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## Mitochondrial DNA diversity and phylogeography of insular Newfoundland red foxes (*Vulpes vulpes deletrix*)

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As the only native insular Newfoundland, Canada, canid from the extinction of the wolf in the 1930s to the recent arrival of coyotes, the red fox (*Vulpes vulpes deletrix*) poses interesting questions about genetic distinctiveness and the postglacial colonization history of the island's depauperate mammalian fauna. Here, we characterize genetic variability at the mitochondrial control region in 189 foxes from the island of Newfoundland, Prince Edward Island, and Labrador. We identified 8 haplotypes (3 new to this study) defined by 11 polymorphic sites, with an average pairwise sequence divergence of  $\sim 0.003$  and haplotypic diversity of 0.56 among localities. A pairwise distribution of control region sequence differences, rho estimate of divergence time, and tests of neutrality (Fu's  $F_S$  and Tajima's  $D$ ) are weakly consistent with a population expansion  $\sim 9,000$  years ago, correlating with retreat of glacial ice from the region. Haplotype composition reflects primarily the Eastern subclade of Aubry and colleagues and supports Aubry's 2-refugia hypothesis that indigenous red foxes in North America are derived from disparate refugia isolated during the Wisconsinan glaciation. Haplotype identity and pattern of population differentiation suggest recolonization of the island of Newfoundland via a northern glacial refugium via Quebec or Labrador rather than an Atlantic or southern route, but provide no mitochondrial genetic evidence to support differentiation of this population of foxes along subspecies lines. We establish a baseline for continued investigations of population demography, genetic structure, and adaptive genetic diversity in island Newfoundland red foxes, a population of interest from both ecological and wildlife disease perspectives.

Key words: haplotypic diversity, mitochondrial control region, population demography, population structure, postglacial recolonization, red fox

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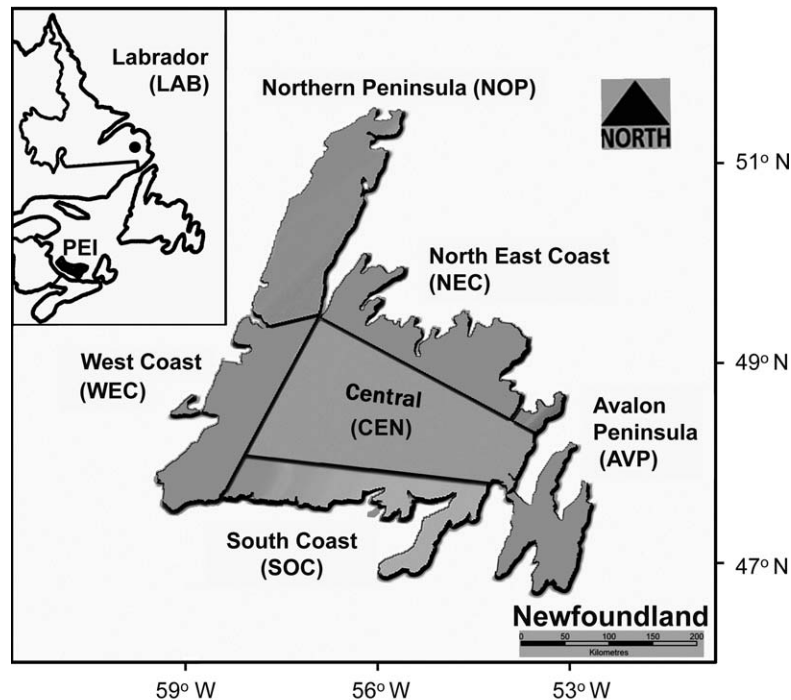
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Widely distributed in North America, Eurasia, northern Africa, and in Australia, where it was introduced in 1858, the red fox (*Vulpes vulpes*) has been recognized as having some 40 subspecies and occupies a broad range of habitats including tundra, semiarid desert, boreal forest, and urban (Larivière and Pasitschniak-Arts 1996). Until recently red foxes in North America were considered both native and nonnative, with native foxes occupying boreal and montane regions, whereas nonnative foxes of European origin were thought to have expanded into agricultural and urban areas (Kamler and Ballard 2002). However, Frey (2013) argued against any substantial contribution of European ancestry to North American populations, a concept supported by mitochondrial DNA (mtDNA) analysis (Statham et al. 2012) as well as historical data. The occurrence of nonnative foxes more likely relates to escapees

from fur farms, whose foxes were originally taken from Prince Edward Island, Canada, or Alaska (Statham et al. 2011, 2012).

