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The Great Lakes Region is a melting pot for vicariant red fox (*Vulpes vulpes*) populations

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During the Pleistocene, red fox (*Vulpes vulpes*) populations in North America were isolated in glacial refugia and diverged into 3 major lineages: the Nearctic-Eastern subclade of eastern Canada, the Nearctic-Mountain subclade of the western mountains, and the Holarctic clade of Alaska. Following glacial retreats, these genetically distinct populations of foxes expanded into newly available habitat. Along with subsequent translocation from fur farms, these expansions have resulted in red foxes now occupying most of the continent. The origin of foxes that colonized the Great Lakes Region, however, remains unknown. Furthermore, it is unclear whether contemporary populations inhabiting this region are the result of natural range expansion or if foxes released from fur farms colonized the landscape in the 1900s. To determine the origin of red foxes in the Great Lakes Region, we collected genetic samples from 3 groups: 1) contemporary wild foxes, 2) historical wild foxes collected before fur farming, and 3) fur-farmed foxes from a contemporary fur farm. We constructed a network of mtDNA haplotypes to identify phylogeographic relationships between the 3 sample groups, and examined genetic signatures of fur-farmed foxes via the androgen receptor gene (AR) associated with tame phenotypes. Historical wild foxes demonstrated natural colonization from all 3 major North American lineages, which converged within the Great Lakes Region, and contemporary wild foxes maintained the historically high genetic diversity. Most contemporary wild foxes also matched haplotypes of fur-farmed foxes; however, AR was not useful in distinguishing fur-farm origins in samples of contemporary wild foxes. Our results show that geographically disparate populations naturally merged in the Great Lakes Region before fur-farmed foxes were introduced. Due to the historically high genetic diversity in the Great Lakes Region, any introductions from fur farms likely contributed to, but did not create, the genetic structure observed in this region.

Key words: captive-bred, Michigan, Minnesota, native, Wisconsin

Understanding continental-scale patterns in a species' phylogeography and identifying the origins of genetically discrete subpopulations can provide insight into historical dynamics (Arbogast and Kenagy 2001), and is important when predicting the effects of environmental changes (Mills et al. 2018). During the Pleistocene glaciations in North America, a large number of taxonomic groups was isolated in southern forested refugia (Shafer et al. 2010), and species diverged into genetically distinct eastern and western lineages separated by xeric grasslands (Topp et al. 2013). Following glacial retreats, many eastern populations expanded northwest through the Great Lakes Region into newly available habitat (e.g., *Lynx rufus*—Loveless et al. 2016). Molecular-based phylogeography, however, has revealed notable exceptions to this general pattern of post-Pleistocene recolonization by vertebrates. For example, careful examination of eastern gray wolves (*Canis lupus lycaon*—Koblmuller et al. 2009) has found a clade unique to the Great Lakes Region.

The red fox (*Vulpes vulpes*), a small-bodied canid common to much of North America, similarly experienced postglacial expansion from isolated refugia in North America, but its colonization history and lineages that occur across most Midwestern states, especially within the Great Lakes Region, are unclear (Frey 2013). Mitochondrial DNA (mtDNA) sequences indicated that red foxes colonized North America from Eurasia via the Bering Land Bridge twice (Fig. 1). The first mitochondrial clade to colonize the continent, known as the Nearctic clade, arrived 279-408K generations ago (Aubry et al. 2009; Kutschera et al. 2013) and split into 2 geographically restricted subclades in southern refugia during the last glaciation. The resulting 3

