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Short Communication

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When recent and evolutionary histories meet: deciphering temporal events from contemporary patterns of mtDNA from fishers (*Martes pennanti*) in north-eastern North America

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Abstract

The current spatial distribution of genetic lineages across a region should reflect the complex interplay of both historical and contemporary processes. Postglacial expansion and recolonization in the distant past, in combination with more recent events with anthropogenic effects such as habitat fragmentation and overexploitation, can help shape the pattern of genetic structure observed in contemporary populations. In this study, we characterize the spatial distribution of mtDNA lineages for fisher (*Martes pennanti*) in north-eastern North America. The history of fishers in this region is well understood and thus provides an opportunity to interpret patterns of genetic structure in the light of known historical (e.g. recolonization from glacial refugia) and contemporary events (e.g. reintroductions, fragmentation and natural recolonization). Our results indicate that fishers likely recolonized north-eastern North America from a single Pleistocene refugium. Three genetically distinct remnant populations persisted through the population declines of the 1800s and served as sources for multiple reintroductions and natural recolonizations that have restored the fisher throughout north-eastern North America. However, the spatial genetic structure of genetic lineages across the region still reflects the three remnant populations.

Key words: Fragmentation – *Martes pennanti* – nucleotide diversity – spatial analysis of molecular variance

Introduction

Patterns of phylogeographic structure are influenced by both historical and contemporary processes. Evolutionary history, including divergence during geographic isolation in glacial refugia and gene flow during postglacial expansion, creates distinct and lasting patterns of genetic structure across the landscape (Avise 1992; Bermingham et al. 1992; Arbogast et al. 2001; Steele and Storfer 2006). In North America, extensive glacial coverage during the Pleistocene and a well-documented path of glacial recession have influenced phylogeographic patterns in predictable ways. For example, in eastern North America, glacial refugia are commonly found south of the Laurentide ice sheet in the southern Appalachian Mountains, and patterns of postglacial recolonization often track glacial recession northward along the Atlantic coast (Wooding and Ward 1997; Sonsthagen et al. 2011). However, patterns of phylogeographic structure do not only reflect evolutionary history, but also have been altered by more recent events including population declines, fragmentation and long distance dispersal achieved through human-mediated translocations (Drew et al. 2003; Hickerson et al. 2010; Smith et al. 2011).

Carnivores are an interesting group in which to study the interplay of historical and contemporary processes in shaping phylogeographic patterns, both because their history in North America is well documented and because they are often particularly sensitive to environmental perturbations (Stone et al. 2002; Wisely et al. 2008; Aubry et al. 2009).

Fishers (*Martes pennanti;* Erxleben, 1777) are medium-sized carnivores native to North America with an extensive and well-documented evolutionary history. During the last glacial maximum in north-eastern North America (~18 000 ybp), fishers were presumed to have persisted in a single refugium that extended from east of the Mississippi River, into the central and southern Appalachians, and along the mid-Atlantic coast (Anderson 1994; Graham and Graham 1994). Fishers expanded into north-eastern North America along the Atlantic coast, likely following the retreat of the Laurentide ice sheet and subsequently expanded westward from coastal regions (Graham and Graham 1994).

Once fishers recolonized northern North America, they were widespread until the 1800s to early 1900s, when extensive trapping, habitat destruction by logging and an extended period of deep snow conditions resulted in severe declines (Hall 1981; Powell and Zielinski 1994; Krohn et al. 1995, 1997; Carr 2005). By the 1930s, only four remnant populations were presumed to exist in north-eastern North America, located in habitat fragments of the Adirondacks of New York, the White Mountains of New Hampshire, the Moosehead Plateau of Maine, and the Cumberland Plateau of New Brunswick Canada (Brander and Books 1973; Hapeman et al. 2011).

Following a period of closed trapping seasons, five major documented reintroductions were initiated in north-eastern North America in an effort to repopulate areas where fishers once thrived (Fig. 1; Berg 1982; Powell 1993; Williams et al. 2000) and augment natural recolonizations into Massachusetts, Rhode Island and eastern Connecticut (originating primarily from New Hampshire; Hapeman et al. 2011). Through these concerted conservation efforts, fishers have regained much of their historical distribution in north-eastern North America.

