Genetic diversity along the core-edge continuum in a species with a continental-scale range, *Canis lupus*

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I. Introduction

The study of genetic trends along the core-edge continuum of a species range dates back to over 60 years ago (Carson 1959) and is of particular interest in contemporary conservation and evolutionary genetics (Eckert et al. 2008). Indeed, edge populations are the focus of conservation efforts due to their novel regimes of natural selection that may lead to new adaptations or even speciation events, the former of which is particularly important in the context of rapidly changing ecosystems as a result of natural and anthropogenic forces (Lesica and Allendorf 1995, Volis et al. 2016). At the same time, edge populations are thought to be smaller in size and host low levels of neutral genetic diversity, making them particularly vulnerable to stochastic events and genetic drift (Lesica and Allendorf 1995).

The Abundant Center Model (ACM; Sagarin and Gaines 2002) postulates that a species' abundance is highest at the center of its geographic range and declines towards the edges. The most popular mechanism proposed is that the availability of favorable conditions for the species declines with increasing distance from the core, until the conditions become so hostile that the species cannot exist (Brown 1984, Pironon et al. 2015). The Central-Marginal Hypothesis (CMH), sometimes called the Core-Peripheral hypothesis or a variation of the two, extends on the ACM, such that the decrease in population size and gene flow towards the edges of a species' range increases the relative effect of genetic drift, driving a decrease in genetic diversity (Eckert et al. 2008). Other factors can amplify this effect and contribute to the formation of the range edge. Immigrants to edge populations may arrive from fewer source populations and are thus less genetically diverse than the total population (Schwartz et al. 2003), and repeated extinction and recolonization events may promote low genetic diversity consistent with the Founder Effect (Mayr 1942, Volis et al. 2016). Furthermore, migrants from the core can help prevent local extinctions on the edge but can also hinder the fixation of alleles beneficial to conditions there (Kirkpatrick and Barton 1997, Volis et al. 2016).

Despite being largely accepted early on, the CMH has sparked discussion as to its universality. A review found near equal support for and against the CMH (Eckert et al. 2008) and outlined frequent issues with the methods in this field, including failing to sample the full extent of the species' range, the absence of statistical testing, and most importantly, a lack of universal definition for core and edge. The latter continues to hinder understanding of the CMH and comparability of results on the subject. While two operational definitions of core and edge have been proposed by Channell and Lomolino (2000a, 2000b) and described by Schwartz et al. (2003), most studies approximate core and edge based on the species' ecology. Furthermore, few studies explain their reasoning or methods for characterizing populations as core or edge, or how the distinction may affect their findings. The result is a wide range of definitions that are largely subjective and could influence the results observed, which may explain some of the discrepancies in findings between studies.

There is also a lack of knowledge regarding changes in large vertebrate genetics along the core-edge continuum. Of the studies using large vertebrates, only a few have focused on species with large-scale continuous ranges that include a variety of habitat types and properties shaping the range edges (Kyle and Strobeck 2002, Schwartz et al. 2003, Rico et al. 2016), with contrasting results regarding the CMH.

Thus, I used gray wolves (*Canis lupus*) to elucidate whether patterns of genetic diversity in large vertebrates with a continental-scale range and high dispersal potential align with the Central-Marginal Hypothesis. The northern edge of the wolf range exceeds 80°N in the high Arctic while the southern edge falls in the temperate zone at mid-latitude, below 35°N. Their range edge is shaped by different features across the landscape, including large water bodies, which make defining the core and edge difficult. Therefore, I also investigated the potential effect of the definitions of core and edge on the patterns of genetic diversity observed.

According to the CMH, a gradual decrease in habitat suitability due to harsh climate and changes in large prey abundance at the northern edge of the range is expected to reduce genetic diversity in wolves. This effect may be weak between continuous wolf populations due to the high dispersal potential and adaptability of wolves, as few migrants per generation are necessary to maintain genetic diversity (Mills and Allendorf 1996**.** Thus, genetic diversity is expected to be lowest for populations that are both in relatively hostile environments that receive limited numbers of migrants from milder core habitats, and separated by the most significant geographic barrier to wolves: water. Because the eastern and western edges of their range are not characterized by gradual declines in habitat suitability, but rather hard boundaries presented by the Atlantic and Pacific Ocean, genetic diversity is not expected to follow the CMH and decrease at these edges.

I used several methods to delineate core and edge populations in wolves based on two published, one reported, and one new dataset. Due to inter-study differences in spatial scale (Fig. 1), sampling method, study period, and genetic markers analyzed, direct statistical comparisons could not be made between them (with the exception of 15 loci shared between the two smaller datasets) but trends in genetic diversity between core and edge groups could be compared and confirmed between datasets (Groot et al. 2016). Using additional datasets, this study provides an important test of, and expansion on, patterns first identified by Carmichael et al. (2008) regarding the genetic diversity of the island and mainland wolves of North America.

II. Materials and methods

Study species

Gray wolves historically occupied one of the largest ranges of any mammal, spanning much of North America and Eurasia. They are habitat generalists found in most habitats with sufficient prey, which are primarily large herbivores such as moose, elk, and caribou, as well as small mammals and fish (Mech 1970, Fuller 1989, Boitani et al. 2018). Their abundance declined and their range was restricted to remote wilderness because of persecution and conflict with humans over livestock, but after a period of increase, the global wolf population is now stable and its range appears to be increasing in some areas (Boitani et al. 2018).

While wolf reintroductions have contributed to their range expansion in areas such as central Idaho and Yellowstone (Forbes and Boyd 1996, 1997), much of the range increase results from their high dispersal potential (Mech and Boitani 2003, Boitani et al. 2018). Individual wolves have been documented to disperse over 500 km straight line distance (Fritts 1983, Mech and Boitani 2003, Treves et al. 2009, Prettyman 2015), with the current record observed being 1092 km (Wabakken et al. 2007). Dispersing wolves can traverse mountains and deserts, as well as waterways by swimming or using ice bridges (Carmichael et al. 2001, Linnell et al. 2005, Muñoz-Fuentes et al. 2009). Most wolves do not disperse over such extreme distances and conditions however, and the typical straight-line dispersal distance is less than 100 km (Kojola et al. 2006, Jimenez et al. 2017).

Sampling location

I used datasets from Carmichael et al. (2007, 2008), Musiani et al. (2007), and McNay (2006) (Fig. 1). I refer to these as the datasets A, B, and C respectively.

The largest dataset, dataset A (Carmichael et al. 2007, 2008), spans the Canadian tundra and boreal forests covering both Canada and Alaska. Islands off the coast of British Columbia and in the High Arctic were also sampled. Dataset B follows and samples both to the north and south of the Canadian tree line (Musiani et al. 2007). The third dataset, C, is localized to a portion of inland Alaska, within Alaska's Game Management Unit 20A (McNay 2006). Their associated sampling methods are described in their respective studies.

Additional samples from Ellesmere Island (Inuit: Umingmak Nuna) were collected and will be discussed here (hereby Dataset D).

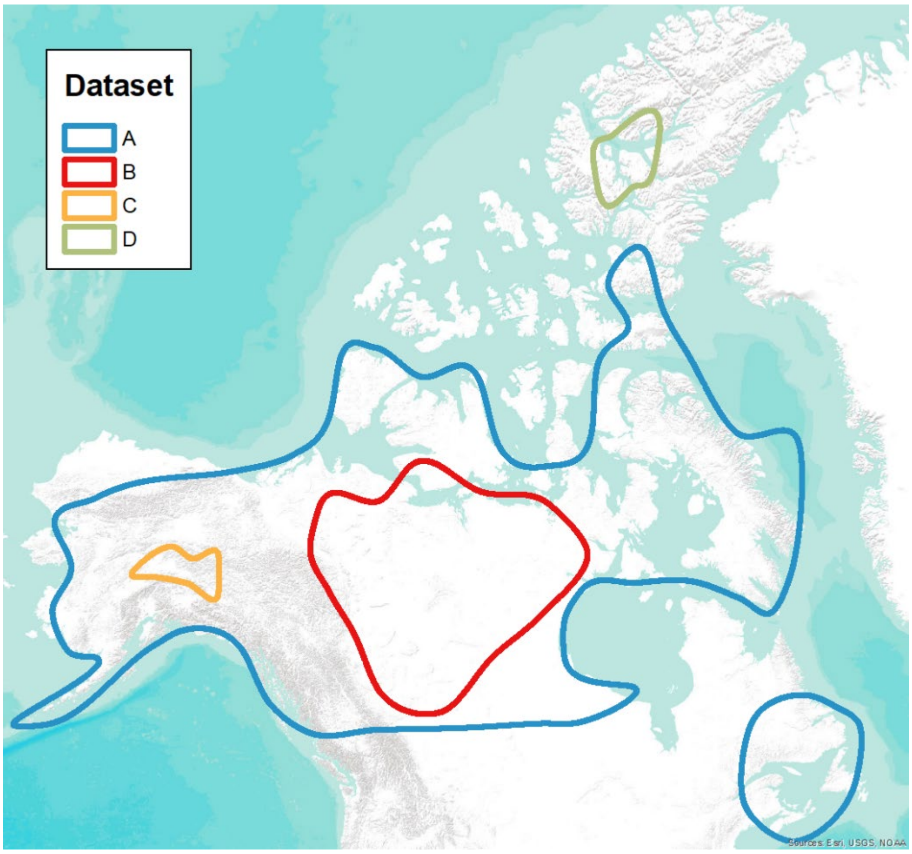


Figure 1. *Sampling area of the four North American Canis lupus microsatellite datasets considered in this study, two of which were previously published (A: Carmichael et al. 2007, B: Musiani et al. 2007), one was used in an agency report (C: McNay 2006), and one is new to this study (D).*

