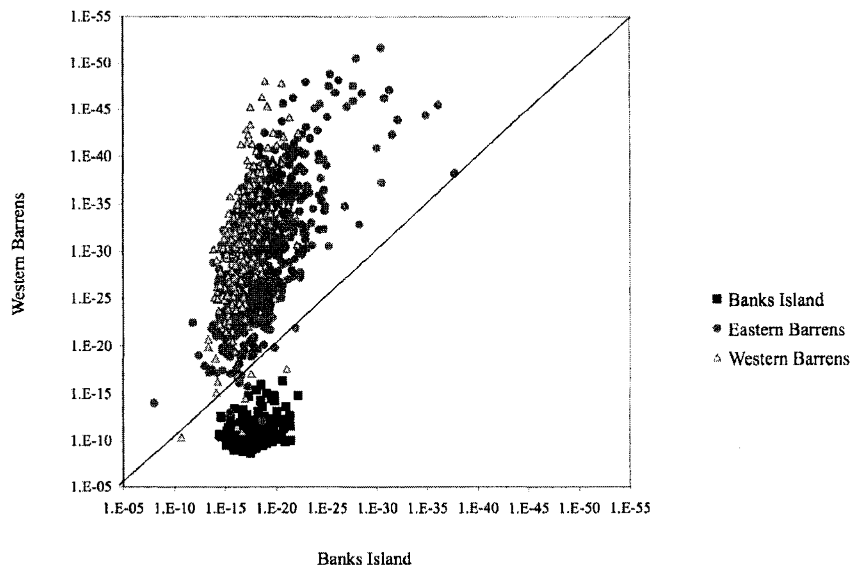

Figure 4-3a Classical assignment between Banks Island and mainland barren ground wolves. Symbols indicate the sampling cluster of each wolf. Individuals are plotted according to the probability that their genotype would arise in each cluster; the diagonal line represents genotypes equally likely in both (for example, hybrid offspring of mainland and island wolves). Here, the absence of overlap in assignment indices is suggestive of high genetic differentiation, despite low level migration between populations.

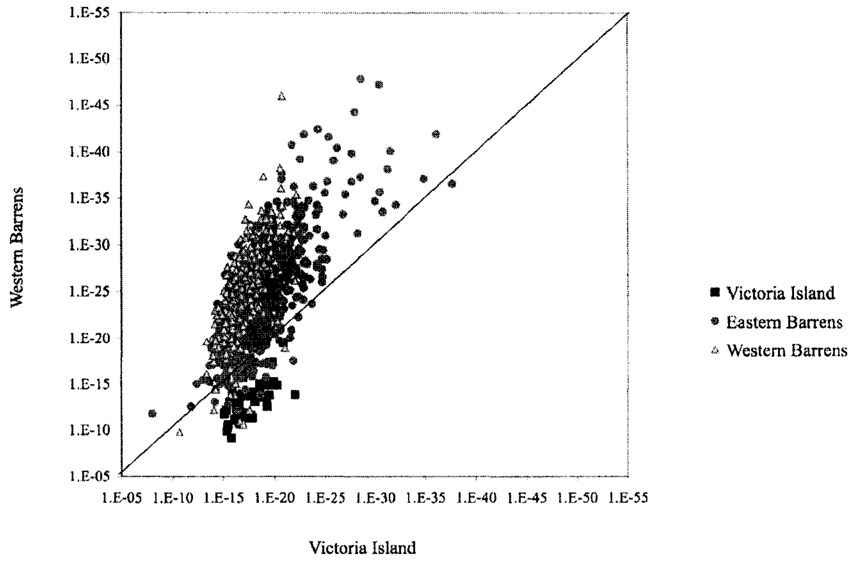

Figure 4-3b Assignment between Victoria Island and mainland barren ground wolves. Increased overlap in assignment indices relative to Banks Island (Fig. 4-3a) indicates higher gene flow between populations, and is supported by a higher number of cross-assigned individuals (potential migrants).

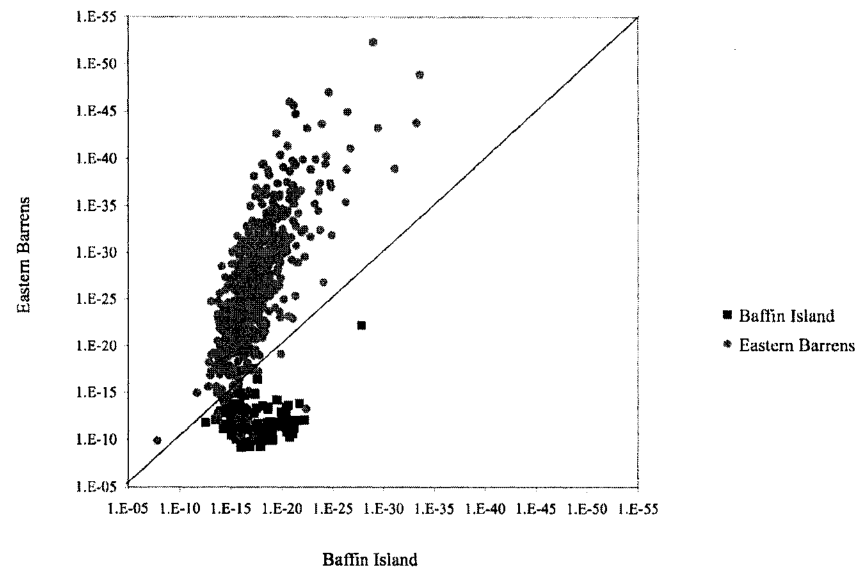

Figure 4-3c Assignment between Baffin Island and Eastern Barrens wolves. Differentiation and migration are similar to that observed for Victoria Island.

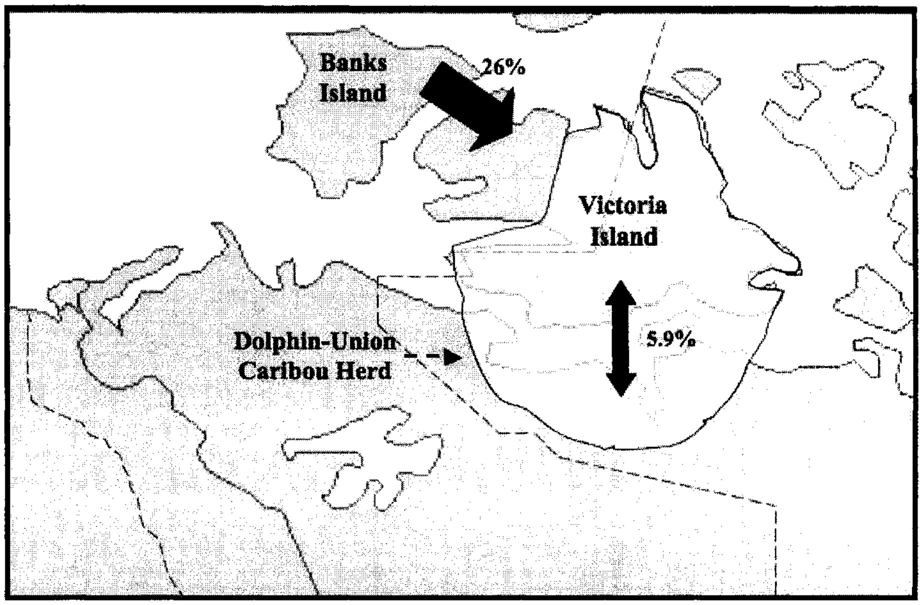

Figure 4-4 Total migration rate between Banks Island, Victoria Island, and mainland barren ground wolf populations as estimated in BAYESASS (comparable data from classical assignment tests is given in the text). The white region overlapping Victoria Island and the mainland is the home range of the Dolphin Union caribou herd, which migrates across the sea ice twice each year.

Chapter 5

Multiple Dispersal Strategies in Svalbard Arctic Foxes

Introduction

Throughout most of their range, arctic foxes (*Alopex lagopus*) are predators that specialize on cyclically abundant lemming populations (Angerbjörn *et al.* 1999; Audet *et al.* 2002; Macpherson 1969). Their strategies for dealing with prey population declines include caching (Samelius & Alisauskas 2000), switching to alternate prey (Roth 2002), and restricted reproduction in resource-poor years (Angerbjörn *et al.* 1995; Macpherson 1969). In addition, North American arctic foxes will migrate over thousands of kilometers to escape large-scale synchrony in lemming crashes (Wrigley & Hatch 1976), often traveling long distances through regions that do not support breeding fox populations, such as boreal forests and sea ice (Andriashek *et al.* 1985; Eberhardt & Hanson 1978). These extensive migrations likely contribute to the maintenance of homogeneous allele frequencies throughout the North American population, which appears to behave as a genetically panmictic unit (Chapter 3).

The Svalbard Archipelago is ecologically distinct from the North American Arctic, but in some areas, arctic foxes are as dense as 1.5 per 10 km^2 (Prestrud 1992a). Aside from small, introduced populations of sibling voles (*Microtus rossiaemeridionalis*) found only near human settlements, small mammals are absent, and alternative prey types dominate the diet of resident foxes: sea birds (*Alcidae* and *Procellariidae*) and geese (*Anser brachyrhynchus* and *Branta lecopsis*), present only during summer and concentrated in traditional coastal nesting areas; rock ptarmigan (*Lagopus muta hyperborea*), dispersed throughout the inland regions; and reindeer *(Rangifer tarandus platyrhynchus*; Eide *et al.* 2004; Prestrud 1992c). While foxes have been observed killing reindeer calves (Prestrud 1992c), they most often scavenge on offal from harvested animals, or from carcasses resulting (primarily) from winter die-offs. The number of carcasses available varies with severity of winter weather conditions, and carcass distribution is spatially stochastic (Jepsen *et al.* 2002).

Home range and behavior of arctic foxes varies with the predictability and richness of their prey populations. North American foxes are territorial primarily during breeding season, abandoning their home ranges in favor of long-distance winter foraging movements (Wrigley & Hatch 1976). Svalbard foxes, in contrast, are territorial year round and defend home ranges as small as 10 km^2 (Eide *et al.* 2004); dispersal distances in a similar habitat ranged between 10-30 km (Angerbjörn *et al.* 2004b). Svalbard foxes might therefore be expected to display greater genetic structuring than their North American counterparts, perhaps over relatively small areas. Alternatively, if the spatio-temporal variation in reindeer availability mimics that of North American lemming populations, extensive foraging movements may promote fox gene flow over large distances, thus suppressing genetic differentiation. The purpose of this study was to determine whether fine-scale genetic structuring exists in the Svalbard fox population, and if so, to identify the social and ecological mechanisms from which it results.

Materials and Methods

Study Area

The Svalbard Archipelago covers approximately 62,700 km^2 and is located in the high arctic between 74° – 81°N and 10° – 30°E; our study focuses on Spitsbergen, the largest island in the archipelago. Arctic foxes and reindeer are the only endemic terrestrial mammals. Foxes in inland areas prey primarily upon reindeer and rock ptarmigan, while those in coastal regions use nesting geese and seabird species which are present in large numbers during the summer months (Eide *et al.* 2005). Human activity is restricted to four small settlements and a few scientific stations. Fur trapping is likely the only anthropogenic influence on resident arctic fox populations, occurring annually from November 1-March 15, and totaling between 80-170 pelts per year. Sixty percent of Svalbard's landmass is covered in permanent snow and glaciers, but since arctic foxes are known to travel over sea ice (e.g., Andriashek *et al.* 1985; Eberhardt & Hanson 1978), physical barriers to dispersal likely do not exist.

Sample Collection and Laboratory Methods

Carcasses of legally harvested foxes were collected from local trappers between fall 1997 and spring 2005. Four additional foxes were also obtained from the 1995-96 harvest year. Trapping location, date, and fox sex were recorded for each individual, and tissue samples preserved frozen for DNA analysis. Fox age was estimated by counting annuli in the cementum of sectioned lower canine teeth (Grue & Jensen 1976; Jensen & Nielsen 1968). Most Svalbard foxes are of the white colour morph, with 3-5% of the population blue phase (Våge *et al.* 2005), and pelt colour of each individual was therefore recorded as well.