Genetic signatures of these more recent restoration efforts are detectable in contemporary fisher populations (Hapeman et al. 2011). However, what remains unclear is the extent to which historical demographic events, such as range expansion and colonization following the Pleistocene, have contributed to present day structure of fisher populations in north-eastern North America. The main objective of this study was to characterize patterns of spatial genetic structure in fishers, focusing on the role of historical processes in shaping contemporary patterns of variation. We predict that contemporary patterns of variation will reflect documented

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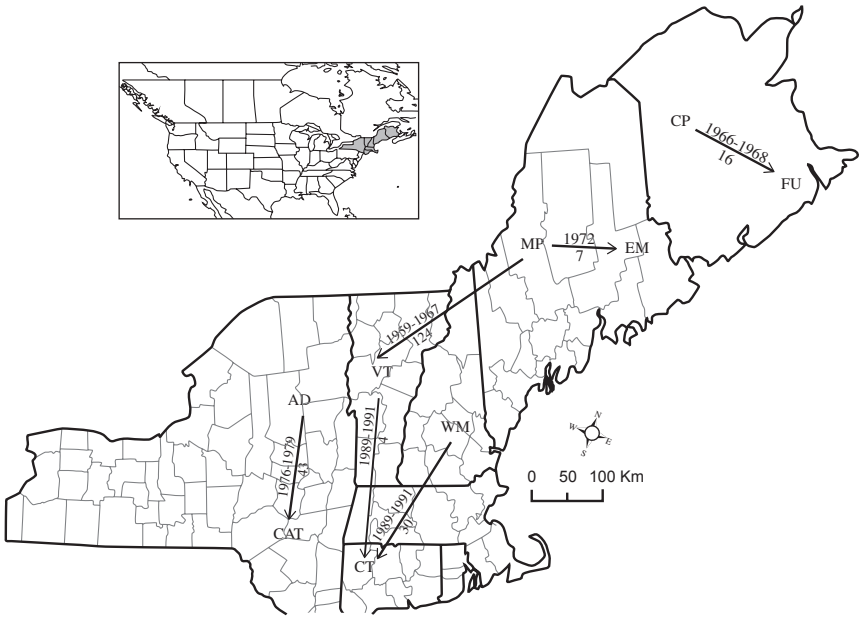

Fig. 1. Locations of source and reintroduced populations of fishers. Arrows point from source populations (AD, Adirondacks; MP, Moosehead Plateau; CP, Cumberland Plateau; WM, White Mountains; VT, Vermont) to reintroduced populations (CAT, Catskills; FU, Fundy; CT, Connecticut; VT; EM, eastern Maine) with dates of the reintroductions above the arrow and the number of animals involved below the arrow. Note that Vermont was first a reintroduced population (with animals released throughout the state) and later used as a source population for a different reintroduction. The map was created using a NAD1983 Projection with dark shadings used to delineate state boundaries.

historical declines and that these signatures will be retained despite more recent demographic recovery of the species in the region.

Materials and methods

Samples

Tissue samples (liver, tongue, or muscle) from fishers were collected between 2001 and 2003 from trappers and state agencies in New York [Catskills (CAT) and Adirondacks (AD; $n = 39$)] and the six New England states: Vermont (VT; $n = 41$), New Hampshire [White Mountains (WM; $n = 52$)], Massachusetts (MA; $n = 22$), Rhode Island (RI; $n = 12$), Maine [Moosehead Plateau (MP) and Waldo County (WA; $n = 75$)] and Connecticut (CT; $n = 28$) (Table 1). Sequences from New Brunswick, Canada [Cumberland Plateau (CP) and the Fundy (FU) region (Wildlife Management Zones 7, 10, 12, 16, 21, 24; $n = 28$)] and eastern Maine (Aroostook and Pisqataquis Counties; EM; $n = 6$) were previously published (Drew et al. 2003). Each sample's collection site was reported as the nearest geographic feature (mountain, lake) or nearest town, which was converted to coordinates in decimal degrees. All maps showing geographic extent were created in ArcGIS 10 using NAD1983 projections. The collection of tissue samples was approved by the University of Vermont institutional animal care and use committee (IACUC 02-074AP).

