

Genetic Structure of Muskrat (*Ondatra zibethicus*) and Its Concordance with Taxonomy in North America

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Abstract

Extrinsic factors such as physical barriers play an important role in shaping population genetic structure. A reduction in gene flow leading to population structuring may ultimately lead to population divergence. These divergent populations are often considered subspecies. Because genetic differentiation may represent differences between subspecies, patterns of genetic structure should reflect subspecies groupings. In this study, we examine the contemporary population genetic structure of muskrat ($n = 331$) and assess the relevance of 4 geographically distinct subspecies designations across northern North America using 9 microsatellite loci. We predicted that patterns of gene flow and genetic structure would reflect the described subspecies. We found evidence of genetic differentiation between western and eastern regions, and muskrats from Newfoundland (NF) showed significantly lower genetic diversity than central regions. A strong isolation by distance pattern was also detected within the eastern cluster. Our results did not differentiate *Ondatra zibethicus spatulus* (northwest) from *O. z. albus* (central), but they suggest a distinction between *O. z. obscurus* (NF) and *O. z. zibethicus* (east). This study highlights the need for more phylogenetic studies in order to better understand intraspecific divergence and the genetic characterization of subspecies.

Key words: genetic structure, isolation by distance, microsatellite loci, *Ondatra zibethicus*, subspecies

The population genetic structure of contemporary populations is influenced by both intrinsic and extrinsic factors. Extrinsic factors include historical events such as Pleistocene glaciations, which have been frequently identified as major influences on gene flow and thus patterns of intraspecific genetic structure (Avice 2000; Lee-Yaw et al. 2008; Grill et al. 2009). Subsequent postglacial colonizations have influenced the geographic distribution of genetic variation of numerous taxa (Durka et al. 2005; Pope et al. 2006; Lee-Yaw et al. 2008; An et al. 2009; Braaker and Heckel 2009). Furthermore, contemporary gene flow and population genetic structure are affected by extrinsic physical barriers such as mountains (Rueness et al. 2003) and bodies of water (Jordan and Snell 2008). Contemporary population structure is also shaped by intrinsic biological factors related to gene flow, such as inherent mobility, the tendency toward philopatry, and sex-biased dispersal (Fraser et al. 2004; Worley et al. 2004). Species with small home range sizes and limited dispersal typically show pronounced population genetic structure (Mossman and Waser 2001; Peakall et al. 2003).

The development of population structure is often a precursor to speciation. Significant genetic differentiation within a population is thus a criterion that has been used to designate subspecies, the lowest taxonomic rank used in systematics. Conceptually, subspecies can be defined as interbreeding populations showing genetic differentiation and are often geographically and morphologically distinct from other populations (Lincoln et al. 1998). Although the taxonomic status of subspecies is debatable (Zink 2004; Phillimore and Owens 2006; Cronin 2007; Patten 2010), it is still widely used (Zink 2004; Phillimore and Owens 2006). Subspecies designations are sometimes viewed as arbitrary, but these biological units can represent intraspecific geographic variations and may be relevant for the conservation of threatened and endangered organisms as well as for understanding the evolutionary history of a species (Johnsen et al. 2006; Phillimore and Owens 2006; Winker 2010). Although it is mainly accepted that subspecies should show some level of genetic divergence, traditional subspecies designations were primarily based on morphological differentiation. However, because the phenotype is the result of a

combination of genetic and environmental effects, morphological differences may also be the result of phenotypic plasticity (Crispo 2008; Pfennig et al. 2010). More recent molecular techniques have helped in the genetic characterization of subspecies and the assessment of their relevance (Johnsen et al. 2006; Hull et al. 2008; Grill et al. 2009). Microsatellite loci have been used to infer phylogenies and resolve evolutionary history uncertainties (Richard and Thorpe 2001). The investigation of both intraspecific phylogeography and subspecies resolution is common in ornithology (Hull et al. 2008; An et al. 2009). Although phylogeographic studies are common, few studies have examined both phylogeography and the relevance of subspecies classification in mammals (but see Cullingham et al. 2008; Latch et al. 2009). The subspecific status of mammal species has rarely been assessed unless these species are of conservation concern (Ramey et al. 2005; Grill et al. 2009).

