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Climate and habitat barriers to dispersal in the highly mobile grey wolf

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Abstract

We reanalysed published data to evaluate whether climate and habitat are barriers to dispersal in one of the most mobile and widely distributed mammals, the grey wolf (*Canis lupus*). Distance-based redundancy analysis (dbRDA) was used to examine the amount of variation in genetic distances that could be explained by an array of environmental factors, including geographical distance. Patterns in genetic variation were also examined using MDS plots among populations and relationships between genetic structure and individual environmental variables were further explored using the BIOENV procedure. We found that, contrary to a previous report, a pattern of isolation with distance is evident on a continental scale in the North American wolf population. This pattern is apparently related to climate and habitat. Specifically, vegetation types appear to play a role in the genetic dissimilarities among populations. When we controlled for the effect of spatial variation, climate was still associated with genetic distance. Further, partitioning of geographical distances into latitudinal and longitudinal axes revealed that the east–west gradient had the strongest relationship with genetic distance. We suggest two possible mechanisms by which environmental conditions may influence the dispersal decisions made by wolves.

Keywords: climate, cline, dispersal, grey wolf, habitat, isolation-by-distance

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Introduction

One of the most vagile predators is the grey wolf. Dispersal distances of several hundred kilometres are common, and movements over 1000 km have been documented (Fritts 1983; Mech *et al*. 1995). The historical range of the grey wolf is the largest of any extant terrestrial mammal and spans most of the Holarctic region. Grey wolves occur in a variety of habitats, from dense forest to open grassland and from the Arctic tundra to extreme desert, avoiding only swamps and rain forests. Long dispersal distances and the ability to thrive in a wide range of habitats suggest that, on a continental scale, geographical distance or habitat distribution should not explain genetic differences among wolf populations. Indeed, previous studies on North American and Eurasian grey wolves showed no pattern of genetic isolation with geographical distance within continental populations (Roy *et al*. 1994; Vilà *et al*. 1999). However, regional studies have demonstrated a significant isolation-by-distance among neighbouring populations (Forbes & Boyd 1997; Carmichael *et al*. 2001). Further, Carmichael *et al*. (2001) showed that a large river, the Mackenzie River in Northwest Territories, Canada, is a barrier to gene flow and suggested that north–south migration of caribou (*Rangifer tarandus*) flanking the river is the driving force governing wolf movements. With the exception of island populations or those recently isolated by habitat loss (Wayne *et al*. 1991; Wayne *et al*. 1992), this was the first study to show that topographical barriers influence genetic structure of wolf populations.

Although the influence of landscape features on population differentiation has long been recognized (Fisher & Ford 1947), only a subset of studies consider it explicitly as an explanatory variable for observed genetic patterns (e.g. Keyghobadi *et al*. 1999; Bockelmann *et al*. 2003). In species that are habitat-restricted or that are habitat-specialists, landscape is expected to have a significant effect on the genetic structure of populations because gaps between suitable habitat patches would serve as barriers to dispersal.

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Similarly, low mobility can also be caused by landscape because some habitats are more difficult to cross. By contrast, we would anticipate that landscape has little effect on population subdivision in highly mobile and cosmopolitan species. In this sense, grey wolves are a model species for testing the prediction that population genetic structure is independent of landscape features. However, the finding that genetic differentiation-by-distance occurs over regional scales in wolf populations, and that it varies according to prey type, suggests subtle patterns of habitat association that may have been missed in previous studies. Here, we use published genetic and ecological data for an array of grey wolf populations across a diversity of habitats to re-examine the potential underlying causes of genetic differentiation in this highly vagile carnivore.

Methods

We used published allele frequency data from studies that examined variation in microsatellite loci and mtDNA restriction fragment length polymorphism (RFLP) profiles among wolf populations (Wayne *et al*. 1992; Roy *et al*. 1994; Forbes & Boyd 1997). Nine microsatellite loci for 11 populations and RFLP profiles based on 21 restriction enzymes for 15 populations were used in these studies. Populations sampled using both RFLP and microsatellites include sites in the contiguous United States and Alaska (Montana, Minnesota, Kenai and Denali; Fig. 1) and in Canada (Vancouver Island and Inuvik; Fig. 1). Other Canadian populations sampled only at microsatellite loci include southern Quebec, northern Quebec, Banff, Hinton and Fort St John (Fig. 1), Finally, populations sampled only at mtDNA RFLP loci included a few populations from Alaska [Nome and Anaktuvik Pass (Fig. 1)] and Canada [central Ontario, western Ontario, Manitoba, Alberta (51°17′ N, 116°58′ W), Yellowknife and Yukon (Fig. 1)]. These localities represent a substantial portion of the current distribution of grey wolves in North America (Fig. 1). Data from reintroduced populations were not included in this analysis.