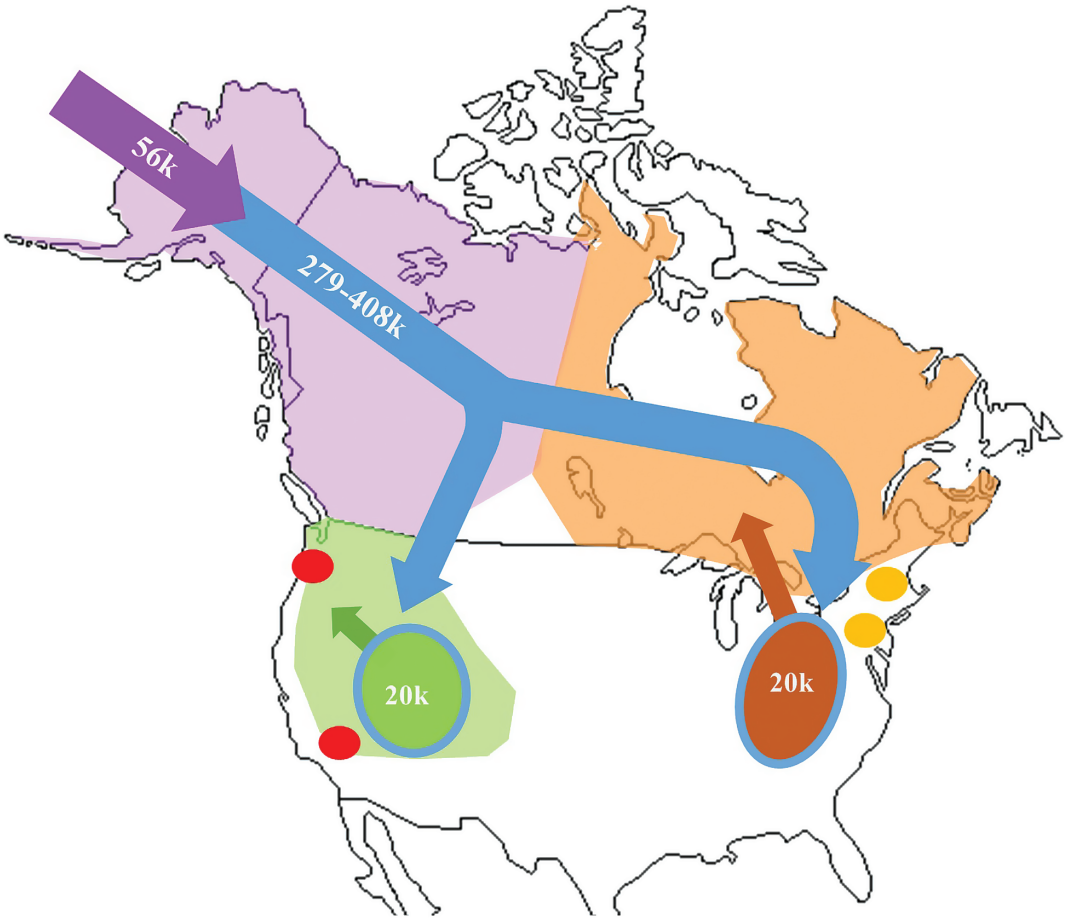

Fig. 1.—Historical range expansion of the red fox (*Vulpes vulpes*) in North America, adapted from Aubry et al. (2009). The first red foxes colonized North America 279-408K generations ago from Asia, and formed the Nearctic clade (blue) while in a northern refugium during the Illinoian glaciation. During the following interglacial period, the Nearctic clade expanded across the continent and populations subsequently became isolated during the Wisconsin glaciation. Climatic changes extirpated Nearctic foxes in their northern refugium and a second colonization by the Holarctic clade (purple) occurred from Beringia 56K generations ago. The Nearctic foxes in southern refugia diverged 20K generations ago into the Nearctic-Eastern (orange) and Nearctic-Mountain (green) geographically restricted lineages. Following glacial retreats, red foxes expanded but remained confined to high elevations until the 1800s when European settlement led to natural expansion by some native populations into the lower elevations of the United States. Also in the 1800s, European foxes brought to North America for hunting established wild populations on the east coast (Kasprowicz et al. 2016), and in the 1900s translocated fur-farmed foxes established wild populations throughout the United States in varying degrees of completely non-native to admixed populations (Sacks et al. 2010; Statham et al. 2012).

Nearctic subclades that inhabit North America today include 1) the ancestral, Nearctic-Widespread lineage; 2) the Nearctic-Mountain lineage of the western mountains (Rockies, Cascade Range, Sierra Nevada); and 3) the Nearctic-Eastern lineage that occupies eastern Canada (Aubry et al. 2009). The second mitochondrial clade, the Holarctic clade, arrived ~56K generations ago and colonized Alaska and western Canada, where Nearctic red foxes were extirpated during the last glaciation (Aubry et al. 2009). In the 1800s, concomitant with human-induced landscape changes to North America, some populations of foxes expanded to lower elevations (Fichter and Williams 1967). The Nearctic-Eastern populations expanded into the southern Atlantic states, while some Nearctic-Mountain populations colonized the lower elevations of the Great Basin and western Oregon (Statham et al. 2012).

