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Research Article

Rapid Homogenization of Multiple Sources: Genetic Structure of a Recolonizing Population of Fishers

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ABSTRACT Fishers (*Martes pennanti*) were extirpated from much of southern Ontario, Canada, prior to the 1950s. We hypothesised that the recent recolonization of this area originated from an expansion of the population in Algonquin Provincial Park, which historically served as a refuge for fishers. To test this hypothesis, we created a sampling lattice to encompass Algonquin and the surrounding area, and we collected contemporaneous DNA samples. We sampled fishers from each of 35 sites and genotyped them at 16 microsatellite loci. Using a Bayesian assignment approach, with no a priori geographic information, we inferred 5 discrete genetic populations and used genetic population assignment as a means to cluster sites together. We concluded that the Algonquin Park fisher population has not been a substantial source for recolonization and expansion, which has instead occurred from a number of remnant populations within Ontario, Quebec, and most recently from the Adirondacks in New York, USA. The genetic structure among sampling sites across the entire area revealed a pattern of isolation-by-distance (IBD). However, an examination of the distribution of genetic structure ($F_{ST}/1-F_{ST}$) at different distances showed higher rates of gene flow than predicted under a strict IBD model at small distances (40 km) within clusters and at larger distances up to 100 km among clusters. This pattern of genetic structure suggests increased migration and gene flow among expanding reproductive fronts. (JOURNAL OF WILDLIFE MANAGEMENT 71(6):1853–1861; 2007)

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KEY WORDS dispersal, fisher, harvest, *Martes pennanti*, migration, population structure, recolonization, refuge, translocation.

The fisher (*Martes pennanti*) was extirpated from much of its geographic range by the 1950s (de Vos 1952, Thompson 2000), but the species has made a striking recovery during the last decade, recolonizing a large portion of its historic range (e.g., Thompson 2000). The disappearance of fishers has been attributed to overharvest, predator control, and habitat loss (de Vos 1952). In Ontario, Canada, a commercial trapping ban was imposed in 1949, and at that time de Vos (1952) considered fishers rare in southern Ontario outside of Algonquin Provincial Park. He suggested that the park acted as a large untrapped refuge for fishers. Since that time, southern Ontario has mostly been recolonized by the species (Thompson 2000, Bowman et al. 2006). The causes and spatial dynamics of this recolonization are largely unknown, although a small proportion of the area has been actively restored through translocations from known sources within the province (see methods for more detail on these translocations; Smith 1979, Berg 1982).

Recent studies of the fisher have found relatively high levels of genetic structure (Kyle et al. 2001, Wisely et al. 2004) that is thought to be due to past fragmentation of populations through overharvest, and low vagility of the species (Kyle et al. 2001). Drew et al. (2003) found that historically translocated fisher populations often were more closely related to sources than to adjacent populations, even many years after the translocation had occurred.

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We examined the genetic structure of contiguous fisher populations in and around Ontario, Canada. The geographic scale of our study was comparable to that of Wisely et al. (2004), except fisher populations in our study were more interconnected and potential borders of genetic groups were mostly impossible to discern a priori. Our objective was to examine the current genetic structure of fisher populations in an area of southern Ontario that has recently been recolonized by the species and to test alternative hypotheses for the mechanism of this recolonization. We wished to identify the source populations, and to assess the genetic diversity and differentiation of fisher populations in this recolonized landscape. Fishers in the area may have originated from the dominant historical refuge of Algonquin Park, which should have produced an expansion front with relatively low levels of genetic variation compared to source or core areas and peripheral populations (Kyle et al. 2001, Wisely et al. 2004). Alternatively, fishers may have recolonized southern Ontario from one or a number of sources other than, or in addition to, Algonquin Park. Possible sources would have to be nearby locations that served as refuges for fishers during widespread population declines of the 1920s and 1930s. These include the Adirondacks in New York, USA (Hamilton and Cook 1955), the Chapleau and Algoma areas of northern Ontario (Fig. 1; de Vos 1952), and some sites in Quebec. The recolonization of fishers from multiple sources throughout the province would result in the contact of expanding

reproductive fronts: a pattern of genetic structure not previously characterized.

STUDY AREA

The study took place in the Great Lakes–St. Lawrence forest region of Canada, a transition zone between the northern boreal forest and the southern temperate forest (Rowe 1972). The dominant forest types that made up the climax forests of our study area were tolerant hardwoods such as sugar maple (*Acer saccharum*), red oak (*Quercus rubra*), yellow birch (*Betula alleghaniensis*), and American beech (*Fagus grandifolia*) along with some softwood species such as hemlock (*Tsuga canadensis*), white spruce (*Picea glauca*), eastern white cedar (*Thuja occidentalis*), balsam fir (*Abies balsamea*), and white pine (*Pinus strobus*). Whereas the northern part of the study area was predominantly forested, the southern part included a large proportion of agriculture, including both livestock and crop production.

Fur harvesting took place throughout the area except for within some parks and protected areas, of which Algonquin Provincial Park was the largest. Trapping took place either on registered trap-lines or with landowner or Crown permissions in areas without trap-lines. All trappers were licensed by the province.