A total of 636 tissue samples was collected. These were first used for population-level comparison of North American and Svalbard arctic fox populations (Chapter 3), and genotyping methods using 12 microsatellite loci are described elsewhere (Chapter 3). For the present fine-scale analysis within the Svalbard fox population, all samples lacking precise harvest coordinates or teeth for aging have been excluded. This study therefore includes unique genotypes from 525 arctic foxes (Fig. 5-1a).

Statistical Analysis

Bayesian clustering analysis has detected complex structure within the Svalbard fox population (Chapter 3) and it was therefore analyzed alone using the admixture model implemented in STRUCTURE (Pritchard *et al.* 2000). Settings were identical to those in Chapter 3: 100,000 burn-in cycles followed by 1,000,000 iterations; no prior population information; a unique level of admixture for each cluster; and a skewed allele frequency distribution with the number of clusters (K) allowed to vary between 1 and 14.

Pairwise relatedness estimators were calculated using SPAGEDI 1.2 (Hardy & Vekemans 2002; Queller & Goodnight 1989). In all analyses, R was estimated among all pairs of foxes captured at the same spatial location, and between all pairs of foxes captured at different spatial locations. Average relatedness and standard errors were estimated by jackknifing across loci, and correlations between pairwise relatedness and geographical distance matrices were tested by permuting spatial locations amongst individuals. All significance levels were adjusted using Bonferroni corrections for multiple tests (initial $P = 0.05$).

Pairwise R was calculated among all 525 foxes in our sample. Individuals were then subdivided according to trapping season (e.g. November 1997 to March 1998) and analysis repeated to test for changes over time. Relatedness within each sex, then between sexes, was estimated after pooling individuals of known sex across years (231 females, 291 males). Juvenile arctic foxes in Svalbard leave their dens as soon as 1.5 months after birth and are sexually mature at 9-10 months, though most do not breed until their second or third year (Frafjord 1992; Prestrud 1992a). To explore differences in structure among foxes at different life stages, young of the year (264 individuals) and young adults (2 years old, 119 individuals) were analyzed separately. Reflecting sample size considerations, mature foxes were pooled into two groups – ages 3-5 (91 individuals) and ages 6-13 (41 individuals) – and analyzed for comparison to their younger counterparts.

Seabirds and geese use traditional nesting areas on Svalbard, and the locations of many of these cliffs have been previously documented; locations of known arctic fox dens on the islands were also available (Fig. 5-1b). Therefore, in addition to the isolation by distance analyses described above, correspondence between fox population structure and locations of potential home ranges and foraging sites was assessed. Reindeer carcasses, while another important food resource for arctic foxes (especially inland and during the winter) have a patchy and unpredictable distribution (Jepsen *et al.* 2002) and are therefore unsuitable for this analysis. Fox capture locations, fox den locations, and coordinates of bird cliffs were plotted on a base map of Svalbard using ARCGIS 9.0 (Environmental Systems Research Institute 1999-2004). The program's "Near" function was then used to identify the single den and bird cliff closest to the harvest location of each arctic fox. Foxes spatially associated with the same den were treated as groups in SPAGEDI, and average *R* calculated among foxes associated with the same den, and between foxes linked to different dens. The analysis was then repeated using corresponding data for bird cliffs. Finally, a subset of the fox samples was divided into categories based on the prey resource areas defined by Eide *et al.* (2004): coastal (seabirds, 92 foxes); rich inland (geese and reindeer, 14 foxes); and poor inland (reindeer only, 15 foxes). Relatedness was compared within each category and for foxes sampled in different resource areas.

Results

Population Structure

Previous STRUCTURE analysis showed the vast majority of foxes in Svalbard belong to the same cluster as all North American foxes (Chapter 3); what little substructure existed was concentrated within the Svalbard population. Examination of the Svalbard population alone recovered similar complexity.

The probability of the data peaked when 12 clusters were assumed (Fig. 5-2a), at which point admixture within each group was also minimized (data not shown). However, there was no correspondence between assigned cluster and fox harvest location, colour, age, or harvest year. The probability distribution for this simulation began to plateau at K = 5, after which probability increased or decreased apparently randomly, and consistency between replicates declined (Fig. 5-2a). It therefore seemed possible that K = 5 was most appropriate under these assumptions, and that further improvements in probability reflected mathematical, rather than biological optimization of the model (Chapter 3). This idea is supported by the fact that, unlike cluster assignments derived under K = 12, results from K = 5 displayed low-level spatial organization (Fig. 5-2b). A significant difference in frequency of cluster assignments among foxes of different colours was also observed ($\chi^2 = 53.67$, df = 4), driven largely by assignment to cluster D, which occurred at 52% for blue foxes and 10% for white.

Fine Scale Structure

Average pairwise relatedness of foxes within spatial locations ranged from -0.0424 to 0.0199 in the yearly subsamples; however, no significant differences existed between years (t test, Bonferroni corrected $P = 0.006$), and average intra-location *R* was 0.0078 in the total set (Table 5-1). In all but one year, relatedness was higher among foxes at the same spatial location than between foxes collected at different locations. While these results suggest spatial structuring of related foxes, average *R* within locations was significantly different from zero in only three tests ($P = 0.005$), and did not differ from inter-location values after Bonferroni correction. Furthermore, slope of the regression between relatedness and geographic distance was significantly negative in the pooled sample alone, and the correlation between distance and *R* was always small (Table 5-1). Low significance of all tests likely resulted from high variance both within and between locations (Table 5-1).

Clustering of related individuals at identical or adjacent locations appeared to be driven by female philopatry (Fig. 5-3a, b). Average relatedness of females within spatial locations was 0.0106 ± 0.0058; *R* of males was 0.0017 ± 0.0049 and not significantly different from zero. Intermediate average *R* was observed for opposite-sex pairs at the same location (0.0075 ± 0.0038). While the correlation between relatedness and geographic distances for females was still very small ($r^2 = 0.003$), the slope of the relationship was significantly negative ($P < 0.001$). Male foxes were equally related within and between locations ($P = 0.25$), and males sampled at different locations were slightly more related than females sampled at different locations (-0.0039 ± 0.0005 vs. -0.0057 ± 0.0005 respectively), also supporting longer or more frequent dispersal among male foxes.

Foxes of all ages were generally unrelated when compared across spatial locations (Fig. 5-4). Within locations, foxes of the same cohort showed greater average *R* than foxes of different ages (0.0102 ± 0.0066 vs. 0.0063 ± 0.002). This result likely stems from a tendency of younger foxes to be spatially associated, although high *R* between young foxes separated by hundreds of kilometers was also observed (Fig. 5-4). In contrast, older foxes (3 years and up) were largely unrelated, regardless of their sampling location (Fig. 5-4).

Foxes spatially associated with the same den had an average *R* of 0.0116 ± 0.0031, while foxes associated with different dens were unrelated (mean *R* -0.0035 ± 0.0004). Similar trends were observed for foxes spatially linked to the same bird cliff (0.0101 ± 0.0026) or with different bird cliffs (-0.0032 ± 0.0003). We found no significant differences in relatedness between foxes sampled in the same resource area (coastal, rich inland, poor inland) versus those sampled in different resource areas ($P = 0.27$), nor any differences among areas sufficient to suggest differential behavior of foxes using different prey species (data not shown). However, small sample sizes for rich and poor inland areas may have hampered this analysis.

Discussion

Composition of the Svalbard Fox Population

While the majority of foxes on Svalbard are genetically indistinguishable from the apparently panmictic North American population (Chapter 3), the remainder fall into several additional clusters which show some correlation to the spatial origin of individuals (Fig. 5-2b). These tendencies could be products of the physical environment, but topographic impediments to fox dispersal most likely do not exist in this region. In addition, foxes spatially associated with the same den, or likely foraging at the same avian nesting ground, had higher relatedness than those associating with different dens or different nesting grounds. While much of the latter pattern may result from centering of home ranges and thus dens in resource-rich patches (Jepsen *et al.* 2002; Prestrud 1992b), the combined result suggests genetic subdivision of Svalbard foxes may arise from fidelity to natal ranges or productive foraging areas, rather than physical inhibition of fox dispersal. However, a spatial correlation so tenuous also suggests dispersal is frequent enough, and extensive enough, to blur any underlying dynamics that may exist.

Differential cluster assignment was observed between white and blue phase foxes in the absence of any spatial isolation (data not shown). Sexual isolation may occur, however, if pelt colour influences mate choice (Musiani 2003, Chapter 3). However, a sexual effect is not sufficient to explain our results; if genetic differentiation arose through assortative mating alone, we would expect two clusters, rather than the five observed here. Furthermore, white and blue foxes have been shown to interbreed in other populations (e.g., Meinke *et al.* 2001). Alternatively, since pelt colour in arctic foxes is genetically determined (Våge *et al.* 2005), it is possible the elevated assignment of blue foxes to cluster D results from linkage between one of our microsatellites and the pelt colour gene.

Svalbard was covered by polar ice during the last glacial maximum, and arctic foxes are believed to have reached the Archipelago only during the last 10,000 years (Fuglei 2000). Given its intermediate location, Svalbard may have been colonized by foxes from North America and Greenland, as well as by foxes of European and Siberian origin. Tagged Svalbard foxes have been found in Siberia (Fuglei 2000) and may have reached Scandinavia in recent years (K. Norén, pers. comm.); these data, combined with recent appearance of the parasite *Echinococcus multilocularis* in the Svalbard fox population, suggest migrants from the eastern and western hemispheres continue to invade Svalbard today (Henttonen *et al.* 2001). Periodic introduction of genes from external sources is an alternative explanation for the mixture of types observed in this region; since blue foxes occur at relatively high frequency in Fennoscandia (Angerbjörn *et al.* 2004a), immigration of blue foxes from Europe, and of white foxes from North America, could also explain the clustering differences observed between colour morphs. This hypothesis may be supported by historical changes in frequency of blue foxes in Svalbard (E. Fuglei, pers. comm.), and could be tested via direct comparison of Svalbard foxes with individuals from North America, Greenland, Europe and Siberia.

Social Structure of Svalbard Foxes

While related foxes were spatially associated, the significance of these associations was low, and high variance in *R* was observed at all spatial scales. As in the clustering analysis, this result suggests long distance dispersal is frequent enough to obscure any underlying tendency of kin to inhabit adjacent regions. Long range movements may be more frequent in resource poor inland areas, or during winters with good grazing conditions, when fewer reindeer carcasses are available to hungry foxes (Fuglei *et al.* 2003). A more extensive comparison of juvenile foxes inhabiting each ecological zone, and analysis of yearly variation in *R* relative to the scale of reindeer mortalities, would help resolve this issue.

Long distance movements are also more likely to occur in young foxes that have not yet obtained a breeding home range (Eberhardt *et al.* 1983); results presented here suggest that young foxes (ages 1-2) occur both in close proximity, and separated by several hundred kilometers (Fig. 5-4). This dichotomy may reflect two alternate dispersal strategies: “staying close” or “going far” (Angerbjörn *et al.* 2004b). In the Swedish population, those foxes staying close during peaks in lemming density are more likely to survive and reproduce while, when lemmings are scarce in natal ranges, foxes going far have a better chance of finding adequate resources for reproduction (Angerbjörn *et al.* 2004b). Aperiodic fluctuations in winter reindeer mortality may support existence of similar contrasting strategies in Svalbard.

In addition, foxes dispersing into territories close to their natal ranges appear to do so with or near their littermates, as we observe spatial correspondence of related individuals in both the young and young adult age categories (1 and 2 years old). Existence of this pattern in potentially reproductive young adults, and the documented tendency of Svalbard foxes to leave their natal dens at an early age (Frafjord 1992) suggests bias due to potential inclusion of pre-dispersal cubs is not solely responsible for this trend. This idea is also supported by the tendency of foxes in other populations to form social groups with multiple adults that are usually closely related (Chapter 7; Anthony 1997; Goltsman *et al.* 2005; Strand *et al.* 2000).

