Molecular Ecology (2003) **12**, 51–62

Conservation genetics of the fisher (*Martes pennanti*) based on mitochondrial DNA sequencing

R. E. DREW,* J. G. HALLETT,* K. B. AUBRY,† K. W. CULLINGS,‡ S. M. KOEPF§ and W. J. ZIELINSKI¶
School of Biological Sciences, Box 644236, Washington State University, Pullman, WA 99164-4236, USA, †USDA Forest Service, Pacific Northwest Research Station, Olympia, WA 98512, USA, ‡NASA-Ames Research Center, Moffett Field, CA 94035-1000, USA, §Department of Biology, San Francisco State University, San Francisco, USA, ¶USDA Forest Service, Pacific Southwest Research Station, Arcata, CA 95521 USA

Abstract

Translocation of animals to re-establish extirpated populations or to maintain declining ones has often been carried out without genetic information on source or target populations, or adequate consideration of the potential effects of mixing genetic stocks. We consider the conservation status of the fisher (*Martes pennanti*) and evaluate the potential genetic consequences of past and future translocations on this medium-sized carnivore by examining population variation in mitochondrial control-region sequences. We sampled populations throughout the fisher's range in North America including five populations unaffected by translocations and two western populations that had received long-distance translocations. Twelve haplotypes showed little sequence divergence. Haplotype frequencies differed significantly among subspecies and between populations within subspecies. Analysis of molecular variance (AMOVA) and neighbour-joining analyses of haplotype relationships revealed population subdivision similar to current subspecies designations, but which may reflect an isolation-by-distance pattern. Populations in Oregon and in Montana and Idaho received several translocations and each showed greater similarity to the populations where translocations originated than to adjacent populations. Additional sequences obtained from museum specimens collected prior to any translocations suggest historical gene flow among populations in British Columbia, Washington, Oregon, and California. Anthropogenic impacts in that region have greatly reduced and isolated extant populations in Oregon and California. Future translocations may be necessary to recover populations in Washington and portions of Oregon and California; our results indicate that British Columbia would be the most appropriate source population.

Keywords: conservation genetics, fisher, *Martes pennanti*, mtDNA, translocation

Received 26 April 2002; revision received 17 September 2002; accepted 17 September 2002

Introduction

Translocations of animals are often used to reintroduce extirpated populations or to augment declining ones (Griffith *et al*. 1989; Wolf *et al*. 1996). Many of the costs and benefits of this approach to conservation have been reviewed by Wolf *et al*. (1996), but the risks involved in relocating and potentially mixing different genetic stocks have received relatively little attention. The identification of conservation units (Moritz 1994; Johnson *et al*. 2001) should, therefore, be an integral part of translocation planning. We address these issues for the fisher (*Martes pennanti*), a medium-sized forest carnivore that is one of the most frequently translocated mammals in North America. Translocations have been used to both augment and reintroduce fisher populations owing to extirpations that resulted from overtrapping and habitat alteration, their potential role as a biological control agent of porcupines (*Erethizon dorsatum*), the high commercial value of their pelts, and their aesthetic appeal (Berg 1982; Powell 1993). In this study, we present an assessment of genetic structure in fisher populations throughout its range

Correspondence: James G. Hallett. Fax: +1 509 335 3184; E-mail: hallett@wsu.edu.
§Present address: Celera Diagnostics, 1401 Harbor Bay Parkway, Alameda, CA 94502 USA.

© 2003 Blackwell Publishing Ltd

in North America and evaluate the potential genetic consequences of past and future translocations.