METHODS

Sampling Design

We centered our sampling around the dominant historical refuge in the study area, Algonquin Provincial Park. Since we were interested in assessing the spatial pattern of any genetic structure that was detected, we spatially stratified our sample collection using as close to a regularly spaced grid as possible. Ontario geographic townships were the principal sampling unit for the study and the smallest resolution possible for individual fisher locations, other than 2 areas where radiotelemetry studies of fishers were ongoing. Townships were not all equal in size, and in some cases, $\geq$2 adjacent townships needed to be grouped together to obtain sufficient sample sizes (Fig. 1). Townships were mostly square or rectangular in shape and about 300 km^2. We selected the minimum distance between sampled townships such that sample sites were not connected by fisher daily movements, but they were connected by dispersers (Arthur et al. 1993, Bowman et al. 2002). Therefore, sampled townships were separated by $\geq$1 township along north–south or east–west axes, whereas they could be adjacent along northwest–southeast or northeast–southwest axes. We sampled fisher populations associated with 2 telemetry studies, 1 in Algonquin Provincial Park and 1 in the Prescott area (AQ and PR in Fig. 1). We also sampled large trapping refugia in 2 neighboring jurisdictions, Gatineau Park in Quebec and Adirondack Park in New York (GA and AD in Fig. 1).

Township sample sites, telemetry study locations, and sites in neighboring jurisdictions (sample sites) were geographic areas of comparable size (about 300 km^2) comprised of differing ecological characteristics (e.g., land cover type) and spatial locations. In total, we sampled 35 sites for fishers (Fig. 1).

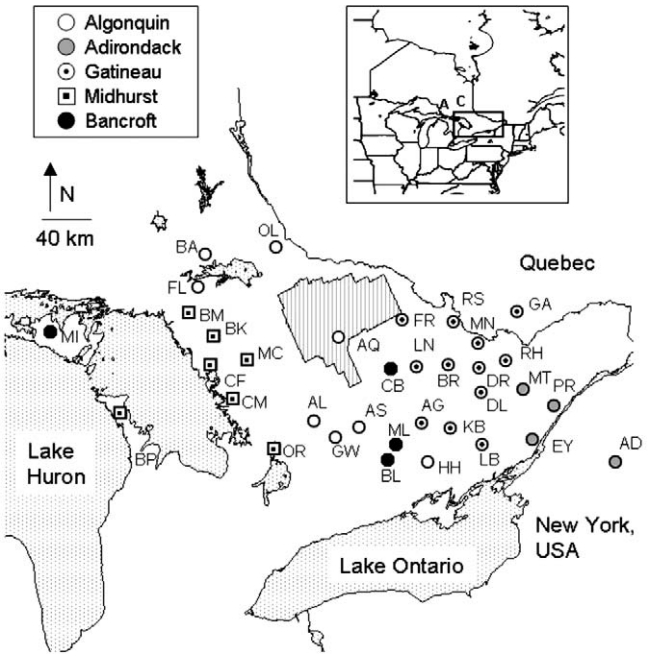

Figure 1. Map of sites in and around Ontario, Canada, where we sampled 769 fishers (*Martes pennanti*) between 2000 and 2003, inclusive. Symbols represent centroids of sample sites, and different symbols represent assignment of sites to 1 of 5 different inferred genetic populations estimated from a Bayesian analysis of 16 microsatellite loci. Two-letter codes are labels for sample sites. Outlines of sample site boundaries are not shown. Algonquin Provincial Park is depicted with shading (around sample site AQ). The study area is within the rectangular box in the inset map of eastern North America. Algoma (A) and Chapleau (C) areas are also depicted on the inset map.

Two isolated sample sites were known to have received translocated fishers during 1979–1982 (Bruce Peninsula [$n = 28$] and Manitoulin Island [$n = 53$]; BP and MI in Fig. 1) from known sources in Ontario, including Algonquin Park, and the Orillia and Bancroft areas (Smith 1979, Berg 1982). A third location (in proximity to sites CM, CF, BK, and MC in Fig. 1) received 97 translocated fishers from Algonquin Park during 1957–1963 (Douglas and Strickland 1987). These are the only known translocations of fishers into the study area.

Tissue Sampling and DNA Extraction

We collected tissue samples from the 2001–2002 fisher harvest in Ontario from Ministry of Natural Resources district offices. Manitoulin Island and Adirondack, New York, samples from the 2000–2001 trapping season were the same used in a 2001 study (Kyle et al. 2007). Samples from the Gatineau park area were provided by C. Pilon at the Société de la Faune et des Parcs du Quebec from their 2001–2002 harvest. We collected samples from Algonquin Provincial Park and Prescott (Koen et al. 2007) and during ongoing fisher telemetry studies started in 2003 (Tully 2006). Additionally, we acquired 8 historic pre–1950s Ontario samples from collections at the Royal Ontario Museum. We sub-sampled small quantities of tissue for

molecular analysis and stored it in 1× lysis buffer (Applied Biosystems, Foster City, CA). We extracted DNA from all tissue types using Promega Magnesil One magnetic bead isolation (Promega Corporation, Madison, WI) on either 2 cm^2 of hide, 0.5 g of muscle, 20–30 hair follicles attached to hide, or 2 cm^2 of blood stored on Whatman-FTS paper (Whatman, Florham Park, NJ). We then quantified extracted DNA by means of Pico Green florescence (Invitrogen, Burlington, ON, Canada) alongside a commercial standard.

