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RESEARCH ARTICLE

Investigating genetic introgression from farmed red foxes into the wild population in Newfoundland, Canada

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Abstract Fur-animal farms can affect the genetic constitution of wild conspecifics through escape and subsequent interbreeding. We studied this problem in red foxes (*Vulpes vulpes*) on the Canadian island of Newfoundland, where a large commercial fox farm (the only large farm on the island) has operated adjacent to the native wild red fox population for >30 years. To test for gene flow from these fur-farm foxes into the wild population, we compared mitochondrial DNA (mtDNA) sequences and nuclear microsatellite genotypes (21 loci) of 93 individuals from the fox farm to those of 79 modern wild foxes sampled from across the island. For reference, we also included 12 historical museum specimens of wild eastern Canadian red fox, all of which were sampled before the introduction of fur farming in the region. Many mtDNA haplotypes were shared among contemporary farmed and wild foxes and the historical eastern Canadian samples, as expected based on the eastern Canadian origin of fur-farming. However, only the fur farm additionally contained haplotypes originating from other parts of North America. More significantly, microsatellite markers, which reflect contemporary gene flow, indicated strong differentiation ($F_{ST} \geq 0.14$, $P < 0.001$) between fur-farm and wild foxes (including the historical samples) and little to no gene flow between them. Admixture and principle components analyses similarly supported clear separation of fur-farm and wild red foxes. Together, these findings indicate that the presence of a large red fox fur farm had little, if any, effect on the genetic constitution of the native wild population in Newfoundland. Tight biosecurity (lack of escapees) or failure of captive-reared foxes to establish in the presence of native wild foxes could explain these findings.

Keywords Fur farm · Microsatellites · mtDNA · PCA · Red fox · *Vulpes vulpes*

Introduction

Genetic admixture between captive-bred organisms and their wild conspecifics is a problem of considerable interest to conservation science as it can threaten the genetic integrity and long-term viability of natural populations (Laikre et al. 2010; Champagnon et al. 2012; Beauclerc et al. 2013; Gil-Sánchez et al. 2015; Le Roux et al. 2015). Such threats potentially include introgression of detrimental genetic variants accumulated in captivity, disruption of locally adapted gene complexes, and, in the most extreme cases, extinction by genetic replacement (Rhymer and Simberloff 1996; Allendorf et al. 2001).

Escapement and release from industrial farms has proven an especially common precedent to invasion by captive-bred animals (Carter and Leonard 2002; McGinnity et al. 2003; Hammershøj et al. 2005; Kauhala and Kowalczyk 2011). In areas where farms occur within the ranges of wild conspecifics, they have the potential to serve

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as continuous sources of introduction and, consequently, increase the likelihood of extensive introgression (Bennett et al. 2010). Genetic introgression into native populations by commercially farmed animals has been documented in birds (Gering et al. 2015), ungulates (Goedbloed et al. 2013; Mager et al. 2013), fish (Bourret et al. 2011), and carnivores (Noren et al. 2005; Kidd et al. 2009; Sacks et al. 2011). On the other hand, the presence of fur farms does not necessarily entail escape, successful establishment in the wild, or genetic integration with existing wild populations (Beauclerc et al. 2013; Gil-Sánchez et al. 2015; Le Roux et al. 2015). The magnitude of the problem apparently depends on the extent of biosecurity of specific farms (i.e., limiting escapement or releases), ecology of the system, and size of the invaded population (Wolf et al. 2001; Beauclerc et al. 2013).

One widely distributed species that is of particular concern with respect to establishment and admixture of industrial farm escapees is the red fox (*Vulpes vulpes*). Red foxes are among the most commonly farmed furbearers in the world, second only to American mink (*Neovison vison*), with farms distributed throughout the northern hemisphere. The first successful red fox fur farms, from which most contemporary fur-farm populations originated, were established on Prince Edward Island, Canada (Fig. 1) in 1894 (Petersen 1914). These farms were initially stocked with wild eastern Canadian individuals (including from Newfoundland), supplemented with individuals imported from Alaska (Balcom 1916; Anonymous 1917; Laut 1921), and at some point incorporated individuals from the Cascade Range in Washington (Statham et al. 2012; Sacks et al. 2016). Selection for desired traits including fur quality, tameness, and successful mating in captivity made the sale of breeding stock a highly profitable business in the early 20th century (Anonymous 1917). Consequently, modern fur-farm foxes all over the world share a common genetic signature, originating from North America, primarily eastern Canada (Statham et al. 2011, 2012).