Ellesmere Island is a 196,235 km² island at the north edge of the Canadian Arctic Archipelago, in the Nunavut territory. The island is largely covered by open tundra and the Arctic Cordillera mountain system, and is characterized by severely cold winters with short, cool summers. The island supports musk oxen (*Ovibos moschatus)*, Peary caribou (*Rangifer tarandus pearyi*), arctic hare (*Lepus arcticus)*, and the northern-most population of Canadian wolves, the only large predators of the Arctic Islands (Mech 1995, 2005, Dick 2017).

For 6-8 months each year, the channels surrounding Ellesmere Island and the Archipelago are covered by land-fast sea ice (Galley et al. 2012), facilitating movement of land-based wildlife between adjacent islands. GPS collared wolves have been documented to move between Ellesmere Island, Axel Heiberg Island to the west, and Devon Island to the south (Anderson et al. 2018). The wolf population is supplemented by occasional migration from more southern islands, and these southern islands receive migrants from the mainland (Carmichael et al. 2007, Musiani et al. 2007, Schweizer et al. 2016)

Sample collection

Wolf genetic samples from Ellesmere Island were collected over three sampling periods: 2005 and 2009-2010 samples were collected by Dr. L. David Mech, and 2014-2017 samples were collected by Dr. Dan MacNulty and Morgan Anderson. During the first two sampling periods, 48 scat samples (2005: 17 adult scats, 2009-2010: 31 pup scats) from around active dens were collected for DNA analysis; 31 were stored in Everclear alcohol, and 17 were stored as dry samples. An additional tissue sample was collected from an adult male wolf that was fitted with a GPS collar for another project. During the 2014-2017 field seasons, scat collection was replaced with tissue sample collection from 10 wolves that were caught and fitted with GPS collars. An additional hair sample was collected from an adult breeding female found deceased in 2015.

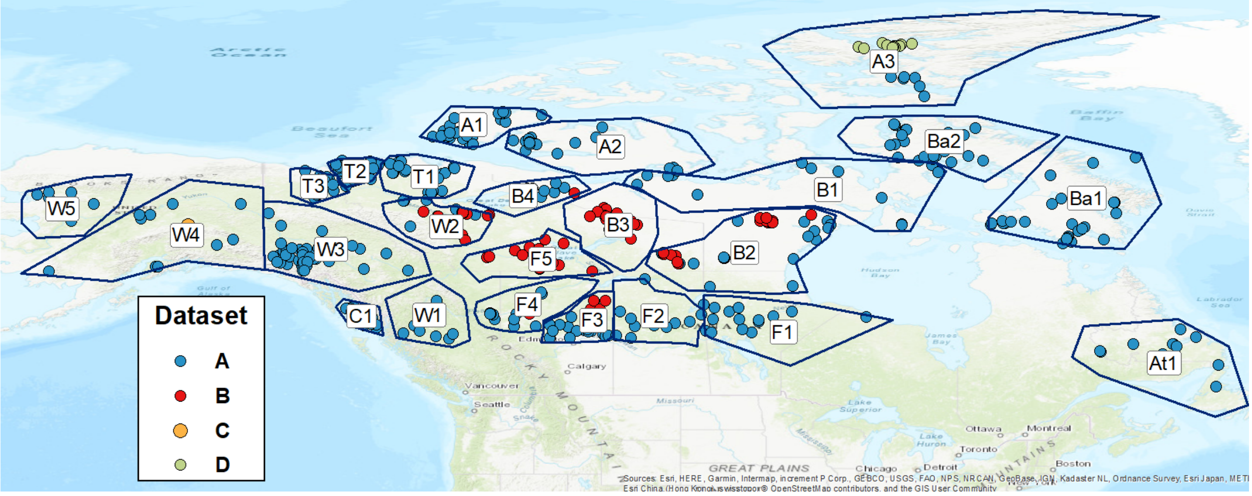
DNA extraction and genotyping

Wildlife Genetics International (Wildlife Genetics International, Nelson, British Columbia) extracted and genotyped the DNA. Prior to DNA extraction, samples stored in Everclear were dried in a 70°C incubator then rotated overnight at 55°C in QIAGEN's ASL buffer. DNA was extracted from scat samples using QIAGEN Stool Minikits, and from the tissue samples using QIAGEN DNeasy Blood and Tissue Kits, following standard protocol (Qiagen 2006, 2012). A total of 22 dinucleotide microsatellite markers developed from the dog genome project, located on different chromosomes and known to amplify strongly in wolves, were genotyped from the samples (C01.251, C02.30, (Ostrander et al. 1993, Breen et al. 2001) CPH9 (Fredholm and Winterø 1995), AHT121 (Holmes et al. 2009), REN145P07, REN183B03, REN262I12, REN85N14, REN181L14, REN210D03, REN316E23, REN69B24, REN105L03, REN233H01, REN112G06, REN68B08, REN144A06, REN297N05, REN199O08, REN94H15, and REN106I06 (Breen et al. 2001)). Samples with low confidence scores were reanalyzed at the problematic markers until they produced high confidence scores for each marker.

Defining populations

Wolf ecology is relatively well understood and the genetic structure of North American populations has been elucidated three times (Carmichael et al. 2007, Musiani et al. 2007, Schweizer et al. 2016). Several factors have been hypothesized to cause the observed genetic clusters of North American wolves: separation by large bodies of water (Carmichael et al. 2001), separation at the tree line (Musiani et al. 2007), prey specialization and ecology linked to migratory caribou (*Rangifer tarandus;* Heard and Williams 1992, Walton et al. 2001), climate (Geffen et al. 2004), and isolation by distance (Geffen et al. 2004). Additionally, correlations between genetic structure and prey type, habitat, and climate have been observed in Eastern European wolves (Pilot et al. 2006)**.** These characteristics defining the genetic clusters allow for the definition of putative populations.

Prior to assigning wolves to core or edge groups, Dr. Marco Musiani and I identified 24 putative populations based on a list of criteria regarding wolf ecology and the spatial distribution of samples. We used the following set of criteria: genetic cluster delimitations (Carmichael et al. 2007, Musiani et al. 2007, Schweizer et al. 2016), land cover (e.g. forest vs tundra), separation by water channels (e.g. Arctic islands), known ecotypes (e.g. coastal wolves), caribou herd migration patterns, and distance (Fig. 2). Six wolves falling outside these populations were removed from the Carmichael dataset. I then defined core and edge groups using the spatial locations of the putative populations based on the following three definitions.



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

Figure 2. *Putative populations of wolves defined using a series of criteria related to wolf ecology and the spatial distribution of GPS telemetry data from the four Canis lupus microsatellite datasets (A: Carmichael et al. 2007, B: Musiani et al. 2007, C: McNay 2006, D: this study). Population names are based on the genetic cluster they fall in.*

Defining Core and Edge groups

The edge region contains features, such as islands that may connect to other islands and mainland via ice bridges during parts of the year, that make it difficult to define a core-edge boundary. Thus, I defined core and edge groups with three approaches, each designed to address limitations of the other approaches.

Definition 1: Geographic position

I first delineated Core and edge populations using water barriers (Fig. 3). I designated populations bordered by less than approximately 50% water as the core and populations bordered by at least 50% water as the edge. I considered populations entirely surrounded by water, i.e. islands, as a separate group ("peripheral"). For mainland populations bordering water and connected to islands via ice bridges for part of the year, I defined a separated group ("pseudo-edge"). The main weakness of this method is the grouping of populations that are ecologically distinct into one group (e.g. coastal BC and Arctic Islands).