Arctic foxes in some populations inherit home ranges from their parents, and may remain in their natal territories until such an opportunity arises (Strand *et al.* 2000). However, home ranges in Svalbard do not usually include adult foxes beyond the dominant breeding pair, perhaps because winter conditions are too severe for single territories to support larger families (Eide *et al.* 2004). The finding of relatively low *R* between young and old foxes at the same spatial location is consistent with these observations. There was also no spatial association of related mature foxes (ages 3 and up). This result may suggest inbreeding avoidance in the primary reproductive population; however, the number of foxes in higher age classes declines both in our sample and in the population, as survival declines sharply with age (Prestrud 1992a), and the absence of spatial structuring may also reflect removal of relatives via trapping or natural death.

In mammalian species where inbreeding avoidance is practiced, it is often achieved via sex-biased dispersal, and male movement with greater female philopatry is observed here. The "going far" strategy may also be more common in male foxes than in females (Fig. 5-3a, b), although differences between males and females were relatively small. These results contrast with data from arctic foxes in Iceland, Sweden, and the total North American population, where no difference in dispersal distance was observed between sexes (Angerbjörn *et al.* 2004b; Chapter 3). Data given here may be more consistent with that from Alaska (Eberhardt & Hanson 1978) and Mednyi Island (Goltsman *et al.* 2005); in the latter study 60% of females remained in their natal ranges, while 91% of males dispersed, irrespective of natal territory richness. It is unclear why dispersal patterns of arctic foxes vary among populations studied to date, but identification of the underlying mechanisms could be an interesting focus of future research.

Conclusions

The results of this study supports frequent occurrence of long-range movements within the Svalbard fox population, as well as into Svalbard from other populations. Since similar levels of spatial structuring occurred within all resource areas, these movements are not restricted to habitats of low productivity. This may mean that cold dry winters with little snow and ice cover – when reindeer have easy access to forage and few reindeer carcasses are thus available to foxes – result in lengthy migrations and mixing of foxes across all resource areas. Spatial structuring is likely also diminished by extensive movements of young male foxes in search of unrelated mates or vacant territories.

The level of relatedness observed, even among foxes at the same spatial location, are lower than those found in other canids (e.g., Jedrzejewksi *et al.* 2005; Kitchen *et al.* 2005; Ralls *et al.* 2001). Isolation by distance was also dramatically reduced relative to a potentially mobile ungulate species, where structure was detected over hundreds of meters, rather than hundreds of kilometers (Coltman *et al.* 2003). These comparisons all demonstrate that, while Svalbard arctic foxes may be somewhat sedentary relative to those subsisting on lemmings, they remain remarkably mobile compared to other species.

Given the high vagility of arctic foxes, it is perhaps surprising that fine-scale genetic structure exists at all; however, results discussed here provide evidence for two mechanisms from which it results – sex-biased dispersal and association of young foxes, potentially littermates, in physical space. Complex social and ecological mechanisms may therefore regulate the spacing and movement of arctic foxes, and these factors may vary over space and time. Future studies, perhaps using non-invasive hair traps to observe fox dispersal over single seasons, may clarify our understanding of these processes. Such investigations could be particularly fascinating for the Svalbard population, which combines characteristics typical of both North American "lemming" and Icelandic stable "coastal" systems. Documentation of potential changes in fox behavior would also be of particular interest for a high arctic population whose ecological backdrop may be dramatically altered through continued climatic warming.

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Table 5-1 Relatedness among Svalbard arctic foxes sampled in different years.

		Spatial Location[§]			**Regression[¥]**		
Harvest Year	**N[†]**	**Within**	**Between**	**Difference**	**Slope**	**Intercept**	**Correlation**
1997-98	81	-0.0027 ± 0.0082	-0.0149 ± 0.0020	0.0122	2.18E-8	-0.0164	3.69E-5
1998-99	66	**0.0084 ± 0.0106**	-0.0223 ± 0.0031	0.0307	-3.32E-7	5.01E-5	0.0057
1999-00	61	-0.0168 ± 0.0097	-0.0166 ± 0.0030	-0.0002	-2.04E-8	-0.0159	6.78E-6
2000-01	23	-0.0424 ± 0.0091	-0.0602 ± 0.0445	0.0178	-3.96E-6	0.0392	0.0293
2001-02	102	-0.0045 ± 0.0067	-0.0107 ± 0.0009	0.0062	-2.50E-7	0.0073	0.0037
2002-03	80	0.0117 ± 0.0099	-0.0137 ± 0.0005	0.0254	-1.99E-7	0.0005	0.0097
2003-04	68	**0.0199 ± 0.0161**	-0.0189 ± 0.0018	0.0388	-2.55E-8	-0.0155	0.0002
2004-05	39	0.0056 ± 0.0132	-0.0344 ± 0.0038	0.0400	3.28E-8	-0.0422	0.0007
Total	525	**0.0078 ± 0.0034**	-0.0027 ± 0.0003	0.0105	**-9.43E-8**	0.0057	0.0022

Values significantly different from zero (p = 0.005) are shown in bold.

[†] Sample size in each harvest year. The total sample includes four additional foxes sampled in 1995-96.

[§] Average *R* ± SE for foxes sampled within or between spatial locations.

[¥] Regression of pairwise relatedness against geographic distance between foxes.

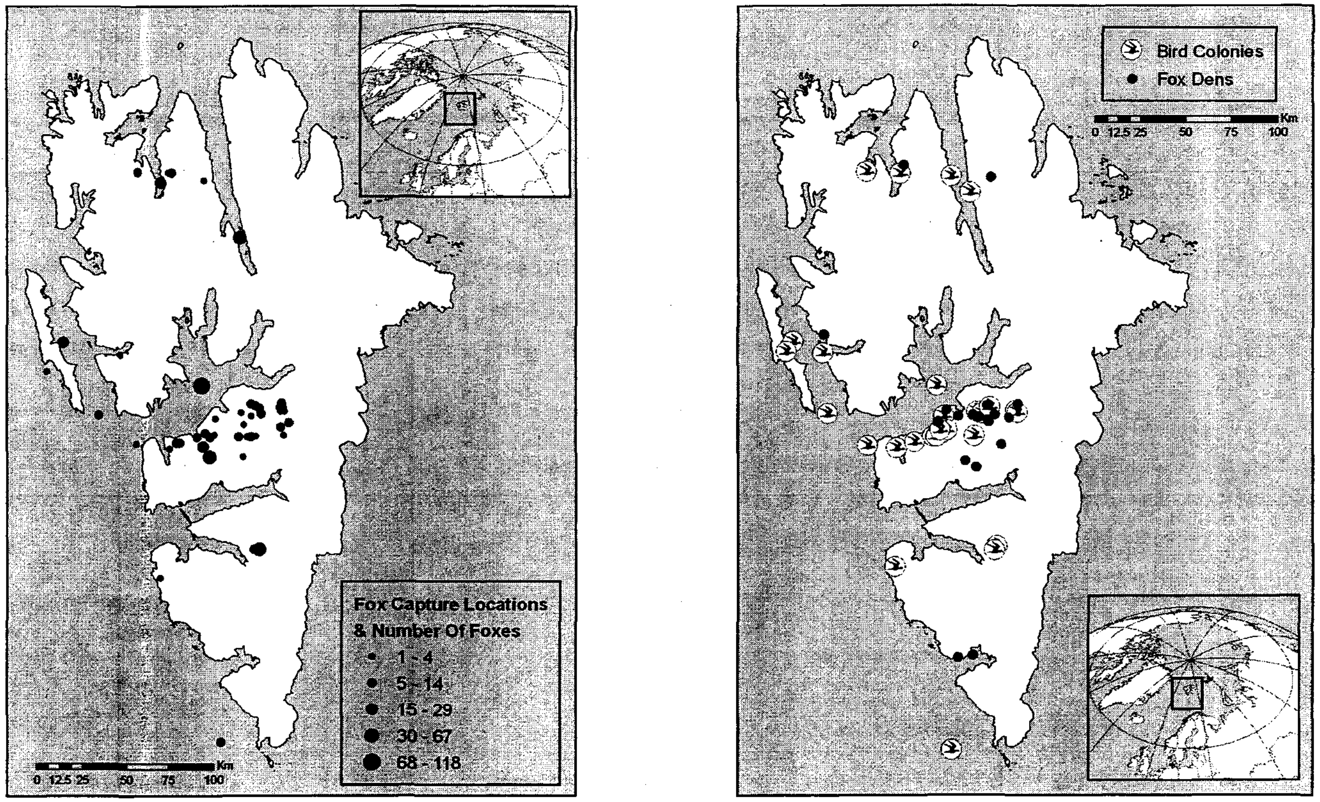

Figure 5-1 **A)** Arctic fox sampling locations, Spitsbergen, Svalbard Archipelago. **B)** Fox dens, seabird, and goose nesting colonies physically closest to sampling locations of each fox in the analysis.

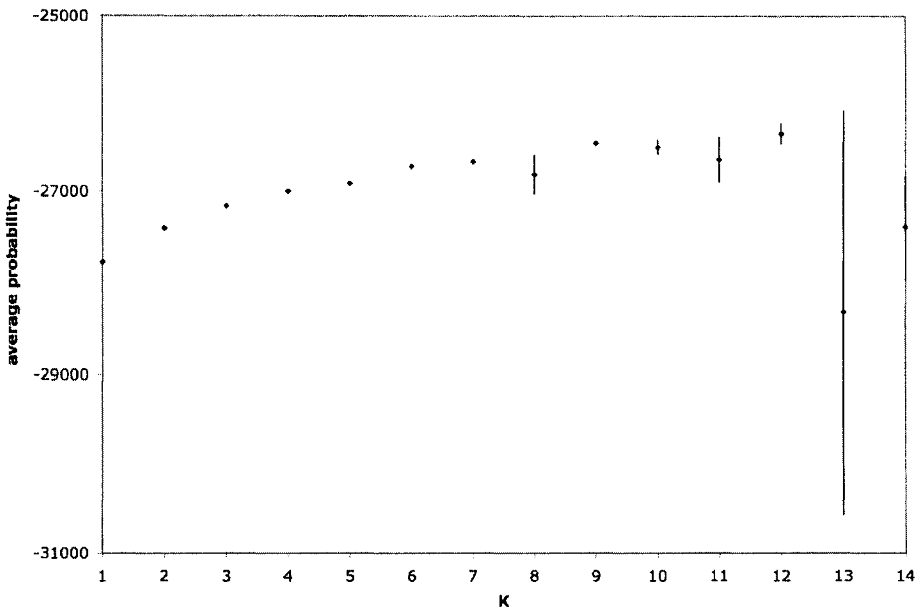

Figure 5-2a Average lnProb(D) for each value of K during Bayesian clustering analysis of fox genotypes. Variance in probability between replicates increased with increasing numbers of clusters. While K =12 had the greatest overall probability, the apparent plateau at K = 5 corresponded to a more reasonable solution for this population.