In the United States, where native and fur-farm fox dynamics previously have been studied, wild populations established from fur farms have impacted native foxes through varying degrees of introgression (Sacks et al. 2011; Statham et al. 2012; Kasprowicz et al. 2016). The crash of fur prices and subsequent industry decline in the southern U.S. by the 1970s presumably resulted in widespread releases or escapes of fur-farm foxes, establishing wild populations in many parts of the country. These nonnative populations tended to become established in urban and agricultural areas or otherwise human-impacted landscapes in much of the U.S. (Aubry 1984; Lewis et al. 1993; Sacks et al. 2011; Statham et al. 2011; Kasprowicz et al. 2016). In contrast, native populations in the U.S. tend to be narrowly restricted to remote, high-elevation habitat of the western mountains or, in an anomalous case, the Sacramento Valley of California (Sacks et al. 2010; Volkmann et al. 2015). One hypothesis put forward to explain the observed distribution of fur-farm relative to native red foxes is that captive-bred populations become established primarily in areas absent of native red foxes, which preferentially mate with other native individuals and hold the competitive advantage over nonnative foxes (Sacks et al. 2011). If true, this contrasts with other North American carnivore species, such as wild American mink, which readily interbreed with fur-farm escapees in the immediate vicinity of farms (Kidd et al. 2009; Beauclerc et al. 2013).

In the present study, we used molecular markers to investigate the putative establishment or introgression of captive-bred red foxes from a large, currently active red fox farm in Newfoundland, Canada into the wild native population. In contrast to most lower-latitude regions of North America, the island of Newfoundland has supported an abundant population of native red foxes since the Last Glacial Maximum (Langille et al. 2014). Newfoundland also maintains a thriving red fox fur-farming industry, which has existed for the past century and continued to operate successfully during the past several decades (Anonymous 1917; Jeffery et al. 2004). Fur-farming on Newfoundland is largely contained on the southern end of the Island on the Avalon Peninsula (Fig. 1). The insular nature of Newfoundland, coupled with the localization of the contemporary fur-farm industry, made this system ideal to investigate the extent of introgression and spread of nonnative alleles. For example, if the wild population was subject to a steady stream of nonnative alleles over the past several decades, admixture analysis would be expected to show successively higher levels of admixture with increasing proximity to the Avalon Peninsula. If, on the other hand, introgression was rare, we would expect to observe admixed individuals only in close proximity to fur-farm locations or not at all.

We compared DNA samples from a large commercial fox farm on the Avalon Peninsula in Newfoundland to wild red foxes sampled at distances ranging from <50 km to 500 km from that location. We first sequenced mitochondrial DNA (mtDNA) to test the presence in wild foxes of maternal ancestry known to be associated with fur farms but foreign to wild eastern Canadian populations (e.g. from Alaska or the western U.S.). Although such phylogenetically divergent haplotypes would provide unambiguous evidence of fur-farm ancestry, many fur farm haplotypes are native to eastern Canada and would thus provide little information on gene flow from captive to wild populations. Therefore, we also used 21 nuclear microsatellites to assign individuals to putative populations and assess population structure. To test for the possibility that modern wild red foxes contained significant introgression from captive-

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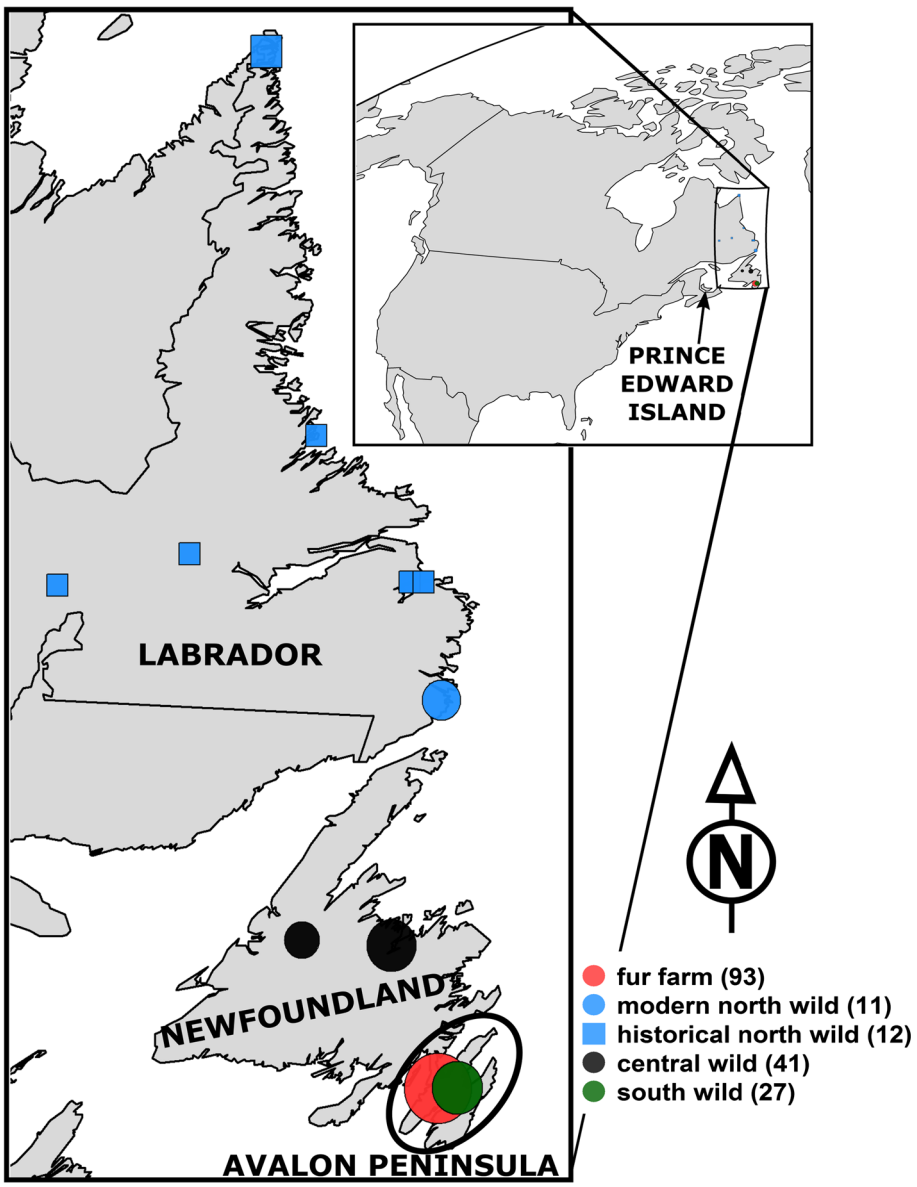