The muskrat (*Ondatra zibethicus*) is a semiaquatic rodent species with a broad geographical distribution across North America. It has been introduced in Europe where it is regarded as an invasive species (Zachos et al. 2007). Although the muskrat is considered an important species in wetland ecosystems (Danell 1996) and a pest with high success of colonization in Europe (Danell 1996; Zachos et al. 2007), population geneticists have largely neglected this species. We only know of one study that looked at population differentiation in Europe using mitochondrial control region sequences (Zachos et al. 2007). Muskrats have small home ranges (Boutin and Birkenholz 1987; Nadeau et al. 1995), they are dependent on watersheds for their food supply and burrows (Willner et al. 1980; Boutin and Birkenholz 1987), and their dispersal abilities are not well known. This restriction to the aquatic environment combined with small home ranges may limit gene flow between populations and lead to genetic structure. Moreover, across North America, natural barriers such as mountains and large bodies of water may act as a barrier to dispersal for this small semiaquatic mammal. There are currently 16 subspecies of muskrat that have been described across North America, of which 5 are present in Canada (Willner et al. 1980). These subspecies were identified based on color and morphological differences (Boyce 1978; Lewis and Johnson 2002). Variation in skull morphology has also been reported between an island population (Newfoundland [NF]) corresponding to the subspecies *O. z. obscurus* and a mainland population (New Brunswick [NB]) corresponding to *O. z. zibethicus* (Rigby and Threlfall 1982). Nonetheless, no extended geographical comparison study has been conducted, and no genetic assessment of this classification has yet been undertaken. The information available concerning variations in muskrat populations is very limited, and here we used these subspecies designations as a null model to assess the presence of geographic variations.

In this study, we examine the contemporary population genetic structure and assess the relevance of the subspecific status of muskrat using microsatellite DNA loci. We also examined genetic diversity among regions. We hypothesized that muskrat populations will show high genetic structure

because of the species' biology (small home range; limited dispersal) and predicted that if the described subspecies represent genetically distinct subgroups, then genetic structure should correspond to existing subspecies designations. We predicted that within the subspecies range, physical barriers such as the Rocky Mountains and large bodies of water would prevent gene flow and isolate populations (e.g., British Columbia [BC], NF, and Prince Edward Island [PEI]).

Methods

Sample Collection

A total of 331 tissue samples were collected for DNA extraction. Two-hundred and ninety-one skin samples were obtained from pelts at the Fur Harvesters Auction Inc., North Bay, Ontario, Canada. These samples were collected from 10 different regions (excluding Ontario [ON]) across North America (Figure 1, Table 1). These animals were trapped between October 2006 and March 2007. Additional tissue samples from ON ($n = 40$) were collected from muskrat carcasses obtained directly from trappers in October and November 2005. Four of the 16 subspecies were represented within our samples: *O. z. spatulus* ($n = 71$), *O. z. albus* ($n = 30$), *O. z. zibethicus* ($n = 205$), and *O. z. obscurus* ($n = 25$) (Figure 1, Table 1). The distribution across North America of the 16 subspecies is described in Willner et al. (1980).

Genetic Analyses

Genomic DNA was extracted using QIAGEN DNeasy tissue kits. Muskrats were genotyped at 12 microsatellite loci (*oz06*, *oz08*, *oz16*, *oz17*, *oz22*, *oz27*, *oz30*, *oz32*, *oz34*, *oz41*, *oz43*, and *oz44*) following the procedures described in Laurence et al. (2009). Only 9 of these 12 microsatellite loci were used in subsequent analyses because 3 of them (*Oz17*, *Oz22*, and *Oz30*) showed inconsistent peak morphology. PCR products were run on an ABI 3730 sequencer, and genotypes were scored using GENEMAPPER 4.0 (Applied Biosystems).

We tested for departure from Hardy–Weinberg equilibrium (HWE) for each region using the software GENEPOP v.4.0.7 (Rousset 2008). Linkage disequilibrium (LD) was tested on all loci using FSTAT v.2.9.3.2. (Goudet 2002). The level of significance for HWE and LD was adjusted by sequential Bonferroni correction to control for multiple tests (Rice 1989). The presence of null alleles and genotyping errors were assessed using the software MICRO-CHECKER v.2.2.3. (van Oosterhout et al. 2004) with a confidence interval of 95% and 5000 randomizations.

Genetic diversity was determined by calculating allelic richness (A), observed (H_o), and unbiased expected (H_e) heterozygosity at each locus. The software HP-RARE (Kalinowski 2005) was used to calculate allelic richness using rarefaction to account for unequal sample sizes. H_o and unbiased H_e were calculated using FSTAT v.2.9.3.2. Differences among regions were tested using a 1-way