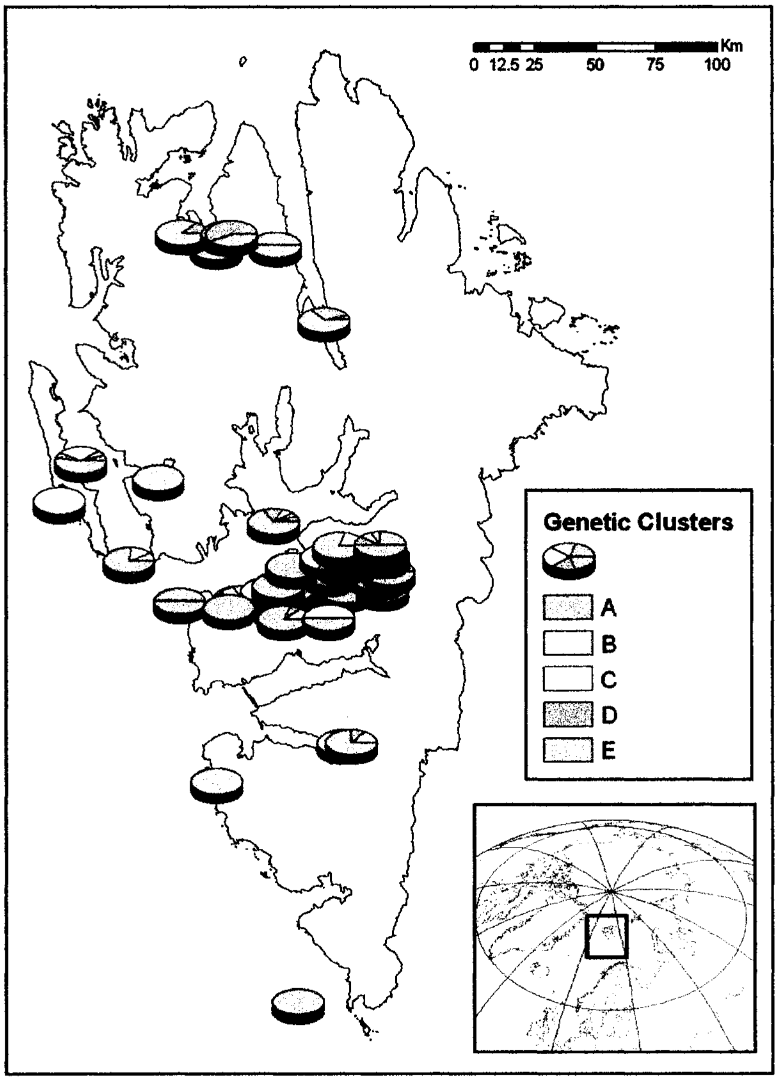

Figure 5-2b Spatial distribution of genetic clusters recovered during STRUCTURE analysis. Proportion of foxes assigning to each cluster is shown for each sampling location. Cluster D was significantly more common ($P < 0.001$) in foxes of the blue colour morph than the white one.

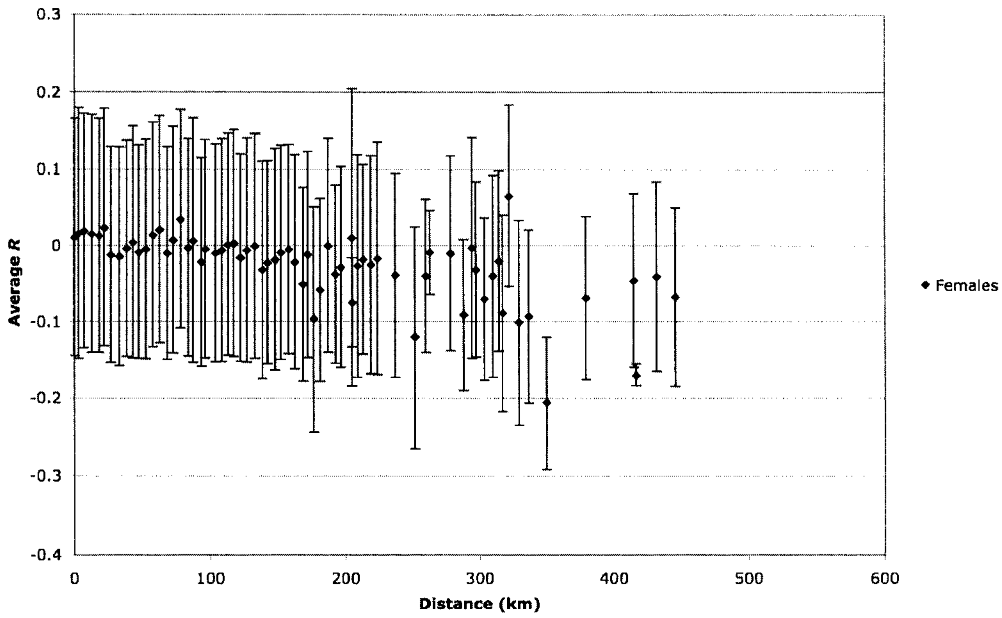

Figure 5-3a Average relatedness of female arctic foxes, with bars indicating one standard error. Averages were calculated over distance intervals totaling 5 km. A significant negative correlation between *R* and distance between individuals was observed.

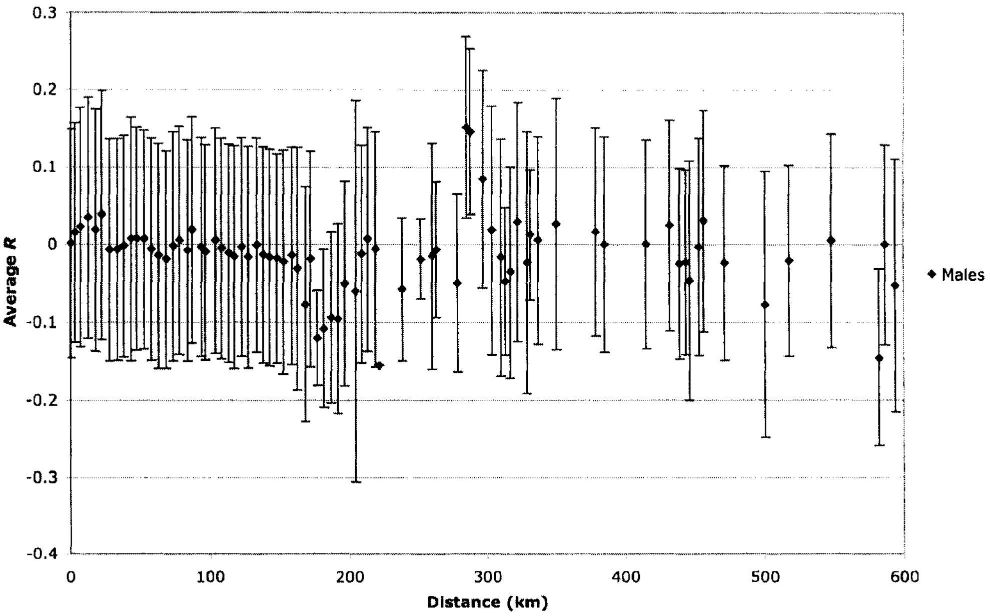

Figure 5-3b Average relatedness of male arctic foxes. No significant isolation by distance was detected.

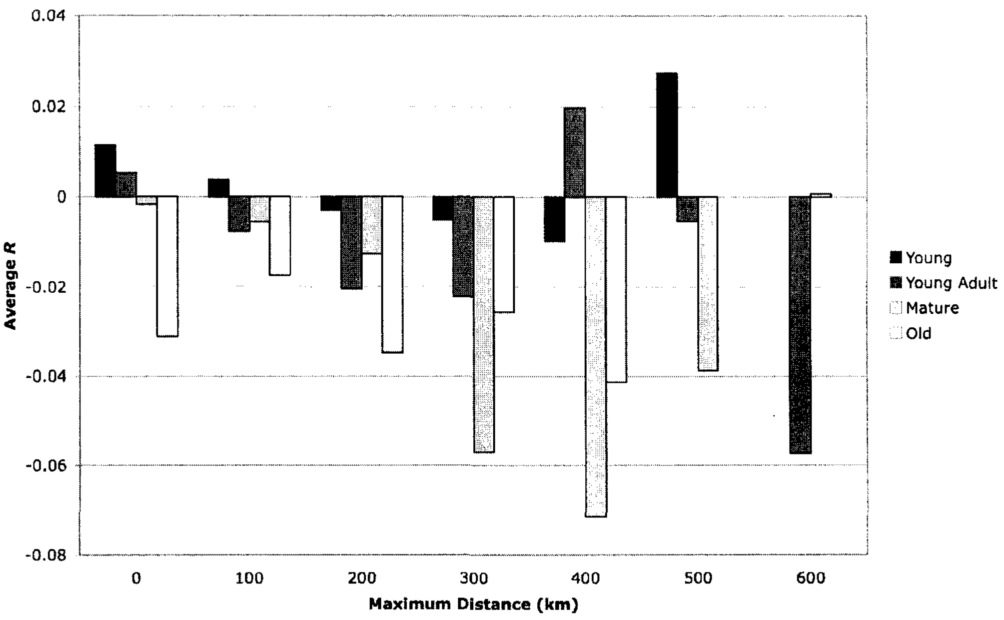

Figure 5-4 Relatedness of foxes in different age classes, averaged over 100 km intervals. Standard errors have been omitted for simplicity but were generally large. Spatial association of related foxes was stronger among young individuals than adult foxes; however, young related males were also found separated by several hundred kilometers.

Chapter 6

Synthesis

Influence of Glaciation on Genetics of Arctic Canids

Skull morphology of North American wolves suggests existence of five distinct subspecies, thought to be the legacy of isolation in as many as five glacial refugia (Brewster & Fritts 1995). However, mitochondrial DNA haplotype distributions are poorly sorted relative to geography (Vilà *et al.* 1999), and museum samples collected from the historical range of wolves in the southern conterminous United States display greater variation than observed in any present population (Leonard *et al.* 2005). While samples of arctic island wolves were not included in these studies, their results are not consistent with the morphologically-derived hypothesis of wolf history; nor is the molecular data presented in this thesis.

With the exception of one unique but rare allele, microsatellite size variation of arctic island wolves was a subset of that found in mainland populations (Chapter 4). In addition, preliminary mitochondrial DNA sequencing revealed no haplotype unique to island populations (Chapter 4). Since gene flow between island and mainland populations does occur (Chapter 2, 4), these results could reflect post-glacial genetic mixing of the previously isolated Pearyland and southern-refugial populations. However, given all molecular data now available, an alternative hypothesis may be more appropriate: that North American grey wolves expanded solely from southern refugia after the onset of the present interglacial. Indeed, long-distance dispersals such as those common in wolves are thought to spearhead post-glacial range expansions, resulting in colonization bottlenecks and reduced heterozygosity at the leading edge of a species' burgeoning distribution (Hewitt 1996). While residual effects of such a history are certainly not the only reasons for reduced variation in arctic island wolves (Chapter 2-4), they might well have contributed to it.

Apparently conflicting signal between morphology and molecular genetics was also recently observed in another canid species, the Asiatic dhole (Iyengar *et al.* 2005). Pelage length and colour have been used to define as many as eleven subspecies of dhole, but Iyengar *et al.* (2005) found no mitochondrial DNA differentiation between any of those included in their recent study. In contrast, the Italian wolf subspecies, *C. l. italicus*, is both morphologically and genetically distinct from neighboring European populations (Lucchini *et al.* 2004). In this case, isolation of wolves on the Italian peninsula may have been initiated by glacial advance and associated climatic changes, then reinforced by habitat fragmentation and persecution by humans (Lucchini *et al.* 2004).

Superimposition of multiple dispersal barriers at a single spatial location limits wolf gene flow more completely than would any of those barriers if encountered separately (Blanco *et al.* 2005, Chapter 3); the Italian example suggests similar outcomes from spatial overlap of individual isolating factors that appear sequentially over time. In contrast to such synergistic effects, the genetics of North American wolves appear to have been shaped by sequential forces with divergent influences throughout their recent history.

Unlike wolves, arctic foxes were not isolated in glacial refugia, but widely distributed throughout the Pleistocene (Dalén *et al.* 2005; Kurtén & Anderson 1980). Since that time, their distribution has contracted northward due to historical climatic warming, is restricted by the southern limit of suitable habitat, and could almost be considered an *inter*glacial refugium for a polar-adapted species. However, while the range of the species has progressively diminished, it remains largely unfragmented, and arctic fox populations surveyed in this thesis are unlikely to have experienced genetic isolation or demographic bottlenecks at any time in their recent history. Morphological and genetic uniformity of arctic foxes throughout North America is therefore unsurprising (Chapter 3). Similar large-scale trends have also been observed in North American coyote (*Canis latrans*) populations (Roy *et al.* 1994), but the historical origin of genetic homogeneity in this species provides an interesting contrast to its origin in *A. lagopus*. Like arctic foxes, coyotes have occupied parts of their range for thousands of years (Sacks *et al.* 2004). However, their range has expanded, rather than contracted, with the distribution of suitable habitat increasing recently enough to suppress coarse-grained genetic differentiation throughout the species (Roy *et al.* 1994; but see Sacks *et al.* 2004).