Fig. 1 Distribution of sampling sites of wild and fur-farm *red foxes* within the study area, spanning Labrador and Newfoundland in Canada. Each point represents a unique sampling location; sizes of the *circles* relate to the sample sizes, which are indicated in *parentheses* in the *legend*. Prince Edward Island is shown on the *inset*, and the Avalon Peninsula (location of fur farm and south wild groups) is circumscribed for reference. (Color figure online)

reared foxes not sampled in the present study, we also compared their mtDNA and microsatellites to those of 12 historical museum specimens of wild eastern Canadian red fox that were sampled during 1882–1915, before the expansion of fur farming to the region.

Materials and methods

Study site and sample collection

We collected 172 modern red fox skin samples for genetic analyses, including 93 from a fur farm and 79 from wild-caught foxes. We also used historical red fox samples (1882–1915) from the National Museum of Natural History ($n = 12$). All skin samples were removed from pelts that had already been prepared for sale by fur-ranchers or trappers; no foxes were captured or killed for our study. Fur-farm samples were sourced from a commercial fur-farming operation on the Avalon Peninsula (M & E Fur Farm) in southern Newfoundland, Canada ("fur farm" $n = 93$). This fur farm was the only large operation on Newfoundland (producing approximately 1500 pelts per year), although several small operations containing one or two breeding pairs were scattered across the island since the 1980s; we had no specific information on the smaller farms or the origins of their foxes. The M & E Fur Farm obtained its original breeding stock ($\sim$20 pairs) in the 1990s from a Norwegian fur-farming operation, although these pairs were presumed (and confirmed in this study) to have originated in North America (Statham et al. 2011, 2012). Wild-caught fox samples originated from

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southern Labrador ("modern north wild" $n = 11$), northern/central Newfoundland ("central wild" $n = 41$), and on the Avalon Peninsula in southern Newfoundland ("south wild" $n = 27$; Fig. 1). Additional information for all samples is available in Online Resource 1.

We extracted genomic DNA from modern skin clips using DNeasy Blood and Tissue kits (Qiagen Inc.) following the manufacturer's protocols. Genomic DNA and modern skin samples were archived at the Mammalian Ecology and Conservation Unit laboratory at the University of California, Davis. We extracted DNA from maxilloturbinal bone in a laboratory dedicated to historical/ancient DNA using a phenol–chloroform extraction protocol as described previously (Wisely et al. 2004; Aubry et al. 2009).

MtDNA amplification and analysis

We sequenced mtDNA both to confirm the presumed North American ancestry of the fur-farm foxes as well as to help assess maternal gene flow from captive to wild populations. We amplified two regions of mtDNA: one 354-bp segment of the cytochrome *b* gene (primers RF14724 and RF15149; Perrine et al. 2007) and one 343-bp segment of the D-loop (primers VVDL1 and VVDL6; Aubry et al. 2009). The PCR mixtures and thermal cycle conditions for both markers were described previously (Perrine et al. 2007; Aubry et al. 2009). We purified PCR products and sequenced them using ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and an ABI 3730 capillary sequencer (Applied Biosystems) for cytochrome *b* (sequenced from primer RF14724) and D-loop (sequenced from primer VVDL1), and trimmed sequences to cover the 354 and 343 bases, respectively, homologous to those used previously (e.g., Aubry et al. 2009). For museum samples, we used mtDNA sequence data available for these samples from a previous study (Aubry et al. 2009).