Genotyping of Microsatellite Loci

We used 19 microsatellite loci previously developed and shown to be polymorphic in closely related species: wolverine (*Gulo gulo*; Gg007, Gg216, Gg443, Gg454, and Gg101), marten (*Martes americana*; Ma002, Ma001, and Ma019), mink (*Neovision vison*; Mvi002, Mvi072, Mvi1341, Mvi1354, Mvi1321, Mvi020, Mvi1302, Mvi1342, and Mvi2243), otter (*Lontra canadensis*; Lut604), and ermine (*Mustela erminea*; Mer041). We chose these markers either because of previous amplification success in fishers (Kyle et al. 2001) or because of their degree of polymorphism in closely related species.

We amplified the 19 microsatellite loci in 8 multiplex polymerase chain reactions (PCR) per individual. We performed amplifications in 10-μL volumes containing 2–5 ng of DNA, 1.5 mM MgCl, 0.2 mM of each dNTP, 1× PCR buffer (Invitrogen), and 0.5 Units of Taq polymerase (Invitrogen). We amplified all samples under the following conditions: 94° C for 5 minutes, 30 cycles of 94° C for 30 seconds, 52° C or 54° C for 45 seconds, 74° C for 30 seconds, and 65° C for 45 minutes. We implemented multiplex reactions to reduce the total number of PCR reactions.

We added multiplex products together in 4 poolings and desalted them through sephadex columns. We visualized each pooling on a MegaBACE 1000 automated genotyper (Amersham Biosciences, Piscataway, NJ) with a size standard (ET-Rox 550; Applied Biosystems) run with each sample to determine base pair length. We scored genotypes, characterized as allele sizes, manually with the Genetic Profiler® v2.2 software package (Amersham Biosciences).

Characterization of Genetic Variation and Population Structure

We evaluated linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE) for each locus using an exact probability test in the GENEPOP 3.3 (Raymond and Rousset 1995) software program. We carried out tests for significant departures from HWE within sites for each locus and globally across all loci. We adjusted for multiple comparisons in linkage disequilibrium and HWE tests using a sequential Bonferonni correction (Rice 1989) and we used only accepted loci in downstream analyses. We generated estimates of F_{IS} per site in the software program GENETIX (Belkhir et al. 1999). We tested globally significant heterozygote deficiencies using GENEPOP 3.3 and by correcting for multiple comparisons (Rice 1989). We estimated allelic diversity (N_A), the effective number of alleles (A_E), and expected heterozygosity (H_E) using the software program GENALEX 6 (Peakall and Smouse 2006). We generated numbers of private alleles using rarefaction in the software program HP-RARE 1.0 (Kalinowski 2005) by randomly sampling 100 genes to account for differences in sample sizes.

We used a Bayesian assignment approach (STRUCTURE version 2.1; Pritchard et al. 2000, Falush et al. 2003) to characterize genetic structure and to assign individuals to their likely population of origin. We adopted a Bayesian approach over traditional allele-frequency–based clustering approaches because a critical question in this study required the identification of the number of remnant populations (Pritchard et al. 2000, Falush et al. 2003), functionality that is limited in the frequency-based methodology. Furthermore, recent empirical studies have supported the performance of Bayesian structure and assignment estimates (Hauser et al. 2006, Latch et al. 2006). We based the results generated on 5 independent runs simulating 1–10 ($K = 1$–10) inferred genetic populations, using a 500,000 burn-in period and 1×10^6 iterations of a Markov chain Monte Carlo simulation. We ran simulation models using no prior information and assumed correlated allele frequencies and admixture for detection of subtle population structure potentially associated with the fragmentation of historically continuous populations (Falush et al. 2003, Evanno et al. 2005). We estimated the number of inferred genetic populations (K) by comparing the estimated ln probability values, plotting the posterior probabilities of all possible K, and selecting the best fit (Pritchard et al. 2000). We used a recent method of detecting K using an ad hoc statistic ΔK (described by Evanno et al. 2005), which is based on the rate of change in the log probability of the data between successive K values, to support the estimated number of populations.

We assigned a sample site to an inferred genetic cluster based on the highest number of individuals with high ancestry ($q_1 \geq 80\%$) and moderate ancestry ($q_1 = 60$–80%) to a given cluster. We classified individuals with <60% ancestry as admixed individuals. We classified individuals with high or moderate ancestries to other clusters as migrants or effective migrants, respectively, where we defined effective migrants as the offspring of a migrant.

We estimated genetic differentiation among clusters by calculating pair-wise F_{ST} (GENETIX 4.0.3; Belkhir et al. 1999). We assessed patterns of isolation-by-distance by using a combination of the software programs IBD (Bohonak 2002) and SPAGEDI (Hardy and Vekemans 2002, Vekemans and Hardy 2004). We applied a Mantel test to assess the overall patterns of genetic structure related to geographic distance. We then employed jack-knifing of loci and permutation of individuals over different distance classes (Hardy and Vekemans 2002) to test for the significance of relationships between genetic and geographic distance of pairs of sample sites. We generated indirect estimates of genetic distance using $F_{ST}/(1-F_{ST})$, which has

Table 1. Variability of 16 microsatellite loci from 769 fishers sampled in and around Ontario, Canada, between 2000 and 2003, inclusive.