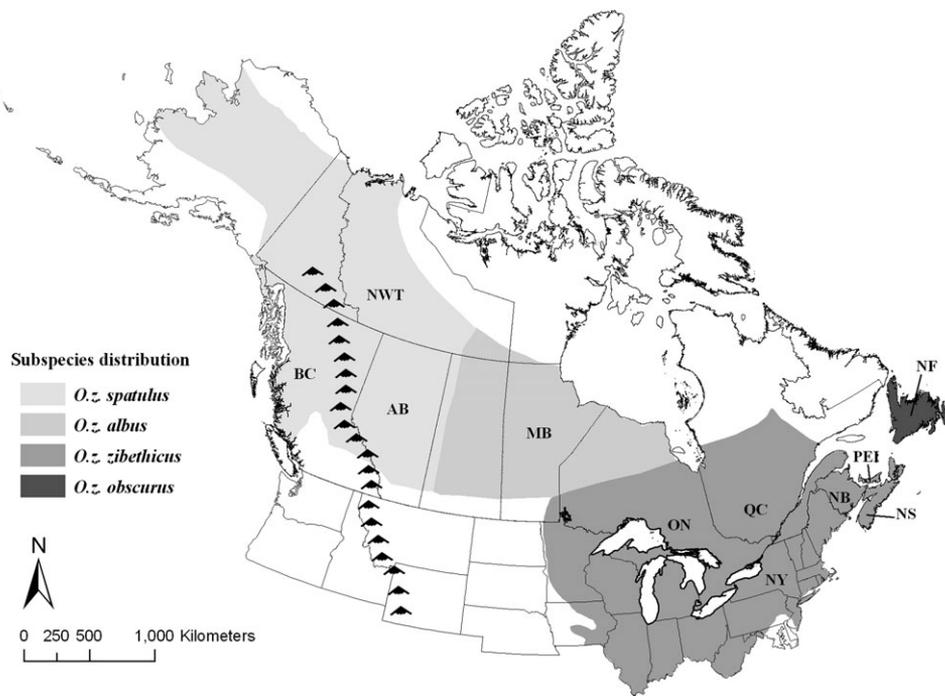


Figure 1. Geographic location of the 11 regions sampled and distribution of the 4 subspecies of muskrat (*Ondatra zibethicus*) studied (from Willner et al. (1980)).

ANOVA and Tukey’s tests for allelic richness and the nonparametric Kruskal–Wallis and post hoc multiple comparison tests for H_o and unbiased H_e (Statistica version 6). Pairwise differentiation (F_{ST}) between regions was determined using FSTAT v.2.9.3.2 (Goudet 2002), and significance of differentiation with Bonferroni correction for multiple tests was tested using 5500 permutations. Pairwise differentiation (F_{ST}) was also determined between the 3 clusters identified. Centroids of the given state or province were used as reference points to estimate the geographic distances between the different regions as no information other than the province or state of origin were

available. We examined the relationship between the natural logarithm of these geographic distances and genetic differentiation ($F_{st}/(1 - F_{st})$) using Mantel tests implemented in FSTAT (10 000 permutations).

Population structure was tested using several approaches. First, we used the Bayesian clustering method implemented by the software STRUCTURE v.2.2 (Pritchard et al. 2000). Five independent runs of each number of subpopulation (K) ranging from $K = 1$ to $K = 15$ were conducted with a burn-in of 1 000 000, followed by 1 000 000 iterations. The runs were performed using correlated allele frequencies model among populations and

Table 1 Genetic diversity of muskrat populations across North America

Location	Subspecies	N	A (n)	H_o	H_e	Loci not in HWE
BC	<i>Ondatra zibethicus spatulatus</i>	11	5.8 (n = 11) ^{a,b,c}	0.56 ± 0.05 ^{a,b}	0.65 ± 0.07 ^{a,b}	—
AB	<i>O. z. spatulatus</i>	20	6.0 (n = 19) ^{a,b,c}	0.62 ± 0.04 ^{a,b}	0.71 ± 0.08 ^{a,b}	oz43; oz44
NWT	<i>O. z. spatulatus</i>	40	6.5 (n = 37) ^{a,b}	0.65 ± 0.03 ^{a,b}	0.67 ± 0.09 ^{a,b}	—
MB	<i>O. z. albus</i>	30	7.2 (n = 30) ^{a,b}	0.71 ± 0.03 ^{a,b}	0.73 ± 0.07 ^{a,b}	oz43
ON	<i>O. z. zibethicus</i>	44	9.2 (n = 43) ^a	0.82 ± 0.02 ^a	0.85 ± 0.04 ^a	oz06
NY	<i>O. z. zibethicus</i>	36	9.3 (n = 36) ^a	0.83 ± 0.02 ^a	0.85 ± 0.04 ^a	oz16; oz43
QC	<i>O. z. zibethicus</i>	40	9.0 (n = 36) ^a	0.82 ± 0.02 ^a	0.84 ± 0.05 ^a	oz41
NB	<i>O. z. zibethicus</i>	40	8.0 (n = 38) ^{a,b}	0.68 ± 0.02 ^{a,b}	0.77 ± 0.08 ^{a,b}	oz08; oz16; oz27
NS	<i>O. z. zibethicus</i>	30	4.6 (n = 30) ^{b,c}	0.60 ± 0.03 ^{a,b}	0.60 ± 0.08 ^{a,b}	—
PEI	<i>O. z. zibethicus</i>	15	4.6 (n = 14) ^{b,c}	0.66 ± 0.04 ^{a,b}	0.63 ± 0.09 ^{a,b}	—
NF	<i>O. z. obscurus</i>	25	2.8 (n = 25) ^c	0.36 ± 0.03 ^b	0.35 ± 0.09 ^b	—