Aubry et al. (2009) showed, using mtDNA sequence data, that native red foxes in North America belong to 2 phylogeographic clades that diverged approximately 400,000 years ago: a Holarctic clade and a Nearctic clade. The Holarctic clade, which constitutes populations found mostly in Alaska and western Canada, originated from a northern refugium established via recolonization of Beringia during the Wisconsinan glacial period. The Nearctic clade was likely established during the earlier Illinoian glaciation, and originated from a southern





**FIG. 1.**—Geographic locations of 8 red fox (*Vulpes vulpes deletrix*) regions sampled in this study: 6 on the island of Newfoundland (Northeast Coast, Central Newfoundland, Avalon Peninsula, West Coast, South Coast, and Northern Peninsula), Labrador (inset), and Prince Edward Island (PEI, inset).

refugium during the Wisconsin. The mitochondrial control region further divides the Nearctic clade into Mountain and Eastern subclades, found primarily in the southwestern mountains and the central–eastern region, respectively, and a Widespread subclade found in both areas, indicative of incomplete lineage sorting. Hence, like other boreal North American mammals, red fox populations comprise multiple evolutionary lineages consistent with long-term isolation in Pleistocene forest refugia (Aubry et al. 2009), but are genetically more complex due to the existence of the Holarctic clade.

The island of Newfoundland, part of the Canadian province of Newfoundland and Labrador and North America's easternmost land mass, is host to only 14 indigenous terrestrial mammal species in 4 orders, including 7 in Carnivora. Most of this fauna colonized the island postglacially, within the last 7,000 years or so (South 1983). Coupled with the island's isolation for much of this period, this raises questions regarding the diversity and distinctiveness of Newfoundland mammal forms. The subspecies of Newfoundland red fox (*Vulpes vulpes deletrix*) has been described as pale and straw-colored relative to mainland counterparts (reviewed by Dodds 1983), and the population also is characterized by silver phase and red- or silver-cross animals. The fox was one of several Newfoundland mammals originally considered distinct enough from both Cape Breton and Labrador populations to warrant subspecies status (Dodds 1983), but its evolutionary origins and genetic affinities with respect to these 2 regions remain unclear. Insular

Newfoundland's population of red foxes is of special interest because it is the only endemic focus in North America of the endoparasitic nematode French heartworm (*Angiostrongylus vasorum*). In addition, the coyote (*Canis latrans*) population in Newfoundland is rapidly expanding (McGrath et al. 2010) and the potential for coyotes to suppress fox populations via competition for territory has been discussed by Levi and Wilmers (2012).

Herein we characterize mitochondrial control region diversity in red foxes from 6 Newfoundland localities and compare it with diversity in 2 mainland locations to characterize genetic diversity and make inferences about population structure, postglacial evolutionary history and route of colonization, and genetic distinctiveness of one of Newfoundland's few native mammal species.

## MATERIALS AND METHODS

**Study area and sample collection.**—A total of 159 red fox tissue samples from 6 sampling regions covering the geographic range of insular Newfoundland (Fig. 1; Table 1) were collected during 2002–2004 by the Animal Health Division of the Government of Newfoundland and Labrador, as part of an insular rabies eradication program, and during 2009–2012 by wildlife trappers from the Salmonier Nature Park. Samples from the mainland locations of Labrador ( $n = 6$ ) and Prince Edward Island ( $n = 24$ ) were provided by Salmonier Nature Park and Dr. Gary Conboy of the Atlantic Veterinary College, respectively. We designated 6 sampling regions on the

**TABLE 1.**—Mitochondrial control region diversity in Newfoundland, Labrador, and Prince Edward Island red foxes (*Vulpes vulpes deletrix*). A) Control region haplotypes defined by 11 polymorphic sites among 189 foxes, and their occurrence in each locality. The total number of individuals of each haplotype is shown in parentheses. Locality abbreviations are: NOP = Northern Peninsula; WEC = West Coast; NEC = North East Coast; CEN = Central; SOC = South Coast; AVP = Avalon Peninsula; LAB = Labrador; PEI = Prince Edward Island. B) Sample sizes and measures of diversity: sample size ( $n$ ), number of haplotypes ( $k$ ), haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ ).

		No. per locality								
		NOP	WEC	NEC	CEN	SOC	AVP	Island	LAB	PEI
A) Haplotype (no.)										
NL1 (74)	T-CCGCGCGAC	7	21	19	15	4	6	72	1	1
NL2 (60)	.-.....A..	14	23	6	5	5	2	55	4	1
PEI (22)	.A.....	0	0	0	0	0	0	0	0	22
NL3 (21)	.-...T..A..	1	3	14	2	0	0	20	1	0
NL4 (4)	C-T.AT.TAGA	3	0	0	0	0	1	4	0	0
NL5 (3)	.-.....A.....	0	0	1	0	0	2	3	0	0
NL6 (3)	.A.....A.....	0	1	1	1	0	0	3	0	0
NL7 (2)	.-.T.....	0	1	1	0	0	0	2	0	0
B) Sample size and measures of diversity per locality										
<i>n</i>		25	49	42	23	9	11	159	6	24
<i>k</i>		4	5	6	4	2	4	7	3	3
<i>h</i> (× 10 <sup>-1</sup> )		6.2	6.0	6.8	5.4	5.6	6.9	6.2	6.0	1.6
<i>π</i> (× 10 <sup>-3</sup> )		6.2	2.3	3.5	2.4	1.7	6.2	3.7	2.0	0.7

island of Newfoundland to correspond approximately to the ecoregions used by Wildlife Division within the province for management purposes (McGrath et al. 2010). Sample use was approved by Memorial University's Institutional Animal Care Committee, in compliance with guidelines of the American Society of Mammalogists (Sikes et al. 2011).