Our goal was to examine whether habitat-related variables explained some of the genetic variation observed among wolf populations and, if so, to see the extent to which this

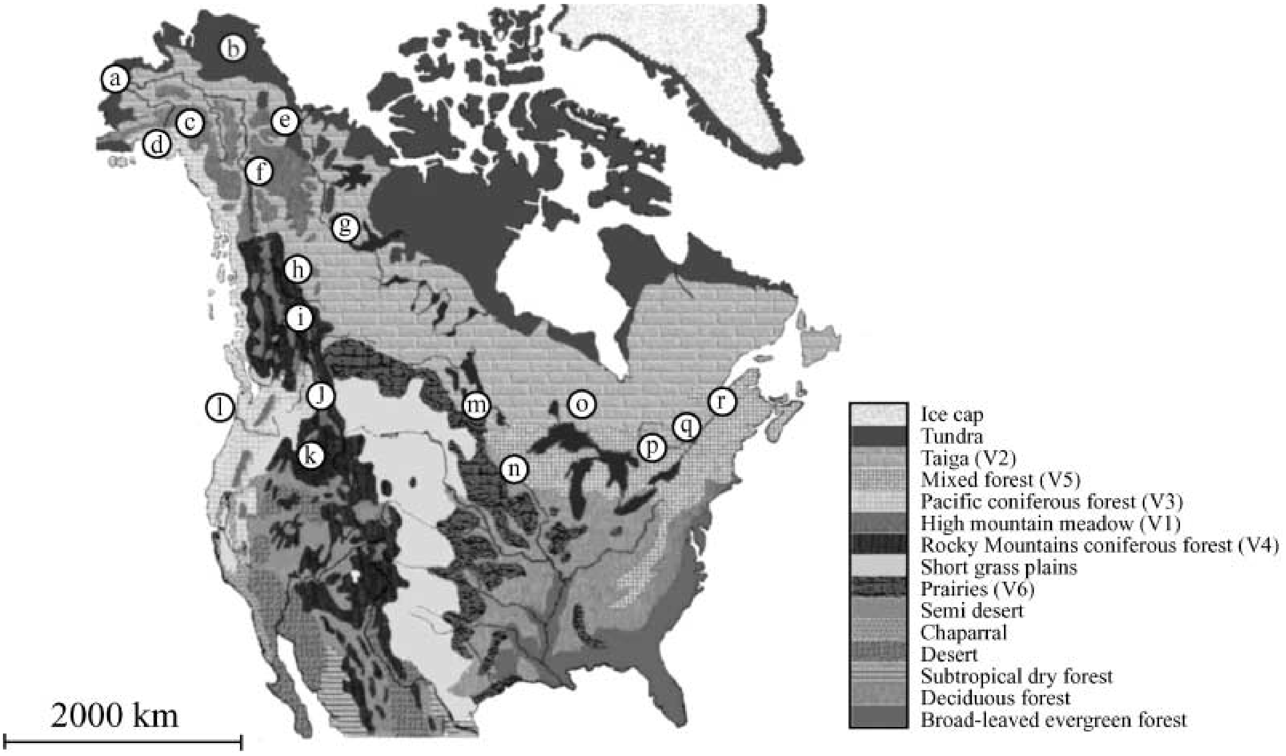


Fig. 1 Map of the main vegetation types in North America, with key to vegetation types presented on the right. Sampling locations for 18 wolf populations are indicated on the map by lowercase letters: a: Nome (64°30′ N, 165°24′ W); b: Anaktuvik Pass (68°10′ N, 149°30′ W); c: Denali (63°43′ N, 148°57′ W); d: Kenai (59°55′ N, 149°58′ W); e: Inuvik (68°35′ N, 133°70′ W); f: Yukon (NWT) (68°10′ N, 137°00′ W); g: Yellowknife (62°27′ N, 114°20 W); h: Fort St John (56°15′ N, 120°49′ W); i: Hinton (53°23′ N, 117°34′ W); j: Banff (51°10′ N, 115°34′ W); k: Montana (48°11′ N, 114°18′ W); l: Vancouver Island (49°40′ N, 125°49′ W); m: Manitoba (51°08′ N, 100°02′ W); n: Minnesota (47°46′ N, 91°58′ W); o: western Ontario (50°17′ N, 89°01′ W); p: central Ontario (45°32′ N, 78°35′ W); q: southern Quebec (47°00′ N, 076°50′ W); r: northern Quebec (47°58′N, 071°25′W). The map was modified from Knight (1968). A coloured version of this figure is available online at http://www.blackwellpublishers.com/xxx.