Locus	Source	Label[a]	N_A[b]	N_E[c]	H_E[d]
Mvis002	Fleming et al. 1999	NED	7.00	2.326	0.570
Mvis072	Fleming et al. 1999	6-FAM	8.00	2.358	0.576
Mer041	Fleming et al. 1999	6-FAM	9.00	3.266	0.694
Lut604	Dallas and Piertney 1998	6-FAM	12.00	5.602	0.822
Gg443	Walker et al. 2001	6-FAM	9.00	4.587	0.782
Gg454	Walker et al. 2001	HEX	7.00	0.814	0.645
Ggu101	Duffy et al. 1998	NED	9.00	2.892	0.654
Ggu216	Duffy et al. 1998	6-FAM	11.00	4.417	0.774
Ma1	Davis and Strobeck 1998	NED	8.00	3.834	0.739
Ma2	Davis and Strobeck 1998	HEX	9.00	3.797	0.737
Ma19	Davis and Strobeck 1998	HEX	9.00	4.907	0.796
Gg007	Davis and Strobeck 1998	HEX	8.00	2.629	0.620
Mvi1321	Vincent, Farid and Otieno (GenBank Accession No. AF480847)	NED	10.00	2.080	0.519
Mvi1341	Vincent, Farid and Otieno (GenBank Accession No. AF480850)	6-FAM	10.00	4.106	0.756
Mvi1342	Vincent, Farid and Otieno (GenBank Accession No. AF480851)	HEX	6.00	3.422	0.708
Mvi1354	Vincent, Farid and Otieno (GenBank Accession No. AF480852)	6-FAM	7.00	2.278	0.561
$\bar{x}$			8.69	3.330	0.677
SD			1.58	1.240	0.098

[a] Fluorescent label used in genotyping.
[b] Obs no. of alleles (N_A).
[c] Effective no. of alleles (N_E).
[d] Exp heterozygosity (H_E).

been validated to show closer concordance between direct and indirect estimates of gene flow than other estimates (Rousset 1997). We used jack-knifing and permutation procedures to generate a distribution of values of [$F_{ST}/(1 - F_{ST})$; Rousset 1997] that would be expected based on isolation-by-distance. We compared these expected values to observed data using multilocus regressions. We carried out this analysis in 3 ways: all sites, comparing only pairs among clusters, and comparing only pairs within clusters.

RESULTS

The majority of samples (i.e., 85%) in this study provided measurable DNA with a PicoGreen assay and required subsequent dilution to optimal concentrations (i.e., 2–5 ng/ul) above the threshold for allelic dropout (Ball et al. 2006). We ran DNA profiles from samples with low DNA template (i.e., Royal Ontario Museum [ROM] historic samples) multiple times to confirm homozygote profiles were not due to allele drop-out. Amplification replication of a subset (>30) of contemporary tissue samples and the historic ROM samples specifically revealed no change in genotype for the optimal sample concentrations (2–5 ng/μl). We observed an allelic drop-out event at 1 locus in each of 2 ROM specimens.

Each locus was polymorphic and within Hardy–Weinberg equilibrium for each site except for Mvi2243 and Mvi020. We removed both loci prior to subsequent analyses, along with locus Mvi1302, which had inconsistent allelic morphology upon examination of electropherograms. The remaining 16 loci were all moderately variable, ranging from 6 alleles to 12 alleles with heterozygosity values ranging from 0.519 to 0.822 (Table 1), and did not exhibit linkage disequilibrium. Four sites significantly departed globally from HWE following a sequential Bonferroni correction ($\alpha = 0.050/35$ sites $= 0.001$): the Bruce Peninsula, Montieth–Christie, Anstruther, and Marmora–Lake. Estimates of F_{IS} indicated a significant global deficiency of heterozygotes in several sites (Table 2).

When we applied a Bayesian analysis of genetic structure and examined the second-order rate of change (ΔK) in likelihood (Ln|P), we identified a strong signal of structure at $K = 2$ and a second signal at $K = 5$ that represented the uppermost level of structure for our dataset (Evanno et al. 2005). The first signal differentiated the Adirondack cluster, including neighboring Ontario sites, from other Ontario and Quebec sites. The second signal included the Adirondack cluster plus 4 additional clusters within Ontario (Fig. 1, Table 3). Our assignment procedure demonstrated that for $K = 5$, there were 4–12 sample sites per cluster. Many sites had individuals that were assigned to other clusters, and were, thus, migrants. Similarly, many sites had admixed individuals (Table 3).

Measurements of genetic variability were comparable across clusters (Table 4) with a narrow range in expected heterozygosity values (0.599–0.679). The main disparity between clusters was in the number of unique alleles, with the Adirondack cluster having the smallest ($N_{UA} = 2$) and Midhurst and Bancroft clusters each having the largest ($N_{UA} = 11$ and 9, respectively).

We estimated pair-wise F_{ST} values among clusters, but with Manitoulin Island and the Bruce Peninsula considered separately due to their isolation and their translocation histories. These 2 sites showed the lowest F_{ST} with their respective inferred cluster compared to other clusters (Table 5). Pair-wise F_{ST} estimates among sample sites were concordant with the grouping of these sites into the 5 inferred clusters (data not shown). We found a high degree of gene flow between certain clusters, most notably between Midhurst and Algonquin ($F_{ST} = 0.019$). The Bancroft cluster had moderate gene flow with both Midhurst and

Table 2. Summary statistics for fishers sampled at 16 microsatellite loci in and around Ontario, Canada, between 2000 and 2003, inclusive.