**Laboratory procedures.**—The DNA was extracted from salivary gland tissues using a QIAamp DNA Mini kit (Qiagen Inc., Toronto, Ontario, Canada) according to the manufacturer's instructions for tissue samples.

A 329-base-pair (bp) fragment of the mitochondrial control region was targeted by polymerase chain reaction amplification with the primers LF15926F and DLH (Kirschning et al. 2007). Polymerase chain reaction was performed in a final reaction volume of 25  $\mu$ l containing 1X Qiagen PCR buffer, 200  $\mu$ M of deoxynucleoside triphosphates (New England Biolabs Inc., Whitby, Ontario, Canada), 400 nM of each primer, 1 U of HotStar Taq DNA polymerase (Qiagen Inc.), and 25–200 ng of DNA template. The amplification profile consisted of an initial denaturation for 5 min at 95°C, followed by 40 cycles of 93°C for 30 s, 50°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 2 min.

All polymerase chain reaction products were sequenced in both directions using BigDye Terminator 2.0 chemistry and electrophoresed on the ABI Prism 3130 DNA Analyzer (Applied Biosystems Inc., Foster City, California). Sequence reads were assembled and edited with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan).

**Data analysis.**—Variable sites and haplotypes among all red fox control region sequences obtained here were identified using MEGA version 5.10 (Tamura et al. 2011), and haplotypes found in this study were compared to the 54 control region haplotypes detailed in Aubry et al. (2009—

GenBank accession numbers FJ830777 - FJ830829 and FJ840491) and also referenced against GenBank using a Basic Local Alignment Tool nucleotide search (BLAST—Altschul et al. 1990). Phylogenetic relationships among all haplotypes found here and those associated with the Nearctic clade of Aubry et al. (2009) were inferred using the best-fit Tamura-3-parameter model (Tamura 1992—determined with the model-selection option of MEGA using default parameters) with invariant sites and the maximum-likelihood algorithm (1,000 bootstrap replicates) in MEGA. A median-joining network was constructed from mutational differences among the control region haplotypes found in this study, as well as haplotypes from the Eastern and Widespread subclades of Aubry et al. (2009), using NETWORK version 4.6.1.1 (Bandelt et al. 1999; Fluxus Technology Ltd., Suffolk, England).

Measures of haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) in each population and over all populations were estimated using Arlequin version 3.5 (Schneider et al. 2000). Population structure was investigated in Arlequin by calculating pairwise and overall measures of  $F_{ST}$  (conventional  $F$ -statistics based on haplotype frequencies with 1,000 permutations to infer significance), and by estimating net number of nucleotide differences between populations ( $d_A$ —Nei and Li 1979). Similarities among populations were further depicted by construction of an unweighted pair-group method using arithmetic averages (UPGMA) dendrogram from the  $d_A$  distance matrix; the probabilities associated with these distances were calculated in Arlequin by 10,000 simulations of the  $d_A$  distance associated with the grouping.

The observed pairwise mismatch distribution was characterized with the raggedness index (Harpending 1994), and evaluated with respect to the expected distribution under a

model of demographic expansion, using Arlequin; significance was inferred with 10,000 bootstrap replicates. The parameters of a demographic expansion were inferred under Arlequin's generalized least-squares approach (Schneider and Excoffier 1999) and interpreted temporally by invoking a mutation rate of 1% per ~56,803 years, as estimated by Aubry et al. (2009—1 mutation in their 342-bp sequence in 16,473 years). The signature of population demographic changes in Newfoundland also was investigated in Arlequin with a test of neutrality, Tajima's  $D$ -test (Tajima 1989—using 10,000 simulated samples) and compared with Fu's  $F_S$  statistic (also with 10,000 simulations), considered a more sensitive metric (Fu 1997). Finally, a rho estimate (calculated with NETWORK) of the average number of mutations separating ancestral and descendent haplotypes also was used to estimate divergence time, using default program parameters and specifying the 2 most common haplotypes as ancestral (Saillard et al. 2000).

## RESULTS

We obtained 329-bp sequences from the mitochondrial control region for 189 red foxes from 6 regions of the island of Newfoundland, Labrador, and Prince Edward Island (Table 1A). Among all sequences, 11 polymorphic sites (9 transitions, 1 transversion, and 1 insertion–deletion [indel]) defined 8 haplotypes (NL1–NL7 and PEI), which differed from each other by 1–9 substitutions. Haplotypes ranged in occurrence from 2 to 74; 2 dominant haplotypes (NL1 and NL2) were found in > 70% of foxes, whereas 2 other haplotypes, designated PEI and NL3, were found in an additional 22% of foxes. All haplotypes were interrogated against GenBank using BLAST and also directly compared to each of the haplotypes reported by Aubry et al. (2009). Three of the haplotypes we found were previously reported by Aubry et al. (2009); our most common haplotype, NL1, is the same as their haplotype 9; the 2nd most frequent, NL2, is identical to 17 of Aubrey and colleagues; and NL6 is the equivalent of their 79. Additionally, our haplotype designated PEI matches haplotype 76 of Statham et al. (2012—GenBank accession HM590005), who found it in 8 foxes in the southeastern United States, and our haplotype NL5 matches haplotype 85 of Statham et al. (2012—GenBank accession HM461968) found in 2 foxes from their fur farm sample.