Table 1. Results of neutrality tests (Fs and D) for four remnant populations of fishers across north-eastern North America. Significant results ($\alpha \leq 0.05$) are in bold. Nucleotide diversity (π) and haplotype diversity (h_D) for each remnant population were calculated in DnaSP version 5.1 (Librado and Rozas 2009)

Population	Fs	D	π	h_D
AD p-value	**6.859**	**2.165**	0.011	0.675
	0.002	**0.014**		
WM p-value	0.733	**−1.299**	0.001	0.176
	0.552	**0.035**		
MP p-value	3.248	0.503	0.003	0.333
	0.064	0.281		
MP & WM p-value	1.609	−0.587	0.003	0.228
	0.157	0.694		
CP p-value	**3.418**	**2.173**	0.008	0.688
	0.02	**0.027**		

p-values are as follows; p (sim Fs ≥ obs Fs); p (D simul ≥ D obs).

Data collection

Tissue samples (1–2 g) preserved in 95% ethanol were washed in distilled water and soaked in Longmire lysis buffer [(Longmire et al. 1997) (0.1 M Tris-HCl, pH 8.0, 0.1 M EDTA, 0.01 M sodium chloride, 0.5% SDS)] for 24 h before being ground to a fine powder in liquid nitrogen (Kilpatrick 2002). The powder was rehydrated in distilled water, and DNA was extracted using either a phenol-chloroform (Blin and Stafford 1976) or modified ammonium acetate protocol (Latch et al. 2006). DNA was quantified using a microplate fluorescence reader and diluted to a final concentration of 10 ng μl^{-1} in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

A 288 base-pair section of domain I, one of the most variable regions of the mtDNA D-loop (Yang et al. 2002; Xiaorui et al. 2011), was amplified by polymerase chain reaction (PCR) in 30 μl reactions using 2.5 μl of template and primers L16022 (Woods et al. 1999) and H16498 (Shields and Kocher 1991) under the following conditions: 3 min at 94°C followed by 33 cycles of 94°C for 15 s, 54°C for 20 s and 72°C for 25 s and ending with 72°C for 30 s. PCR products were purified with PEG (polyethylene glycol) precipitation using the methods of Maniatis et al. (1982). Sequencing reactions (15 μl) were performed using Big Dye (version 3.1) following the protocol provided by the manufacturer (Applied Biosystems, Inc, Foster City, CA, USA) and visualized on an ABI 3700 DNA Analyzer (Life Technologies, Grand Island, NY, USA) at the Purdue University Core Genomics Center or on an ABI 310 (Life Technologies) at the DNA Facility of the Vermont Cancer Center (University of Vermont). Both forward and reverse sequences were collected for half of the samples. Samples with only forward sequences were resequenced in the reverse direction if they yielded any ambiguous peaks, and 10% of the samples were randomly chosen from the data set and resequenced in both directions to verify accuracy of sequence data.

Data analysis

Trimmed sequences of 254 base pairs were aligned against published sequences of *Martes pennanti* (Drew et al. 2003; GenBank accession numbers AY143663-AY143674, referred to here as haplotypes 1–12)

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using the program SEQUENCHER 4.1 (Gene Codes Corporation). Haplotypes from our sample were mapped to visualize the distributions across the area sampled, and one novel sequence of each haplotype was submitted to GenBank (accession #s KF696675-KF696680).