Recent human translocations of foxes through fur farming, however, complicate the historic patterns of fox phylogeography (Frey 2013). The first red fox fur farm in North America was established on Prince Edward Island in eastern Canada in the early 1900s, and their breeding stock was shipped to fur farms globally (Statham et al. 2011) and are still bred today (Merson et al. 2017). As a result, contemporary farmed foxes are North American in origin but represent several Nearctic-Widespread and Nearctic-Eastern haplotypes, plus at least 2 Holarctic haplotypes and 1 Nearctic-Mountain haplotype that were supplemented to captive breeding stock by farms in western states of the United States (Statham et al. 2011, 2012; Kasprowicz et al. 2016; Lounsberry et al. 2017). Release of captive-bred red foxes from fur farms occurred across much of the United States (Aubry 1984; Bailey 1992; Lewis et al. 1999), including the Great Lakes Region. In particular, the states of Wisconsin, Minnesota, and Michigan produced more than one-half of fox pelts in the United States in the 1930s (Ashbrook 1938) and likely had a number of

intentional and unintentional red fox releases. Translocated foxes established non-native populations in the 1900s in areas of low elevation across the United States (Statham et al. 2012; Kasprowicz et al. 2016). Holarctic individuals that were translocated to eastern states via fur farming successfully colonized human-dominated landscapes of the east coast while native Nearctic-Eastern foxes persist in natural areas along the Appalachian Mountains (Kasprowicz et al. 2016). Nearctic-Eastern individuals that were translocated to western states via fur farming similarly colonized lower elevations outside their native range while native Nearctic-Mountain foxes persisted in high elevations (Sacks et al. 2010, 2016; Statham et al. 2012). Given that landscapes in the Great Lakes Region are largely agricultural and increasingly urban, both of which are associated with non-native fox populations of fur-farming origin (Merson et al. 2017), contemporary wild populations may possess genetic characteristics of captive breeding.

Our goal was to describe red fox phylogeography and colonization in the Great Lakes Region. To do so, we collected genetic samples from 1) contemporary wild foxes, 2) historical foxes collected before the peak of fur farming in the 1930s, and 3) captive-bred foxes from a fur farm, and compared their mtDNA haplotypes. We hypothesized that the Great Lakes Region was originally colonized by eastern populations of red foxes, but that the native lineage became displaced by released fur-farmed foxes that established populations after the fur-farming industry declined. We predicted historical foxes to exhibit Nearctic-Eastern haplotypes, indicating natural range expansion by eastern refugial populations. We also predicted both contemporary wild and fur-farmed foxes to exhibit haplotypes that overlap with fur-farmed foxes sequenced in previous studies, suggesting that fur-farmed foxes fostered by human settlement replaced native foxes that had colonized the Great Lakes Region following glacial retreats. To identify whether contemporary haplotypes denote fur-farmed origin or ancient Nearctic ancestry that predates fur farming, we also examined a functional gene that has been linked with aggressiveness in canids (Gronek et al. 2008; Konno et al. 2011), hypothesizing that this gene is lacking in foxes of captive-bred origin.

Materials and Methods

Sample collection.—We collected biological samples from 3 discrete groups of red foxes in Wisconsin: contemporary wild, historical wild, and fur-farmed foxes. Samples from contemporary wild foxes ($n = 48$) were obtained from carcasses collected at the North American Fur Auction in Stoughton, Wisconsin ($n = 31$), in Madison, Wisconsin ($n = 12$—Mueller et al. 2018), and by the Wisconsin Department of Natural Resources ($n = 5$). Samples from historic wild foxes in Wisconsin, Minnesota, and the Upper Peninsula of Michigan (from 1896 to 1930; $n = 24$), were obtained from the University of Wisconsin Zoological Museum, University of Kansas Natural History Museum, and University of Michigan Museum of Zoology, respectively. (Appendix I). We scraped tissue samples from skulls or cut tissue from pelts, and extracted dentine from the roots of teeth following protocols in Pauli et al. (2015). We collected samples from fur-farmed foxes ($n = 44$) as hair follicles provided by the Autumn River Farm in Juneau, Wisconsin.