Number of individuals (N) sampled per region and putative subspecies from Willner et al. (1980). Allelic richness (A), observed heterozygosity (H_o), and unbiased expected heterozygosity (H_e) are indicated with standard error. n, Minimum number of individuals in a sample that were genotyped successfully at all loci. Regions with the same superscript letters did not differ ($P > 0.05$; post hoc multiple comparison tests).

admixture model. The most probable K was estimated by comparing the likelihood ($\text{Ln}P(D)$) with the different values of K and by selecting the largest ΔK (Evanno et al. 2005) with an average estimated proportion of ancestry for each inferred cluster of 0.80 minimum at that K . This method is not applicable when $K = 1$ (Evanno et al. 2005); therefore, for each round, we first looked at the higher estimate of $\text{Ln}P(D)$ to verify if it was at $K = 1$ (Coulon et al. 2008). This procedure was repeated for each second-order cluster until no further subdivision was detected. To assign each individual to a specific cluster, we averaged the q values across the 5 runs. Each individual with a $q < 0.6$ were unassigned and left out of subsequent steps (see Coulon et al. 2008).

We also performed a principal component analysis (PCA) using the adegenet package in R (Jombart 2008) to visualize the genetic relationships among regions.

Results

Genetic Variation

Significant deviations from HWE occurred at 1–3 loci for 6 out of 11 regions after Bonferroni correction (Table 1). No LD was observed after Bonferroni correction. No evidence of allelic dropout or scoring error due to stuttering was detected. Null alleles may be present based on homozygous excess at 2 loci (oz_{27} and oz_{44}). We performed the analyses without these 2 loci and did not detect any differences between the results; therefore, we maintained these loci in our analyses.

Genetic diversity was significantly different between regions as determined by an ANOVA for allelic richness (\mathcal{A}) ($H = 45.41$, $P < 0.001$) and a nonparametric Kruskal–Wallis test for H_o ($H = 29.08$, $P = 0.0012$) and H_e ($H = 39.43$, $P < 0.001$) (Table 1). The island of NF had the lowest allelic richness and also showed the lowest observed and expected heterozygosity (Table 1), whereas central populations were significantly more diverse (ON, New York State [NY], and Quebec [QC]; post hoc multiple comparison tests for all 3 measures of diversity [$0.001 < P < 0.013$]) (Table 1). PEI and Nova Scotia (NS) also showed significantly less allelic richness than central populations ($0.003 < P < 0.012$).

Pairwise F_{st} between the regions were all statistically significant ($P \leq 0.05$) and ranged from 0.019 to 0.461 (Table 2) with a global F_{st} of 0.167. NF showed the highest degree of differentiation from the different mainland populations ($F_{st} = 0.260$ – 0.461). We found a significant positive relationship between geographic distances and genetic distances (Mantel $r = 0.156$, $P = 0.003$), indicating a pattern of isolation by distance (IBD).

Population Structure and Subspecies Status

Following Evanno et al. (2005), the most likely number of clusters suggested by Bayesian analysis implemented in STRUCTURE was $K = 2$ (Figure 2a). These genetic clusters corresponded to eastern (ON, NY, QC, NB, PEI, NS, and

NF) and western (BC, Alberta [AB], North West Territories [NWT], and Manitoba [MB]) regions (Figure 2b). The average estimated proportion of ancestry for each inferred cluster at that K was high for both eastern and western groups (0.969 and 0.988, respectively). These 2 clusters were run separately in STRUCTURE until no further subdivision was detected. The eastern group was subdivided into 5 clusters (Figures 3 and 4). Most samples from the mainland eastern regions were distributed into different clusters, and no clear groupings could be identified on the mainland. Within the eastern region, NF was identified as a separate cluster. All individuals from NS and PEI were also assigned to their own cluster but were grouped with 13 and 7 individuals from NB, respectively (Figures 3b and 4). For the western cluster, $\text{Ln}P(D)$ was the maximum at $K = 1$, and the average proportion of ancestry was lower than 80% for all K values beyond $K = 1$ (71% for $K = 2$, 63% for $K = 3$, and 55% for $K = 4$). Therefore, we considered that only one cluster was detected for the western regions.