could be detected over and above the spatial variation in genetic dissimilarities due simply to geographical distances among sites (e.g. Borcard *et al*. 1992). To do this, we performed a partitioning of genetic distance matrices using distance-based redundancy analysis (dbRDA, Legendre & Anderson 1999; McArdle & Anderson 2001). This is a form of multivariate multiple regression which can be performed directly on a distance or dissimilarity response matrix of choice. Although partial Mantel tests (Smouse *et al*. 1986) have been used to perform partial regression analyses for genetic distances (e.g. Malhotra & Thorpe 2000), there has been recent debate concerning the validity of such an approach for different situations (e.g. Legendre 2000; Raufaste & Rousset 2001; Castellano & Balletto 2002; Rousset 2002). The problem arises due to the lack of independence of individual distances in a distance matrix. Although a simple Mantel test overcomes this issue by the use of permutations, a permutational approach does not necessarily solve problems introduced by several uncontrolled nuisance parameters in the case of more than one regressor (i.e. partial tests). Thus, we do not use a Mantel approach here, but rather use the distance-based multivariate approach of McArdle & Anderson (2001). The important point is that, for dbRDA, the individual distances are not treated as a single univariate response variable, as in the Mantel test, but rather the individual sites are the units of observation for analysis, about which we have calculated distances using an entire set of genetic variables. The distance matrix is therefore treated as information regarding multivariate response data. Taking this multivariate approach avoids the problems associated with the partial Mantel test.

We calculated two traditional measures of genetic variation, F_{ST} and Nei's unbiased genetic distance ($D_{(Nei)}$) using FSTAT (version 2.9.3; Goudet 1995) and ARLEQUIN (version 2000; Schneider *et al*. 2000), respectively. Predictor variables used for the analysis were considered separately in seven different groups or sets (Table 1). First, latitude and longitude were considered together as predictors describing geographical distances among sites. Three continuous variables described different aspects of temperature at each site (annual maximum temperature, average annual temperature and annual minimum temperature). Another quantitative variable was the average annual rainfall at each site. Clearly, average minimum and maximum temperatures are likely to be highly related variables. We decided to include both because it is unclear which may be more important. We expect annual minimum temperature to be a better predictor of movements between populations if cold-adapted wolves are intolerant of warmer regions. Similarly, desert-adapted wolves may not move into cool or moist areas, making annual maximum temperature or rainfall a good predictor of the potential for dispersal. The remaining environmental characteristics were categorical, including habitat type, the presence of a water barrier, climate and vegetation. Habitat type had two states: zero for open habitat (e.g. tundra) and one for closed habitat (e.g. forest). Similarly, the variable indicating the presence of a water barrier took the value of 1 for Vancouver and 0 for all other sites. Finally, we used climate and vegetation maps

Table 1 Sets of predictor variables used in statistical analyses

Set	Variables in the set
Distance	Latitude Longitude
Temperature	Maximum annual temperature Minimum annual temperature Average annual temperature
Rainfall	Mean annual rainfall
Habitat type	Indicator variable identifying whether the site is located in open (e.g. grassland) or closed (e.g. forest) habitat
Water barrier	Indicator variable identifying whether the site is an island (Vancouver Island only)
Climate	Categorical indicator variables for seven different climate regimes *Microsatellite data*: C1 — marine cool temperate, C2 — extreme continental warm temperate, C3 — transitional warm temperate, C4 — continental warm temperate, C5 — subarctic polar, C6 — continental cool temperate, and C7 — extreme continental cool temperate *RFLP profile*: C1 — continental warm temperate, C2 — extreme continental warm temperate, C3 — dry extreme continental warm temperate, C4 — marine cool temperate, C5 — continental cool temperate, C6 — extreme continental cool temperate, and C7 — subarctic polar
Vegetation	Categorical indicator variables for 6 different vegetation zones: V1 — high mountain meadows and scrubs, V2 — boreal coniferous forest (Taiga), V3 — Pacific coniferous forest, V4 — Rocky Mountains coniferous forest, V5 — mixed forest, and V6 — tall grass with groups of trees (prairie)

to assign wolf populations to habitat zones (Knight 1968; NCDC, TD 9641 Clim 81, 1961–1990 Normals). There were seven climate categories and six vegetation categories (Table 1). It is important to note that, for several of the categorical variables, only one population was indicated (e.g. the presence of a water barrier occurred only for Vancouver). Thus, due to the lack of replication, it is not possible to extend inferences to specific climate or vegetation characteristics *per se*. That is, for example, if the Vancouver population appears to be quite different from the other populations genetically, this cannot be used to infer that water barriers in general are necessarily the cause for differences observed.