Laboratory procedures.—We extracted DNA from contemporary wild and fur-farmed foxes using a DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland) following the manufacturer's protocol. DNA from historical samples from the University of Wisconsin Zoological Museum were extracted at the University of California-Santa Cruz Paleogenomics Lab, and DNA from the remaining historical samples were extracted in a pre-PCR ultra-clean room at the University of Wisconsin-Madison following previously developed protocols (Pauli et al. 2015). We sequenced 2 sections of mtDNA: 354 base pairs (bp) from the cytochrome-*b* (*Cytb*) gene using primers RF14724 and RF15149, and 343 bp from the D-loop region using primers VVDL1 and VVDL6 (Aubry et al. 2009). PCRs were conducted in a 25-µl reaction using a PCR Core kit (Qiagen), 5 µl bovine serum albumin (BSA), 0.625 µl of each forward and reverse primer, and 1 µl template DNA. The reaction profile involved an initial denaturation of 94°C at 30 s. Then 40 cycles at 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s, followed by a final extension at 72°C for 10 min. Due to degradation in historical samples, we analyzed smaller overlapping fragments using primer pairs RF14724 and RFCYTB3R, and RFCYTBBF and RF15149 for *Cytb* (Perrine et al. 2007) and primer pairs VVDL1 and VVDL4, and VVDL5 and VVDL6 for the D-loop (Statham et al. 2014). PCRs for historical samples included 2 µl DNA template, 4 µg/µl BSA to neutralize inhibitors, HotStar Plus Taq (Qiagen) with an initial denaturation of 5 min, and the reaction profile included a touchdown from 56°C to 50°C for the first 5 cycles. We cleaned PCR product with ExoSapIT to remove the excess fragments and primers. Sequencing was performed by the University of Wisconsin-Madison Biotechnology Center. We confirmed the quality of sequences in FinchTV 1.4.0 (Geospiza, Inc., Seattle, Washington) and trimmed and aligned sequences using MEGA7 (Kumar et al. 2016). We used the Basic Local Alignment Search Tool (BLAST) in MEGA7 to assign sequences to red fox haplotypes from previous studies (e.g., Statham et al. 2012) stored in GenBank. We combined *Cytb*/D-loop mtDNA haplotypes into a single 696-bp haplotype indicative of each fox's lineage. Following previous studies, we weighted the *Cytb* portion twice as much as the D-loop (Sacks et al. 2010) to construct a median-joining network in PopArt (Leigh and Bryant 2015). In 15 of 20 historic samples, only partial fragments of *Cytb* or the D-loop could be amplified and haplotypes could not be determined. Therefore, we used discriminating base pair sites that were recovered to match each sample to Holarctic or Nearctic lineages.

The diverse lineages represented in fur farms make introgression with wild fox genomes difficult to detect using mtDNA haplotypes alone (Merson et al. 2017). However, their differentiation could be improved by examining a functional gene potentially affected through captive breeding. Commercially farmed foxes were deliberately selected to be nonaggressive toward humans, and are observably tamer than wild ones (Trut 1999; Statham et al. 2011). In the androgen receptor gene (AR)

on the X chromosome, short alleles indicating increased AR function are linked to aggressiveness in red foxes (Gronek et al. 2008) and other animals (Konno et al. 2011; Butovskaya et al. 2015; Song et al. 2017), and can potentially distinguish contemporary foxes of wild versus captive-bred origin. Because AR-associated aggressive behavior was only recognized in males (Konno et al. 2011), we first identified male samples by amplifying a zinc finger (ZF) protein gene fragment with a TaqI digestion site on the Zfy gene. We used primers forward ZFKF 203L and reverse ZFKF 195H to amplify 195 bp including the TaqI digestion site (Ortega et al. 2004) on all samples of unknown sex. PCR amplifications were run using a Taq PCR Core kit (Qiagen) under the same PCR conditions as in Ortega et al. (2004), but with an annealing temperature of 52.2°C. PCR products were digested in a 20-µl reaction volume with 5 U restriction enzyme Taq$^{\alpha}$I and 1 µl NEB Buffer (New England BioLabs, Ipswich, Massachusetts), 0.2 µl BSA, 10 µl PCR product and ddH_2O. The digestion was incubated for 3 h at 65°C and products were run on a 3% agarose gel at 40 V. Female samples yielded 1 band of 195 bp, whereas male samples contained the restriction site and yielded 2 bands at 195 and 152 bp.

We extracted DNA from only male samples to analyze AR length. We amplified the repeat region in exon 1 of AR using primers designed on sequences in dogs: Q2F and Q2R (Maejima et al. 2005). PCR was conducted in a 10-µl reaction using a PCR Core kit (Qiagen) following manufacturer's protocol with Q-solution, and with 0.5 units Taq polymerase and 1 µl template DNA. The thermocycler profile followed Maejima et al. (2005) except for annealing temperature at 62°C. For historic samples, we increased the reaction size to 25 µl, added 4 µg/µl BSA to counteract inhibitors, and decreased the annealing temperature to 60°C. PCR product was submitted to the University of Wisconsin-Madison Biotechnology Center for sequencing in both forward and reverse directions. We confirmed and trimmed the sequences in a similar process as described for mtDNA above. After aligning sequences, we determined AR haplotypes by counting codon repeats and applied Fisher's exact test to detect significant differences in allele frequency between the sample groups.