Cluster[a]	Sample site[b]	ID	N[c]	N_A[d]	SE	A_E[e]	SE	H_E[f]	SE	F_{IS}[g]
Midhurst	Bruce Peninsula	BP	25	4.31	0.31	2.91	0.16	0.640	0.020	**0.158**
	Carling–Ferguson	CF	8	3.56	0.26	2.58	0.20	0.567	0.040	0.035
	Blair–Mowat	BM	26	5.19	0.34	3.12	0.28	0.634	0.035	**0.111**
	Burton–McKenzie	BK	16	4.81	0.29	3.03	0.22	0.645	0.024	0.054
	Conger–Freeman	CM	15	4.63	0.34	2.86	0.21	0.615	0.034	**0.129**
	Montieth–Christie	MC	26	5.19	0.42	3.30	0.26	0.659	0.035	**0.183**
	Orillia–Ramara	OR	17	4.63	0.26	3.02	0.20	0.644	0.026	**0.119**
Algonquin	Falconer	FL	22	4.56	0.38	3.16	0.24	0.657	0.025	0.053
	Badgerow	BA	22	5.06	0.40	3.25	0.29	0.649	0.034	0.025
	Olrig Cluster	OL	14	4.75	0.41	3.10	0.27	0.639	0.031	0.051
	Algonquin telemetry study*	AQ_	20	4.75	0.34	3.09	0.22	0.647	0.028	**0.076**
	Anson–Lutterworth	AL	25	4.88	0.42	3.14	0.24	0.648	0.030	0.029
	Anstruther	AS	24	4.31	0.31	2.91	0.26	0.615	0.034	0.081
	Galway	GW	20	4.31	0.34	2.87	0.25	0.615	0.034	−0.004
	Hungerford–Huntington	HH	14	4.00	0.24	2.60	0.20	0.582	0.030	**0.110**
Bancroft	Carlow–Bangor	CB	20	4.38	0.34	2.82	0.19	0.620	0.025	−0.020
	Marmora–Lake	ML	32	5.19	0.28	3.04	0.26	0.631	0.034	**0.134**
	Belmont	BL	7	3.56	0.34	2.54	0.29	0.542	0.040	**0.148**
	Manitoulin Island	MI	40	4.56	0.30	2.84	0.15	0.631	0.020	**0.066**
Gatineau	Fraser–Richards	FR	21	4.69	0.36	2.95	0.21	0.630	0.030	0.017
	Lyndoch	LN	19	4.94	0.32	3.19	0.27	0.641	0.039	**0.098**
	Angelsea–Grimsthorpe	AG	16	4.19	0.25	2.79	0.26	0.595	0.036	0.050
	Kennebec	KB	23	5.06	0.35	3.14	0.28	0.639	0.035	**0.089**
	Brougham	BR	23	5.19	0.36	2.94	0.20	0.632	0.027	0.009
	Dalhousie	DL	20	4.88	0.30	2.88	0.27	0.599	0.041	0.012
	Loughborough–Bedford	LB	31	4.81	0.26	2.85	0.26	0.599	0.038	0.004
	Ross	RO	19	5.06	0.32	2.96	0.23	0.626	0.033	**0.094**
	McNab	MN	24	4.88	0.30	2.69	0.22	0.588	0.035	**0.089**
	Darling	DR	22	5.44	0.42	2.92	0.22	0.626	0.029	**0.100**
	Gatineau, Quebec*	GA	18	5.19	0.38	3.19	0.26	0.655	0.028	0.009
Adirondack	Ramsey–Huntley	RH	20	5.19	0.38	2.89	0.25	0.611	0.037	0.067
	Montague	MT	21	4.38	0.34	2.63	0.21	0.584	0.032	0.123
	Escott–Yonge	EY	20	3.94	0.30	2.55	0.21	0.558	0.044	0.031
	Prescott telemetry study*	PR	48	4.50	0.32	2.32	0.20	0.513	0.045	**0.055**
	Adirondack, NY*	AD	22	4.56	0.29	2.76	0.19	0.607	0.034	**0.083**

[a] We grouped sites into inferred clusters based on the results of STRUCTURE (Pritchard et al. 2000).
[b] Most sample sites were Ontario townships (except *). Hyphenated sites indicate pairs of adjacent townships combined to obtain a sufficient sample. ID = sample site abbreviation.
[c] Sample size (N).
[d] Obs no. of alleles (N_A).
[e] Effective no. of alleles (A_E).
[f] Exp heterozygosity (H_E).
[g] F_{IS} values demonstrating significant global heterozygote deficiency are in bold.

Algonquin clusters (F_{ST} = 0.030 and F_{ST} = 0.026, respectively; Table 5). In contrast, the Adirondack cluster was differentiated ($F_{ST} > 0.100$) from all clusters other than Gatineau, with which it shared moderate gene flow ($F_{ST} =$ 0.045). The Gatineau cluster, in fact, shared moderate gene flow with all clusters ($F_{ST} = 0.034$–0.053).

There was a pattern of isolation-by-distance over the entire study area ($Z = -3{,}493.3$, $r^2 = 0.142$, $P < 0.001$). An analysis of the distribution of genetic structure between all pairs of sites regardless of cluster assignment revealed higher gene flow at distances <100 km than we expected based on isolation-by-distance, and less gene flow than we expected at 400 km (Fig. 2). When we considered differences within and among clusters, we found higher gene flow between pairs of points within clusters than among clusters (Fig. 3). Much of the deviation in gene flow from values expected based on isolation-by-distance was due to pair-wise comparison of sites among clusters (Fig. 3). For example, the among-cluster analysis closely resembled the comparison between all pairs of sites regardless of cluster assignment.