As in North America, the genetics of the Svalbard arctic fox population appear to have been influenced by glaciation and the shifting distribution of suitable habitat (Chapter 5). Arctic foxes did not reach Svalbard until the retreat of the northern ice cap around 10,000 years ago (Fuglei 2000). The Archipelago is approximately midway between fox populations in the eastern and western hemispheres, and multiple genetic clusters observed within Svalbard foxes may reflect post-glacial colonization of the islands by individuals originating on different continents (Chapter 5). The Channel Islands off California's coast were colonized by mainland grey foxes (*Urocyon cinereoargenteus*) around the same time arctic foxes reached Svalbard, but unlike Svalbard, these southern islands are surrounded by open water year round (Goldstein *et al.* 1999). Hence, severe genetic isolation of this southern population has resulted in rapid evolution of a species (*Urocyon littoralis*) that is morphologically, behaviorally, and genetically distinct from its mainland ancestor (Roemer *et al.* 2001); furthermore, foxes from each Channel Island are genetically identifiable, almost without exception, to their island of origin (Goldstein *et al.* 1999). In contrast, Svalbard arctic foxes likely remain connected to large continental populations; the vast majority of Svalbard foxes still assign to the single North American genetic cluster, and the remainder may soon be shown to align with currently unsampled European and Siberian populations (Chapter 3, 5).

Ecological Influences on Genetics of Arctic Canids

Habitat type (coastal or inland) did not appear to influence amount or direction of genetic exchange in Svalbard arctic foxes (Chapter 5). In wolves, however, genetic differentiation increased between barren ground and boreal forest populations, suggesting habitat transitions reduce gene flow in this species (Chapter 3). These results corroborate previous suggestions that wolves may become "imprinted" for recognition of suitable habitat prior to dispersal from their natal packs (Geffen *et al.* 2004), and that natal habitat-biased dispersal (NHBD) results in genetic isolation between populations where no physical impediments to movement likely exist.

NHBD is thought to provide two major evolutionary advantages. It allows individuals to identify suitable habitat quickly, thus reducing the risks associated with dispersal. It also increases dispersers' chances of survival and reproduction by "matching" them with home ranges to which they are already adapted (Davis & Stamps 2004). Furthermore, by increasing spatial isolation between populations, NHBD reinforces local adaptations, thus potentially increasing genetic diversity of the species as a whole (Davis & Stamps 2004). Among canids, NHBD was first suggested for *C. latrans* (Sacks *et al.* 2004). While relatively uniform over broad areas, finer-scale studies of California coyotes discovered genetic structuring corresponding to habitat types (Sacks *et al.* 2004). While not specifically identified as such, NHBD may also occur in red foxes (*Vulpes vulpes*) in Switzerland (Wandeler *et al.* 2003). In this study, the strongest potential barriers to dispersal lay between two rural populations, but genetic differentiation was greater between rural and urban foxes. While drift due to founder effects likely increased genetic distinction of urban red fox populations (Wandeler *et al.* 2003), it is quite probable that urban-born foxes settling in urban areas would be more likely to survive and reproduce than immigrants from rural areas, and vice versa.

For NHBD to occur frequently enough to impact a species' genetic structure, changes in habitat must be consistently perceived by the majority of dispersing individuals; consistent perception is encouraged when habitat breaks are signaled by multiple cues (Sacks *et al.* 2004). For northern wolves, vegetation cover (tundra versus forested regions) may be the most obvious indicator of suitable habitat. However, type of prey species encountered may also signal a desirable home range (Sacks *et al.* 2005). Data presented here suggest that predator-prey interactions influence genetic structuring of wolves at all spatial scales.

Given the migratory nature of their primary prey, the barren ground caribou, it is not surprising that tundra wolves as a group experience higher gene flow than forest wolves relying on sedentary ungulate species (Chapter 3). In the Western Arctic, spatially variable distribution of prey also appears to influence gene flow between island and mainland populations of wolves (Chapter 2, 4). Since muskoxen are plentiful on Banks and Victoria Islands (Larter & Nagy 2001), and caribou plentiful on the mainland, wolves in general may have little incentive to attempt movements across winter pack ice in the Northwest Passage. However, when the Dolphin-Union caribou are involved in just such movements, wolves pursuing these caribou may migrate incidentally; behavior of prey may thus facilitate gene flow into the otherwise isolated island metapopulation (Chapter 2, 4). The orientation of gene flow among island wolves might also be driven by ungulate populations that decline or increase on various islands at various times (Chapter 4).

Fine-scale structuring of wolf populations within single islands also seems to be influenced by spatial variation in prey abundance. Banks Island and Baffin Island wolves may form northern and southern subpopulations which focus about distinct caribou herds (Clark 1971) or high-density muskoxen populations (Chapter 2, 4). Learned hunting behavior may thus encourage NHBD of wolves in these (and possibly mainland barren ground) populations; observation of denning wolves on Baffin Island has suggested that individuals search for caribou along historically productive trajectories (Clark 1971), and dispersing wolves might therefore choose to settle near prey populations with familiar habits.

As with wolves, the genetics of arctic fox populations appear strongly linked to dynamics of their prey. A recent study of social structure in Mednyi Island arctic foxes demonstrated the influence of resource richness within territories on sex ratio of litters: because this population is characterized by strong female philopatry, mothers in poor territories tend to produce a higher proportion of male offspring (Goltsman *et al.* 2005). Since sex ratio influences the number of juvenile foxes dispersing from their natal ranges, resource variation could thus influence population genetics of arctic foxes over a very fine scale. Intrapopulation effects on genetics of arctic foxes were also found in the present work: related arctic foxes in Svalbard tend to forage at the same seabird nesting grounds (Chapter 5).

At a broad-scale, spatio-temporal variation in lemming population density has likely selected for the ability of the arctic fox, a relatively small carnivore, to travel over vast distances when food resources have locally declined (Audet *et al.* 2002; Dalén *et al.* 2005). Shifting ungulate densities in the Canadian Arctic Archipelago might drive directional movement of wolves among islands (Chapter 4); differential lemming density would be expected to have a similar influence on inter-island movements of arctic foxes. In all regions, long-distance fox movements surely lead to long-range gene flow, therefore playing a key role in maintenance of genetic homogeneity across the North American range of the species (Chapter 3). In Svalbard, where small mammals are largely absent (Eide *et al.* 2004), long-distance dispersal might remain adaptive for inland foxes scavenging on reindeer carcasses of uncertain availability, and is the most likely explanation for observation of related foxes separated by a wide range of physical distances (Chapter 5).

Implications for Conservation of Arctic Canids

All island and mainland arctic fox populations, in both North America and Svalbard, appear to be highly variable and connected by extensive gene flow (Chapter 3). Therefore, genetic factors should not limit persistence of this species in the short term. However, continued climate warming will present arctic foxes with additional challenges. The northern distribution of red foxes is limited by low winter temperatures and associated resource scarcity (Hersteinsson & Macdonald 1992; Prestrud 1991); resulting spatial separation between red and arctic foxes allows arctic foxes to avoid interference competition with their larger cousins (Bailey 1992; Dalén *et al.* 2004; Tannerfeldt *et al.* 2002). As red foxes expand into the north, arctic foxes may persist only on High Arctic islands – in both North America and Svalbard – that red foxes cannot reach. Loss of variation and increased genetic isolation of these island populations may then be observed (Dalén 2005; Dalén *et al.* 2005). However, survival of island arctic foxes will be more immediately dependent on the availability of alternative prey, as they may not be able to escape climate-associated crashes in local lemming (Krebs *et al.* 2002), or Svalbard reindeer, populations. However, if seabirds and geese continue to nest in these areas, arctic foxes are likely to persist, if perhaps in smaller numbers and only in coastal regions.

Influence of warming climate on North American ungulate populations is also a concern for arctic island wolves, as sufficient alternate prey for wolves does not exist in these areas (Mech 2005). While musk-oxen populations have increased on some islands, they have declined in other areas, and Peary caribou are endangered throughout the arctic islands (COSEWIC 2004; Gunn *et al.* 2006). Arctic island wolves, like Vancouver Island wolves (Roy *et al.* 1994), Scandinavian wolves (Flagstad *et al.* 2003), island dholes (Iyengar *et al.* 2005), Channel Island foxes (Roemer *et al.* 2001), and Phillip Island red foxes (Lade *et al.* 1996), display lower genetic variation than neighboring mainland populations (Chapter 2-4). They are also differentiated from mainland populations (Chapter 2, 4), with gene flow restricted to two relatively narrow corridors, one at the northern tip of Baffin Island and one via Victoria Island (Chapter 2, 4). Climate change may therefore also restrict gene flow into island wolf populations. Reduction of ice in the Northwest Passage, perhaps further aggravated by increased shipping traffic through the region (Charron 2005; Johnston 2002), could interfere with the movements of the Dolphin-Union caribou herd and thus with island-mainland wolf gene flow in the Western Arctic. While genetic exchange among island populations is currently high (Chapter 2, 4), continued influx of new variation from large, stable mainland populations may be critical to maintenance of healthy island wolves (Vilà *et al.* 2003). Commercial activity in the Northwest Passage should therefore be seasonally regulated to minimize impact of movements of caribou and thus wolves in this region. Future climate change may reduce total genetic variation of *C. lupus* as a species; northward movement of the tree line (Grace *et al.* 2002) over the long term will erode habitat boundaries currently segregating wolf populations, thus decreasing NHBD and encouraging genetic homogenization of populations whose regional adaptations had previously made them distinct. On the other hand, advance of the treeline will reduce spatial separation between denning wolves and calving caribou, and may thus increase survival of wolf cubs in tundra areas (Heard & Williams 1992).

Given the increasing ecological and genetic challenges arctic canids may face, anthropogenic influences could tip their balance to either persistence or extirpation. Both wolves and arctic foxes are harvested throughout their North American ranges (Van Zyll de Jong & Carbyn 1999), and harvest quotas may need to be regularly assessed should genetic health of these species begin to decline, or prey populations become dangerously depleted. As natural resource extraction increases human disturbance in remote northern areas, the impact of these activities on local canids and their prey systems will need to be assessed (McLoughlin *et al.* 2004). For two reasons, the arctic island wolf may represent the greatest canid conservation challenge. Wolves prey upon island populations of Peary caribou, and their continued presence may be in conflict with preservation of these endangered ungulates (Gunn *et al.* 2006). Secondly, while conservation is facilitated by political recognition of a population's unique taxonomic status, genetic data now available do not support a subspecific designation for arctic island wolves. However, wolves and arctic foxes are the only canid species found throughout the Arctic Archipelago, and the only major mammalian carnivores in this terrestrial ecosystem. Their unique ecological role is therefore worthy of preservation (Kyle *et al.* 2006; Wayne & Jenks 1991).

Conclusions and Future Research

This thesis extends previous genetic investigations of wolves in North America and is the first study of its kind for arctic foxes in this region. As such, it provides a baseline for investigating future changes in these populations, particularly those in sensitive arctic island ecosystems. However, several interesting questions still remain. The taxonomic status of northern wolves in general, and island wolves in particular, could be better resolved with further mitochondrial DNA sequencing, and perhaps parallel Y chromosome studies. Fine-scale genetics of arctic foxes could also be examined over a longer time frame, to determine whether the amount and direction of fox gene flow can be directly linked to annual changes in lemming population, or reindeer carcass availability. Future observation of potentially climate-mediated changes in genetics of northern canids could provide many fresh insights into the dynamics of arctic ecosystems.

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Chapter 7

Addendum 1 – Free Love in the Far North: Plural Breeding, Polygyny, and Polyandry of Arctic Foxes (*Alopex lagopus*) on Bylot Island, Nunavut[2]

Introduction

Molecular genetic techniques have begun to reveal complexities in mammalian mating systems that were not apparent from observational studies of social behavior. For example, many canid species were thought to form territorial groups consisting of a dominant, mated pair and a collection of subordinates, often presumed to be offspring or relatives (Geffen *et al.* 1996). However, recent comparisons of microsatellite DNA fingerprints between juveniles and adults of their social group have challenged such simple structures in a number of species (e.g. Baker *et al.* 2004; Kitchen *et al.* 2006; Sillero-Zubiri *et al.* 1996).