We converted chromatograms to fasta format based on quality using default parameters in TraceTuner v4.0 (Denisov et al. 2004). For both cytochrome *b* and D loop independently, we then created a basic local alignment search tool (BLAST, Altschul et al. 1990) database of all known red fox haplotypes available on GenBank, performed a command-line BLAST search against this database to determine haplotypes for each sample, and parsed the results using a custom Bash script (https://github.com/zlounsberry/UCDMammalianEcologyAndConservation/tree/master/LounsberryEtAl2016_NewfoundlandRF/AB1_To_Fasta). We then compared sequences to their top BLAST hits in MEGA6 (Tamura et al. 2013) and checked any discrepancies manually using chromatogram quality in Bioedit (Hall 1999). We finalized haplotypes when sequences had no bases mismatching those of reference haplotypes, except in cases of novel unambiguous haplotypes. After assigning each individual a cytochrome *b* and D-loop haplotype, we combined haplotypes for each individual into a single concatenated haplotype. Using these concatenated sequences and omitting samples that were missing data at either mtDNA marker, we constructed a haplotype network based on frequencies using the 'pegas' package in R (Paradis 2010).

Microsatellite amplification and analysis

We genotyped modern individuals at 21 nuclear microsatellite loci—Vv-AHT133, Vv-AHT140, Vv-c01.424, Vv-RF08.618, Vv-RFCPH2, Vv-RF2001, Vv-FH2004, Vv-FH2010, Vv-RF2054, Vv-FH2088, Vv-FH2289, Vv-FH2328, Vv-FH2380, Vv-RF2457, Vv-AHTh171, Vv-CPH11, Vv-CPH18, Vv-CXX-468, Vv-CXX-602, Vv-FH2848, Vv-REN54P11—using previously published primers (Wandeler and Funk 2006; Moore et al. 2010; Sacks et al. 2010). The PCR mixtures for modern samples contained 1 μL of DNA diluted 1:10 in sterile water. We used fluorescently labeled (6-FAM, VIC, NED, PET; Applied Biosystems) forward primers and performed PCR in three multiplex groups as described by Moore et al. (2010) using the Qiagen multiplex PCR kit with "Q-solution" and a thermal profile according to the manufacturers recommended protocols; annealing temperature was 58 °C. We electrophoresed PCR products along with an internal size standard Genescan 500 LIZ (Applied Biosystems) on an ABI 3730 capillary sequencer (Applied Biosystems). Historical foxes were genotyped at a subset of 14 loci that have been used previously in low-quality museum samples (Vv-AHT133, Vv-AHT140, Vv-c01.424PET, Vv-RF08.618, Vv-RFCPH2, Vv-RF2001, Vv-FH2004, Vv-FH2010, Vv-RF2054, Vv-FH2088, Vv-FH2289, Vv-FH2328, Vv-FH2380, Vv-RF2457; Sacks et al. 2010). Each museum sample was amplified in a 17-μL reaction containing 1× Abgene PCR buffer IV, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1× bovine serum albumin, 0.15–0.80 μM PCR primers, and 0.7 units of Abgene Taq polymerase. Template DNA was used at full concentration and denatured for 10 min at 95 °C before being mixed with PCR reagents (while held at 85 °C for 10 min), and then run with 33 cycles of 95 °C for 60 s, 62 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 30 min.

We scored alleles using STRand software (Toonen and Hughes 2001). With the exception of the historical samples, which were included regardless of missing data, individuals missing a genotype at more than one locus were excluded from subsequent analyses. For each locus, we calculated observed and expected heterozygosities and performed a test for population differentiation (F_{ST}) in Arlequin version 3.5 (Excoffier and Lischer 2010). We tested for locus-by-locus deviations from Hardy–Weinberg

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equilibrium using a probability test (F_{IS}, Robertson and Hill 1984) and from gametic equilibrium using a log-likelihood ratio statistic in Genepop On The Web (http://genepop.curtin.edu.au/; Raymond and Rousset 1995).

To assess levels of admixture between fur-farm and wild foxes, as well as among geographic samples of wild foxes, we used two independent microsatellite-based approaches. First, we used a Bayesian clustering approach with an admixture model and allele frequencies correlated among populations implemented in Structure v2.3.4 (Pritchard et al. 2000; Falush et al. 2003) to assign individuals to genetic clusters (K). Using 1,100,000 MCMC iterations (discarding the first 100,000 as burn-in), we tested 3 iterations of each value of $K = 1$ through $K = 10$ and used output files to determine the most likely value of K using the Delta-K method (Evanno et al. 2005) implemented in the web portal STRUCTURE HARVESTER (Pritchard et al. 2000; Earl and von Holdt 2012). To assess confidence in individual assignment, we also estimated a 95% credibility interval for each assignment. We performed these analyses on the modern wild and fur-farm groups together using all 21 markers, as well as on both groups separately to assess levels of within-group clustering. Because historical samples were only genotyped using 14 of the 21 total markers used in this study, we conducted a second set of analyses limited to these 14 markers and including historical samples.