There were three response matrices of interest: (1) F_{ST} distances obtained using microsatellite data (11 sites), (2) Nei's unbiased distances obtained using microsatellite data (also 11 sites) and (3) F_{ST} distances obtained using RFLP profiles (15 sites). Each of these response matrices were analysed as follows. First, the relationship between the response matrix and each set of predictor variables (shown in Table 1) was analysed separately (marginal tests) using dbRDA. For these analyses, *P*-values were obtained using 9999 unrestricted but simultaneous permutations of the rows and columns of the distance matrix. Second, partial dbRDA was performed for each of the sets of predictor variables, having first fitted latitude and longitude (i.e. geographical distance) as covariables in the analysis. This examines the extent to which any of the sets of predictor variables explains significant genetic variability among the sites over and above that explained by geographical distance alone. Although previous authors have suggested that this approach of 'partialling out' the spatial component of the variation should include more than just the latitude and longitude (i.e. they generally include a polynomial of these variables up to order 3, Borcard *et al*. 1992), we did not wish to make the spatial model overly complex. This was because we had only hypotheses about interpoint geographical distances among sites and also because fitting such complex models is not feasible or reasonable when the number of observations is so limited (i.e. our response data sets had only 11 or 15 observation sites). The *P*-values for partial tests were obtained using 9999 permutations of the rows and columns of the multivariate residual matrix under the reduced model (e.g. Freedman & Lane 1983; Anderson & Legendre 1999). All dbRDA analyses were performed using the program DISTLM (Anderson 2003). Due to the small number of observations for all of these statistical analyses, we balanced the possible lack of power to detect effects by interpreting any *P*-values less than 0.10 as providing some evidence against the null hypothesis.

To visualize patterns in the relationships among the sites in terms of the genetic variation of wolf populations, we used nonmetric multidimensional scaling on each of the genetic distance matrices (MDS, Kruskal & Wish 1978). The relative distances among the sites in these ordination plots indicate the relative genetic distances among wolf populations among the sites, with plots having a stress value less than 0.20 providing interpretable information concerning intersite relationships (e.g. Clarke 1993).

We were also interested to examine which subset of individual predictor variables may provide the best model of differences in genetic structure among populations. To do this, we used the BIOENV procedure of Clarke & Ainsworth (1993). Although this approach was proposed originally to relate multivariate ecological species abundance data to environmental variables, it is an appropriate nonparametric procedure in the present context as well. The basic idea is to calculate the value of Spearman's rank correlation coefficient (ρ) between the distances in the response distance matrix (in our case, a matrix of genetic distances) with a distance matrix calculated as the Euclidean distance among one or more predictor variables. Thus, the BIOENV statistic ρ is akin to a nonparametric version of a simple Mantel correlation between two distance matrices: one based on genetic distance (D_G) and one based on a set of predictor variables (D_P). The BIOENV procedure calculates the value of ρ using every possible combination of predictor variables until it finds the 'best' fit (i.e. that combination of predictor variables whose Euclidean distance matrix D_P yields the highest value of ρ). Obviously, the number of calculations required here becomes very large with increases in the number of variables to be fitted. Thus, one can limit the procedure by examining the best one-variable fit, the best two-variable fit, and so on. We implemented the BIOENV procedure and identified, for each of the three response matrices, the best one, two, three, four and five-variable fits. Note that the value of ρ (unlike R^2 in multiple regression) does not necessarily increase with increases in the number of predictor variables. Note also that it is not valid to then perform a permutation test (as might be employed for a simple Mantel test) on the resulting fits, as the fact that a selection criterion was used to choose the environmental variables contributing to D_P will increase the type I error of a simple test for relationship. Thus, the results of the BIOENV are indicative of sets of variables which may potentially be most useful in distinguishing populations on the basis of their genetic structure. As pointed out by Clarke & Ainsworth (1993), BIOENV is best thought of as an exploratory procedure. All MDS plots and BIOENV analyses were performed using the PRIMER computer package (Clarke & Gorley 2001).

We were concerned that high weight that may be given to the east–west axis (longitude) may reflect the presence of hybridized wolves in northeastern North America (Lehman *et al*. 1991; Roy *et al*. 1994; Wilson *et al*. 2000). Thus, we re-analysed each data set using the BIOENV procedure but where sites from Minnesota, Ontario and Quebec were excluded from the analysis. We also examined the relationship of each dissimilarity matrix with the variable of longitude alone (using DISTLM with 9999 permutations) after removing these sites.

Results

We detected significant isolation with distance for all three data sets: the results were clearest for F_{ST} using RFLP profiles ($P = 0.0094$), whereas evidence was less strong using either F_{ST} from microsatellite data ($P = 0.0878$) or Nei's genetic distance ($P = 0.0603$) (Table 2). The other set of variables that had a significant relationship with genetic distance in all three cases was that for vegetation (Table 2). Thus, vegetation types appear to play a role in the genetic dissimilarities among populations. In the case of F_{ST} based on RFLP profiles, there was also a significant relationship detected for climate variables ($P = 0.0429$) and habitat type ($P = 0.0437$).