Definition 2: Distance from the centroid

One of the operational definitions of core and edge used by Channell and Lomolino (2000b) is based on the distance from the centroid of the species' range to the closest edge, where half of that distance is considered the cutoff between core and edge. Using the closest geographic edge to the wolf centroid, Hudson Bay, resulted in a cutoff radius that was too restrictive given the distance from coast to coast. Instead, I obtained the centroid of each population and all populations combined to measure the distances separating each population centroid to the centroid of all the populations (Fig. 4). I modified Channell and Lomolino's method such that the cutoff for core and edge was half the distance from the centroid of the populations to the centroid of the most distant population (i.e. the Atlantic population At1, centroid-centroid distance = 3284 km).

I used the centroids of the population delineations rather than centroids of the data points for three reasons: first, wolves likely occurred in areas of their population that they were not sampled in. Second, some wolves in the Canadian tundra are migratory and their sampling location does not account for their presence across their putative population. Finally, while some data points are exact sampling locations, others are approximations that depend on the sampling methods of their respective studies.

I further divided the core and edge groups by island and mainland, given evidence that the island wolves have lower genetic diversity compared to mainland wolves (Carmichael et al. 2008). Dataset B was only represented in one group; as no trends could be observed from one group, it was not used for this definition.

The issue with this definition is that the centroid of our populations, which is based on the spatial extent of the data used, is not the true centroid of the North American wolf range. The true centroid is located farther south-east, as wolves inhabit parts of Southern Canada, Eastern Canada, and the USA that were not sampled.

Definition 3: Latitudinal position

The final grouping, done to address the presence of wolves living south of the extent of the datasets used, was based on latitude. I defined five latitudinal groups, with the highest and lowest group spanning ten degrees each. The three intermediate groups each spanned five degrees (Fig. 5). When populations overlapped several latitudes, the latitudinal group containing the centroid of the population was used. As Southern Canada and the USA were not sampled, the groups thus formed a core-edge continuum from south to north.

Genetic diversity

Scoring errors and the presence of null alleles were detected using MICROCHECKER v2.2.3 for each population (Van Oosterhout et al. 2004). I also tested markers in each dataset and population for deviations from Hardy-Weinberg Equilibrium (HWE) and for Linkage Disequilibrium (LD), using the exact probability test in Genepop v4.2 (Rousset 2008). Error rates were adjusted using a Bonferroni correction adapted to the number of markers per dataset.

Allelic richness (AR) and Nei’s unbiased expected heterozygosity (He; Nei and Roychoudhury 1974) were used as measures of genetic diversity. Both were used as allelic richness measures the number of alleles in a population standardized by sample size, and is a measure of the raw amount of variation at a locus, while expected heterozygosity accounts for both the number of alleles and the evenness of allele frequencies. I pooled populations falling into each defined core or edge group and calculated both AR and He for the pooled groups. I also calculated He and AR for individual populations for the creation of linear mixed effects models. AR was calculated using the rarefaction method implemented in FSTAT v2.9.4 (Goudet 2003), and He was obtained using Genetix v4.05.2 (Belkhir et al. 2004). Wilcoxon Mann-Whitney tests (Sokal and Rohlf 1995), with Bonferroni corrections for multiple testing adapted to each dataset, were used to test for significant differences in AR and He between groups in R v3.5.2 (R Core Team 2018). Due to the different marker sets used by different authors, significant differences in genetic diversity were only tested within datasets, and between datasets C and D at 15 shared loci. Graphs were made using R packages ggplot2 (Wickham 2019) and cowplot (Wilke 2019).

Allelic Richness models

In order to confirm and clarify the effects seen in the grouped core and edges, and to determine how the individual ungrouped populations were affected by their spatial location, I modeled allelic richness using the largest dataset, A. I made three sets of models used to test three questions arising from results of the grouped analysis. Expected heterozygosity, which was strongly correlated to AR, was not modeled.

I obtained the AR at each of the 15 markers for each population (n=23), as well as the grouping relative to proximity to water (definition 1), the distance from the centroid (definition 2, km), and the latitude (definition 3, degrees) of each population. Distance and latitude were scaled and centered for better comparison of their effect, and allelic richness was modeled using linear mixed-effects models with a Gaussian distribution.

The first set of models was created to determine if water barriers (definition 1) had an effect on mainland edge populations, or if results following definition 1 were strictly based on whether populations were on the mainland or islands. Three univariate models for allelic richness were created using package lme4 (Bates et al. 2015), with 3 levels of complexity for the fixed effect. The first only distinguished whether populations were on islands or the mainland ("position model"), the second distinguished between the four groups defined in core-edge definition one ("edge model"), and the third included island, mainland, and grouped edge and pseudo-edge into a simplified edge group ("simplified edge model"). The genetic markers and population ID were included in the models as random effects. The second set of models was made to determine if distance from the centroid (definition 2) had an effect on population level genetic diversity, and if it interacted with the mainland or island position of a population. Again, I created three models. The first modelled allelic richness as a function of distance from the centroid only. The second added the mainland-island position of a population, and the third expanded onto that with the interaction of distance and position. Unlike the geographic group models, only the genetic marker was included as a random effect. Using population ID was considered redundant, as each distance from the centroid measurement was unique to and based on the population. I made the third set of models to determine if latitude (definition 3) had an effect on population level genetic diversity beyond the exclusive presence of islands in the northern most groups, and if latitude interacted with the mainland-island position of a population. The three models were identical to the distance from the centroid models, with latitude substituted in for distance. Likewise, only the genetic marker was included as a random effect, as latitude measurements were based on the population.

Finally, I created the null model for allelic richness, with the population ID and the genetic marker as random effects. For each set of models, I selected the best model as the model with the lowest second-order Akaike Information Criterion (AICc) compared to the null model and others in its set. The contribution of each explanatory variable was tested with an analysis of deviance test. The overall best model was then chosen from all the models and the marginal effects were plotted using R package sjplot (Lüdecke 2019).

III. Results

As the A, B, and C datasets had already been cleaned by their respective authors, no scoring errors were found. A summary of the datasets can be found in Table 1.

DNA extraction for dataset D identified four wolves from 2005, five pups from 2009-2010, and the male radio-collared wolf. All 11 wolves were identified from the 2014-2017 samples. No scoring errors were detected with MICROCHECKER.

One locus in dataset B was found to have a high frequency of null alleles across all populations, and was removed. Null alleles did not appear consistently across all populations for dataset A, so all alleles were retained. No sign of null alleles was detected for the D dataset, and one locus showed signs of null alleles in the C dataset, but at a low frequency- as such, all loci were kept. Neither datasets A or B showed consistent deviations from Hardy-Weinberg Equilibrium (HWE) across all markers or populations. Likewise, no loci pair was consistently found to be in linkage disequilibrium (LD) across populations. Dataset D had no markers out of HWE, but 4 markers out of HWE were removed from dataset C. Both datasets C and D had pairs of markers in LD (C: 56 pairs, D: 1 pair) but these were retained as they are known to not be physically linked. The genetic correlation observed in these datasets was most likely due to the sampling of related individuals in a small geographic area.

Table 1. *Summary of datasets used. The C and D datasets shared 15 markers that were used to for statistical comparisons of genetic diversity.*

Dataset	**A**	**B**	**C**	**D**
Markers retained	15	13	18	22
Number of wolves (n) retained	1902	384	119	21
n of the smallest population	9	15	119	21
n of the largest population	614	198	119	21
Population(s) represented	All but B3 and F5	F3, F5, W2, B2, B3, B4	W4	A3

Genetic diversity in core/edge groups

When comparing the genetic diversity of groups based on geographic position (core-edge definition 1) in dataset A, no difference in either allelic richness (AR) or expected heterozygosity (He) was found between the three mainland groups: core, edge, and pseudo-edge (Fig. 3). Similarly, no difference was found between the core and pseudo-edge represented by dataset B: in mainland Canadian and Alaskan wolves, there is no support for the Central-Marginal Hypothesis when core and edge groups are defined by water borders.

The islands however, represented by the peripheral group, have significantly lower He than the edge (b) and lower AR than the core and edge groups (c) in the A dataset. This was also evidenced in the C and D datasets representing the edge and periphery respectively. When population are grouped, genetic diversity in the peripheral group is lower than core and edge groups, but not the pseudo-edge.