We initially combined samples (n = 303) from all eight sampling localities into four putative groups of populations based on their geographical location (Fig. 3; see also Table S1). Sampling localities for these putative groups included remnant populations, documented reintroduced populations and areas of the north-east naturally recolonized by fishers. We initially assessed the randomness of haplotypes found among populations using the χ^2 test for haplotypic data from Hudson et al. (1992). Significance of the χ^2 statistic was determined by 10 000 permutations in the program DNASP 5.0 (Librado and Rozas 2009). We used the simulated annealing approach of Dupanloup et al. (2002) to conduct a spatial analysis of molecular variance (SAMOVA) to address whether our genetic data support the previously reported remnant population structure in north-eastern North America (Brander and Books 1973; Williams et al. 2000; Hapeman et al. 2011). The SAMOVA identifies how sequence variation is partitioned within and among populations, and groups populations together in such a way that among-group variation (Φ_{CT}) is maximized. We ran the analyses in two ways; first, we used all samples in the four putative groups as a direct comparison with the genetic structure previously identified from the same data set using microsatellites (Hapeman et al. 2011). We treated the additional data from Drew et al. (2003) as the fourth group consisting of two populations in New Brunswick, Canada, located in and around the CP remnant population and the reintroduced population in the Fundy Bay area. Our preliminary results from this first analysis helped guide a second analysis that included only a subset of samples from the data (n = 187) that were associated with the remnant populations and excluded samples from all other areas (see Fig. 2: Populations 1–4 and also Table S1). We ran the SAMOVA analyses in SAMOVA 1.0 (Dupanloup et al. 2002), with the number of initial conditions set to 100 and the number of geographical groupings varied between 2 and 8. We used our results from the remnant only population analysis as a comparison to those from the full data set to visualize how historical population structure of fishers changed following their demographic recovery in the north-east. The associated p-values for the observed variance components and fixation indices (Φ_{CT}, Φ_{ST} Φ_{SC}) were calculated relative to randomized data sets using a nonparametric randomization of the data using 100 000 bootstrap replicates. We further examined population structure in the full data set and in the remnant only subset (n = 187) using principle coordinate analysis (PCoA) in the program GENALEX 6.5 (Peakall and Smouse 2012). We first calculated Nei's genetic distance (Nei 1972) between pairs of populations, and the results were then used as the input for the PCoA.

Nucleotide (π) and haplotype (h_D) diversities were calculated for each of the four remnant populations following Nei (1987) using DNASP version 5.1 (Rozas et al. 2003). Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989) were indirectly used to detect changes in population sizes in each of the four remnant populations and were chosen for their power to detect departures from the null hypothesis of a selectively neutral population and for their complementarity in the ways in which they use the data to test the hypotheses (Drummond and Suchard 2008; Ferretti et al. 2010). Although not formal tests for bottlenecks, neutrality tests used in combination can provide useful insight into demographic events that influence genetic patterns in populations. Fu's Fs and Tajima's D were estimated using ARLEQUIN version 3.0 (Excoffier et al. 2005). The significance and confidence intervals for Fu's Fs were calculated in ARLEQUIN, and confidence intervals from Simonsen et al. (1995) were used to assess significance of the estimates of Tajima's D for each population.

We used the program NETWORK 4.6.1.1 (fluxus-engineering.com) to establish median-joining networks (Bandelt et al. 1999) for our data set and also combined our D-loop data with haplotypes from Drew et al. (2003) to provide a complete representation of all D-loop haplotypes that have been observed in north-eastern fishers to date and to provide a richer context for interpreting phylogeographic patterns identified in the data set.

Results

Nine variable nucleotide sites (Table 1) were observed among the seven different haplotypes (Fig. 3 and Table S1) from the overall sample of fishers (n = 303) from north-eastern North America. Three haplotypes (D, E, F) were new to this study and have not been found outside of our study area while four (A, B, C, G) were described previously (Drew et al. 2003; accession numbers AY143665, AY143667, AY143669, AY143670). One