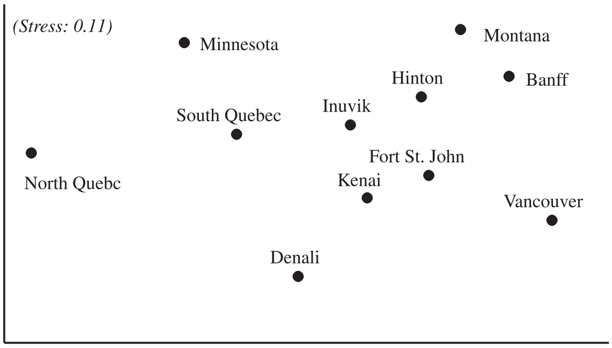
When the spatial variation was taken into account (by fitting latitude and longitude as covariables in the analysis), a significant relationship between genetic variation and climate was still detected in the case of Nei's distance ($P = 0.0921$) or F_{ST} based on RFLP profiles ($P = 0.0842$), but not for F_{ST} from microsatellite data ($P = 0.1552$) (Table 2). None of the other sets of environmental variables showed a statistically significant relationship with genetic structure, whether considered alone or after fitting latitude and longitude as covariables (Table 2). In some cases, the values of multivariate *F*-statistics were relatively large for conditional tests, but *P*-values did not result in rejection of the null hypothesis. This was due essentially to a lack of power with such small sample sizes (i.e. either 11 or 15 populations), so clearly analyses based on greater numbers of wolf populations would be desirable, if possible, in future studies.

The BIOENV procedure found that longitude, a water barrier and several climate and vegetation indicators together had the strongest relationship with rank genetic distance (Table 3). The procedure also indicated that the strongest relationship between genetic structure and any single variable was longitude in the case of either F_{ST} on RFLP profiles or Nei's genetic distance. A strong east–west gradient was also evident in MDS plots (Fig. 2b–c), where

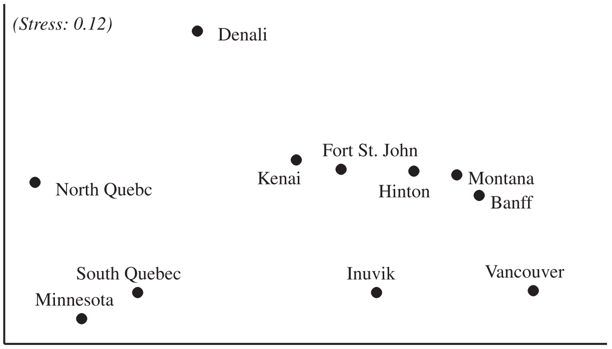
Table 2 Tests for relationships between the genetic structure of wolf populations at different sites and several individual sets of predictor variables, using the dbRDA multivariate *F*-statistic. On the left are the marginal tests of individual sets, on the right are the partial (conditional) tests, where the variables of latitude and longitude have been included as covariables in each analysis. *P*-values less than 0.10 are highlighted in bold. The column headed '%var' indicates the percentage of the multivariate genetic variation explained by the particular set of predictor variables

Marginal tests				Conditional tests			
Variable set	*F*	*P*	%var	Variable set	*F*	*P*	%var
F_{ST} (microsatellite)							
Distance	1.822	**0.0878**	31.29				
Temperature	0.794	0.6795	25.39	Temperature	1.169	0.3969	28.32
Rainfall	1.136	0.3679	11.20	Rainfall	2.282	0.1459	16.89
Habitat type	0.683	0.6625	7.05	Habitat type	0.555	0.5672	5.04
Water barrier	1.669	0.1789	15.65	Water barrier	1.872	0.1841	14.50
Climate	1.594	0.1953	78.81	Climate	6.553	0.1552	67.24
Vegetation	2.393	**0.0168**	70.53	Vegetation	1.770	0.2775	51.31
Nei's distance							
Distance	2.221	**0.0603**	35.71				
Temperature	0.706	0.7208	23.24	Temperature	1.103	0.4282	25.60
Rainfall	1.037	0.4171	10.33	Rainfall	1.992	0.1670	14.24
Habitat type	0.838	0.5268	8.52	Habitat type	0.527	0.5806	4.50
Water barrier	1.687	0.3721	15.79	Water barrier	1.674	0.2225	12.41
Climate	1.454	0.2241	77.23	Climate	11.451	**0.0921**	63.50
Vegetation	3.597	**0.0047**	78.25	Vegetation	3.011	0.1196	53.61
F_{ST} (RFLP)							
Distance	4.929	**0.0094**	45.10				
Temperature	0.793	0.6117	17.77	Temperature	0.984	0.4431	13.56
Rainfall	0.324	0.7160	2.43	Rainfall	0.765	0.4052	3.57
Habitat type	3.783	**0.0437**	22.54	Habitat type	2.145	0.1713	8.96
Water barrier	1.394	0.3329	9.68	Water barrier	1.411	0.2730	6.24
Climate	2.725	**0.0429**	73.16	Climate	3.625	**0.0842**	45.86
Vegetation	4.112	**0.0116**	75.51	Vegetation	1.684	0.2647	34.44