To visualize relationships among multilocus genotypes in 2-dimensional space, we also used a distance-based approach to investigating genetic clustering. We analyzed global diversity using the first two axes of a principal component analysis (PCA) implemented in the 'adegenet' package in R (Jombart 2008). This approach was also useful to supplement Bayesian clustering because it did not impose admixture models or assumptions of Hardy–Weinberg equilibrium on the focal population(s). We performed PCA on each of the same sub-groups that we used for the Bayesian clustering (i.e., modern-only with 21 loci and both modern and historical samples using 14 loci).

Results

mtDNA amplification and analysis

We successfully amplified both of our target mtDNA regions in modern samples from the fur farm ($n = 92$) and the wild ($n = 78$), shown in Table 1 along with 8 haplotypes from historical foxes from the wild. All 10 haplotypes observed in the modern sample were of North American origin, including three (F-17, F-79, F-9) that also occurred in the historical sample (Fig. 2). The most common haplotype found in the modern fur-farm and wild groups (F-17) also was a common haplotype in the historical eastern Canadian samples (Fig. 2). Two haplotypes, O-24 and E-86, were found exclusively in the fur farm group. The O-24 haplotype was previously shown to be native to Washington State (Aubry et al. 2009; Sacks et al. 2010). The E-86 haplotype, ultimately clustering with other eastern haplotypes (in the "eastern subclade;" Aubry et al. 2009), had been reported previously both from another fur farm (Statham et al. 2011, 2012) and within a population from the eastern U.S. that was partly composed of fur-farm ancestry (Kasprowicz et al. 2016). We discovered two novel cytochrome *b* haplotypes (F6, F7) and two novel D-loop haplotypes (274, 275) in wild foxes, all of which clustered in the eastern subclade (Genbank accession Nos. KX766409–KX766412).

Microsatellite analysis

We successfully amplified ≥20 microsatellite markers in 147 modern foxes, 75 from fur farms and 72 from the modern wild group, as well as 8–14 loci in 9 historical museum specimens (Table 2; Online Resource 1). Among modern samples (using all 21 loci), Bayesian clustering showed the strongest support for a split between the fur-farm and wild populations ($K = 2$; Fig. 3a). At larger K values (e.g., $K = 3$–10), only the split between fur-farm and wild groups was consistent across levels of K, with no signals of admixture between these groups (Online Resource 2). The 95% credibility intervals of wild foxes overlapped 100% assignment to the wild cluster in all but one case, which, after correction for multiple comparisons (i.e., n = 72), was not statistically meaningful. Moreover, this individual was from central Newfoundland, rather than southern Newfoundland, where true admixture would have been most likely.

Among the combined modern and historical samples (14 loci), we observed similar results. The most well-supported value for K was $K = 2$, and all 95% credibility intervals overlapped 0% (fur-farm foxes) or 100% (wild foxes) assignment to the wild cluster, consistent with no admixture between fur-farm and wild populations (Fig. 3b). At higher values of K, historical individuals tended to group with foxes from Labrador (the modern northern wild group), consistent with where they were collected (Online Resource 2).

The PCA showed consistent clustering between fur-farm and wild foxes, providing the same qualitative results as the admixture analysis (Fig. 4). The genetic distance between fur-farm and wild foxes was highly significant both in the 21-locus modern-only sample ($F_{ST} = 0.15$, $P < 0.001$) and the 14-locus sample that included historical specimens ($F_{ST} = 0.14$, $P < 0.001$).

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Table 1 Mitochondrial cytochrome *b* and D-loop (concatenated) haplotype frequencies in fur-farm and wild foxes from Newfoundland, Canada

Haplotype	n	E-86	F-17	F-274	F-275	F6-17	F7-9	F-79	F-85	F-9	O-24
fur farm	92	2	86	–	–	–	–	–	2	–	2
modern north wild	10	–	4	1	–	–	–	1	–	4	–
historical north wild[a]	8	–	3	–	–	–	–	1	–	4	–
central wild	41	–	7	6	3	4	1	1	–	19	–
south wild	27	–	20	–	–	–	–	–	5	2	–
total	178	2	120	7	3	4	1	3	7	29	2

[a] Four of the 12 historical specimens used in this study had incomplete sequences and are not shown here, but partial sequences were consistent with the same three haplotypes represented here