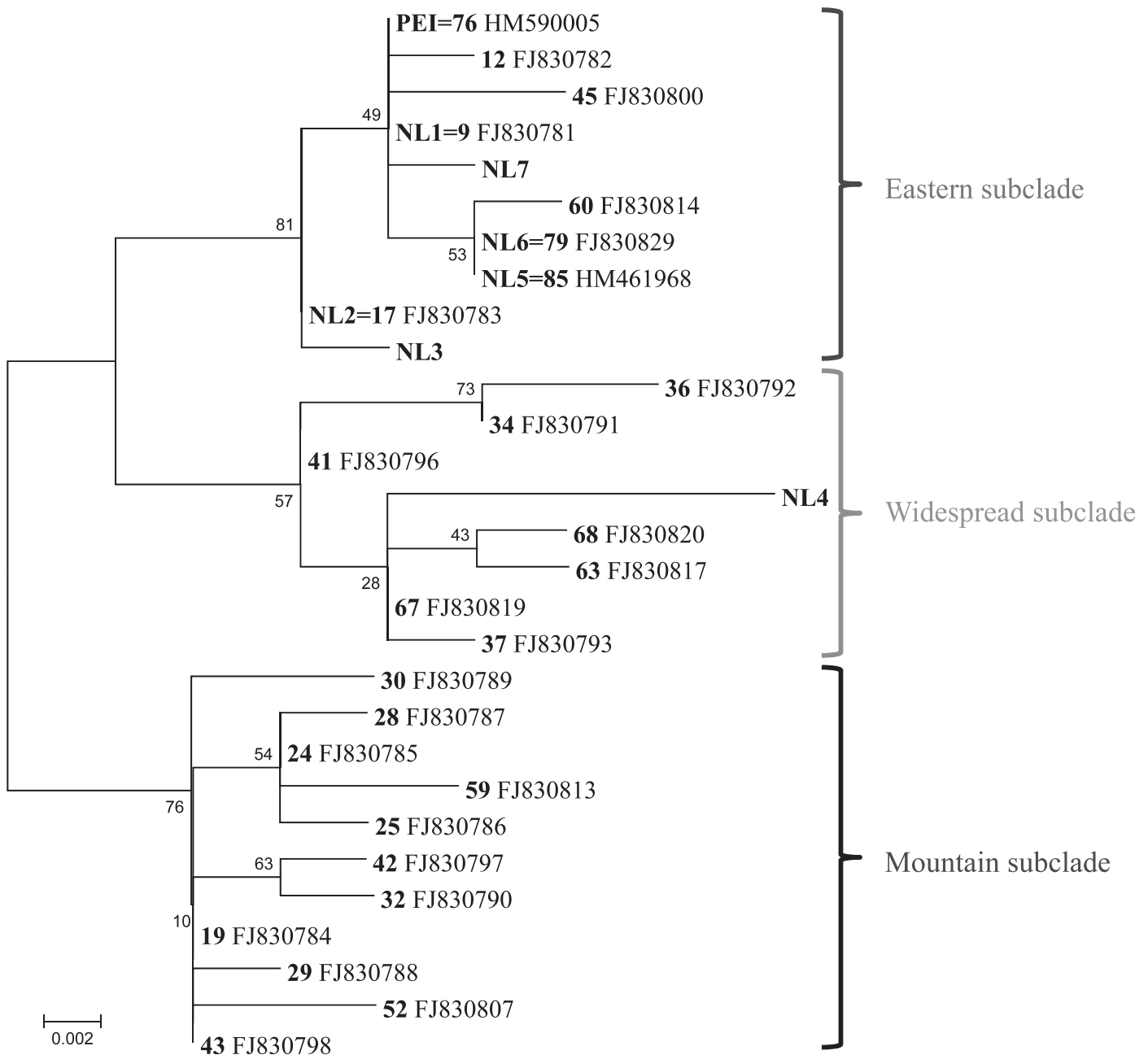
Phylogenetic relationships among Nearctic clade haplotypes depicted in Fig. 2 show that all haplotypes except NL4 are part of the Eastern subclade of Aubry et al. (2009), whereas NL4 belongs to the Widespread subclade. Common haplotypes NL1 and NL2 were shared among all localities studied here, whereas the 3rd most frequent haplotype (PEI), which differs from NL1 by only 1 insertion, was found only in Prince Edward Island. NL3 was found in 5 of the 8 localities; and the remaining rarer haplotypes NL4–NL7 were restricted to 2 or 3 localities. NL1 and NL2 are both relatively basal within the Eastern subclade of the phylogeny, analogous to their central locations in the median-joining network (Fig. 3) among haplotypes from this study and the Eastern and Widespread

subclade haplotypes of Aubry et al. (2009). The median-joining network (Fig. 3), depicting the haplotype frequencies in Newfoundland, Labrador, and PEI, supports the common widespread haplotypes as basal.