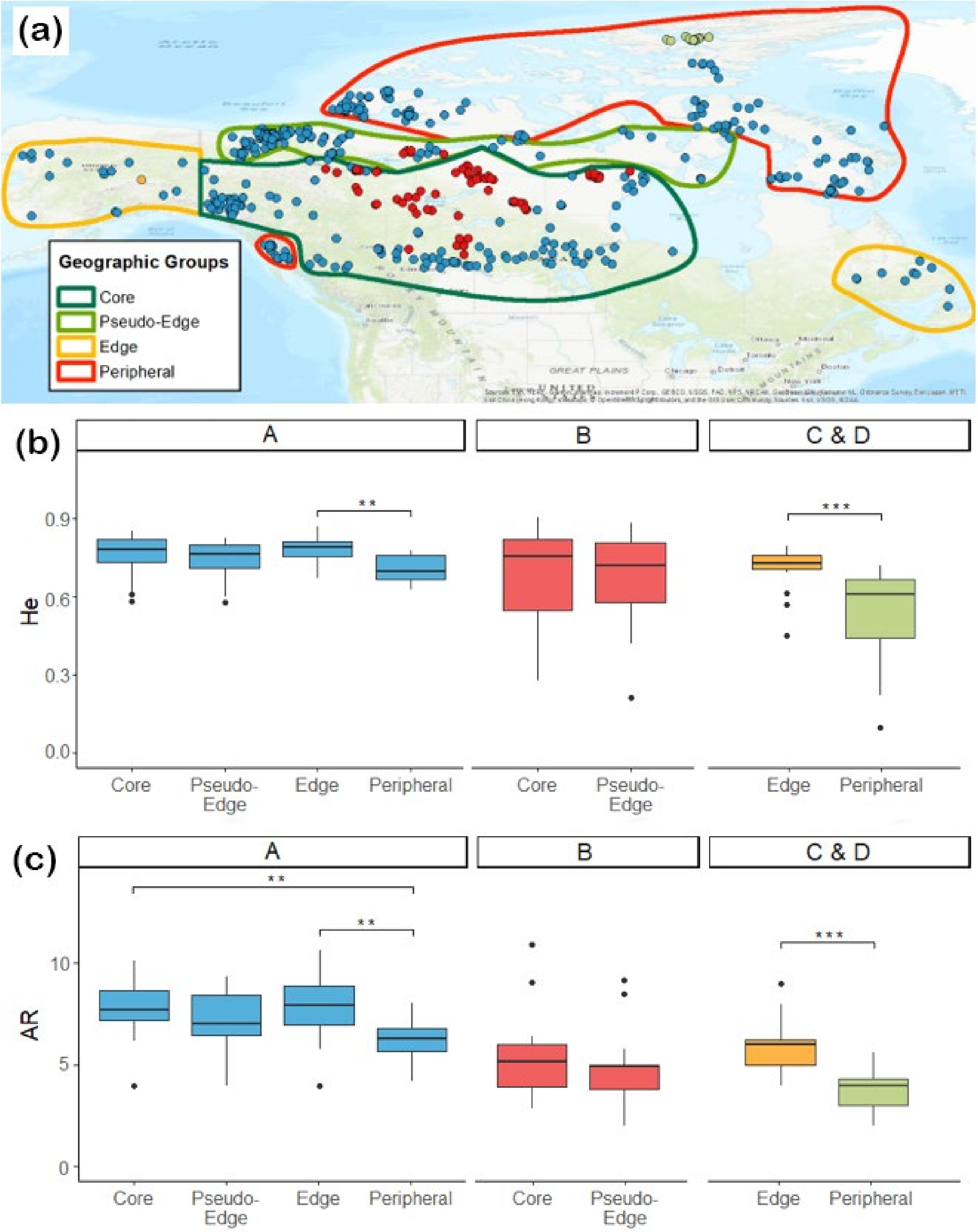


Figure 3. Core-Edge definition 1: Geographic groups based on water barriers. *(a) Distribution of Canis lupus microsatellite datasets within core-edge groups. (b) Nei's unbiased expected heterozygosity, He and (c) allelic richness, AR for core, edge, pseudo-edge, and peripheral groups (** < 0.01, *** < 0.001). Data and boxplot colors represent the datasets used (orange = C, green = D).*

Under the centroid method (definition 2), dataset A revealed no difference between mainland core and edge groups, which is not consistent with the Central-Marginal Hypothesis (Fig. 4). Analysis using dataset A also revealed that mainland core and edge groups had greater He and AR than both the island core and edge. Additional comparisons using datasets C and D support the difference between the mainland edge and island edge. Furthermore, separating islands by distance from the centroid revealed that the island group far from the centroid showed significantly lower expected heterozygosity than islands close to the centroid ($p = 0.0075$), which is consistent with the Central-Marginal Hypothesis. Allelic richness, however, did not differ.

Grouping populations by latitudinal grouping (definition 3) revealed that both measures of genetic diversity were stable across low, medium-low, and medium-high latitudinal groups, as evidenced by datasets A and B (Fig. 5). Dataset A also showed a decline in both He and AR at high latitudes, and further decline at very high latitudes. The C and D datasets supported the decline from medium-low to very high latitudes, which they represented respectively. These changes are in support of the Central-Marginal Hypothesis when considering a core-edge gradient from South to North, with high overall genetic diversity in the southern core groups, and declining diversity in high and very high latitude edge groups.

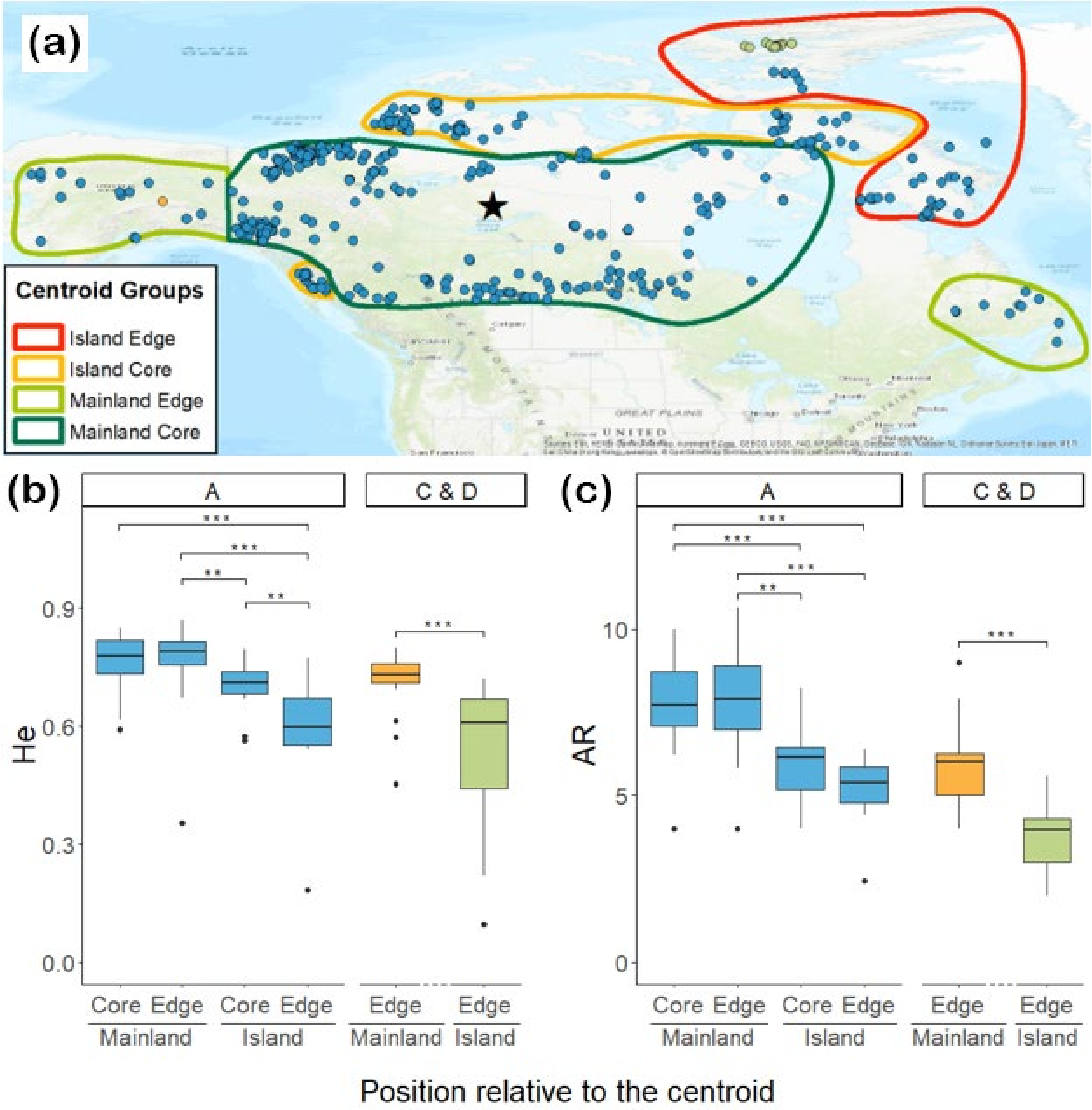


Figure 4. Core-Edge definition 2: Position relative to the centroid. *(a) Distribution of Canis lupus microsatellite datasets used within core-edge groups. (b) Expected heterozygosity, He, and (c) Allelic Richness, AR, of mainland and island groups relative to their distance from the centroid of the populations (** < 0.01, *** < 0.001). Data and boxplot colors represent the datasets used (orange = C, green = D).*