Results

We obtained complete *Cytb*/D-loop sequences from 93 samples, including 43 from contemporary wild foxes, 5 from historical wild foxes, and 45 from fur-farmed foxes. We also obtained 20 partial sequences, including 5 from contemporary wild foxes, 14 from historical wild foxes, and 1 from a fur-farmed fox (Tables 1 and 2). We identified 10 distinct full-locus haplotypes, of which only 2 D-loop haplotypes (84a and 12a; GenBank accession nos. MG777945–6) were novel. All haplotypes were assigned to the phylogenetically distinct lineages that had been previously described (Aubry et al. 2009). Although we only sampled 1 fur farm for our study, all haplotypes detected in our fur-farmed samples (F-9, F-12, F-17, E-88, and O-24) matched haplotypes found in fur farms from previous studies (Statham et al. 2012; Lounsberry et al. 2017).

The *Cytb*/D-loop median-joining network of contemporary samples in Wisconsin is represented by all 4 North American lineages (Fig. 2). Three contemporary haplotypes (F-9, F-12, and O-24) were also found in the fur farm we sampled. Two contemporary haplotypes (G-73 and G-38) were reported in fur farms from previous analyses (Statham et al. 2011, 2012; Kasprowicz et al. 2016), and 2 other contemporary haplotypes (A-273 and F3-9) were presumably translocated for fur farming in previous analyses (Sacks et al. 2016; Merson et al. 2017). Three contemporary haplotypes were also historically present in Wisconsin (F-12, F-9, and G-38); however, all 3 haplotypes were also found in fur farms. Because most contemporary and fur-farmed haplotypes overlap, all but 3 contemporary haplotypes could have arisen from releases from fur farms.

All 4 lineages occurred in both historical and contemporary samples, but their spatial distribution suggests that different lineages occurred in different geographic regions (Fig. 3). Historically, Holarctic foxes occurred in presettlement forests of northern Wisconsin, while Nearctic-Widespread and Nearctic-Eastern foxes occurred in presettlement oak savannas and prairies in southern Wisconsin. All of these lineages were detected in their historical regions in contemporary samples as well. Four partial Nearctic-Mountain haplotypes were detected in historical wild samples from Minnesota, including 1 partial Nearctic-Mountain haplotype that was detected in a previous analysis (included in Fig. 3; Statham et al. 2012). Similarly, we found the Nearctic-Mountain subclade in contemporary wild samples from western Wisconsin.

In 45 male red foxes, we detected 5 alleles with 9, 10, 10T, 11, and 12 repeats in exon 1 of AR (Table 3). Two wild samples with 9 repeats were shorter than alleles previously detected in red foxes (Gronek et al. 2008). Allele frequency proportions differed between historical wild, contemporary wild, and fur-farmed samples ($P < 0.001$). Allele frequencies in historical wild and contemporary wild foxes did not differ ($P = 0.09$), although both differed from allele frequencies in fur-farmed foxes ($P < 0.001$ and $P = 0.025$, respectively). Notably, only

Table 1.—Occurrence of 6 cytochrome-*b* haplotypes in 100 red foxes (*Vulpes vulpes*) from Wisconsin. Three sample groups of foxes (contemporary wild, historical wild, and fur-farmed) and the clade of each haplotype are shown.

	Nearctic-Widespread	Nearctic-Eastern			Nearctic-Mountain	Holarctic
	A	E	F	F3	O	G
Contemporary wild	17		12	2	2	15
Historical wild	1		4			3
Fur-farmed		13	27		4	

Table 2.—Occurrence of 13 D-loop haplotypes in 98 red foxes (*Vulpes vulpes*) around the Great Lakes Region. Three sample groups of foxes (contemporary wild, historical wild, and fur-farmed) and the clade of each haplotype are shown. *Haplotypes novel to this study.

	Nearctic-Widespread		Nearctic-Eastern					Nearctic-Mountain				Holarctic	
	84a*	273	9	12	12a*	17	88	19	24	59	271	38	73
Contemporary wild	3	12	3	9	2			1	2			5	8
Historical wild			5	1		5				1	1	4	
Fur-farmed			30	4		2	4		4				