We were able to identify 3 distinct clusters using PCA (Figure 5). The first principal axis differentiated muskrats from western and eastern regions (eigenvalues for component 1 = 0.616; axis 1 explained 13.6% of the variation), whereas the second axis further differentiated the eastern regions by separating NF muskrats from the eastern cluster (eigenvalues component 2 = 0.331; axis 2 explained 7.3% of the variation). The third component (eigenvalue = 0.227; 5% of the variation) did not further differentiate the 3 clusters. However, when examining the eastern cluster, NS appeared to differentiate from the central regions (Figure 5). When examining the eastern regions only, using the PCA (results not shown), we found that NS as well as PEI were differentiated from the central regions on the third component (eigenvalues component 3 = 0.183; axis 4 explained 4.4% of the variation).

Our Bayesian clustering analysis grouped the 2 subspecies *O. z. spatulus* and *O. z. albus* within the western cluster (Figure 4). Within the 5 clusters identified for the eastern regions, one of them consisted of the *O. z. obscurus* samples, whereas the 4 other clusters corresponded to *O. z. zibethicus* (Figure 4). The PCA analysis showed a similar clustering pattern of the 4 subspecies. The pairwise F_{st} between the 3 genetically defined clusters were all statistically significant ($P \leq 0.05$) with F_{st} between west/east = 0.146, F_{st} between east/NF = 0.215, and F_{st} between west/NF = 0.343.

Discussion

Genetic Diversity and Genetic Structure of Populations

We found evidence of genetic structure of muskrat across northern North America with genetic differentiation between western and eastern regions. Our results also indicate a lower genetic diversity for the NF samples with a significant differentiation from the mainland. Within the eastern regions, muskrat genetic structure reflects a clinal pattern rather than clustered groupings (hierarchical structure analysis and PCA),

Table 2 Pairwise F_{st} (from Weir and Cockerham 1984) between regions (top half of the table)

	BC	AB	NWT	MB	ON	NY	QC	NB	PEI	NS	NF
BC		0.074	0.047	0.063	0.156	0.158	0.173	0.210	0.289	0.326	0.461
AB	*		0.058	0.072	0.152	0.158	0.167	0.207	0.276	0.300	0.420
NWT	**	**		0.041	0.169	0.178	0.184	0.215	0.276	0.298	0.403
MB	**	**	**		0.119	0.126	0.127	0.167	0.243	0.264	0.369
ON	**	**	**	**		0.019	0.026	0.057	0.116	0.157	0.269
NY	**	**	**	**	**		0.019	0.054	0.115	0.156	0.278
QC	**	**	**	**	**	**		0.037	0.113	0.131	0.270
NB	**	**	**	**	**	**	**		0.070	0.082	0.260
PEI	**	**	**	**	**	**	**	**		0.199	0.406
NS	**	**	**	**	**	**	**	**	**		0.342
NF	**	**	**	**	**	**	**	**	**	**	

All values were statistically significant after correction for multiple tests (bottom half of the table; * $P \leq 0.05$, ** $P \leq 0.01$).

which seems to be the result of a strong IBD at this broad spatial scale. Our results showed some degree of concordance between the genetic structure of muskrat and the existing subspecies designations. However, one of the subspecies (*O. z. albus*) was undifferentiated.

Genetic diversity in muskrat varies among regions and ranged from low (i.e., NF) to high (i.e., ON). Muskrats from NF showed significantly lower genetic diversity than central

populations. The genetic diversity in this island population may be less than mainland populations due to a founder effect generated by glacial retreat, which has often been observed in northern regions (Hewitt 2000). Island populations are also expected to have lower genetic diversity and show strong genetic differentiation from mainland populations because of restricted gene flow due to geographic isolation combined with genetic drift (Jordan