In wolf-like canids, multiple paternity of single litters (African wild dog, *Lycaon pictus*, Girman *et al.* 1997; Ethiopian wolf, *Canis simensis*, Gottelli *et al.* 1994; SilleroZubiri *et al.* 1996) and plural breeding within social groups (*L. pictus*, Girman *et al.* 1997; grey wolf, *Canis lupus*, Meier et al. 1995) have been documented using genetic methods. More recently, polygyny (males breeding with multiple females), polyandry (females breeding with multiple males), multiple paternity (single litters with multiple sires), and plural breeding (multiple breeding females in a social group) have also been identified in fox-like canids such as red foxes (*Vulpes vulpes*, Baker *et al.* 2004), island foxes (*Urocyon littoralis*, Roemer *et al.* 2001) and swift foxes (*Vulpes velox*, Kitchen *et al.* 2006), suggesting that complex mating patterns occur throughout the canid lineage.

The arctic fox (*Alopex lagopus*) is a small canid adapted to arctic and alpine climates that are characterized by spatio-temporal resource variability (Angerbjörn *et al.* 1999; Eide *et al.* 2005; Eide *et al.* 2004; Prestrud 1991). In contrast to other canids, arctic foxes are territorial primarily during the breeding season, and tend to form smaller social groups (Audet *et al.* 2002; Baker *et al.* 2004). Mating systems have never been genetically tested in this species, however, a recent ecological study (Strand *et al.* 2000) documented families of up to four adults, with or without young, including a group which consisted of a male, cubs, and two lactating female adults. Complex breeding structures are therefore expected for arctic foxes.

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For the present study, we collected DNA samples from arctic foxes trapped at dens on Bylot Island, Nunavut (Fig. 7-1). The combination of social group data and microsatellite fingerprinting techniques allowed us to explore mating patterns in this species.

Materials and Methods

Study area

Our study was conducted on the south plain of Bylot Island (73°N, 80°W) in Sirmilik National Park, Nunavut, Canada (Fig. 7-1). The area is characterized by large upland mesic plateaus covering 90% of the landscape (Massé *et al.* 2001) and intersected by several valleys filled with moist lowland habitats. More than 20,000 greater snow geese (*Chen caerulescens atlantica*) breed in this area annually (Reed *et al.* 2002), and many other migratory bird species are present during the arctic fox denning season (Lepage *et al.* 1998). The brown lemming (*Lemmus sibericus*) and the collared lemming (*Dicrostonyx groenlandicus*) are the primary prey of foxes on Bylot Island; foxes also use goose eggs and chicks, especially when rodent populations decline (Bety *et al.* 2001).

Field Methods and Sample Collection

In the summer of 2003 we performed an extensive den survey by foot and snowmobile over approximately 425 km^2 (Fig. 7-1). The study site was delimited to the west by Navy Board Inlet, and to the north and east by semi-deserts where arctic fox dens appeared to be rare or absent. More dens are present to the south but logistical reasons prevented us from surveying this area.

The position of every fox den discovered was recorded using a Global Positioning System. In 2004, each den was visited two or three times to identify those inhabited by reproductive foxes. Arctic foxes were observed at 18 dens, but adults moved cubs to new dens on at least three occasions; therefore, no more than 15 litters existed in the study area. Between June 19 and July 26, foxes were trapped at eight occupied dens using collapsible live traps (Tomahawk cage traps #205, Tomahawk Live Trap Company, WI) placed directly on the den, or padded leghold traps (Softcatch #1, Oneida Victor Inc. Ltd., OH) positioned within 100 m. Traps were kept under continuous surveillance or visited at least every 12 hours, depending on the site. We anesthetized captured adults by injecting 15 mg Telazol (Fort Dodge Animal Health, IA) into the upper rear leg muscle; juveniles were manipulated without chemical immobilization using a large fabric bag. Each individual was measured, weighed, sexed and tagged on both ears using a unique set of colored and numbered plastic tags (Dalton Rototags). Twenty to 40 summer hairs were collected from the back or flank of each animal and stored dry for genetic analysis.

Capture techniques and immobilization procedures were approved by the Université du Québec à Rimouski Animal Care Committee (permit # CPA15-02-01) and field research was approved by the Joint Park Management Committee of Sirmilik National Park of Canada (permit # SNP-2004-003).

Microsatellite DNA Fingerprinting

DNA was extracted using a DNeasy tissue protocol (QIAGEN, Germany). Twelve independently-assorting (Chapter 3), biparentally-inherited microsatellite loci were PCR-amplified from each individual using primers designed from domestic dogs (CPH5, CPH9, CPH15, Fredholm & Wintero 1995; CXX671, CXX733, CXX745, CXX758, CXX771, Mellersh et al. 1997; CXX140, CXX147, CXX173, CXX250, Ostrander et al. 1993) and labeled with fluorescent tags (FAM, TET, or HEX; Applied Biosystems, California). Single-locus amplifications of CPH5, CPH9, CXX140, CXX147, CXX250, or CXX745 contained 0.16 μmol each primer, 0.12 mmol dNTPs, 2.5 mmol $MgCl_2$, 1 x PCR buffer (50 mmol KCl, 10 mmol Tris-HCl, pH 8.8, 0.1% Triton X100), 1 U of Taq polymerase, and approximately 40 ng of template in a total volume of 15 μl. For multiplex reactions of CXX173/CXX671, CPH15/CXX758, or CXX733/CXX771 we increased dNTP concentration to 0.16 mmol dNTPs, and $MgCl_2$ to 2.7 mmol. All amplifications were conducted in Eppendorf Mastercycler ep thermocyclers (Eppendorf AG, Germany) using: 2 minutes at 94°C; 3 cycles of 45 sec at 94°, 30 sec at 50°, 10 sec at 72°; 30 cycles of 35 sec at 94°, 35 sec at 50°, 5 sec at 72°; and 30 min at 72°. Reaction products were pooled and separated on an ABI 377 Sequencer (Applied Biosystems) and genotypes assigned using GENESCAN 3.1 and GENOTYPER 2.0 software (Applied Biosystems). Each genotype was also manually checked for accuracy.

We used GENEPOP version 3.4 (Guo & Thompson 1992; Raymond & Rousset 1995) to test conformance to Hardy-Weinberg Equilibrium among adult foxes in our sample prior to parentage analysis.

Assignment of Parentage and Calculation of Relatedness

Our sample of 49 individuals contained only seven adult foxes, a small proportion of the total population. Due to low adult sample size, population allele frequency estimations are likely inaccurate. Therefore, probabilistic or likelihood-based assignments of parentage could not be performed with confidence. We used instead an inclusion-exclusion test based on simple Mendelian heredity of co-dominant microsatellite markers, whereby offspring inherit one allele at each locus from each parent.

Each adult fox was assumed to belong to the social group associated with its den of capture. Genotypes of resident adults were tested against the genotypes of cubs found at their den; adults who shared at least one allele at every locus with a cub were included as potential parents of that cub. At den 106 one adult male and one adult female were captured; paternal alleles were checked against offspring after maternal alleles had been identified (i.e., adults were treated as a parental set). Although Baker *et al.* (2004) and Roemer *et al.* (2001) considered single-locus mismatches adequate for full parental exclusion, we interpreted them as "potential exclusion" to allow for the possibility of germ-line mutation. Mismatches at two or more loci were interpreted as full exclusion (Kitchen *et al.* 2006).

Female foxes are likely to be spatially associated with their own cubs, or cubs of their social group only (Strand *et al.* 2000). However, as in other canids, male foxes may fertilize females of other social groups, and may therefore sire offspring found at other dens (Baker *et al.* 2004; Kitchen *et al.* 2006). For any cub whose resident male had been excluded as a father, and for dens where no adults were sampled, all other sampled males were tested as potential fathers. For dens with no sampled adults, the number of unique alleles observed at a single locus was used to estimate the minimum number of parents required to produce the observed offspring.

Relatedness coefficients (R, Queller & Goodnight 1989) are indices of the proportion of alleles identical by descent between two individuals, accounting for the frequencies of those alleles in the population. A pair of individuals with R between -1 and 0 are less related on average than two randomly chosen individuals, while those with R between 0 and +1 are more related than average; $R \approx 0.5$ is expected for first degree relationships (parent-offspring or full sibling), while $R \approx 0.25$ is predicted for half-siblings or other similarly related pairs. The midpoint 0.375 can be used as a cutoff to distinguish between first and second degree relatives (Blouin *et al.* 1996). Pairwise R was calculated between all foxes using SPAGeDi version 1.2 (Hardy & Vekemans 2002). Average values and standard deviations (SD) were also calculated among foxes at each den. Again, due to small sample size, allele frequencies could not be estimated with confidence. Therefore, R values were not used to draw conclusions, but to provide additional support for conclusions based on inclusion/exclusion analysis, and should be considered approximate.

Results

Sampling and Genotyping

Two adult females, four adult males, and 42 juvenile foxes were sampled from a total of eight dens. An additional male, BY08, was sampled near the greater snow goose nesting colony near the den sites, but was genetically excluded as a potential father for all juveniles in the study.

One cub from den 145 could not be genotyped due to poor quality DNA and was excluded from further analysis. Genotyping of the remaining 48 foxes was 99% complete, and no fox was typed for fewer than 11 loci. Among adult foxes, no microsatellite deviated significantly from Hardy-Weinberg Equilibrium. Taken together, these results suggest that null alleles were rare or absent in our sample.

On three occasions, we observed adult foxes moving cubs between dens. The translocations occurred late in the denning season, when cubs were older and thus heavier. However, there was no correlation between average cub mass and genetic inference of complex breeding patterns (Fig. 7-2). Moreover, during a trapping session at a given den, we never observed a juvenile previously marked at another den. Therefore, pups sampled at each den most likely represent offspring of single social groups. The genetic data presented here could still support a number of possible mating configurations, but we present the most parsimonious solutions, involving the smallest number of possible parents for each litter.

A visual summary of our results is presented in Fig. 7-2; genotypes of all 48 foxes are given in Table 7-1.

Single Breeding Pairs

Adult foxes were not sampled at dens 108, 112, or 327. However, despite the fact that 9 of 12 loci had more than five alleles in the adult sample, the cubs from each den contained no more than four unique alleles at any locus; therefore, a single male-female pair would be adequate to explain offspring at each den. Relatedness among cubs averaged 0.53 ± 0.14 at den 108, 0.54 ± 0.14 at den 112, and 0.4 at den 327, supporting the status of each litter as full siblings. However, no male in our sample shared one or more alleles per locus with any of these cubs, and therefore their paternity is unknown.

One adult male and one cub were sampled from den 137. The male was included as a possible father of this cub.

Plural Breeding with Polygyny

At den 010, a single male BY15 was captured and included as a father for all six cubs. Though no adult females were sampled, a minimum of two mothers would be required; at both locus CPH9 and CXX733, the cubs totaled three unique alleles in addition to the paternal alleles. Furthermore, cubs BY35 and BY42 shared $R = 0.55$, but were related at a half-sibling level to the other four cubs in the litter ($R = 0.26 \pm 0.11$).

One adult female and one unrelated adult male (R = -0.13) were sampled at den 106, which contained four juvenile foxes. The adult male was included as a father for all four cubs (R = 0.48 ± 0.17); the female was included for cubs BY20 and BY40 (R = 0.4 ± 0.09), but excluded for BY23 and BY32 by 2 loci (R = 0.27 ± 0.15). Average relatedness between the two pairs of cubs was 0.47 ± 0.14, which could suggest that two breeding females, BY21 and the putative 106A (Fig. 7-2), were sisters mated by the same male fox.

Plural Breeding, Polyandry, and Multiple Paternity

Adult male BY04 was included as a father for two of the five pups found at den 145 (R = 0.45 ± 0.03), but excluded at eight or more loci for the remaining three: a second male would thus be required to explain these juveniles. This social group may also have included two adult females; at locus 173, offspring attributed to BY04 contained putative maternal alleles 124 and 130, while one cub attributed to the second, unknown male was homozygous for allele 128 (Table 7-1). Polyandry and multiple paternity with a maternal germ-line mutation is possible, but plural breeding of two mated pairs may be more likely.