Haplotypic diversity (Table 1B) was similar across all locations (~60%) except Prince Edward Island, where only 2 haplotypes were found among 24 individuals; it was highest in the Avalon Peninsula sample and lowest in the Central region. By way of contrast, nucleotide diversity (Table 1B) was markedly higher in the Northern and Avalon Peninsula samples (0.0062 versus ~0.0025), consistent with the presence of the divergent haplotype NL4 in those locations. There were no obvious geographic trends to the measures of diversity, although they were generally lower in the Central and South Coast regions.

Overall  $F_{ST}$  among all 8 red fox sampling localities was 0.257 ( $P = 0$ ) calculated from haplotype frequencies and 0.252 ( $P = 0$ ) calculated from pairwise differences, indicating substantial population genetic structure. Among the 6 island Newfoundland localities these estimates remained significantly positive but were reduced to 0.0796 ( $P = 0$ ) and 0.0599 ( $P = 0.0068$ ). Pairwise estimates of population differentiation ( $F_{ST}$  and  $d_A$  [Table 2]) reflect this, indicating a high degree of differentiation of the Prince Edward Island sample and also significant differentiation of the North East Coast sample from the Northern Peninsula and Central regions ( $F_{ST}$ ) and Northern Peninsula from Central ( $d_A$ ). Many of the other estimates of  $F_{ST}$  or pairwise differentiation were also fairly high (0.05–0.23) but not significant after correction for multiple comparisons (i.e.,  $P > \sim 0.003$ ). The level of differentiation among localities is depicted in the UPGMA dendrogram of  $d_A$  estimates (Fig. 4); clearly Prince Edward Island is quite distinct, whereas the Northern Peninsula and Labrador are similar to each other, as are the South and West Coasts, and Central and Avalon Peninsula regions, and all of the latter to the North East Coast.

Tajima's  $D$  within insular Newfoundland was negative but not significantly different from 0 ( $D = -0.95$ ;  $P = 0.183$ ); results for Fu's  $F_S$  were similar ( $F_S = -0.17$ ;  $P = 0.511$ ). When the localities were examined separately Tajima's  $D$  was typically negative (except for the South Coast sample) but not significantly so ( $P > 0.240$ ) with the marginal exception of the Avalon Peninsula sample ( $P = 0.083$ ). When localities were examined separately Fu's  $F_S$  statistic also was typically negative but never significantly different from 0 (all  $P > 0.264$ ). The mismatch distribution for the pooled insular Newfoundland samples was significantly different from expected under a model of demographic expansion ( $P = 0.0346$ ), and the raggedness index ( $RI$ ) also was significantly larger than expected under demographic expansion ( $RI = 0.105$ ;  $P = 0.0029$ ). However, a model of demographic expansion could not be rejected for most regions investigated individually nor were raggedness indexes larger than expected ( $P > 0.05$ ; except the West Coast population, for which  $P = 0.0442$  for the model and  $P = 0.015$  for the raggedness index). Thus, although the pattern of results



**FIG. 2.**—Maximum-likelihood phylogeny inferred using the Tamura 3-parameter model with invariant sites among haplotypes identified in this study (labeled NL and PEI) and those in the Nearctic clade (Eastern, Widespread, and Mountain subclades) from Aubry et al. (2009). Note that NL1 is the same as haplotype 9, NL2 as 17, and NL6 as 79 of Aubry et al. (2009), whereas PEI matches haplotype 76, and NL5 matches haplotype 85 of Statham et al. (2012). PEI differs from NL1 by 1 insertion–deletion (indel) and NL5 differs from NL6 by 1 indel; these haplotypes appear identical because MEGA treated indels as missing data. Numbers at nodes represent percentage support from 1,000 bootstrap replicates, and the inset shows the distance scale.

suggests population expansion, the statistical support for it is equivocal, perhaps reflecting the evolving population structure in the time since recolonization. The  $\tau$  parameter of the putative demographic expansion was 1.000 with 95% confidence limits of 0.748–1.459. Using the estimated mutation rate  $\mu$  of 1% per 56,803 years, and the relationship  $\tau = 2\mu t$  (Rogers and Harpending 1992), this corresponds to a

time of expansion of  $t$  of  $\sim 8,633$  years ago with a range of 6,457–12,595 years ago. The rho estimate of age was similar at 8,986 years. Notably, the rho estimate of age is considered insensitive to population demography (Saillard et al. 2000), but because we do not have clear statistical support for expansion, the confidence interval remains uncertain, as does that around the  $\tau$  parameter.