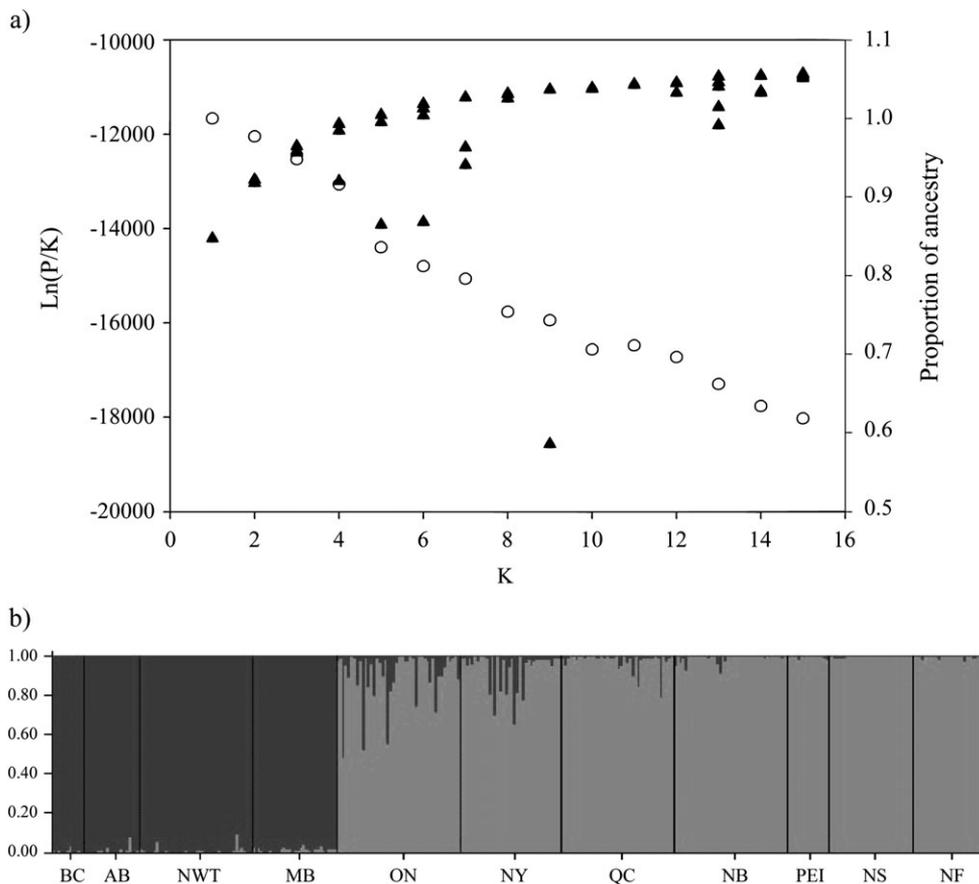


Figure 2. (a) $\ln(P/K)$ (Δ) and proportion of ancestry (\circ) using 5 runs at each K from 1 to 15. (b) Assignment of individuals to each cluster for $K = 2$ using STRUCTURE. Individuals are grouped based on their sampling region.

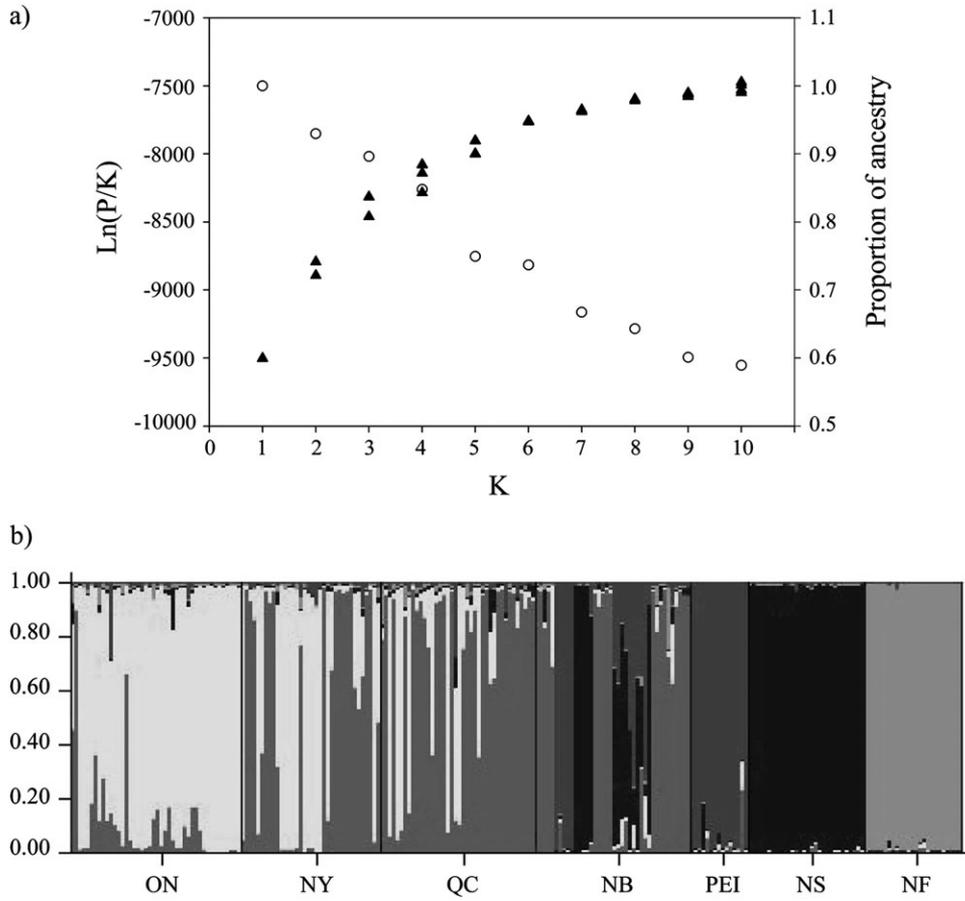


Figure 3. (a) $\ln(P/K)$ (Δ) and proportion of ancestry (\circ) for the eastern samples using 5 runs at each K from 1 to 10. (b) Assignment of individuals to each cluster for $K = 5$ using STRUCTURE. Individuals are grouped based on their sampling region.