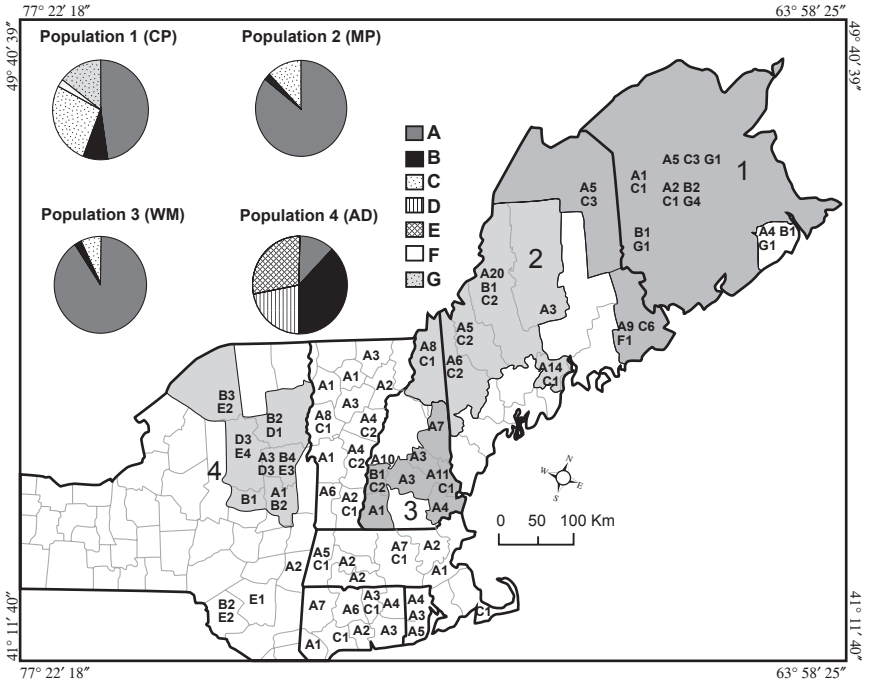

Fig. 2. Map showing four population model used in the spatial analysis of molecular variance (SAMOVA) for the analysis of the subset of data (n = 187). For this analysis, MP and WM (2 & 3) were combined. Different shadings (white and grey) and numbers are used to indicate proposed populations. Haplotypes are labelled using alphabetical codes with adjacent numbers representing the number of each haplotype (reference Figure 4). Pie charts show the relative proportions of each haplotype in each of the four remnant populations (Populations 1–4).

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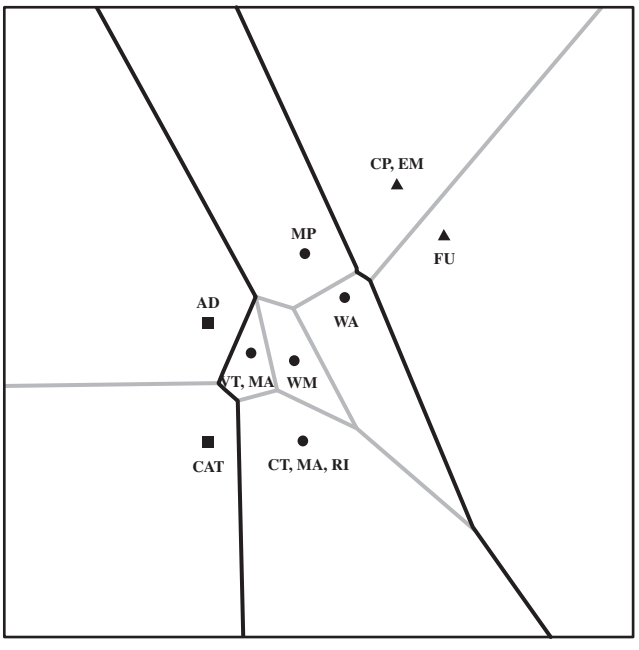

Fig. 3. Results from spatial analysis of molecular variance (SAMOVA) analysis of the entire data set (n = 303) showing nine sampling localities. Model assumed k = 4 groups of populations as a comparison to population structure identified by Hapeman et al. 2011 using microsatellite data. The maximum among-group variation supported only three genetically distinct groups, and these are delineated by dark lines.

common haplotype (A) occurred across all of north-eastern North America except in most parts of New York state (AD and CAT), whereas unique haplotypes were found in New Brunswick (haplotype F in EM and haplotype G in CP, FU, and EM) and New York (haplotype D in AD and haplotype E in AD and CAT) (Fig. 2).