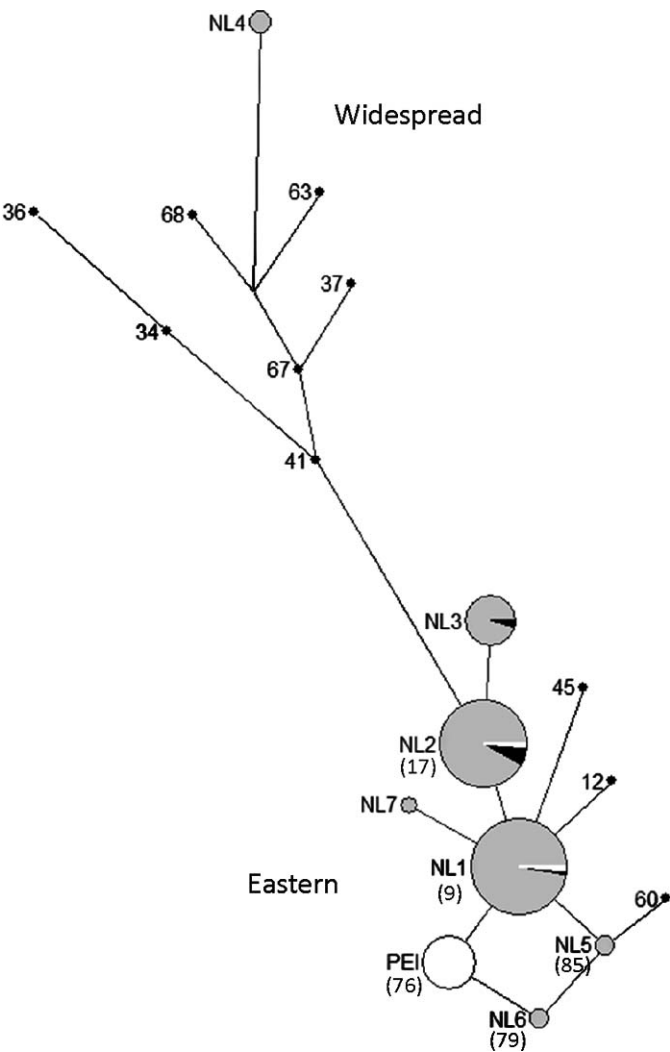


FIG. 3.—Median-joining network of the 8 control region haplotypes found in this study among 189 Newfoundland, Labrador, and Prince Edward Island red foxes (*Vulpes vulpes deletrix*), as well as additional haplotypes in the Eastern and Widespread subclades of Aubry et al. (2009). For the haplotypes found in this study, the sizes of the circles correspond to the number of individuals, and gray, white, and black to Newfoundland, Prince Edward Island, and Labrador, respectively. Numbers in parentheses indicate the Aubry et al. (2009) or Statham et al. (2012) equivalent. The length of the branches corresponds approximately to the number of mutational differences between haplotypes.

DISCUSSION

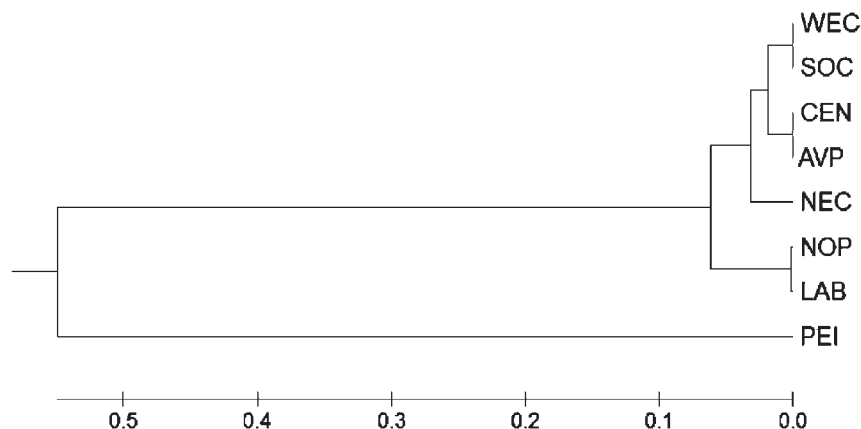
Herein we characterized genetic variability of the mitochondrial control region in red foxes from 6 geographic regions in Newfoundland to make inferences about postglacial recolonization history, genetic distinctiveness, diversity, and population structure of this subspecies. We show that mitochondrial control region diversity is weakly consistent with recent postglacial expansion to the island and colonization via a northern route, but do not find in the mtDNA any substantial evidence to support the genetic subdivision of Newfoundland foxes relative to the mainland.

*Postglacial recolonization history of red foxes in Newfoundland.*—With respect to the continental phylogeography of red foxes in North America as investigated by Aubry et al. (2009), the control region haplotypes found in Newfoundland, Labrador, and Prince Edward Island in this study are primarily Eastern subclade haplotypes, with 1 representative of the Widespread subclade. This is as expected from the work of Aubry et al. (2009) and lends additional support to the 2-refugia hypothesis tested therein, which postulates that central and eastern populations of red foxes derived from a southern refugium distinct from western montane populations. The additional haplotypes found here may simply not have been sampled by Aubry et al. (2009), may have increased in frequency in the locations sampled here by genetic drift, or may have evolved regionally. Indeed the 3rd most frequent Prince Edward Island haplotype in this study was identified by Statham et al. (2012) in 8 foxes in the southeastern United States. The next most frequent haplotype, NL3, widespread in Newfoundland, may have increased in frequency on the island due to drift and be rare enough outside the island not to have been sampled previously. Another possible explanation for the new haplotypes found here is that we have sampled fox farm escapees or their descendants; in fact our haplotype NL5 matches a farmed fox haplotype of Statham et al. (2012).