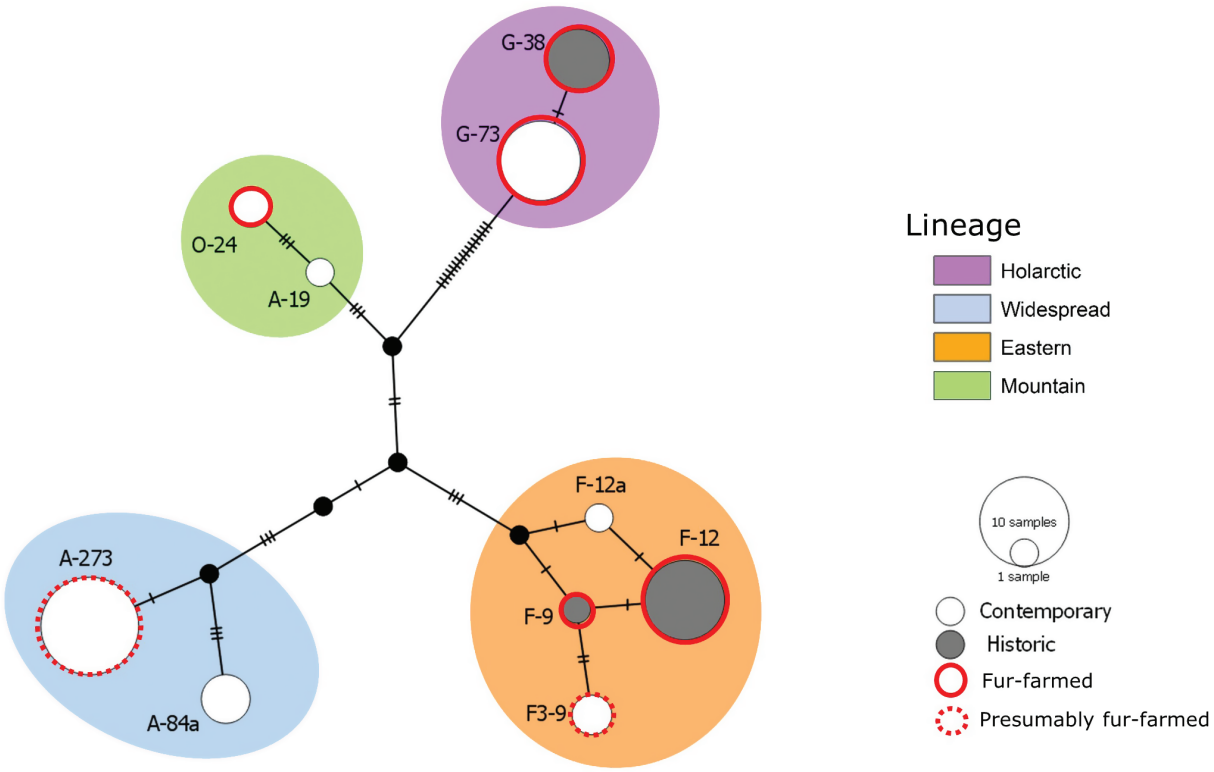

Fig. 2.—Cytochrome-*b*/D-loop median-joining network based on a 696-bp haplotype for 43 specimens of contemporary wild red foxes (*Vulpes vulpes*). The node size is proportional to the number of samples belonging to each haplotype, bars represent mutational differences, and colors indicate haplotype assignment to known subclades. Three contemporary haplotypes that match haplotypes found in historical samples are shaded. Contemporary haplotypes that were found in fur farms in previous studies are highlighted in red.

short alleles, 10 and 10T, were detected in fur-farmed samples. When comparing Holarctic and Nearctic foxes for both historical wild and contemporary wild individuals, samples did not differ in allele frequency ($P = 0.17$).

Discussion

The historical and contemporary genetic diversity suggests that the Great Lakes Region is the intersection between distant North American subclades of red foxes. While much is known about phylogeography of red foxes in montane regions of North America, the origin of foxes that colonized the Great Lakes Region were heretofore unclear. Only one other study explored the origin of red foxes in this region, using a small sample size ($n = 5$—Statham et al. 2012). Those authors identified a few haplotypes that matched fur-farmed foxes and concluded that those individuals were likely translocated by humans via fur farms. Our historical samples, though, revealed that even before fur farming became a likely source of individuals (i.e., pre-1930s), all 4 lineages of foxes occupied the region. Thus, our findings suggest that all 4 lineages colonized the Great Lakes Region before fur farming was established. The historical and contemporary diversity of haplotypes indicates that a westward expansion by the Nearctic-Eastern subclade, eastward expansion by the Nearctic-Mountain subclade, and southern expansion by the Holarctic clade created a natural diversity of fox haplotypes within the Great Lakes Region.