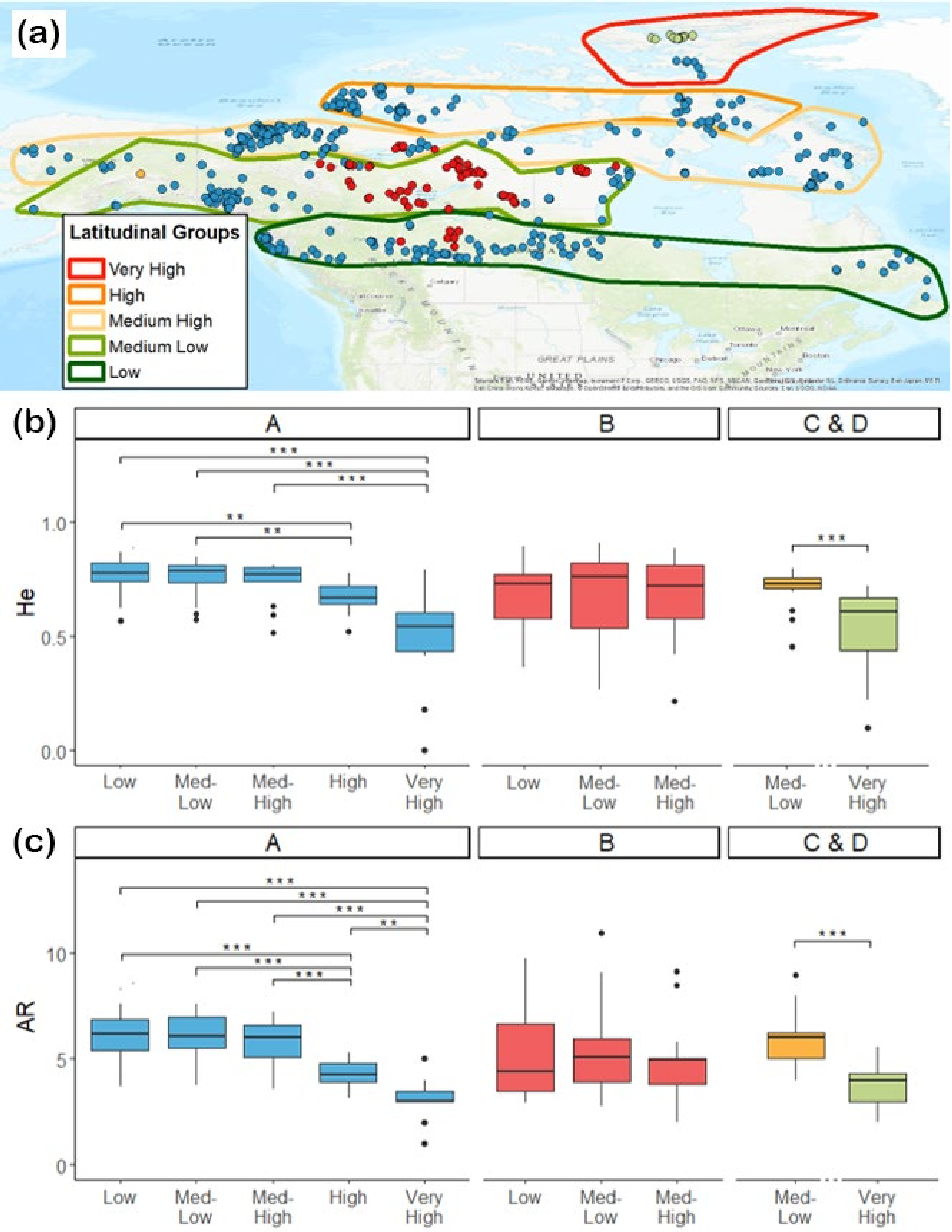


Figure 5*. *Core-Edge Definition 3: Latitudinal position. *(a) Distribution of Canis lupus microsatellite datasets within core-edge groups. (b) Expected heterozygosity, He, and (c) allelic richness, AR for latitudinal groups of wolf groups (** < 0.01, *** < 0.001). Data and boxplot colors represent the datasets used (orange = C, green = D).*

Allelic Richness Model

All three of the water-defined group (core-edge definition 1) models had lower AICc values and thus outperformed the null model. Of the three, the best models were the ones that separated the mainland core and mainland edge, though the decision to group (“simplified edge model”) or separate (“edge model”) the edge and pseudo-edge did not result in a significantly better model (Table 2).

Distance from the centroid alone, and latitude alone, could not explain trends in allelic richness: both models had higher AICc values than the null model. Adding island-mainland position to the distance model improved it significantly, though addition of the interaction between distance and position did not result in further improvement in the AICc. For models using latitude, the addition of the island-mainland position and the interaction improved the models’ AICc significantly.

Table 2. *Summary of the best models of allelic richness per hypothesis. The effect of explanatory variables (p-value results) was calculated using an analysis of deviance test. While two models for geographic position, and two models for distance from the centroid were not significantly different, only the models with lowest AICc for their category are shown.*

Model	**Random effects**	**Fixed effects retained**		**Coefficient**	**p-value**	**AICc**
Geographic position	Marker ID, Population ID	Simplified Edge	Core (intercept)	5.370	< 0.001	793.72
			Edge	-0.257		
			Peripheral	-1.592		
Distance from the centroid	Marker ID	Distance		-0.113	0.019	804.25
		Position	Mainland (intercept)	5.227	< 0.001	
			Island	-1.409		
Latitude	Marker ID	Latitude		-0.0122	0.007	804.89
		Position	Mainland (intercept)	5.238	< 0.001	
			Island	-1.296		
		Latitude : Island		-0.187	0.011	

The models with the lowest AICc overall were the models based on geographic position that distinguished between core and edge, whether or not edge and pseudo-edge were grouped (Fig. 6). In these models, mainland edge and pseudo-edge populations have slightly lower expected allelic richness than mainland core populations.

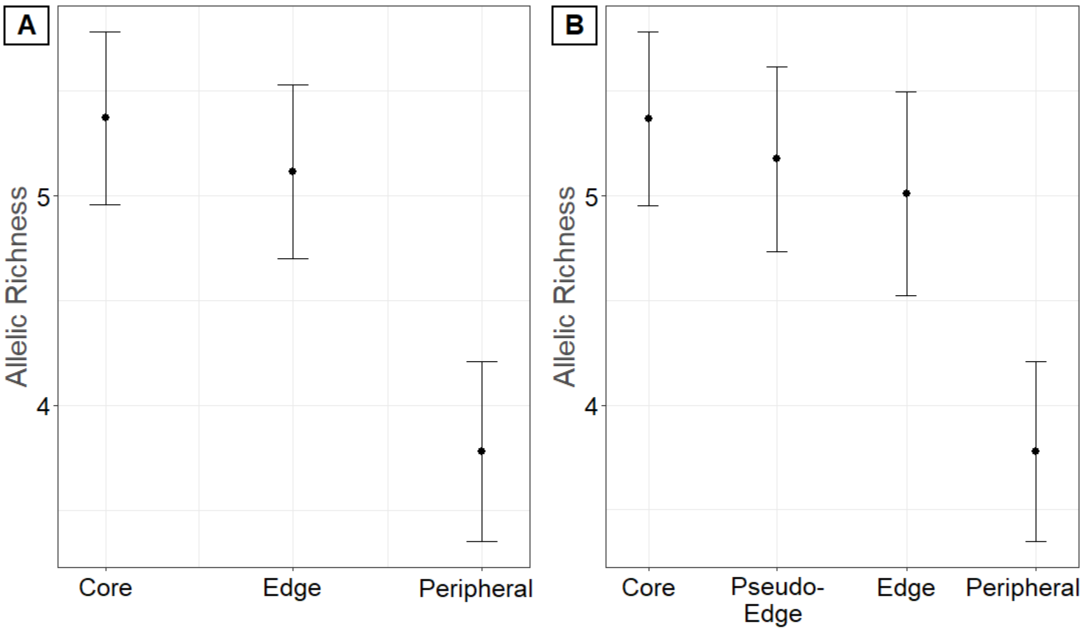


Figure 6. *Marginal effect of geographic position relative to water barriers on allelic richness, with simplified edge (A) and edge (B) positions as fixed effects. The two models had similar AICc values.*