(a) F_{ST} (microsatellites)



(b) Nei's distance



(c) F_{ST} (RFLP)

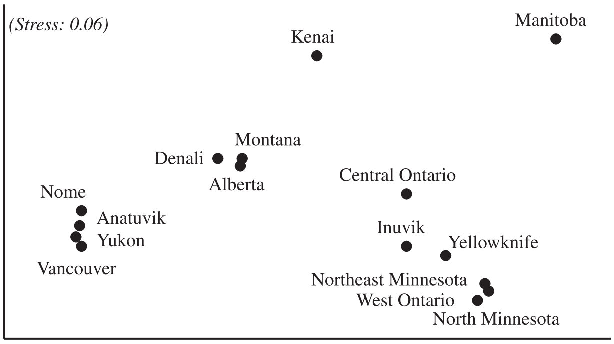


Fig. 2 Non-metric MDS plots of wolf populations at different locations on the basis of genetic distances using (a) F_{ST} from microsatellite data, (b) unbiased Nei's genetic distance or (c) F_{ST} from RFLP profiles. Stress values are indicated.

eastern populations (e.g. Quebec, Minnesota or Western Ontario) were clearly separated from western populations (e.g. Vancouver, Banff, Nome, etc.). In contrast, there was no clear evidence of any strong north–south gradient. For example, the populations of Yukon and Vancouver are quite closely associated with one another genetically according to the F_{ST} measures on RFLP profiles (Fig. 2c), and latitude was never chosen by the BIOENV procedure (Table 3).

When considering the F_{ST} measures on microsatellite data, several climate and vegetation variables were highlighted by the BIOENV procedure as providing the best relationship of any combination of environmental variables with the genetic structure of wolf populations (Table 3). The important climate and vegetation categories were: the continental warm temperate zone that distinguished North Quebec from the other sites (C4), the continental cool temperate zone that distinguished Denali from the other sites (C6), the Rocky Mountains coniferous forest that distinguished Banff, Hinton and Montana from the other sites (V4; Fig. 1), the mixed forest that distinguished Minnesota from the other sites (V5; Fig. 1) and the water barrier that distinguished Vancouver from the other sites (Table 4). These patterns were clearly seen on the MDS plot as well, where North Quebec, Denali, Minnesota and Vancouver are populations occurring around the outside perimeter of the plot and Montana, Banff and Hinton form a cluster in the upper right-hand corner (Fig. 2a).

For Nei's genetic distance, results were similar, however, the cluster of Banff, Montana and Hinton was no longer distinct (see Fig. 2b and note also that V4 was not chosen by BIOENV). Instead the variable that distinguished North and South Quebec together as different from the rest was highlighted as important (V2; Taiga; Fig. 1), a pattern which was also apparent in the MDS plot.

For the F_{ST} distance calculated from RFLP profiles, several clusters of populations were evident on the MDS plot (Fig. 2c). This suggested that there may be strong genetic similarities among certain populations, even though they may be separated by substantial geographical distance. For example, Nome, Yukon, Anatuvik and Vancouver form a tight cluster on the plot. Another tight cluster is formed by the populations from Denali, Montana and Alberta, and so on. The important variables identified by BIOENV were: the extreme continental warm temperate zone that distinguished Minnesota-N, Minnesota-NE and Western Ontario from the other sites (C2), the continental cool temperate zone that distinguished Yukon from the other sites (C5), the marine cool temperate zone that distinguished Kenai from the other sites (C4), the prairies that distinguished Manitoba from the other sites (V6; Fig. 1) and, once again, the water barrier that distinguished Vancouver from the other sites (Table 4). Patterns seen in the MDS plot are consistent with these results, with Minnesota-N, Minnesota-NE and Western Ontario forming a tight cluster and with populations from Manitoba and Kenai clearly separated from the other populations (Fig. 2c).