Haplotypes found among populations exhibited a significant departure from a random distribution based on the χ^2 results from DNASP 5.0 ($p < 0.001$). The maximum among-group variation from our SAMOVA analyses of the entire data set (assuming k = 4 groups) occurred with models of k = 3 genetically distinct groups ($\Phi_{CT} = 0.368$, $p < 0.001$ +/−0.0001) consisting of New York (AD and CAT), New England (WM, MP, VT, CT, and WA) and New Brunswick (CP, FU, EM) (Fig. 3). Group structure did not change between analyses using the full data set and the analysis with only the subsample of the data ($\Phi_{CT} = 0.339$, $p < 0.001$ +/−0.0001). In addition, a smaller but significant amount of genetic differentiation was found among populations within groups ($\Phi_{SC} = 0.018$, $p < 0.001$). The first two axes from the PCoA of the full data set explained 90.78% and 9.22% of the variation (Fig. 4), while the first two axes of the four remnant population data set explained 88.64% and 11.36% and separated populations in a similar manner to the full data set.

The AD remnant population was characterized by four haplotypes of relatively equal frequency (A, B, D, E; Fig. 2). Haplotypes A and B were also found in the other remnant populations, but haplotype A was at much higher frequency outside of the AD remnant population, and haplotype B was at a much lower frequency. The CP remnant population contained individuals of five distinct haplotypes (A, B, C, F, G; Fig. 2). The MP and WM remnant populations each had three haplotypes (A, B, C) and very similar haplotype frequency distributions (Fig. 2). Outside of remnant populations, haplotypes A and C were common throughout the region, although A was uncommon and C was not found in New York state (AD and CAT; Fig. 2). Nucleotide diversities and haplotype diversities were higher in remnant

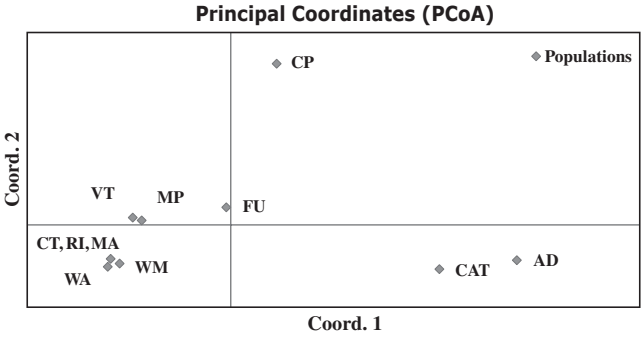

Fig. 4. Principle coordinate analysis (PCoA) of all samples (n = 303) from nine sampling localities in our study area.

populations outside of New England (AD and CP) as opposed to those within New England (WM and MP; Table 1).

The MP population produced values of Fs and D that were consistent with a population at mutation-drift equilibrium. The WM population yielded a non-significant Fs value, but a negative D that suggests recent population expansion. The AD and CP populations showed the opposite pattern. Very high positive values of Fs and D provide strong evidence that these populations experienced recent population subdivision or bottlenecks (Table 1).

The median-joining network of haplotypes for our data set was characterized by 10 mutations (Fig. 5). Observed haplotypes were spread throughout the network and did not exhibit long branches and singletons radiating from a central haplotype (star phylogeny) characteristic of a recent expansion from a small number of haplotypes (Avise 2000) (Fig. 5). When haplotypes from our data were combined with data from Drew et al. (2003), it resulted in 18 mutations within the network with several ambiguous loops (Fig. 6).

Discussion

Our genetic data are largely consistent with previous reports of population structure in north-eastern North America (Brander and Books 1973; Williams et al. 2000; Hapeman et al. 2011). However, the results of our analyses supported the distinctiveness of only three of these remnant populations (AD, CP, and WM/MP; Fig. 3), with very little genetic differentiation between the two remnant populations in New England (WM and MP). Fishers located in the WM and MP have previously been found to be distinct populations using microsatellites (Hapeman et al. 2011), a marker that exhibits a faster rate of sorting of genetic variation than mtDNA. Therefore, it is likely that incomplete lineage sorting of mtDNA lineages resulted in an inability to differentiate these two remnant populations.