We were able to extract and amplify DNA from 5 specimens obtained from the Royal Ontario Museum and originating in 1949 and 1950 from locations in central Ontario (Table 6). Three of the samples were most highly assigned to the Midhurst cluster, and 2 were most highly assigned to Gatineau. Adirondack was the second-highest assignment for 4 of the 5 samples. None of the samples had probabilities of assignment to Bancroft or Algonquin higher than 0.07.

DISCUSSION

Our results were not consistent with the hypothesis that Algonquin Provincial Park has served as the main source of fishers during the recolonization of southern Ontario. Rather, the recolonization appears to have originated from multiple sources suggesting that multiple remnant populations persisted in the region through the lows of the 1950s.

Table 3. Estimated ancestry for 5 inferred genetic clusters of fishers sampled at 16 microsatellite loci in and around Ontario, Canada, between 2000 and 2003, inclusive.

Inferred cluster[a]	Sample site	ID[b]	≥80 assigned cluster	≥60–80 assigned cluster	<60 admixed	≥60–80 other clusters	≥80 other clusters
Midhurst	Bruce Peninsula	BP	0.880	0.120	0.000	0.000	0.000
	Carling–Ferguson	CF	0.125	0.250	0.250	0.125	0.250
	Blair–Mowat	BM	0.115	0.192	0.500	0.154	0.038
	Burton–McKenzie	BK	0.063	0.250	0.438	0.188	0.063
	Conger–Freeman	CM	0.333	0.133	0.467	0.067	0.000
	Montieth–Christie	MC	0.154	0.269	0.346	0.154	0.077
	Orillia–Ramara	OR	0.059	0.176	0.529	0.118	0.118
Algonquin	Falconer	FL	0.545	0.091	0.318	0.000	0.045
	Badgerow	BA	0.364	0.182	0.182	0.182	0.091
	Olrig Cluster	OL	0.286	0.000	0.429	0.143	0.143
	Algonquin study	AQ	0.300	0.050	0.450	0.150	0.050
	Anson–Lutterworth	AL	0.240	0.120	0.280	0.280	0.080
	Anstruther	AS	0.333	0.083	0.333	0.167	0.083
	Galway	GW	0.700	0.150	0.050	0.050	0.050
	Hungerford–Huntington	HH	0.429	0.000	0.286	0.143	0.143
Bancroft	Carlow–Bangor	CB	0.182	0.091	0.545	0.182	0.000
	Marmora–Lake	ML	0.375	0.125	0.281	0.063	0.156
	Belmont	BL	0.571	0.143	0.286	0.000	0.000
	Manitoulin Island	MI	0.600	0.275	0.100	0.025	0.000
Gatineau	Fraser–Richards	FR	0.048	0.143	0.571	0.095	0.143
	Lyndoch	LN	0.053	0.263	0.421	0.263	0.000
	Angelsea–Grimsthorpe	AG	0.250	0.000	0.625	0.063	0.063
	Kennebec	KB	0.348	0.261	0.304	0.087	0.000
	Brougham	BR	0.182	0.136	0.591	0.045	0.045
	Dalhousie	DL	0.200	0.050	0.500	0.150	0.100
	Loughborough–Bedford	LB	0.290	0.419	0.065	0.161	0.065
	Ross	RO	0.263	0.105	0.421	0.105	0.105
	McNab	MN	0.500	0.167	0.250	0.083	0.000
	Darling	DR	0.091	0.318	0.409	0.136	0.045
	Gatineau, Quebec	GA	0.222	0.111	0.444	0.000	0.222
Adirondack	Ramsey–Huntley	RH	0.300	0.050	0.400	0.150	0.100
	Montague	MT	0.429	0.429	0.048	0.095	0.000
	Escott–Yonge	EY	0.850	0.050	0.100	0.000	0.000
	Prescott study	PR	0.938	0.042	0.021	0.000	0.000
	Adirondack, NY	AD	0.364	0.318	0.227	0.000	0.091

[a] Ancestry to a cluster required the highest proportion of individuals be assigned at high or moderate levels to the cluster.
[b] ID = sample site abbreviation.

We identified 5 clusters that may have resulted from 5 different refugia. Two of these, the Algonquin and Adirondack clusters, can likely be assigned to the Algonquin and Adirondack refuges, respectively. The Gatineau cluster may have resulted from a refuge in Quebec. The Midhurst and Bancroft clusters suggest the presence of additional, unexpected refuges (in addition to Algonquin Park) within Ontario. We discuss some of these issues below.

Assessing Source Populations

The recent appearance of fishers in southeastern Ontario has created public interest because the agricultural environment is atypical for fishers and some residents perceive fishers to be a potential nuisance. Abundance data demonstrate that fishers have only recently recolonized the agricultural southeast (Bowman et al. 2006). Our genetic assignment test revealed that these new colonists were highly assigned to

Table 4. Measurements of genetic variability of fishers sampled at 16 microsatellite loci in and around Ontario, Canada, between 2000 and 2003, inclusive, and assigned to each of 5 inferred populations.

Cluster	N[a]	N_A[b]	SE	N_{UA}[c]	Private allelic richness[d]	SE	H_E[e]	SE
Midhurst	133	6.88	0.41	11	6.11	0.33	0.679	0.025
Algonquin	161	6.25	0.44	5	5.55	0.39	0.659	0.029
Bancroft	99	6.19	0.34	9	5.69	0.32	0.650	0.026
Gatineau	236	6.75	0.39	4	6.02	0.31	0.659	0.028
Adirondack	131	5.88	0.38	2	5.53	0.32	0.599	0.037

[a] Sample size (N).
[b] $\bar{x}$ obs no. of alleles (N_A).
[c] No. of unique alleles (N_{UA}).
[d] Estimated using rarefaction to correct for sample size (Kalinoski 2005).
[e] $\bar{x}$ exp heterozygosity (H_E).