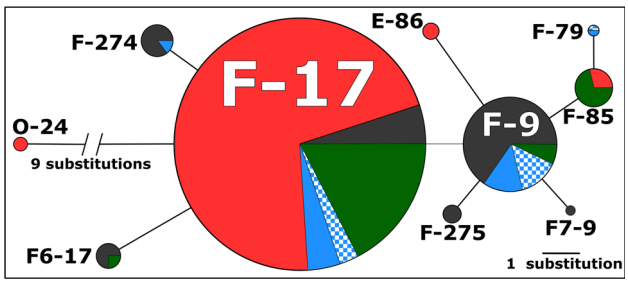

Fig. 2 Haplotype network based on frequencies showing relationships between concatenated cytochrome *b* and D-loop sequences of fur farm (*red*) and wild (modern north = *solid blue*, historical north = *checkered blue*, central = *gray*, south = *green*) *red foxes* in Newfoundland, Canada. The size of the *circle* represents the frequency of occurrence ranging from n = 1 (F7-9) to n = 121 (F-17). In total, 8 historical samples collected from the wild during 1882–1915 are reflected in this figure. (Color figure online)

Discussion

Our results indicated little, if any, discernable admixture between red foxes from a large-scale Newfoundland fur-farming operation and wild populations of otherwise native red foxes. Establishment of captive fox populations into or adjacent to wild populations, while problematic elsewhere in North America, did not appear to be a pervasive issue in Newfoundland.

Red fox mtDNA haplotypes can be useful in diagnosing potential fur farm ancestry in parts of North America (Sacks et al. 2011; Kasprowicz et al. 2016). In general, red fox mtDNA haplotypes are predictably distributed, with populations endemic to an area often having genetic signatures reflecting their respective native ancestry (Aubry et al. 2009; Statham et al. 2012). Also, North American haplotypes are easily discerned from European haplotypes, which have been well characterized and shown to differ by deep phylogenetic divergence (Statham et al. 2014; Kasprowicz et al. 2016). In our focal fur farm, two samples had haplotypes from Washington State (O-24), implying that not all of the stock from this farm was of eastern Canadian origin. However, the remaining haplotypes sampled were either known to occur in the region (as well as southern latitudes within the eastern U.S. in the cases of E-86 and F-9; Kasprowicz et al. 2016) or were recently derived from these haplotypes. For example, many fur farm foxes shared haplotypes F-17 and F-85 with the wild population. These haplotypes also matched two haplotypes, NL2 and NL5, respectively, that were based on a partially homologous fragment of mtDNA previously reported in wild Newfoundland red foxes (Langille et al. 2014). Although these haplotypes also had been associated with fur-farming previously (Statham et al. 2011, 2012), one of these haplotypes (F-17) also was found in wild Newfoundland red foxes sampled before fur-farming was introduced to the island (Aubry et al. 2009). Thus, ultimately, mtDNA was not directly informative about admixture in the current study, but served primarily to confirm the North American origin of fur-farm stock imported from Norway.

More informatively, the use of microsatellite markers indicated little-to-no gene flow from fur farm foxes into the wild population. For the most part, genotypes showed strong assignment to their respective genetic cluster based on Bayesian clustering analyses, PCA, and F_{ST}. Although the 95% credibility interval of the estimated ancestry fraction of one wild fox did not overlap 0 or 1 in the modern-sample Bayesian analysis (i.e., seemingly consistent with admixture), the physical location of that sample argued against admixture. Specifically, the apparently admixed fox was sampled in the center of Newfoundland, >100 km from the fur farm, whereas all wild foxes on the Avalon Peninsula, directly adjacent to the fur farm, showed strong differentiation from the fur-farm group. It seems more plausible that the apparent admixture in the one individual reflected a type I error, which was expected to occur in 5% of the sample on the basis of chance alone. For example, the historical wild foxes could not possibly have had any fur-farm ancestry because they predated the fur farms (i.e., they were a priori known to be 100% wild), yet the face-value estimates of their fur-farm ancestry fraction ranged up to nearly as high as the one modern wild individual in question. Nevertheless, regardless of whether or not one modern individual was or was not actually

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Table 2 Microsatellite characteristics of fur-farm (n = 75) and wild-caught (modern n = 72; historical n = 9) populations of red foxes in Newfoundland, Canada