IV. Discussion

To an extent, North American wolves appear to follow the patterns of decreasing genetic diversity at the range edge described by the Central-Marginal Hypothesis. This is most apparent when looking at islands, which, for wolves, are a type of range edge. Whether they are grouped together as the periphery using proximity to water barriers (definition 1), or separated based on distance from the centroid (definition 2), islands have consistently low genetic diversity compared to the mainland. Patterns of genetic diversity relating to the Central-Marginal Hypothesis on the mainland, however, are less clear. When populations were grouped, no core-edge difference was found with definitions 1, 2 or 3 (latitude), the latter of which had a few islands grouped into the mainland-centric groups. Models also revealed that latitude and distance alone did not exert a strong effect on allelic richness at the population level. Models based on core-edge definition 1, however, revealed that the delineation of mainland core and edge populations as a function of water barriers was stronger than the model that only differentiated between the mainland and islands. Model coefficients revealed that the mainland edge populations sampled were slightly less genetically diverse than the mainland core populations.

The genetic patterns observed on the mainland are consistent with what is known about wolf ecology. Wolves have high dispersal potential (Mech and Boitani 2003, Boitani et al. 2018), and migration between populations homogenizes genetic diversity across the landscape. Edge populations without migratory connectivity to the core and other edge populations may lose alleles due to drift at a greater rate than populations with high migratory connectivity (Lacy 1987), but despite the potential for natal-biased dispersal in wolves (Geffen et al. 2004, Carmichael et al. 2007, Schweizer et al. 2016), only a small number of migrants between populations per generation are needed to prevent the loss of neutral genetic diversity (Mills and Allendorf 1996). Because wolves can disperse long distances and over or around many known physical barriers (Mech et al. 1995, Ciucci et al. 2009), it is reasonable to expect that a few migrants per generation successfully move between populations, thus preventing a sharp drop in genetic diversity in mainland edge populations compared to the mainland core.

Additionally, the Abundant Center Model, and by extension the CMH, is based on the underlying assumption that habitat suitability declines gradually towards the edge, and that gene flow towards the edge subsequently decreases (Sagarin and Gaines 2002, Eckert et al. 2008). The lack of consistent difference in genetic diversity between core and edge groups defined by definitions 1 and 2, and the weak decrease in genetic diversity in individual edge populations based on model findings, is probably linked to the fact that the eastern and western edges of wolf range are delimited abruptly by oceans and seas rather than by a gradual decrease in habitat suitability. Wolves are habitat generalists (Mech 1970), so with the exception of heavily human modified spaces, they can potentially thrive throughout Canada and could migrate fairly easily between gradually changing habitats. Because the base assumption of decreased habitat suitability along the edge is not met, the mainland edge does not, as a group, appear to experience the decreased genetic diversity predicted based on the CMH, and only experience slight decreases in genetic diversity at the population level. The differences in results between the grouped Wilcoxon analyses and the ungrouped linear mixed models indicate that while individual populations on the edges may have slightly lower allelic richness than that of the mainland core, the mainland edge as a group contains populations with different alleles, and as such has the same genetic diversity as the core group.

Unlike the mainland, the island wolf populations show a clear decrease in genetic diversity both when grouped based on all three definitions and as individual populations, which is consistent with the findings of a previous study by Carmichael et al. (2008). As these island populations are part of the range edge, aspects of both the CMH and Island biogeography theory (IBT; MacArthur and Wilson 1967) can be used to explain the results observed.

The underlying assumption of the CMH regarding decreased habitat suitability along the edge of a species range appears to hold true for the Arctic Islands. Climatic conditions grow increasingly hostile further north, and the availability of typical cervid prey declines and is replaced by more dangerous prey, like musk oxen, or by small, fast prey, like arctic hares (Mech 2005). These conditions likely hinder successful immigration from areas with different prey types and milder climates. In turn, this decreased habitat suitability to southern wolves makes these populations more vulnerable to stochastic events, and they have in the past been subject to demographic and genetic bottleneck events (Mech 2005, Carmichael et al. 2008). Sharp habitat changes and differing prey types in the coastal islands compared to inland British Columbia appear to be the main factor limiting migration between the populations (Muñoz-Fuentes et al. 2009, Stronen et al. 2014). As with the Arctic islands, different habitat type and conditions may make the coasts and coastal islands relatively hostile to inland wolves, decreasing gene flow between the two and driving the subsequent decrease in genetic diversity.

Island biogeography theory predicts low migration rates as a result of increased distance between suitable habitats (MacArthur and Wilson 1967). Although the distance between the coastal islands and the mainland are on the order of 1 to 10 km, the lack of sea ice means that the only way for wolves to disperse is by swimming (Muñoz-Fuentes et al. 2009), which can contribute to limiting gene flow. For arctic islands, the separation between adjacent islands ranges from around 50 to over 500 km, so dispersal between islands is limited to the months when ice is present (Galley et al. 2012). Populations that are further from the mainland or from other islands, especially in the Arctic, are likely to have the lowest genetic diversity due to low migration rates, which result from the increased difficulty of dispersing long distances over an unsuitable matrix during a limited time period. The coastal islands were grouped into one population for the purpose of this study, so the effect of increasing isolation could not be tested. However, the effect of increasing isolation was observed in Arctic islands with the centroid and latitude groupings, where the more distant island populations had lower genetic diversity for at least one of the two measures.

With regards to the effect of the definition of core and edge, there was no difference in genetic diversity of core and edge groups derived from the three definitions used. Islands were consistently less diverse than the mainland, and mainland core and edge groups did not differ significantly. However, analysis of individual populations using mixed effects models revealed that neither the distance from the centroid nor the latitude alone explained more variation in genetic diversity than did the null model. As such, genetic patterns observed using the latitudinal and centroid-based groupings were most likely due to the mainland-island position (definition 1) of a population. These results highlight the importance of explicitly defining what constitutes the core and edge of a species' range, especially if the delineation is based on hypotheses regarding the species' ecology, and the need to consider how conclusions drawn are affected by the chosen delineation. Alternatively, testing multiple definitions of core and edge based on species ecology and operational definitions may help clarify what drives the resulting patterns of genetic diversity.

This new analysis focusing on how the genetic diversity of a local wolf population is affected by its position relative to the species' range edge is of conservation interest for Canadian and Alaskan wolves. On the whole, mainland edge populations do not appear to experience strongly reduced neutral genetic diversity compared to mainland core populations. Wolves are found in relatively large numbers in Alaska and the Atlantic regions of Canada, with over 7000 wolves in each region (Mech and Boitani 2003) and are relatively well connected to the core populations. Provided they remain well-connected and population numbers remain stable, they are not currently at risk of strong genetic drift and loss of genetic diversity. Island populations, on the other hand, have low neutral genetic diversity as a result of decreased dispersal activity, and changing habitat type and prey availability.

The conservation value of coastal wolves has previously been established (Muñoz-Fuentes et al. 2009) and this study supports their findings. Coastal wolves have low neutral genetic diversity, but their unique ecology and adaptations to coastal life, as well as the low immigration rates from inland populations, make them a hotspot for evolutionary changes and strong candidates for protection (Lesica and Allendorf 1995). Indeed, neutral genetic diversity does not necessarily correlate with diversity at selectively important genes (Volis et al. 2016), hence the importance of protecting islands and their evolutionary potential.