Human-mediated reintroductions and natural recolonizations from the remnant populations have left distinct genetic signatures on the region. All populations outside the remnants harboured a subset of haplotypes found in the remnants, consistent with previous findings that these remnants were the sole sources of genetic variation in the region (Hapeman et al. 2011). In addition, overall genetic variation was higher in the remnant populations than in more recently re-established populations. Such a pattern is expected because expanding populations have only subsets of the variation found in the ancestral range (Nei et al. 1975; Ibrahim et al. 1996; Templeton 1998).

We expected that remnant populations would exhibit signatures of population expansion as a result of the movement of individuals from these areas to other parts of the region following their recovery; however, we found this signature for only

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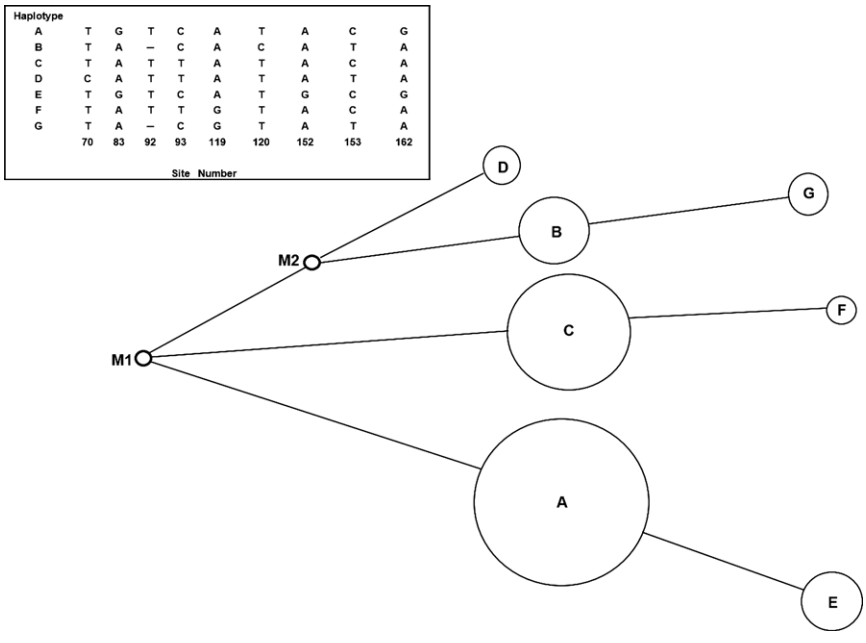

Fig. 5. Median-joining network (Bandelt et al. 1999) from NETWORK 4.6.1.1 (fluxus-engineering.com). Circles with single-letter codes (A–G) represent haplotypes found in samples of fishers (n = 303) across north-eastern North America, and open circles represent inferred intermediates (M1-M2). Circles are proportional in size to number found in the total sample. The inset indicates the haplotypes with variable sites aligned against a published sequence of *Martes pennanti* (Drew et al. 2003; GenBank accession AY143665) and the (-) symbol for site 92 represents an indel.

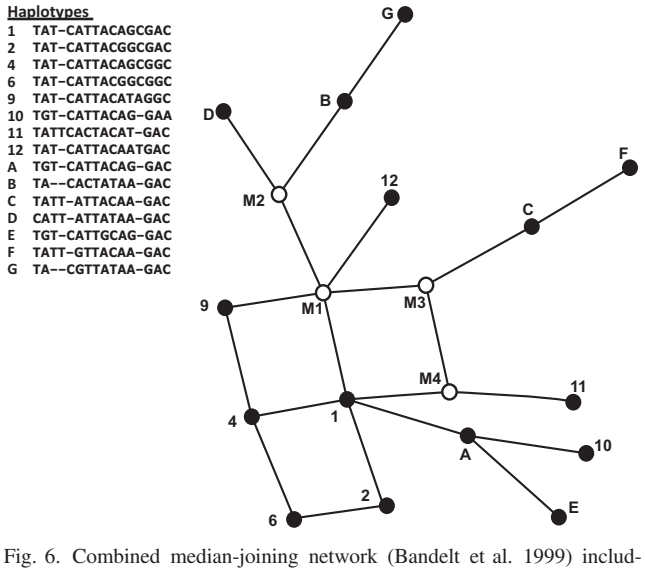

Fig. 6. Combined median-joining network (Bandelt et al. 1999) including data from Drew et al. (2003). Circles with single-letter codes (A–F) represent haplotypes found in our sample of fishers (n = 303) across north-eastern North America, while circles with numbers are from Drew et al. (2003; GenBank accession AY143665-AY143674; 1–12). The inset indicates the variable sites found among the sequences, and the (-) symbol represents an indel.