The pattern of mitochondrial control region diversity in Newfoundland red foxes demonstrates the signature of their recent postglacial colonization history on the island. The dominance of the median-joining network by 2 widespread, common haplotypes and several closely related, less-frequent, and geographically restricted haplotypes is typical of recently

TABLE 2.—Measures of pairwise population differentiation among red foxes sampling localities of foxes (*Vulpes vulpes deletrix*), inferred from control region sequences. Below diagonal:  $F_{ST}$ ; above diagonal:  $d_A$ . Values in boldface type are significant at  $\alpha = 0.05$  after sequential Bonferroni correction for multiple comparisons. Locality abbreviations are as in Table 1.

	NOP	WEC	NEC	CEN	SOC	AVP	LAB	PEI
NOP		0.085	0.125	<b>0.211</b>	0.068	0.110	0.003	<b>1.367</b>
WEC	0.008		0.056	0.029	−0.033	0.044	0.041	<b>1.034</b>
NEC	<b>0.168</b>	<b>0.107</b>		0.060	0.076	0.062	0.089	<b>1.081</b>
CEN	0.163	0.062	0.049		0.027	−0.038	0.217	<b>0.819</b>
SOC	−0.041	−0.059	0.129	0.070		0.048	0.019	<b>1.068</b>
AVP	0.115	0.056	0.054	−0.013	0.048		0.255	<b>0.886</b>
LAB	−0.062	0.005	0.161	0.230	−0.038	0.174		<b>1.434</b>
PEI	<b>0.593</b>	<b>0.555</b>	<b>0.527</b>	<b>0.637</b>	<b>0.699</b>	<b>0.633</b>	<b>0.717</b>	



**FIG. 4.**—Unweighted pair-group method using arithmetic averages (UPGMA) dendrogram of  $d_A$  distances among populations of red foxes (*Vulpes vulpes deletrix*) in Newfoundland, Labrador, and Prince Edward Island, using control region sequences. Location abbreviations are given in Table 1. The probability associated with the distance between NOP/LAB and WEC/SOC/CEN/AVP/NEC is  $P = 0.0029$ , between NEC and WEC/SOC/CEN/AVP is  $P = 0.0127$ , and between WEC/SOC and AVP/CEN is  $P = 0.0303$ , based on 1,000 replicates in Arlequin.

founded populations. Low levels of nucleotide diversity but high haplotype diversity represent the signal of past genetic bottleneck events, where most haplotypes became extinct followed by a population expansion (Avise 2000; e.g., Ojeda 2010). The pairwise mismatch distribution among control region sequences within the 6 island localities is suggestive of a demographic expansion  $\sim 8,600$ – $9,000$  years ago, corresponding well with the expected time frame for retreat of the last glacial ice from Newfoundland and indeed the region in general. The presence of the more divergent Widespread subclade haplotype NL4, albeit at low frequency, may inflate this time estimate somewhat, rendering the expansion slightly more recent. Tajima's  $D$  and Fu's  $F_S$  were negative, albeit not significantly so, consistent with a recent population expansion. The population genetic data obtained here, the first for this species from this part of its range, thus clearly supports the status of the red fox as one of Newfoundland's indigenous mammals (Dodds 1983). We cannot say with certainty whether the putative population expansion occurred after foxes had colonized the island or before, as part of the general range expansion northward, but it seems likely to have been before, as the slightly longer time frame indicated by the rho estimate suggests.

Relationships among populations, patterns of diversity, and population structure suggest a possible route of colonization to the island. The close relationship between Labrador and the Northern Peninsula population establishes the latter as a likely point of entry, followed by expansion to the Northeast Coast, then the Avalon, Central, South, and West Coast regions. Lower measures of both haplotypic and nucleotide diversity in the southern part of the island (West Coast, South Coast, and Central regions) could suggest that these locations were colonized more recently, or that they maintain smaller effective population sizes. The Avalon Peninsula diversity is unexpectedly high under the scenario presented, partly due to the presence of the divergent Widespread subclade haplotype NL4

in this location. One possible explanation for this is a secondary introduction into this region, such as fox farm escapement.