Table 5. Pair-wise values of F_{ST} for fishers from reintroduction sites (Manitoulin Island and Bruce Peninsula) and from 5 genetically inferred populations sampled in and around Ontario, Canada, between 2000 and 2003, inclusive.

Cluster	MI	BP	MID	ALG	BAN	GAT	ADIR
Manitoulin Island (MI)	0.000						
Bruce Peninsula (BP)	0.091	0.000					
Midhurst (MID)	0.046	0.074	0.000				
Algonquin (ALG)	0.052	0.082	0.019	0.000			
Bancroft (BAN)	0.027	0.106	0.030	0.026	0.000		
Gatineau (GAT)	0.061	0.118	0.034	0.053	0.042	0.000	
Adirondack (ADIR)	0.136	0.183	0.097	0.130	0.132	0.045	0.000

the Adirondack cluster. Genetic differentiation was moderate to weak across almost all clusters when Adirondack was excluded (F_{ST} = 0.019–0.054), but strong between Adirondack and all other clusters (F_{ST} = 0.045–0.136). Further, F_{ST} values at distance classes corresponding exclusively to pair-wise comparisons with sample sites in the Adirondack cluster (400 km) had less gene flow than expected based on distance. Taken together, these results suggest that the Adirondack fisher population has recently expanded into Ontario from New York, where Adirondack Park has historically acted as a refuge for fishers (Hamilton and Cook 1955). The St. Lawrence River does separate the landscapes but it is not uncommon for fishers to swim. Seton (1929) gives an account of a fisher swimming across a lake a mile wide and mentions that fishers often were known to swim rivers and lakes in the Adirondacks.

The Algonquin cluster was likely the result of diffusive population spread from Algonquin Park. The spread of fishers from the refuge would have been similar to the expansion of fishers from the Chapleau Crown Game Preserve during the 1950s (de Vos 1951). A similar process may have occurred in Quebec, with fishers spreading into Ontario and forming the Gatineau cluster. Refuges for fishers also existed in more northern areas of Ontario, such as the Algoma highlands, on the eastern shore of Lake Superior (Fig. 1; de Vos 1952). Two of the 3 historical fisher samples from the Algoma highlands assigned to the Midhurst cluster with ≥87% assignment. The third sample assigned 47% to the Midhurst cluster and 48% to the Gatineau cluster. This suggests that the Midhurst cluster may have originated from individuals moving south from Algoma or it may have been contiguous with Algoma at one time.

Manitoulin Island and some mainland Ontario sites both were assigned to the same genetic cluster. This is undoubtedly a result of the translocation of fishers that took place from Bancroft to Manitoulin Island during the 1979–1982 program (Berg 1982, Douglas and Strickland 1987, Thompson 2000). Our finding that Manitoulin Island fishers still clustered with those in Bancroft is consistent with the results of Drew et al. (2003), who found that historically translocated fisher populations in the western United States were more similar to source populations than to adjacent populations. It was surprising that fishers in the Bancroft area have remained genetically distinct from adjacent

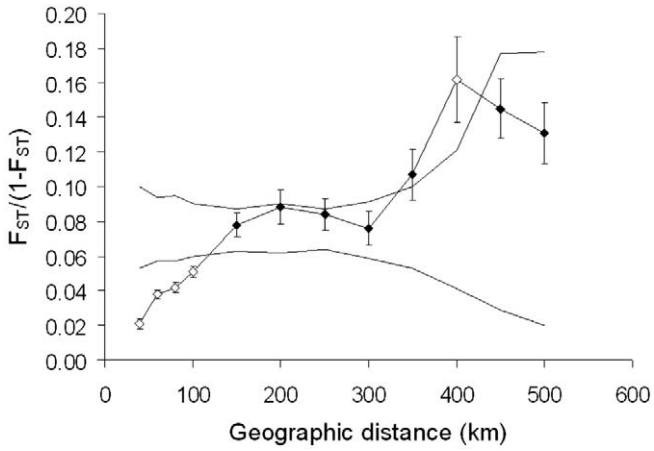

Figure 2. Estimated gene flow between pairs of sample sites at different distance classes where we sampled fishers at 16 microsatellite loci in and around Ontario, Canada, between 2000 and 2003, inclusive. We determined confidence limits (95%) using 10,000 permutations of individuals by location and 10,000 bootstraps of loci. Closed symbols indicate nonsignificant values of genetic structuring and open symbols indicate significant genetic structuring following a progressive Bonferroni correction. We made pair-wise comparisons in this analysis between all sample sites irrespective of their inferred ancestries.