Adult female BY07 was sampled at den 101 and included as a mother for five of the nine cubs found there. The genotype of the remaining four cubs was 123/123 at locus 250; the female's genotype was 125/133 (Table 7-1). Average R between BY07 and these excluded cubs was 0.23 ± 0.02, supporting potential exclusion of BY07 as a mother. Since average R between these two groups of cubs was 0.20 ± 0.24, we suggest that two females, one unsampled (101C in Fig. 7-2), birthed litters at this den. This hypothesis is supported by field observation of an unidentified female suckling cubs at den 101 (M-A Giroux, pers. obs.). No sampled male was included as the father of any cub at den 101, however, the cubs attributed to female BY07 possessed three putative paternal alleles at both locus CXX733 and CXX758. Given the distribution of presumed paternal alleles in all cubs, we suggest that one unknown male, 101B mated with unknown female 101C and with female BY07. BY07 must also have bred with a second unknown male, 101A (Fig. 7-2 and Table 7-1). Plural breeding and polyandry with multiple paternity are therefore represented at this den.

Discussion

Field studies indicate that social structure in arctic foxes is variable and can be complex (Audet *et al.* 2002; Goltsman *et al.* 2005; Hersteinsson & MacDonald 1982; Korhonen & Alasuutari 1994; Strand *et al.* 2000). While groups including multiple adults have been observed, previous studies often suggest that breeding is restricted to the dominant pair (e.g., Eide *et al.* 2004; Korhonen & Alasuutari 1994; Kullberg & Angerbjörn 1992); our work provides preliminary genetic evidence for plural breeding, polygyny, and polyandry with multiple paternity in the arctic fox.

Breeding season territoriality of arctic foxes has been linked to spatio-temporal resource abundance in a social group's home range (Eide *et al.* 2004). Arctic foxes are known to cache lemmings and bird eggs for winter consumption (Careau *et al.* 2006; Eide *et al.* 2004; Samelius & Alisauskas 2000) and subordinate animals of social groups, who do not often provision cubs directly (Strand *et al.* 2000), have been observed caching food within the territory of the breeding pair (Eide *et al.* 2004). This behavior suggests a possible energetic advantage to the dominant pair that may permit formation of larger social groups (Eide *et al.* 2004); plural breeding may thus be elevated in resource-rich habitats. Our study was conducted in an area of Bylot Island which includes both lemmings and avian nesting grounds, and it is worth noting that 3 out of 4 dens with potential plural breeding (101, 106, and 145) were clustered near the snow goose nesting colony.

Explanations for polyandry and its associated multiple paternity are varied, including both material benefits and genetic advantages such as assurance of compatibility between maternal and paternal genomes (Zeh & Zeh 2001). Multiple paternity also allows a female to increase the genetic variation contained in a single season's reproductive output. This increase in variation might result in an increased probability that at least one cub in a litter will be optimally adapted to its current environment, or better equipped to deal with changes in its environment over time. The reproductive output of arctic foxes is closely tied to the productivity of their habitat in any given year (Angerbjörn *et al.* 1995), and multiple paternity may provide an additional adaptive advantage to both recurrent ecological fluctuations and incipient climate-induced changes in the polar habitat of the arctic fox.

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Table 7-1 Microsatellite DNA fingerprints of all arctic foxes sampled on Bylot Island. For each cub, presumed paternal alleles are given in bold and presumed maternal alleles in italics. Outlined alleles imply multiple female parents, shaded alleles imply multiple male parents.

Den	Age and Sex	Fox	5	9	15	140	147	173	250	671	733	745	758	771
?	Adult	BY08	113	151	157	142	177	126	127	207	250	273	-	102
	Male		131	157	157	144	177	128	133	211	250	277	-	102
010	Adult	BY15	**125**	**151**	**153**	**146**	**171**	**122**	**127**	**203**	**250**	**271**	**239**	**100**
	Male		**133**	**157**	**157**	**148**	**179**	**124**	**129**	**209**	**256**	**277**	**245**	**106**
010	Juvenile	BY25	**125**	**151**	**157**	**148**	**179**	**122**	123	**209**	**256**	**271**	241	**106**
	Male		131	$\boxed{155}$	161	148	179	**124**	**127**	209	$\boxed{262}$	**277**	**245**	106
010	Juvenile	BY35	**125**	**151**	**153**	**148**	**179**	**122**	123	**203**	**256**	**277**	**239**	96
	Male		131	$\boxed{157}$	159	148	183	122	**129**	**209**	$\boxed{262}$	277	241	**106**
010	Juvenile	BY42	131	**151**	**157**	144	**171**	**122**	123	207	**250**	**277**	**239**	96
	Male		**133**	**157**	159	**146**	**179**	122	**129**	**209**	$\boxed{256}$	277	241	**106**
010	Juvenile	BY45	**125**	**151**	**157**	**146**	**179**	**122**	**127**	**209**	$\boxed{\mathbf{250}}$	**271**	**239**	**106**
	Male		**133**	$\boxed{151}$	161	**148**	179	126	**129**	209	$\boxed{\mathbf{254}}$	275	**245**	106
010	Juvenile	BY47	**125**	**151**	**157**	**146**	**171**	**122**	123	**203**	**250**	**271**	**245**	**100**
	Male		131	**157**	161	**148**	**179**	**124**	**127**	**209**	$\boxed{262}$	275	245	**106**
010	Juvenile	BY48	131	**151**	**157**	**148**	**179**	**124**	123	**209**	**250**	**271**	-	96
	Male		**133**	$\boxed{155}$	159	148	179	126	**127**	209	$\boxed{262}$	**277**	-	**106**

Den	Age and Sex	Fox	5	9	15	140	147	173	250	671	733	745	758	771
101	Adult	BY07	*121*	*151*	*157*	*152*	*177*	*124*	*125*	*211*	*246*	*279*	*228*	*100*
	Female		*129*	*155*	*157*	*154*	*179*	*126*	*133*	*213*	*260*	*283*	*241*	*108*
101	Juvenile	BY10	*121*	*155*	*157*	148	*179*	122	*125*	207	*260*	275	*224*	*100*
	Female		127	155	159	*152*	183	*126*	*133*	*213*	270	*279*	*228*	100
101	Juvenile	BY16	*121*	*151*	*157*	148	*177*	122	*133*	209	*246*	*279*	*228*	*108*
	Female		*129*	*155*	157	*152*	*179*	*126*	133	*211*	250	*283*	254	108
101	Juvenile	BY19	*121*	*155*	*157*	146	*177*	122	*125*	209	[illegible]	275	224	*100*
	Female		127	155	159	*152*	185	*124*	*133*	*213*	*260*	*283*	*228*	100
101	Juvenile	BY22	127	153	*157*	146	*179*	*126*	123	207	*246*	275	*241*	*100*
	Female		*129*	*155*	159	*154*	183	126	123	*211*	252	*279*	241	*108*
101	Juvenile	BY43	*121*	*151*	*157*	148	*177*	122	123	207	*246*	275	*241*	*100*
	Female		127	*155*	159	*154*	183	*124*	123	*213*	270	*279*	241	100
101	Juvenile	BY14	127	*151*	*157*	146	*177*	122	123	209	*260*	273	*241*	*100*
	Male		*129*	155	159	*152*	185	*124*	123	*211*	270	*283*	241	100
101	Juvenile	BY28	*121*	*155*	*157*	146	*179*	122	123	207	*246*	275	*228*	*108*
	Male		127	155	159	*154*	183	*126*	*133*	*211*	[illegible]	*279*	*241*	108
101	Juvenile	BY44	*121*	*155*	*157*	146	*177*	122	123	209	[illegible]	273	*228*	*100*
	Male		127	155	159	*154*	183	*126*	*133*	*211*	*260*	*283*	*241*	*108*
101	Juvenile	BY49	127	*155*	*157*	146	*179*	122	123	207	*246*	275	*241*	*108*
	Male		*129*	155	159	*152*	183	*126*	123	*211*	252	*283*	241	108

Den	**Age and Sex**	**Fox**	**5**	**9**	**15**	**140**	**147**	**173**	**250**	**671**	**733**	**745**	**758**	**771**
106	Adult	BY21	*121*	*151*	*157*	*148*	*177*	*122*	*133*	*203*	*250*	*267*	*234*	*104*
	Female		*133*	*155*	*165*	*148*	*179*	*122*	*133*	*209*	*256*	*277*	*241*	*108*
106	Adult	BY01	-	**155**	**157**	**146**	**177**	**124**	**125**	**209**	**250**	**273**	**222**	**108**
	Male		-	**155**	**159**	**148**	**183**	**124**	**135**	**213**	**252**	**281**	**234**	**112**
106	Juvenile	BY23	131	*155*	**157**	**146**	*177*	124	*133*	*203*	*250*	*267*	**222**	*108*
	Female		131	**155**	*165*	*148*	**177**	**124**	**135**	**209**	**252**	**281**	*234*	**112**
106	Juvenile	BY32	131	*155*	**157**	*148*	*177*	*122*	*133*	*203*	**250**	**273**	**222**	*104*
	Female		131	**155**	*165*	**148**	**183**	**124**	**135**	**209**	254	*277*	*241*	**108**
106	Juvenile	BY20	113	*151*	**157**	**146**	*177*	*122*	*133*	*209*	*250*	*277*	**222**	*104*
	Male		*121*	**155**	*165*	*148*	**177**	**124**	**135**	**213**	**252**	**281**	*234*	**112**
106	Juvenile	BY40	*121*	*151*	**157**	*148*	**177**	*122*	*133*	*209*	*250*	**273**	**222**	*104*
	Male		131	**155**	*165*	**148**	*179*	**124**	**135**	**209**	**252**	*277*	*241*	**112**
108	Juvenile	BY02	121	155	157	146	177	122	125	203	250	267	222	104
	Female		129	155	165	148	183	122	131	211	250	277	245	104
108	Juvenile	BY11	123	151	157	148	179	122	125	203	250	277	222	102
	Female		131	155	159	148	183	122	133	209	256	279	241	104
108	Juvenile	BY29	123	151	157	148	183	122	125	203	250	277	222	104
	Female		131	155	159	148	183	122	133	209	250	279	241	104
108	Juvenile	BY31	123	155	159	146	179	122	133	203	250	277	234	104
	Female		129	155	165	148	183	122	133	211	250	279	241	108
108	Juvenile	BY33	121	151	157	146	179	122	131	203	250	267	234	104
	Female		131	155	157	148	183	122	133	211	250	277	245	108
108	Juvenile	BY09	121	155	157	146	177	122	133	203	250	277	234	104
	Male		131	155	159	148	183	122	133	211	256	279	241	108
108	Juvenile	BY37	123	151	157	148	177	122	125	203	250	277	222	102
	Male		131	155	165	148	183	122	133	211	250	279	241	104

Den	Age and Sex	Fox	5	9	15	140	147	173	250	671	733	745	758	771
112	Juvenile	BY18	119	151	157	136	177	126	125	203	250	271	239	96
	Female		133	151	159	148	179	128	127	213	250	277	241	100
112	Juvenile	BY27	127	151	157	136	171	126	127	207	250	271	241	104
	Female		133	155	157	148	177	128	133	207	252	277	241	108
112	Juvenile	BY30	119	151	157	136	171	126	127	207	250	267	241	96
	Female		133	151	159	148	177	128	133	213	252	271	241	108
112	Juvenile	BY38	127	151	157	136	171	126	127	203	250	271	241	100
	Female		133	155	159	148	177	126	133	207	250	277	241	104
112	Juvenile	BY39	127	151	157	136	171	126	127	207	250	267	241	104
	Female		133	155	157	148	177	126	133	213	250	271	241	108
112	Juvenile	BY46	127	151	157	136	177	126	125	207	250	271	239	96
	Female		133	151	157	148	179	128	127	207	250	277	241	100
112	Juvenile	BY24	127	151	157	136	177	126	125	203	250	267	239	96
	Male		133	155	159	148	179	128	127	213	252	271	241	108
137	Adult	BY34	**131**	**151**	**157**	**142**	**177**	**126**	**125**	**207**	**250**	**267**	**222**	**98**
	Male		**131**	**151**	**159**	**148**	**183**	**128**	**133**	**211**	**258**	**273**	**241**	**104**
137	Juvenile	BY03	117	**151**	**157**	**142**	**177**	122	123	**207**	**250**	**267**	**222**	**98**
	Male		**131**	155	157	144	**183**	**128**	**133**	207	**258**	277	230	106