one of the remnant populations (WM). In direct contrast to our expectations, AD and CP exhibited an excess of intermediate frequency alleles, consistent with recent population declines or population subdivision. It is feasible that both AD and CP have experienced very recent population declines given their reported history. It is also possible that genetically differentiated individuals have been moving into the remnant populations bringing new alleles that have increased frequencies above what would be expected through new mutation alone. In the Adirondacks, for example, haplotype A is only found on the eastern edge of the population and could be the result of immigration from Vermont or Connecticut.

It has been proposed that fishers were located within a refugium located in the central and southern Appalachians (Graham and Graham 1994) during the Pleistocene. The limited genetic diversity, absence of strong geographical associations between closely connected clusters of haplotypes in our network and the geographical ubiquity of two haplotypes (A and B) found on opposite tips of the network are consistent with expansion from a single glacial refugium (e.g. Ball et al. 1988; Ferrando et al. 2004; Tiedemann et al. 2004; Wisely et al. 2008). Deep phylogeographic patterns were not found in our data set, and similar results have been found in a number of carnivore species including raccoons (Cullingham et al. 2008), wolverines (Tomasik and Cook 2005), and badgers (Ethier et al. 2012). The absence of a strong phylogeographic pattern could be the result of populations that only recently diverged or that exhibited substantial gene flow in the past. This is not surprising as carnivores tend to exhibit high vagility which would facilitate gene flow among populations (Wayne and Koepfli 1996). Additional sampling to verify the location of this southern refugium would need to utilize specimens from natural history collections, because fishers were extirpated in the Appalachians and occur there now as a result of reintroductions.

Fishers are thought to have expanded into north-eastern North America following the Pleistocene and then moved west, reaching the Pacific states approximately 5000 years ago. Barton and Wisely (2012) found a similar pattern of northward expansion of striped skunks (*Mephitis mephitis;* Schreber, 1776) into New England between 8500 and 4500 years ago. Phylogeographic patterns in our study of fishers are largely consistent with this proposed movement. Several haplotypes found in our study and also in other studies of fishers (Drew et al. 2003; Vinkey et al. 2006) have been found in populations further west in North America (HapB and HapC from Vinkey et al. 2006). This more likely reflects the westward movement of fishers following the Pleistocene than more recent reintroductions.

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We recognize that recurring mutations within the D-loop have been reported (Knaus et al. 2011) and can make interpretations of phylogeographic history challenging. For example, the fossil record indicates that fishers may have arrived in the eastern part of north-eastern North America first (8500 ybp in New York and New Brunswick; Graham and Lundelius 1994) and later re-established other areas of the north-east. If the fossil record is accurate, we might expect to find unique haplotypes and high levels of genetic diversity in these populations (Posada and Crandall 2001) relative to other areas of the north-east that were colonized later. Our data matched these expectations; however, this same pattern of genetic diversity could be explained by the local loss of haplotypes during the near extirpation of fishers from New England. Without further sampling, we cannot discriminate between these two alternative mechanisms.

Overall, the phylogeographic structure of fisher populations in the north-east is similar to what has been found in many carnivore species. Deep phylogeographic patterns are not present yet historical processes are evident in the data. Spatial genetic structure of current populations indicates that recent events such as reintroductions and natural recolonization have promoted gene flow among remnant populations and re-established fishers in areas where they were previously extirpated. Despite evidence for gene flow both into and out of remnant populations, genetic differentiation among remnant populations across the region has been retained.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of haplotypes (A–G) used in this study sorted by state and county. Population membership is given for samples used in the subset data set (n = 187) and all samples' (n = 303) models in the SAMOVA analysis.

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