and Snell 2008). Another possible explanation of this difference in genetic diversity may be related to the size of the area sampled and therefore to the proportion of the population sampled. However, as this information was not available to us, it is not possible to know if there are any differences in the size of the areas sampled. Although not significant, a trend of lower genetic diversity was observed in regions such as BC and NS. In previous studies, lower genetic diversity at the periphery of species range has also been observed because of lower gene flow in these regions (Schwartz et al. 2003; Eckert et al. 2008). The decrease of genetic variability at the periphery of a species' range may also be related to the smaller effective population size found at the margin of the distribution (Johansson et al. 2006).

The Bayesian clustering analysis distinguished populations from eastern and western Canada. However, our results suggested that the eastern cluster is further substructured and NF is separated from the rest of the eastern regions. These results were supported by the PCA. Genetic differentiation between eastern and western regions may be the result of 2 scenarios of muskrat's colonization of northern regions after the retreat of the ice sheets. Muskrat's postglacial colonization may have come from distinct glacial

refugia, as it has been observed for several species (Hewitt 2000; Schaschl et al. 2003; Lee-Yaw et al. 2008), in which case genetic differentiation must have been maintained through time as a result of low gene flow between these regions. On the other hand, the differentiation between eastern and western regions may also be explained by genetically similar colonizers that have diverged due to low gene flow between these 2 regions. Postglacial colonization from glacial refugia and signs of past barriers are usually examined using mitochondrial DNA (Avice 2000; Schaschl et al. 2003; Lee-Yaw et al. 2008; Flanders et al. 2009). In contrast, because of their high mutation rate, microsatellite loci (nuclear DNA) have been used to characterize genetic differentiation between populations that are the result of more recent events (Pope et al. 2006; Rowe and Beebe 2007). Additional analyses using mitochondrial DNA may help us to detect the effects of historical processes and therefore help us to better understand postglacial colonization patterns and gene flow between contemporary populations. Ruess et al. (2003) found strong genetic differentiation between eastern and western lynx (*Lynx canadensis*) populations across Canada. The authors have attributed this genetic separation to the presence of an

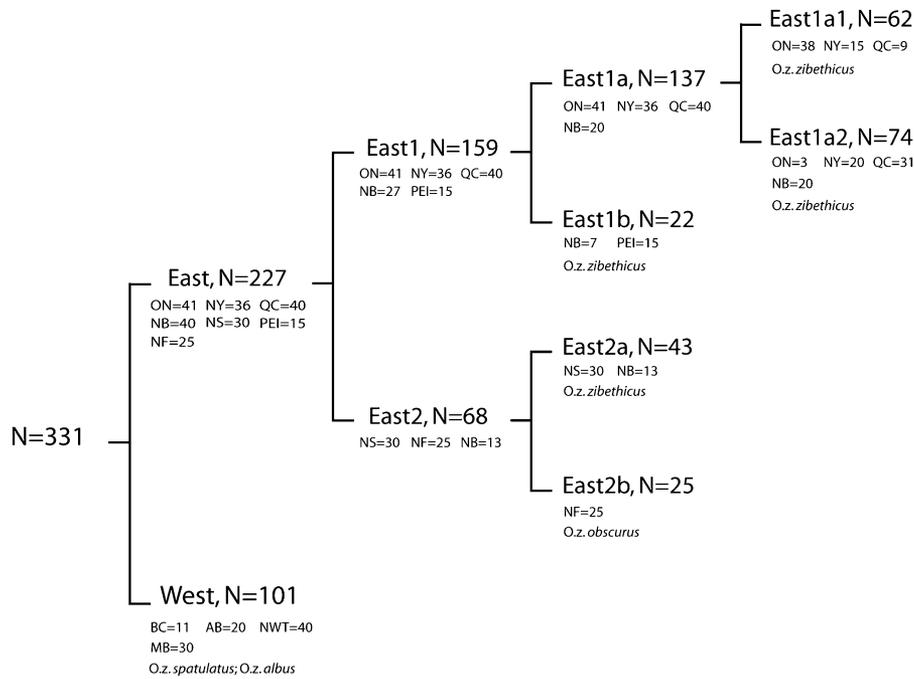


Figure 4. Results of structure analyses for the eastern regions following a hierarchical method. Associated subspecies are indicated for the final clusters.

invisible barrier between these regions that could be related to differences in climatic conditions (continental vs. Atlantic climates). Hull et al. (2008) have also reported a separation between eastern and western red-shouldered hawk (*Buteo lineatus*) populations in North America due to unsuitable habitat preventing gene flow. The broad-scale genetic

pattern observed for the muskrat may be the result of limited local dispersal due to a heterogeneous landscape and hence restricted gene flow (Pope et al. 2006) as well as limited dispersal due to behavior such as philopatry (Worley et al. 2004). Although no clear barrier can be identified for muskrat populations, unsuitable habitat such as variable water levels may increase mortality and limit recruitment (Virgl and Messier 1996) and may lead to reduced dispersal and hence reduced gene flow.