Analyses performed after removing sites in Ontario, Minnesota and Quebec indicated that longitude was no longer among the most important of the variables discriminating genetic variation in wolf populations (BIOENV analyses, Table 3). Instead, the BIOENV analysis generally picked out more of the climate variables as being relevant. However, even after these sites were removed, longitude alone still explained a significant portion of the genetic

Table 3 Results of the BIOENV procedure, showing the best fit obtained, for all populations and when eastern populations were excluded, in the case of one, two, three, four or five predictor variable subsets for each genetic distance matrix

	All populations		Eastern populations excluded	
No. of variables	Spearman's rho (ρ)	Variables chosen	Spearman's rho (ρ)	Variables chosen
F_{ST} (microsatellite)				
1	0.517	C4	0.567	C6
2	0.569	C4, V5	0.725	WB, C6
3	0.625	C4, C6, V5	0.784	WB, C2, C6
4	0.678	WB, C4, C6, V5	0.784	WB, C2, C4, C6
5	0.713	WB, C4, C6, V4, V5	0.784	WB, C2, C3, C4, C6
Nei's distance				
1	0.582	Longitude	0.608	C6
2	0.583	Longitude, C6	0.716	WB, C6
3	0.654	C6, V2, V5	0.749	WB, C5, C6
4	0.677	WB, C6, V2, V5	0.764	WB, C2, C5, C6
5	0.708	WB, C4, C6, V2, V5	0.764	WB, C2, C3, C5, C6
F_{ST} (RFLP)				
1	0.351	Longitude	0.508	V6
2	0.505	C2, V6	0.603	C4, V6
3	0.553	C2, C4, V6	0.703	C4, V2, V6
4	0.545	WB, C2, C4, V6	0.703	C1, C4, V2, V6
5	0.546	WB, C2, C4, C5, V6	0.703	C1, C2, C4, V2, V6

Table 4 Values for the variables chosen by the BIOENV routine as given in Table 3

Site	Water barrier	C4	C6	V2	V4	V5
Microsatellite data						
Banff	0	0	0	0	1	0
Denali	0	0	1	0	0	0
Fort St John	0	0	0	0	0	0
Hinton	0	0	0	0	1	0
Inuvik	0	0	0	0	0	0
Kenai	0	0	0	0	0	0
Minnesota	0	0	0	0	0	1
Montana	0	0	0	0	1	0
North Quebec	0	1	0	1	0	0
South Quebec	0	0	0	1	0	0
Vancouver	1	0	0	0	0	0
	Water barrier	Longitude	C2	C4	C5	V6
RFLP profile data						
Alberta	0	116.97	0	0	0	0
Anaktuvik	0	149.50	0	0	0	0
Central Ontario	0	78.60	0	0	0	0
Denali	0	148.97	0	0	0	0
Inuvik	0	133.70	0	0	0	0
Kenai	0	149.97	0	1	0	0
Manitoba	0	100.50	0	0	0	1
Minnesota-N	0	92.80	1	0	0	0
Minnesota-NE	0	91.98	1	0	0	0
Montana	0	114.32	0	0	0	0
Nome	0	165.40	0	0	0	0
Vancouver	1	125.80	0	0	0	0
Western Ontario	0	89.30	1	0	0	0
Yellowknife	0	114.35	0	0	0	0
Yukon	0	137.00	0	0	1	0

variation among wolf populations, using F_{ST} distance on RFLP profiles ($F_{1,9} = 2.76$, $P = 0.0908$), F_{ST} on microsatellite data ($F_{1,6} = 3.67$, $P = 0.0040$) or Nei's genetic distance ($F_{1,6} = 4.26$, $P = 0.0167$). Thus, the east–west gradient still has a strong detectable relationship with genetic structure, even if the potential hybrid wolves from these regions were excluded from the analysis.

Discussion

Conceptually, geographical distance must be an important obstacle to dispersal, because at some geographical scale distance prevents the exchange of individuals between populations. Thus, a pattern of isolation with distance is predicted in populations at mutation-drift equilibrium that also exhibit finite dispersal (Slatkin 1993; Hutchison & Templeton 1999). Furthermore, distance may be associated with topographical barriers that restrict exchange of individuals (Perez *et al*. 2002). However, isolation-by-distance is often not detected. For some studies, the absence of isolation with distance may be due to the effects of scale, especially if the distance between sampled populations is not much greater than the genetic neighbourhood distance (Wright 1969). Alternatively, historical effects or dispersal corridors between the studied populations may counteract the development of a pattern consistent with isolation-by-distance (e.g. Taberlet *et al*. 1998). In large North American wolf-like canids, dispersal corridors exist between the majority of extant populations where natural habitat can be found. Considering the high mobility of grey wolves, a pattern of isolation with distance is not expected. A continental-level study supported this expectation by showing an absence of correlation between genetic differentiation and distance (Wayne *et al*. 1992; Roy *et al*. 1994); however, a correlation was apparent on a smaller geographical scale (Forbes & Boyd 1997).

None the less, the use of geographical distance as a sole factor explaining population subdivision can be misleading. Imagine a series of populations that are located on a north–south axis. In such a configuration, isolation-by-distance encompasses a geographical distance component that includes climatic differences. In this case, the correlation between genetic and geographical distance does not reflect the influence of distance alone. In contrast, a series of locations configured on the east–west axis may generate an absence of isolation-by-distance because the axis consists of a single climatic regime or habitat type, allowing for unimpeded dispersal. Our previous continental wolf study included both east–west and north–south axes, thus a north–south pattern of isolation with distance may have been obscured.