Den	Age and Sex	Fox	5	9	15	140	147	173	250	671	733	745	758	771
145	Adult	BY04	**121**	**151**	**159**	**142**	**177**	**126**	**125**	**205**	**246**	**267**	**228**	**100**
	Male		**121**	**155**	**165**	**144**	**187**	**126**	**127**	**207**	**250**	**277**	**241**	**102**
145	Juvenile	BY05	131	**151**	[illegible]	[illegible]	167	128	**125**	**207**	**250**	271	[illegible]	[illegible]
	Female		133	157	[illegible]	152	171	130	133	209	254	279	249	104
145	Juvenile	BY06	**121**	**151**	153	140	179	**126**	123	**207**	**246**	**277**	-	**100**
	Female		129	151	**165**	**142**	**187**	130	**127**	207	**250**	279	-	**102**
145	Juvenile	BY13	131	**155**	[illegible]	140	174	128	[illegible]	203	**250**	[illegible]	[illegible]	104
	Female		133	157	[illegible]	152	179	128	[illegible]	209	254	279	[illegible]	104
145	Juvenile	BY12	**121**	**155**	**159**	140	167	124	**125**	**207**	**250**	**277**	**228**	**100**
	Male		129	155	161	**144**	**177**	**126**	125	207	250	279	243	100
145	Juvenile	BY26	131	**151**	[illegible]	[illegible]	179	124	123	**207**	**246**	271	[illegible]	[illegible]
	Male		133	157	[illegible]	[illegible]	183	128	**125**	209	**250**	[illegible]	[illegible]	104
327	Juvenile	BY36	113	151	155	148	175	122	123	203	250	271	239	102
	Female		133	151	157	148	179	128	127	207	250	275	245	104
327	Juvenile	BY41	119	151	153	146	175	122	123	203	250	271	241	102
	Male		133	155	157	146	183	128	127	207	256	275	245	104

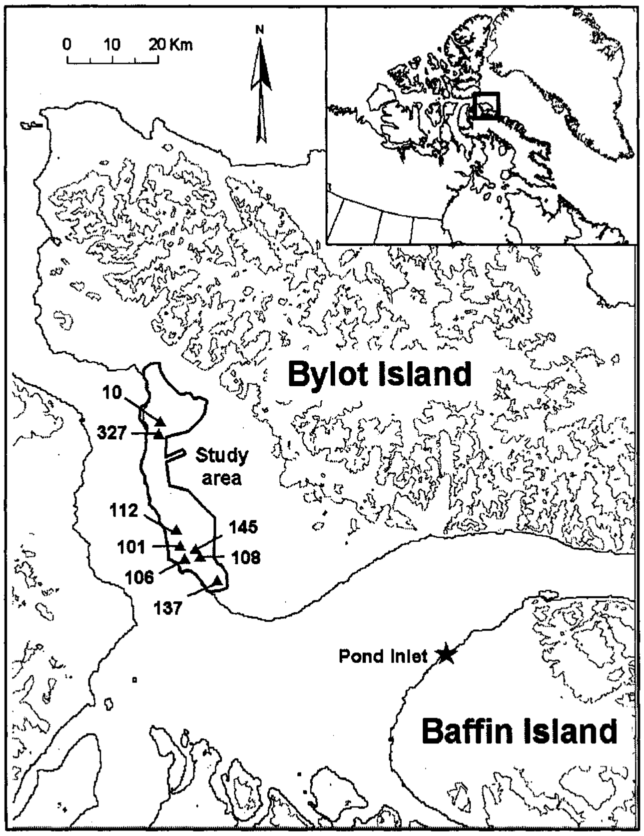

Figure 7-1 Study area on Bylot Island (73°0'N, 80°0'W), Nunavut, Canada. Triangles represent occupied arctic fox dens and den numbers are given.

Den	Male	Female	Offspring	Mean ± SD
010	BY15	? 010A	BY25 BY45 BY47 BY48	1965.00 ± 79.69
		? 010B	BY35 BY42	
101	? 101A	BY07	BY10 BY16	950.00 ± 96.82
	? 101B		BY19 BY28 BY44	
		? 101C	BY22 BY43 BY14 BY49	
106	BY01	BY21	BY20 BY40	1467.50 ± 67.02
		? 106A	BY23 BY32	
108	? 108A	? 108B	All	1346.43 ± 116.75
112	? 112A	? 112B	All	1235.71 ± 24.40
137	BY34	? 137A	BY03	2900
145	BY04	? 145B	BY06 BY12	1324.17 ± 177.16
	? 145A	? 145C	BY05 BY13 BY26	
327	? 327A	? 327B	All	1900.00 ± 70.71

Figure 7-2 Summary of inclusion/exclusion analysis. Each den is represented by a horizontal box. Sampled individuals are labeled with the letters BY; unsampled individuals predicted by exclusion analysis are indicated by ?'s and coded with den numbers and letters. Horizontal lines within den boxes indicate presence of multiple same-sex parents at a den. For example, in Den 101, putative male 101A was included as a father for cubs BY10, and BY16, with all other cubs arising from putative male 101B. BY07 was included for cubs BY10, BY16, BY19, BY28, and BY44 at this den, while a second, unsampled female would be required to explain the remaining cubs. Mean and standard deviation of cub weight at time of capture are given by Mean ± SD.

Chapter 8

Addendum 2 - Monozygotic Twinning in a Wild Carnivore Species

Introduction

Monozygotic twins have been extensively studied in humans (e.g. Hrubec & Robinette 1984) but have rarely been documented in other mammals (Gleeson *et al.* 1994). In fact, we could find no published record confirming monozygotic twinning in any wild carnivore species. Here we present what may be the first evidence of such twinning in a caniform carnivore, the grey wolf (*Canis lupus*).

Materials and Methods

Two thousand twenty-five (2,025) wolf tissue samples were collected from hunters, fur auction houses, and museums; the sampling distribution spanned most of the North American Arctic (Chapter 3). Samples were stored frozen and extracted using a DNeasy tissue protocol (QIAGEN, Germay). Fourteen microsatellite loci (CPH5, CPH16 (Fredholm & Wintero 1995); CXX140, CXX173, CXX250, CXX251, CXX377 (Ostrander *et al.* 1993); CXX618, CXX671, CXX733, CXX745, CXX758, CXX781, CXX2079 (Mellersh *et al.* 1997)) were amplified using the polymerase chain reaction and primers originally designed for domestic dogs. DBX and DBY, pseudoautosomal markers for molecular sex identification, were also amplified from each sample (Seddon 2005). PCR amplification, gel electrophoresis conditions, and analysis of genotypes are described in Chapter 3.

Matching genotypes were identified using the Excel Microsatellite Toolkit (Park 2001), and assignment tests (Paetkau *et al.* 1995) performed with the calculator available at http://www2.biology.ualberta.ca/jbrzusto/Doh.php . The probability that two randomly chosen individuals would possess the particular genotype shared by potential twin wolves TU9291 and SHS9201 was the probability of the complete genotype, and was calculated using the allele frequencies from Banks Island, the putative population of origin. The probability that full siblings would share this genotype was calculated using allele frequencies from Banks Island and the expectations of identity by descent.

Results and Discussion

The database contained 101 pairs of wolves with identical genotypes, but 100 of these pairs were clearly the result of multiply-sampling the same individual (in most cases, one sample had been obtained from a fur house and its match directly from a hunter).

The genetic identity of the 101st pair, male wolves TU9291 and SHS9201, was of particular interest. Wolf TU9291 was sampled in Tuktoyaktuk (mainland Northwest Territories, Canada) on February 19, 1992, while SHS9201 was collected in Sachs Harbour, Banks Island (Northwest Territories, Canada) nine months later. Assignment tests (Paetkau *et al.* 1995) indicate these wolves share a genotype that originated on Banks Island, rather than in a mainland wolf population (Fig. 8-1), implying that TU9291 migrated from Banks Island to the mainland after freeze-up of sea ice between these regions. This represents a straight-line movement of at least 375 km; the actual distance migrated may have been much longer (Chapter 4).

Three factors suggest that genetic identity of wolves TU9291 and SHS9201 was not simply due to error. The individuals were sampled nine months apart, in separate towns, by separate researchers, and thus could not have been confused during initial collection (J Nagy, pers. comm.). Both samples consisted of whole skulls submitted by local hunters, eliminating the possibility that a hunter could mistakenly submit two samples from a single wolf. Finally, the 14-locus genotypes used here were a composite of data from Carmichael *et al.* (2001, Chapter 2) and from amplifications of additional loci performed using fresh DNA extractions of the original source material (Chapter 3); laboratory error producing spurious identity is therefore unlikely. Individuals TU9291 and SHS9201 thus appear to be two distinct and genetically identical wolves.

The probability that two randomly chosen wolves from Banks Island would share the relevant genotype was 3.5E-12. At 3.6E-05, chance identity of non-twin siblings is more likely, but would not be expected given the estimated census size of 200 wolves in this population (Chapter 2). Since Banks Island wolves are less variable than their mainland counterparts (Chapter 2, 4), a mating between related individuals could also produce offspring that are genetically indistinguishable at the loci used here without being monozygotic twins. However, incest is rare in wolves (Smith *et al.* 1997) and there is no genetic evidence of inbreeding in the Banks Island population (data not shown). In 1999, Neff *et al.* found one set of verified monozygotic twins during pedigree analysis of approximately 200 domestic dogs. The occurrence of one set of monozygotic twin wolves in a sample of over 2000 individuals is therefore reasonable, and seems the most likely explanation for the apparent identity of TU9291 and SHS9201.

Most interesting of all is the observation that these presumptive identical twins experienced divergent life histories, with one individual remaining in its natal island population while the other undertook a migration over sea ice to the mainland. Bayesian estimation suggests a total migration rate of only 1.7% between Banks Island and northern mainland wolf populations (Chapter 4), underscoring the uniqueness of this event.

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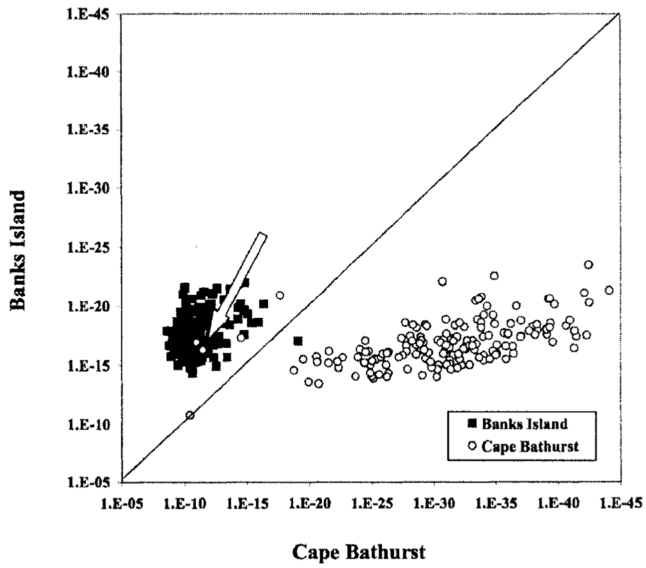

Figure 8-1 Assignment results for two northern Canadian wolf populations. Symbol colour represents sampling population of each individual. Wolves are plotted according to the probability their genotype would arise in Banks Island, the sampling location of SHS9201, or Cape Bathurst, the mainland wolf population in which TU9291 was found. The arrow indicates the superimposed white and black points corresponding to these putative monozygotic twin wolves.