Our results show the presence of an IBD pattern. Bayesian clustering method is not well suited for resolving IBD patterned data (Frantz et al. 2009) as most individuals show mixed membership in multiple groups (Pritchard et al. 2000). Bayesian clustering techniques can detect clusters when IBD alone is present (Frantz et al. 2009) and therefore simulate the effect of barriers. In our study, the IBD pattern observed was based on the geographic distances using the centroids of regions because the exact geographic locations were not available and therefore should be taken with caution. Moreover, the clustering of muskrats from western regions, eastern regions, and NF was detected using the PCA. Further sampling with a more precise location of each sample would be necessary in future studies in order to assess if the population structure observed is the result of IBD rather than the effect of barriers to gene flow or a combination of both.

Subspecific Status of Muskrat

From a taxonomic point of view, our results suggest at least 3 genetically distinct clusters: a western group including *O. z. spatulatus* and *O. z. albus*, an eastern group comprised of *O. z.*

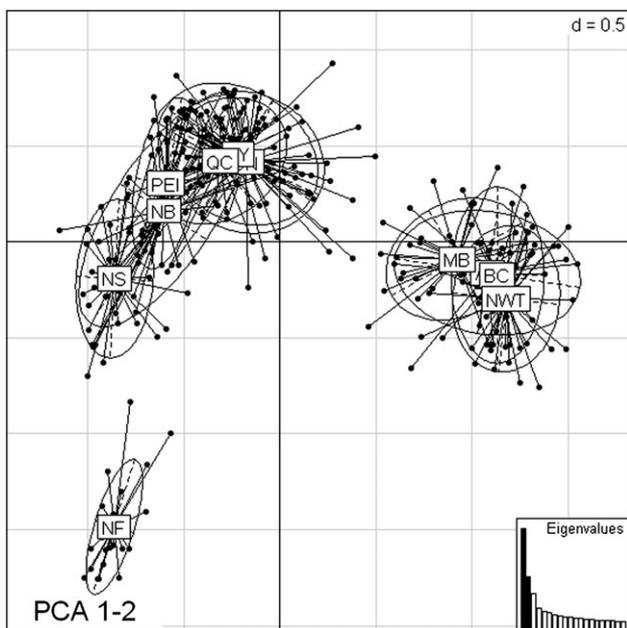


Figure 5. PCA of the first 2 principal components. Each ellipse groups 95% of the individuals for each of the 11 regions.

zibethicus, and NF comprised of *O. z. obscurus*. Contrary to our predictions, the populations from MB were not clearly differentiated from the more western populations (e.g., AB and BC), and we found little support for the distinction between the subspecies *O. z. spatulus* and *O. z. albus* using microsatellite data. This lack of genetic differentiation between populations may indicate common ancestry, high levels of gene flow, or the combination of both (Johnsen et al. 2006). If muskrat populations from western regions and from the prairies were 2 distinct subspecies as suggested by Willner et al. (1980), it is possible that recent gene flow between these regions has reduced the genetic distance between these populations. In concordance with the current subspecific status of muskrat in North America, our results do support the presence of a subspecies in NF (*O. z. obscurus*) showing a clear genetic differentiation from the mainland eastern cluster (*O. z. zibethicus*). Although we recognize that the use of subspecies is subjective, we use these designations as geographical variations of muskrat populations. These biological units may be useful for conservation programs, and, as in the case of a widespread species like the muskrat, these subspecific variations may also be interesting in order to understand the evolutionary history of the species.

A caveat to our conclusions is that some samples could be assigned to other subspecies for the western regions (e.g., NWT could be assigned to *O. z. albus* at its southeastern border). First, this does not affect our conclusion regarding genetic structure of muskrat populations in Canada because all samples within each western province clustered together. Second, the subspecies names we assigned to each population cluster are arbitrarily based on the most widespread subspecies in the province.

The assessment of the subspecific status of this species would benefit from the analysis of other molecular markers such as mitochondrial DNA. Moreover, the use of neutral markers may not reflect the effects of selective adaptation; selection may be strong enough to overcome gene flow, thus leading to morphological differences despite gene flow (Ballentine and Greenberg 2010; Patten 2010). A diagnosis on the relevance of the described subspecies based only on genetic variations is insufficient, and a more thorough analysis of morphological traits would also be necessary (Patten 2010).

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