One of the questions pertaining to indigenous mammalian diversity in Newfoundland has been the putative source or route of recolonization from the neighboring mainland. These are called the Northern and Southern routes by Wilkerson (2010). In the Northern Route scenario, animals in the southern refugium migrated northward as the ice sheet retreated, into Quebec and then Labrador, where they crossed the Strait of Belle Isle to the island. The Strait of Belle Isle is  $\sim 18$  km across and remains frozen with sea ice for much of the winter. In the Southern Route scenario, additional refugia existed along the east coast of North America, including the now submarine banks associated with Sable Island and the Grand Banks (Pielou 1991). Under this hypothesis, animals could have populated Newfoundland and the other Atlantic provinces from these coastal refugia when the ice retreated. The evidence here supports the Northern Route, because Newfoundland red foxes from the Northern Peninsula share genetic affinity with Labrador foxes. The Prince Edward Island sample is strongly differentiated here from all other locations due to the presence of a distinct haplotype that differs from the common haplotype NL1 by a single insertion. Altogether, the genetic evidence is mounting that Newfoundland's larger mammalian fauna arrived via Labrador or Quebec, as has been also discussed for black bears (*Ursus americanus hamiltoni*—Paetkau and Strobeck 1996; Marshall et al. 2011) and woodland caribou (*Rangifer tarandus caribou*—Wilkerson 2010).

*Population structure and subspecies status of Newfoundland red foxes.*—Both measures of differentiation ( $F_{ST}$  and  $d_A$ ) were significantly greater than 0 in all comparisons with Prince Edward Island due to the prominence of the private haplotype in that location. In Newfoundland, only Northern Peninsula and Central are significantly differentiated using  $d_A$ , whereas North East Coast is significantly differentiated from both West

Coast and Northern Peninsula with  $F_{ST}$ . Many intrainland pairwise measures are moderate to high but not significant, whereas others ( $d_A$ : South Coast–West Coast and Avalon Peninsula–Central;  $F_{ST}$ : South Coast–West Coast–Northern Peninsula–Labrador) are approximately 0. This difference between the 2 measures in grouping localities probably reflects the dominance of South Coast and West Coast by haplotypes 1 and 2 and their consequently lower levels of diversity; the presence of haplotype 6 in West Coast and Central may serve to group those localities in the  $d_A$  dendrogram.

The lack of strong significant population differentiation among pairs of island localities is consistent with the recent postglacial history of the island and the insensitivity of mtDNA to small-scale or recent population events, a conclusion also drawn by Kirschning et al. (2007) in a study of Serbian red foxes over a similar geographical scale, which failed to identify genetic evidence for 2 barriers to gene flow (the Danube and Tisza rivers). On the other hand, Inoue et al. (2007) were able to demonstrate genetic evidence of an older, effective barrier to gene flow in Japan, corresponding to Blakiston's Line, formed in the middle Pleistocene. Similarly, in North America, Aubry et al. (2009) reported high and significant measures of differentiation on a continental scale corresponding to different refugial clades. However, high adaptability and dispersal capabilities of red foxes may preclude the development of significant structure even over the longer term, as was shown by Teacher et al. (2011) for European red foxes. Microsatellites or other fast-evolving loci that could reveal more subtle or recent patterns of structure remain to be investigated.

Between Labrador and Newfoundland,  $F_{ST}$  and  $d_A$  were numerically moderate to high in comparisons to Avalon Peninsula, Central, and North East Coast samples but were in no cases significantly different from 0. Hence, the mtDNA data provide no genetic evidence to support the subspecific status of Newfoundland red foxes. Similarly, neither Newfoundland caribou (Wilkerson 2010) nor black bears (Paetkau and Strobeck 1996) could be conclusively differentiated from Labrador samples on the basis of mtDNA. Nonetheless, for Newfoundland marten (*Martes americana atrata*) microsatellite markers revealed strong differentiation of the island population relative to the mainland (Kyle and Strobeck 2003), despite lack of mtDNA structuring (Hicks and Carr 1992). Microsatellites again may reveal a different perspective for red foxes, although red foxes could travel to the island on ice bridges or drifting ice from Nova Scotia or Labrador, as was the case for coyotes and still is for arctic fox (*Vulpes lagopus*—Blake 2006), which would maintain ongoing gene flow even at finer scales.

**Future directions.**—One of the motivations for our work with Newfoundland red foxes is to establish contemporary baseline levels of genetic diversity at neutral and putatively adaptive genetic loci for continued investigations of population demography, genetic structure, and adaptive genetic diversity in this population. Of interest are potential changes in red fox

population sizes with respect to future expansion of the coyote population, and also the capacity for red foxes to spread French heartworm beyond its currently restricted geographic range in North America. With their potential for dispersal, even when densities are low (Allen and Sargeant 1993), foxes can spread diseases over long distances. The lack of strong evidence of mtDNA structuring in red foxes in Newfoundland suggests that barriers to host gene flow are not the cause of the geographically restricted incidence of *A. vasorum*. Because there is no identifiable genetic barrier in the fox population, the potential for future spread of the parasite via movement to mainland regions of North America exists and points to the need for continued genetic monitoring of Newfoundland's red fox population, although climate, intermediate host availability, or other factors also may influence movement. Nonetheless, we note that the historical and maternal focus of mtDNA markers emphasize the need for finer scale assessments of population structure with more highly polymorphic nuclear markers such as microsatellites to track such movement patterns.

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