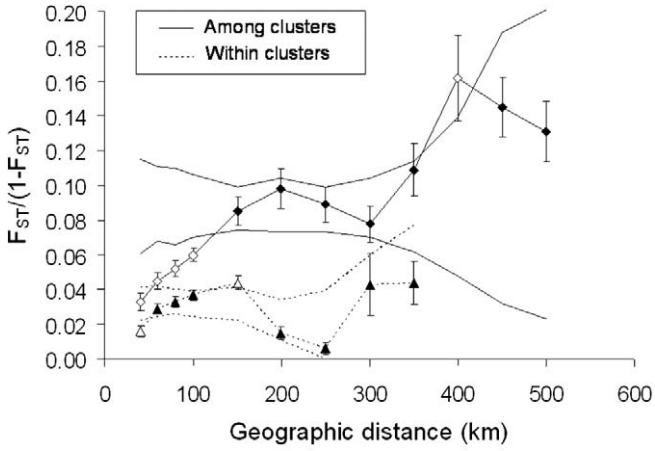

Figure 3. Estimated gene flow between pairs of sample sites at different distance classes where we sampled fishers at 16 microsatellite loci in and around Ontario, Canada, between 2000 and 2003, inclusive. We made pair-wise comparisons of sample sites, which accounted for inferred ancestry. We made comparisons between sites both within (dotted line) and among (solid line) inferred population clusters. We determined confidence limits (95%) using 10,000 permutations of individuals by location and 10,000 bootstraps of loci. Closed symbols indicate nonsignificant values of genetic structuring and open symbols indicate significant genetic structure.

Table 6. Historical fisher specimens from the Royal Ontario Museum, Canada, profiled at 16 microsatellite loci.

Specimen	Location[a]	Yr	Age	Sex	Highest assignment[b]	Second assignment[b]
20157	Nipissing	1949	Ad	F	Gatineau 0.76	Adirondack 0.13
20188	Algoma	1950	Ad	F	Midhurst 0.87	Adirondack 0.09
20184	Algoma	1950	Ad	M	Midhurst 0.92	Adirondack 0.04
20127	Algoma	1950	Ad	M	Gatineau 0.48	Midhurst 0.47
20069	Sudbury	1949	Ad	M	Midhurst 0.61	Adirondack 0.30

[a] All locations are counties in Ontario, Canada.
[b] Assignments are probabilities of membership in inferred genetic population, based on a Bayesian analysis of microsatellites.

populations given their proximity. De Vos (1952) indicated that some fishers were still being trapped in the Bancroft area in 1951. It appears that this location has persisted as a refuge since the 1950s and that the population has differentiated from surrounding remnant populations.

Genetic Diversity and Differentiation

Genetic diversity was high in each cluster, with consistent allele numbers, allelic richness, and expected heterozygosity values (Table 4). Kyle et al. (2001) found similar heterozygosity values across their study area ranging from 0.56 to 0.68 (0.62 ± 0.04), but Wisely et al. (2004) found much lower values in the fragmented populations along the Pacific coast, ranging from 0.16 to 0.42 (0.28 ± 0.10). This suggests that most fisher populations in our study were not experiencing inbreeding pressure, with one probable exception being the Bruce Peninsula, which demonstrated a relatively high estimate of F_{IS} and low immigration. Other sites also had high F_{IS} levels (Table 2), but it is important to interpret these results in the context of recolonizing populations. The high F_{IS} levels observed within several sample sites more likely reflected a Wahlund effect of overlapping individuals from different subpopulations (Weir and Cockerham 1984).

We observed relatively similar numbers of private alleles among the clusters following a rarefaction correction for sample size differences. The presence of unique or private alleles is an important indicator of genetic distinctiveness in a population, and it supports the finding of multiple refugia within Ontario inferred using the assignment tests. The partitioning of genetic variation into multiple refugia during the period of population fragmentation has important implications for the short- and long-term recovery of fishers in Ontario. In particular, the presence of multiple refugia has likely maintained relatively higher levels of genetic variation than predicted for a single source. Recent contact among expanding reproductive fronts may have counteracted loss of genetic variation within any one cluster by increasing the homogenization of allelic variation through gene flow.

Despite the overall pattern of isolation-by-distance, there was a complex relationship between genetic structure and geographic distance that we associated with expanding reproductive fronts. We suggest that the early phase of genetic homogenization at contact zones of expanding fisher populations was evident from the high gene flow at short distances among genetic clusters. Such a pattern would arise from contacting expansion fronts if these fronts have high productivity, which is expected due to the availability of naïve prey. Populations may reach their intrinsic rate of increase along such fronts (e.g., Caughley 1970). Bowman et al. (2006) analyzed abundance of a recolonizing fisher population in eastern Ontario (encompassing sites EY and PR in Fig. 2) during 1995–2004 and suggested that the population increase observed might be explained by this expansion front hypothesis. A nonexclusive alternative cause of high productivity could be hybrid vigor due to heterosis (Rhymer and Simberloff 1996). The rapid homogenization suggested by our results may have conservation implications as genetic connectivity appears to be accelerated at contact zones of recolonizing populations and suggests that multiple source populations may more rapidly facilitate the reestablishment of a connected meta-population.

MANAGEMENT IMPLICATIONS

Our finding of high gene flow at short distances among genetic clusters suggests that these populations were productive and rapidly homogenizing along expansion fronts. The apparent high productivity along these fronts may have been due to the availability of naïve prey, heterosis, or both. This suggests that managers planning to restore populations through translocations should consider using animals from multiple sources, and should also consider having multiple receiver landscapes within a single restoration initiative. Such a design would take advantage of potential heterosis and increase the number of expansion fronts.

A perception by the public of high fisher population levels in southern Ontario during recent years has resulted in increased fisher harvest quotas. Fur managers should recognize that the recent population trends have been due to an expansion, and that this will have an end. Annual rates of fisher population growth should be monitored and harvest quotas adjusted to avoid overharvest when expansion fronts have passed.

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