Likewise, Arctic Islands are of particular conservation value. Arctic Island wolves live in harsh conditions, and time-limited migration northwards could allow for local adaptation to these challenging conditions. This is especially true of Ellesmere wolves; as the northern limit of North American wolf range, Ellesmere wolves are likely to have selectively important genetic mutations facilitating life at the edge, allowing the species to persist at very high latitudes. Yet comparisons using three of the four datasets found a sharp decline in neutral genetic diversity between mainland wolves and Ellesmere, and to a lesser extent, between more southern arctic islands and Ellesmere, confirming past results found by Carmichael et al. (2008). Because of their low neutral genetic diversity, Ellesmere wolves are also particularly vulnerable. Indeed, past bottlenecks have decreased genetic diversity significantly (Mech 2005, Carmichael et al. 2008) and make this population particularly at risk of genetic drift and other problems associated with small populations, like inbreeding depression. Although diversity is partly maintained by ongoing migration from the south (Carmichael et al. 2008), climate change is shortening the period during which land-fast sea ice connects Arctic Islands and allows migration (Galley et al. 2012). This puts the population at increased risk of local extinction via drift in the case of a demographic bottleneck event. If the climate continues to warm, human intervention may become necessary to preserve Ellesmere and other Arctic island wolves, which, as the only large predator in their ecosystem, fill a unique ecological niche (Carmichael et al. 2008).

Limitations and future direction

The four separate datasets provided consistent, clean results, but the use of three different sets of markers hindered the ability to combine datasets, resulting in small samples in some populations. This was not a problem in the first part of the analyses with grouped populations. The models however, used individual populations from dataset A, meaning that certain populations, most often in the edge and periphery, are under-sampled and results should be interpreted with caution. Additionally, comparing datasets C and D provided consistently the same result regardless of definition, because each only span one population and could not be supplemented with other more expansive datasets sharing the same markers. This issue of differing markers hindering the understanding of predator population genetics by preventing comparison of results has been addressed before, as has the need to standardize methods and markers chosen in studies for each species (Groot et al. 2016). Standardizing methods and markers would allow for the production of continental-scale results, with the possibility of supplementing datasets over time and space (Groot et al. 2016). These measures would create stronger results and improved knowledge regarding population history and dynamics, and in turn create better educated conservation strategies.

Finally, none of the four datasets used in the study sampled southern Canada or the contiguous United States. This sampling limitation resulted in some biases in the definitions of core and edge, especially for definition 2 (distance from the centroid). It also did not allow for the examination of the southern edge of wolf range, which is of interest as the only edge that is currently expanding and not defined by water barriers. As such, future studies including the southern edge of wolf range would provide additional information regarding trends in genetic diversity in mainland edge populations and the causes for such trends.

V. Conclusion

Due to their ability to disperse long distances and over many known barriers, Canadian and Alaskan wolves experience relatively stable levels of genetic diversity across the mainland core-edge continuum, though individual edge populations appear to have slightly lower genetic diversity than core populations. The observed drop in genetic diversity on islands is likely due to increased dispersal resistance due to water barriers, habitat differences between the mainland and islands, and different prey availabilities creating unsuitable habitats for migrating mainland wolves. These unique features make these populations particularly important to wolf conservation, as wolves on these islands are adapted to local conditions and may act as potential hotspots for evolution, yet they are vulnerable to genetic drift and stochastic events as a result of limited gene flow. This is especially true of the High Arctic regions, as warming temperatures may continue to decrease the time available for migration over sea ice and prevent rescue by immigrants from the mainland. Despite the wolf's adaptability and ability to disperse long distances, human intervention may one day become necessary in order to preserve these populations living at the edge.

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VII. Supplementary Material

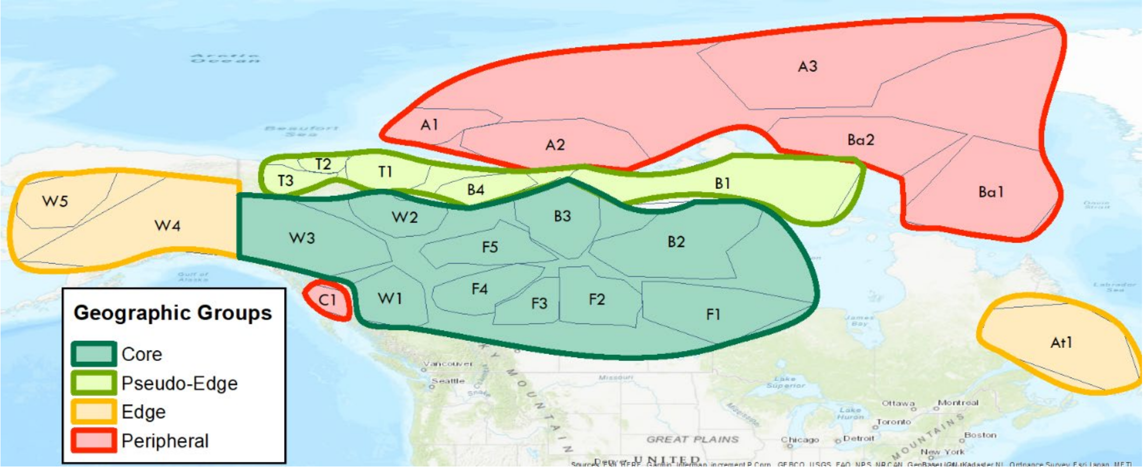
Population and core-edge group categorization

Annex 1. *Summary of criteria used to delineate each population.*

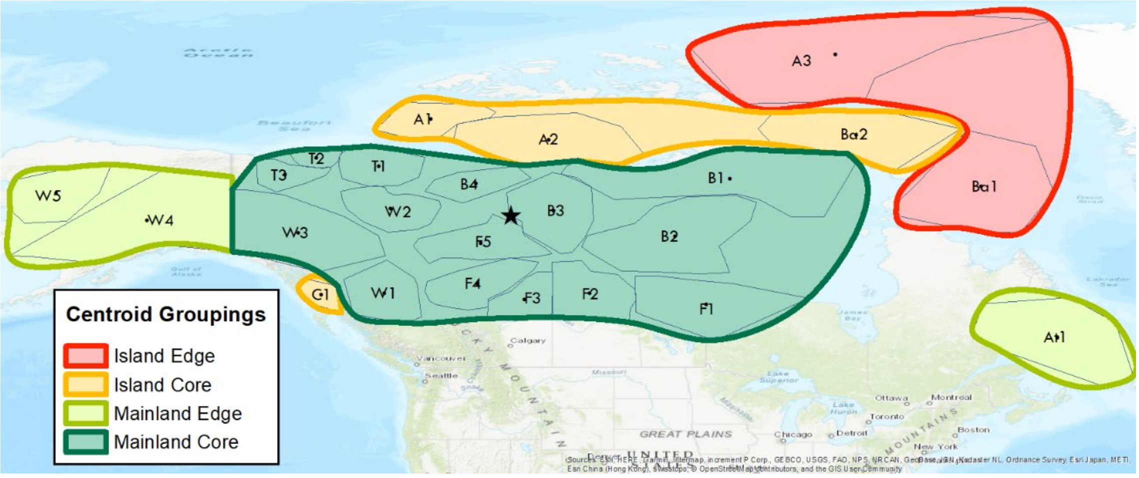
Population	Land cover	Genetic cluster	Water barriers	Caribou migration patterns	Ecotypes	Distance	Country & Province
A1		x	x				
A2		x	x				
A3		x	x				
At1		x				x	
B1		x		x			
B2	x	x		x			
B3	x	x		x			
B4	x	x	x	x			
Ba1		x	x			x	
Ba2		x	x			x	
C1		x	x		x		
F1	x	x				x	x
F2	x	x				x	x
F3		x				x	x
F4		x				x	
F5	x	x				x	
T1	x	x					
T2	x	x	x				
T3	x	x	x				
W1		x					
W2	x	x	x				
W3	x	x				x	x
W4	x	x				x	x
W5	x	x			x		