We have found that contrary to our previous work, a pattern of isolation with distance is evident in a wider survey of North American wolf populations. This pattern appears to be related to climate zones (Fig. 1). There was significant variability in the genetic structure of wolf populations that could be explained by climactic categories over and above that which could be explained by geographical distance alone. These findings are consistent with observations that grey wolves vary in size and colour, especially when comparing northern and southern populations (Young & Goldman 1944; Gipson *et al*. 2002). Some wolf populations are totally white in colour and large (e.g. 80 kg, Elsmeer Island; Mech 1988), whereas others are reddish or dark and small in size (e.g. 37 kg; Mexican wolf; McBride 1980). Our results suggest that such phenotypical differences may be maintained by environmentally induced restrictions in gene flow that allow genetic drift and probably natural selection to cause both genetic and morphological differentiation among wolf populations.

The importance of climate as an explanation for population genetic structure has been more widely invoked for plants than animals (Sork *et al*. 1999). The low dispersal ability of plants allows for adaptation to local environmental conditions (Sork *et al*. 1999). Climatic and phytogeographical factors may also restrict movements of small terrestrial species (e.g. King 1987; Arter 1990) or species that are associated with specific host plants (e.g. Keyghobadi *et al*. 1999). The impact of such factors on highly mobile animals is much harder to envision. An environmental cline can provide a mechanism for differentiation (e.g. Endler 1980; Lande 1982; Turelli *et al*. 2001) and a recent model of speciation suggests that evolutionary branching can occur along clines of moderate steepness given the presence of intraspecific competition (Doebeli & Dieckmann 2003). Competition, both inter- and intraspecific, may be intense in larger carnivores (Van Valkenburgh & Wayne 1994), and in wolves, intraspecific strife between wolf packs is one of the largest sources of natural mortality (Mech 1994). Consequently, a model of differentiation along a cline could conceivably apply to North American wolves.

Additionally, we suggest that developing grey wolves may become imprinted with regard to climate and habitat. The tenure of wolves in their natal pack can be long, as young wolves are often recruited as helpers (Mech 1988; Mech 1999), and could drive the development of hunting skills for prey in local habitats. When young wolves disperse, often in their second or third year (Gese & Mech 1991), they may direct their movements toward familiar landscapes. Dispersing wolves that select familiar ground have a better chance of survival (Gese & Mech 1991). Such behaviour may account for why wolves hunting migrating caribou appear to be differentiated from nearby resident wolves that hunt nonmigratory game (Carmichael *et al*. 2001). Ecotypes of this nature have been described in killer whales (*Orcinus orca*; Saulitis *et al*. 2000) but not in large terrestrial carnivores (but see Rueness *et al*. 2003).

Gipson *et al*. (2002) have shown that the proportion of white wolves increases from less than 2% in the United

States to over 90% in the taiga and the Arctic of Canada and Greenland. These pelt differences suggest that latitudinal mixing of populations is restricted, and that geographical distance should account for much of the genetic variation among populations along that cline. Contrary to the above expectation, our results indicate that longitudinal contrasts explained more of the genetic variation between populations than latitude. The lack of correlation between genetic variation and north–south axis is not a consequence of low resolution because our sampling spanned nearly 4000 km north to south, from the High Arctic to the US border.

We suggest two possible mechanisms underlying the observed correlation between an east–west axis and genetic variation in North American wolf populations. First, North America has several dominant topographic divisions that extend several thousand kilometres on a north–south axis (e.g. Pacific Coast range, Rocky Mountains, Great Plains; see Fig. 1). Each of these regions creates a broad longitudinal band of unique vegetation zones. The reluctance of dispersing individuals to move between these major zones would generate an isolation-by-distance pattern on an east–west axis. Second, wolf packs usually do not follow migratory caribou but maintain year-round resident territories. However, during years when prey densities are low, up to 17% of all wolf packs follow migratory caribou and then return to their original territory for denning (Ballard *et al*. 1997). The caribou migration is a well-documented phenomenon that occurs across Alaska and Canada (Kelsall 1968). The pursuit of migratory caribou by wolves across large distances would diminish the possibility of having a north–south genetic cline in wolf populations along the migration route because wolf populations along this gradient could mix, whereas the dominance of north–south movements could reduce dispersal east and west (Carmichael *et al*. 2001). Specific data on long-range dispersal in relation to landscape features for wolves and caribou are needed to test these hypotheses. In conclusion, our results indicate the need for future genetic studies to assess the potential importance of climate and habitat as underlying causes of genetic patterns of differentiation.

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