Locus	Fur farm				Modern				Historical			
	H_e	H_o	F_{IS}	No. alleles	H_e	H_o	F_{IS}	No. alleles	H_e	H_o	F_{IS}	No. alleles
Vv-AHT133	0.27	0.19	0.31	5	0.52	0.46	0.09	6	0.46	0.11	0.91	3
Vv-AHT140	0.51	0.52	0	8	0.62	0.65	0.03	8	0.72	0.78	0	6
Vv-C01.424	0.46	0.44	0.32	6	0.56	0.53	0.04	7	0.71	0.83	−0.14	4
Vv-RF08.618	0.7	0.71	0.16	7	0.72	0.68	0.1	7	0.74	0.29	0.56	4
Vv-RFCPH2	0.54	0.56	0.18	8	0.79	0.78	0.05	8	0.76	0.44	0.52	4
Vv-RF2001	0.18	0.16	0.31	7	0.43	0.42	0.48*	7	0.57	0.56	0.03	3
Vv-FH2004	0.22	0.24	−0.06	3	0.55	0.47	0.07	3	0.42	0.11	0.83	2
Vv-FH2010	0.48	0.41	0.11	5	0.67	0.65	0.02	5	0.5	0.5	0	2
Vv-RF2054	0.5	0.52	−0.05	8	0.75	0.68	−0.01	9	0.85	0.63	0.28	8
Vv-FH2088	0.75	0.39	0.38*	5	0.69	0.54	0.07	5	0.72	0.63	0.06	4
Vv-FH2289	0.44	0.27	0.39*	2	0.5	0.56	0.02	2	0.2	0.2	0	2
Vv-FH2328	0.7	0.75	−0.05	13	0.89	0.78	0.15*	14	0.8	0.6	0.17	5
Vv-FH2380	0.66	0.67	−0.03	6	0.67	0.62	0.04	6	0.81	0.71	0.1	5
Vv-RF2457	0.71	0.62	0.12	12	0.85	0.66	−0.01	12	0.87	0.43	0.36*	8
Vv-AHTh171	0.13	0.13	−0.07	3	0.32	0.25	0.14	3	–	–	–	–
Vv-CPH11	0.3	0.31	−0.04	4	0.71	0.6	−0.02	4	–	–	–	–
Vv-CPH18	0.75	0.79	0	7	0.83	0.75	0.12	7	–	–	–	–
Vv-CXX-468	0.58	0.51	0.07	5	0.58	0.49	0.09	5	–	–	–	–
Vv-CXX-602	0.53	0.57	−0.03	11	0.88	0.84	0.02	11	–	–	–	–
Vv-FH2848	0.46	0.45	−0.02	8	0.78	0.68	−0.01	8	–	–	–	–
Vv-REN54P11	0.63	0.67	−0.06	8	0.68	0.65	−0.05	8	–	–	–	–
Average	0.5	0.47	0.09	6.71	0.66	0.61	0.07	6.9	0.65	0.49	0.26	4.29

For each locus, H_e expected heterozygosity; H_o observed heterozygosity; *No. alleles* the number of alleles

* Significant deviations from Hardy–Weinberg equilibrium after sequential Bonferroni correction

admixed, the relatively strong differentiation between the fur-farm and wild sample overall ($F_{ST} \geq 0.14$) clearly indicated that gene flow from the fur-farm to the wild population was at most minimal.

The rarity of fur-farm ancestry in this free-ranging Canadian population stood in contrast to other studies of red fox in North America, particularly those within the contiguous U.S. (e.g., Sacks et al. 2011, 2016). Several scenarios could explain the apparent absence of genetic impact on the native Newfoundland population. First, the focal farm may have possessed adequate biosecurity measures to prevent animals from escaping in significant numbers. A study of mink in Ontario found that the individual identity of a mink farm was a more important predictor of captive-bred genotypes than either farm density or proximity to native populations, indicating that not all farms posed equivalent risks for invasion (Beauclerc et al. 2013). Further, the rise of wild populations in the contiguous U.S. coincided with the crash of fur prices and subsequent industry decline in the U.S., which raises the possibility that whole-sale releases (rather than occasional escapes) were chiefly responsible for the prevalence of captive-bred populations in the contiguous U.S. (Sacks et al. 2016).

A second possibility is that escapes have occurred regularly, but native red foxes resist interbreeding with farmed foxes despite their recent shared ancestry. This hypothesis is consistent with the existence of an apparently stable hybrid zone in the Sacramento Valley of California between native and nonnative red foxes (Sacks et al. 2011). Sacks et al. proposed that the monogamous mating structure of canids promoted strong mate selectivity, creating higher resistance to interbreeding between divergent canid populations than for other (e.g., mustelid; Beauclerc et al. 2013) carnivores. For red foxes, as well as other canid species, high rates of admixture frequently coincide with areas of low native density, where fewer mating opportunities can act to reduce discrimination (Randi 2008; Sacks et al. 2011; Stronen and Paquet 2013; Kasprowicz et al. 2016). In this scenario, the density and distribution of a native red fox population may be critical factors in determining invasion success. Unlike in the western U.S. where native red foxes have narrowly restricted ranges, or the eastern U.S., where red fox are recent colonizers following post-