Annex 2. *Population assignment to core and edge groups.*

(A) *Core-edge definition 1: Grouping relative to proximity to water barriers.*

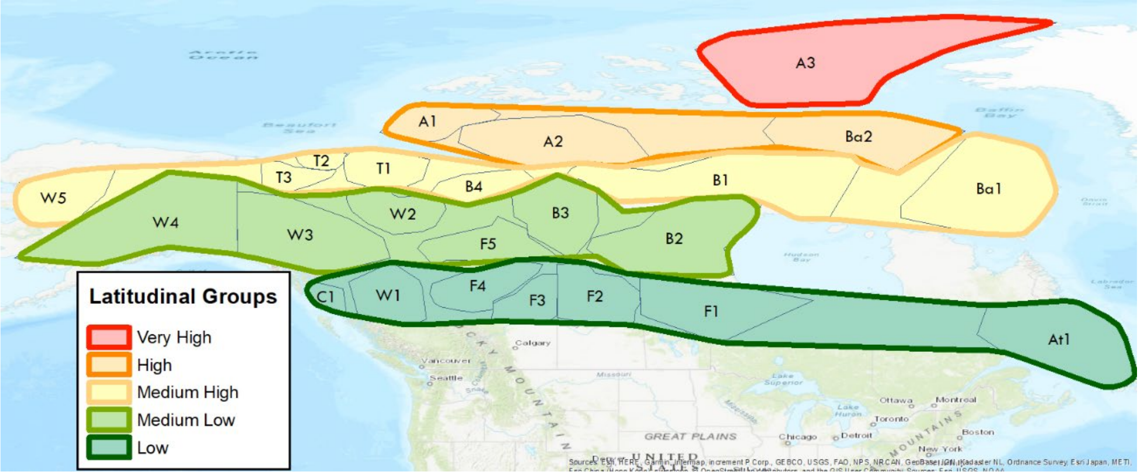


(B) *Core-edge definition 2: Grouping relative to distance from the overall centroid (star) and mainland-island position.*



Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community

(C) *Core-edge definition 3: Grouping relative to latitude.*



Sample sizes

***Annex 3**. Genetic cluster position and sample size (number of wolves) for each population.*

Cluster name (Carmichael et al. 2008)	Modified cluster came and code	Population	Dataset A	Dataset B	Dataset C	Dataset D
Arctic Island	**Arctic Island (A)**	**A1**	163			
		A2	52			
		A3	11			21
Atlantic	**Atlantic (At)**	**At1**	20			
Eastern Barrens	**Barrens (B)**	**B1**	47			
		B2	614	198		
		B3		93		
		B4	13	15		
Baffin Island	**Baffin Island (Ba)**	**Ba1**	73			
		Ba2	43			
Coastal	**Coastal (C)**	**C1**	35			
Forest	**Forest (F)**	**F1**	69			
		F2	143			
		F3	41	28		
		F4	66			
		F5		27		
Western Barrens	**Tundra (T)**	**T1**	80			
		T2	137			
		T3	82			
Western Woods	**Woods (W)**	**W1**	17			
		W2	39	23		
		W3	135			
		W4	13		119	
		W5	9			
		Total	1902	384	119	21

***Annex 4.** Sample size of the datasets in each group based on proximity to water.*

	A	B	C	D
Core	1124	369		
Pseudo	359	15		
Edge	42		119	
Peripheral	377			21
Total	1902	384	119	21

Annex 5. *Sample size of the datasets for groups based on distance from the centroid.*

		A	C	D
Mainland	**Core**	1535		
	Edge	42	119	
Island	**Core**	198		
	Edge	127		21
Total		1902	119	21

Annex 6. *Sample size of the datasets for groups based on latitude.*

	A	B	C	D
Low	391	28		
Med-Low	801	341		
Med-High	441	15	119	
High	258			
Very High	11			21
Total	1902	384	119	21

P-values

Annex 7. *P-values for comparisons of water-defined groups for dataset A (Bonferroni adjusted alpha = 0.0083). Top values are comparisons of allelic richness, bottom values are comparisons of expected heterozygosity.*

	Core	Pseudo-Edge	Edge	Peripheral
Core		0.468	0.967	0.002
Pseudo-Edge	0.513		0.481	0.023
Edge	0.740	0.384		0.003
Peripheral	0.041	0.068	0.008	

Annex 8. *P-values for comparisons of water-defined groups for dataset B (alpha = 0.05). Top values are for comparisons of allelic richness, bottom values are for expected heterozygosity.*

	Core	Pseudo-Edge
Core		0.448
Pseudo-Edge	0.879	

Annex 9. *P-values for comparisons of core and edge groups relative to the centroid for dataset A (Bonferroni adjusted alpha = 0.0083). Top values are for comparisons of allelic richness, bottom values are for expected heterozygosity.*

		Mainland		Island	
		Core	Edge	Core	Edge
Mainland	**Core**		0.901	0.001	< 0.001
	Edge	0.678		0.002	< 0.001
Island	**Core**	0.016	0.007		0.056
	Edge	< 0.001	< 0.001	0.008	

Annex 10. *P-values for comparisons of latitudinal groups for dataset A (Bonferroni adjusted alpha = 0.005). Top values are for comparisons of allelic richness, bottom values are for expected heterozygosity.*

	Low	Med-Low	Med-High	High	Very High
Low		1	0.461	< 0.001	< 0.001
Med-Low	0.775		0.513	< 0.001	< 0.001
Med-High	0.436	0.567		< 0.001	< 0.001
High	0.005	0.005	0.006		0.004
Very High	< 0.001	< 0.001	0.001	0.013	

Annex 11. *P-values for comparisons of latitudinal groups for dataset B (Bonferroni adjusted alpha = 0.0167). Top values are for comparisons of allelic richness, bottom values are for expected heterozygosity.*

	Low	Med-Low	Med-High
Low		0.579	0.920
Med-Low	0.801		0.448
Med-High	0.960	0.801	

Annex 12. *P-values for the comparison of genetic diversity at 15 loci shared by datasets C and D, for all three definitions (alpha= 0.05).*

	p-value
Allelic richness (AR)	< 0.001
Expected heterozygosity (He)	< 0.001

VIII. Abstract

According to the Central-Marginal Hypothesis, declining habitat suitability and population size is expected to decrease genetic diversity towards the species' range edge. However, North American grey wolves (*Canis lupus*) are adept dispersers and occupy a nearly continental-scale range, which may allow their genetic diversity to remain stable throughout the range. I used four microsatellite datasets from Canada and Alaska to investigate whether patterns of genetic diversity in a species with a continental-scale range and high dispersal potential vary along the core-edge range continuum, and how the methods used to delineate core and edge may affect the results. I delineated core and edge groups using three definitions: proximity to water barriers, distance from the range centroid, and latitude, and compared the allelic richness and expected heterozygosity between these groups. Linear mixed-effects models of allelic richness were built for each definition and compared to determine which definition best explained patterns of genetic diversity at the population level. Results for all datasets showed that islands consistently had lower genetic diversity than the mainland. The mainland edge as a group had similar genetic diversity to the core group for all definitions, but models showed that individual edge populations had slightly lower allelic richness than core populations when defining the edge based on proximity to water. The four datasets used did not extend into southern Canada and the USA, so future studies on the south of the species' range, which appears to be expanding and is not currently defined by water, would determine what factors other than proximity to water explain genetic patterns on the mainland.

IX. Résumé

Selon l'Hypothèse Centrale-Marginale, la diminution de la qualité de l'habitat et de la taille de la population devrait entraîner une baisse de la diversité génétique vers le bord de l'aire de répartition d'une espèce. Cependant, les loups (*Canis lupus*) sont de grands migrateurs et occupent un territoire qui couvre une grande partie du continent Nord-Américain, ce qui pourrait leur permettre de maintenir une diversité génétique stable sur l'étendue du territoire. J'ai utilisé quatre jeux de données microsatellites provenant du Canada et de l'Alaska pour déterminer si la diversité génétique de cette espèce avec un territoire étendu et un potentiel de dispersion élevé varie le long du continuum du centre au bord de l'aire de répartition. J'ai aussi étudié l'effet des méthodes utilisées pour délimiter le centre et le bord sur les résultats. J'ai délimité le centre et le bord selon trois définitions: la proximité de l'eau, la distance entre le centroïde et le bord, et la latitude ; puis j'ai comparé la richesse allélique et l'hétérozygotie attendue entre ces groupes. Des modèles linéaires à effets mixtes ont été construits pour la richesse allélique avec chaque définition et utilisant les populations individuelles. J'ai comparé les modèles pour déterminer quelle définition explique le mieux les tendances de diversité génétique. Les résultats sur tous les jeux de données et définitions montrent que les îles présentent systématiquement une diversité génétique plus faible que le continent. Quand les populations du bord du continent sont rassemblées en un seul groupe, celui-ci à une diversité génétique égale à celle du groupe centre pour toutes les définitions. Toutefois les modèles montrent que les populations individuelles du bord ont une richesse allélique légèrement inférieure à celle des populations centrales lorsque la définition est basée sur la proximité de l'eau. Les quatre jeux de données utilisés ne s'étendent pas vers les États-Unis et le sud du Canada, donc de futures études sur le bord sud de l'aire de répartition, qui semble s'élargir et n'est pas limité par l'eau, détermineraient quels facteurs autres que la proximité de l'eau pourraient expliquer les tendances génétiques sur le continent.