The presence of fur farms in the Great Lakes Region complicates the colonization history of contemporary populations. Three contemporary haplotypes (F-12, F-9, and G-38) were historically present in Wisconsin and are currently present in fur farms, making it impossible to ascertain whether those contemporary samples were of native origin or released from fur farms. Furthermore, not all haplotypes of fur-farmed foxes have

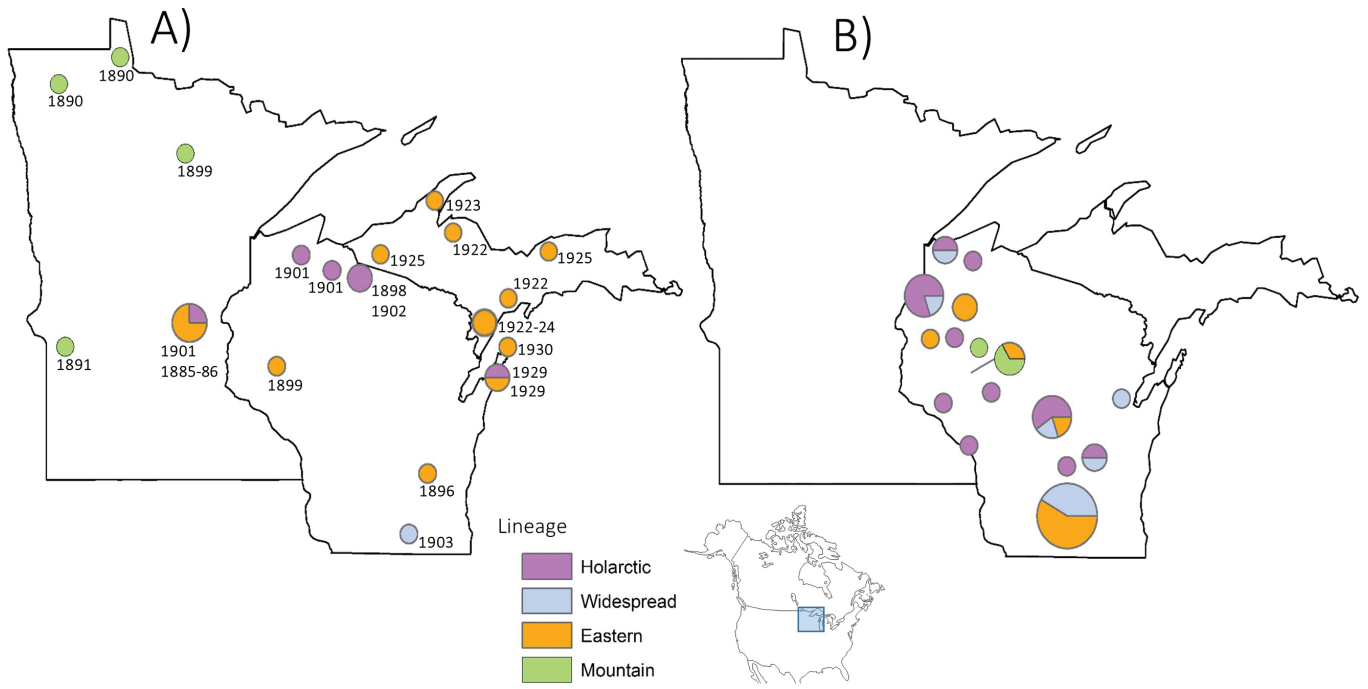

Fig. 3.—Geographic distribution of red fox (*Vulpes vulpes*) subclades based on both full and partial cytochrome-*b*/D-loop sequences in historical wild and contemporary wild sample groups. Circle size is proportional to the number of samples from each location, and circle color represents the sample's lineage. (A) Historical samples represent wild red foxes in the Great Lakes Region before the peak of fur-farming in 1930, and the year each specimen was collected is indicated. Five samples of historical wild foxes from Minnesota from Statham et al. (2012) are also included. Historically, Holarctic foxes occurred in northern forests, and Nearctic-Widespread and Nearctic-Eastern foxes occurred in southern prairies, while Nearctic-Mountain foxes occurred to the west. (B) Contemporary samples represent wild red foxes in the same region collected after the peak of fur farming. Contemporarily, Holarctic foxes mostly remain in northern Wisconsin, while Nearctic foxes predominate in the southern part of the state, with some admixture in central Wisconsin.

Table 3.—Allele frequency of glutamine repeats in the androgen receptor gene (AR) in 45 male red foxes (*Vulpes vulpes*). Samples are represented in 3 groups of foxes (contemporary wild, historical wild, and fur-farmed) from Wisconsin.