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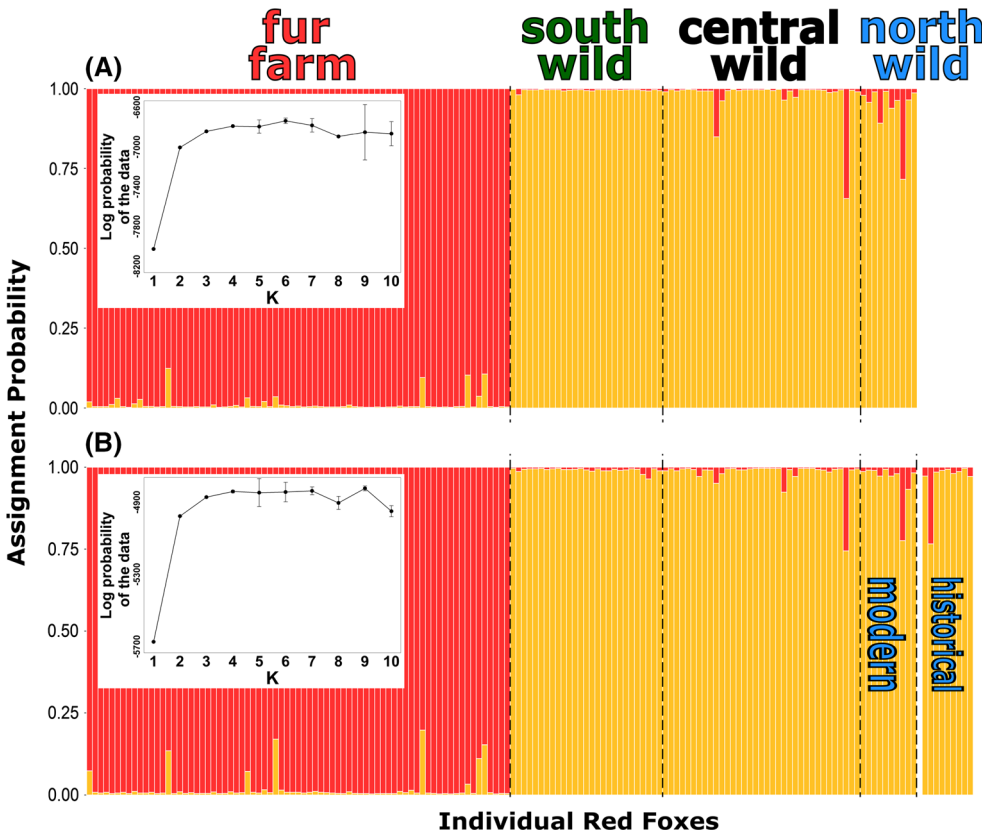

Fig. 3 *Bar plots* showing probability of assignment for individual wild and fur-farm *red foxes* (represented by a *vertical bar*) at the most highly supported value of putative populations, $K = 2$, inferred by STRUCTURE: **a** modern foxes using full 21-locus genotypes; **b** modern and historical (1882–1915) foxes using 14 microsatellite loci. Individuals are sorted by geography (separated by *dashed lines*) for visualization. For each *barplot*, the log-probability of the data for all values of $K = 1$ through $K = 10$ is included as an *inset*. (Color figure online)

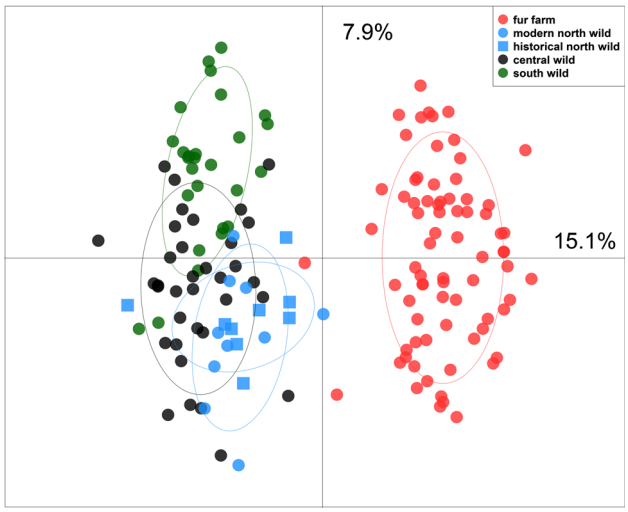

Fig. 4 Principal component analysis (PCA) showing differentiation of fur farm and wild *red foxes* in Newfoundland, Canada, using 14 microsatellite loci. *Colors indicate* population identifiers ($n_{fur\ farm} = 75$, $n_{modern\ north\ wild} = 10$, $n_{historical\ north\ wild} = 9$, $n_{central\ wild} = 35$, $n_{south\ wild} = 27$). Variance explained by each principal component is given as text on its respective axis. *Circles* represent 95% inertia *ellipses* for each population identifier. (Color figure online)

European settlement, Newfoundland has presumably possessed a robust native red fox population since the late Pleistocene (Langille et al. 2014).

Whatever the cause, the red foxes in Newfoundland's only major fur farm have not impacted substantially the genetic composition of native populations. Although this study represents a single case, it was the first to investigate putative introgression from an extant red fox fur farm into a pre-existing wild population. Our findings were consistent with the hypothesis that establishment of nonnative populations or substantial introgression from captive-reared red foxes is less likely to occur in the presence of a thriving native populations. Nevertheless, we cannot rule out the role of tight biosecurity as an equally plausible explanation for our findings. Additional studies over larger regions of northern North America involving multiple fur-farms would be necessary to generalize our findings.

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