Allele	9	10	10T	11	12
Contemporary wild	1	3	10	1	4
Historical wild	1		1		2
Fur-farmed		13	9		

been identified, which means that haplotypes of wild foxes that do not match haplotypes of fur-farmed foxes are not necessarily evidence of natural colonization. Two of the common haplotypes that we detected in wild foxes in Wisconsin (A-273 and F3-9) have not been previously detected in fur farms. However, foxes with these haplotypes were found outside their natural range in southern California and Colorado, presumably due to human introductions (Sacks et al. 2016; Merson et al. 2017). Therefore, although contemporary A-273 and F3-9 haplotypes have not yet been detected in fur farms sampled in our and previous studies, it is possible that they were introduced to the Great Lakes by fur farmers.

We did not expect the Nearctic-Mountain lineage to expand eastward to the Great Lakes Region due to potential barriers preventing biota from the Rocky Mountains from dispersing into the plains (Topp et al. 2013). Indeed, we detected Nearctic-Mountain haplotype O-24 in both contemporary and fur-farmed samples, but not in historical samples. Therefore, it seems more likely that the contemporary O-24 foxes stemmed from releases from fur farms than natural expansion. However, while O-24 was possibly introduced through fur farms, 3 other D-loop Nearctic-Mountain haplotypes (19, 59, and 271) have not yet been detected in any fur farms but we found them in our wild samples. In particular, A-19 is one of the most common wild haplotypes in the Intermountain West (Volkmann et al. 2015) and so its presence on the contemporary landscape is most likely due to natural postglacial expansion. By identifying A-19 in the Great Lakes Region, it appears that some Nearctic-Mountain individuals dispersed further east than previously believed, and have the capacity to colonize low-elevation habitats.

Unintentional and intentional release of captive-bred animals can have detrimental effects on the native population by swamping local adaptive genotypes and population structure (Tymchuck et al. 2007; Laikre et al. 2010). For example, fur-farmed foxes admixing with native populations could alter anti-predatory responses and dispersal capacity (Champagnon et al. 2012). Indeed, admixture between native and fur-farmed foxes exists in lower elevations of the Rocky Mountains and potentially threatens genetic integrity of montane populations (Merson

et al. 2017). We did not detect differences in AR length between historically native and fur-farmed foxes, and contrary to our predictions, only short alleles associated with aggressive personality traits were detected in fur-farmed samples. Thus, AR did not indicate tame behavior selected through captive breeding on fur farms, and AR was not a useful signature of introgression by fur-farmed foxes in wild foxes. Regardless, further research is needed to understand genetic differences between wild and farmed foxes that may be maladaptive in a wild environment. For example, expression of the gene HTR2C, which differs between tame and aggressive farmed foxes (Kukekova et al. 2011), or other allelic associations with aggression found in canids (Vage et al. 2010) should be studied. Even if fur-farmed foxes do not mate with native foxes, these captive-bred foxes still could compete with wild conspecifics (Champagnon et al. 2012). In California, native and introduced fur-farmed red foxes tended to avoid interbreeding (Sacks et al. 2010). Native and reintroduced foxes that colonized the Great Lakes Region might also demonstrate assortative mating that can lead to highly structured subpopulations (Grauer et al. 2017). However, due to the historical genetic diversity of red fox populations in the Great Lakes Region, fur-farm introductions from divergent genetic stocks might not be particularly consequential. Indeed, given that reintroduced individuals match the historical patterns of genetic diversity, it is likely that such releases simply reinforce preexisting genetic variation and structure (Groombridge et al. 2012).

In summary, we found that red foxes historically colonized the Great Lakes Region from geographically disparate subclades resulting in a melting pot. Today, the Great Lakes Region retains high levels of genetic diversity, although possibly reinforced by release of fur-farmed foxes. Future research should identify genetic and behavioral differences between wild and farmed foxes, explore additional genes under selective pressure, and assess how captive-bred adaptations may affect fitness of native populations.

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Appendix I
Accession Numbers of Museum Specimens Used in Our Analyses

University of Wisconsin Zoological Museum: UWZM7046, UWZM8628, UWZM4345, UWZM7047, UWZM7050, UWZM8629, UWZM8630, UWZM8631, UWZM8634, UWZM8638, UWZM11853. University of Michigan Museum of Zoology: UMMZ54947, UMMZ54949, UMMZ54952, UMMZ56488, UMMZ56494, UMMZ57756, UMMZ57760. University of Kansas Natural History Museum: KU1624, KU1626. National Museum of Natural History: USNM188069, USNM188070, USNM19969, USNM110812, USNM107765 (Statham et al. 2012).