In the 20th century, the fisher underwent a rapid population decline in the USA due primarily to habitat loss and overtrapping (Powell 1993; Powell & Zielinski 1994). In the midwestern and north-eastern USA, where trapping of fishers has been strictly regulated and where forest has reclaimed extensive areas of abandoned farmland, fisher populations have recovered in many areas from both natural recolonization and reintroduction programmes initiated by state wildlife agencies (Powell 1993; Buskirk & Powell 1994). Similarly, fisher population declines in the northern Rocky Mountains have resulted in a series of reintroduction efforts in Montana and Idaho since the early 1960s (Berg 1982; Jones 1991; Roy 1991; Heinemeyer 1993). The decline in fisher populations and the resulting conservation concerns regarding the status of extant populations have been most evident in the Pacific states (Washington, Oregon and California). Although fishers in Washington and Oregon have been protected from trapping for > 60 years, fishers are extremely rare in both states and have probably been extirpated from most of their historical range in this region (Aubry & Houston 1992; Gibilisco 1994; Powell & Zielinski 1994; Lewis & Stinson 1998; Aubry & Lewis 2003). Translocations have not occurred in Washington, but fishers from several source populations were released in Oregon in 1961 and from 1977 to 1981 (Berg 1982; Aubry & Lewis 2003). In California, evidence from remote camera and trackplate surveys indicate that the fisher has been extirpated in the central and northern Sierra Nevada, resulting in a 50% contraction of their historical range and the apparent isolation of two remnant populations by a distance of > 400 km (Zielinski *et al*. 1995). There are no records of fishers being translocated into California.

Successful translocations may result in a gene pool that is a mixture of native and introduced genotypes or a complete replacement of native material with introduced (Storfer 1999). If the introduced genetic material disrupts coadapted gene complexes (Templeton 1986) or lacks traits that are adaptive to the local environment, the population could be driven to extinction by outbreeding depression (Greig 1979). Indeed, it has been suggested that western fishers may have locally adaptive behaviours that eastern fishers lack (Roy 1991), although this possibility has not been explored empirically. If such adaptations exist, inappropriate translocations could have unforeseen detrimental effects on target populations. Although fishers have been translocated in many areas throughout their range, only one study has attempted to assess the potential effects of long-distance translocations on the genetics of fisher populations (Williams *et al*. 2000). This research was limited to translocations within the eastern range of the fisher, however, and showed little genetic subdivision among populations. Further study of translocated populations, particularly those in the Pacific states, is an essential next step.

In response to observed declines of the fisher, petitions to list populations in the Pacific states under the Endangered Species Act (hereafter the Act) have been submitted three times in the last 12 years. The first two attempts were rejected because of insufficient data on the habitat requirements and demographic parameters of fishers in the western USA (US Fish & Wildlife Service 1991) and failure to demonstrate the presence of defined conservation units [i.e. a specific form of management unit called a 'distinct population segment' (DPS) under the Act, US Fish and Wildlife Service and National Marine Fisheries Service 1996]. Although Pacific coast fishers were given subspecific status (*M. p. pacifica*) by Goldman (1935), the validity of his designations was questioned by later workers (Grinnell *et al*. 1937; Hagmeier 1959; Powell 1993). This uncertainty regarding subspecific differentiation in fishers was cited in the second decision, which concluded that '… the continuity of the fisher's range … between Canada and the United States, provides for genetic exchange throughout North America' (US Fish & Wildlife Service 1996). The third petition to list fisher populations in the Pacific states was submitted in November 2000 (Greenwald *et al*. 2000); as of this writing a decision on this petition is pending.

We focus on the criteria used by the US Fish and Wildlife Service to define 'distinct population segments.' Alternative approaches, such as the Evolutionarily Significant Unit (ESU) concept (Ryder 1986) may be difficult to define (Fraser & Bernatchez 2001) and to apply in practice (Moritz 1994), and some have argued for abandoning the ESU terminology altogether (Crandall *et al*. 2000). To be considered a DPS under the Act, the population must be: (i) discrete and (ii) biologically and ecologically significant. Discrete populations are geographically isolated from other populations within the species by physical, physiological, ecological or behavioural factors. Biological and ecological significance is determined by a variety of potential factors including (but not limited to) the following: it occurs in a unique or unusual ecological setting, its loss would result in a significant gap in the range of the species, or it differs markedly from other populations in its genetic characteristics (US Fish and Wildlife Service and National Marine Fisheries Service 1996).

Several components of our study have important implications for the conservation of fishers in North America. The designation of DPSs among extant fisher populations remains a key consideration for developing conservation strategies, including additional translocations. To establish a genetic basis for designating DPSs, we test for the presence of genetic structure in fishers by examining geographical and temporal patterns of sequence variation in the rapidly evolving control region of the mitochondrial DNA (mtDNA) genome. The maternally inherited mtDNA

genome is more sensitive to genetic drift (Harrison 1989; Amos & Hoelzel 1992) and can be more readily retrieved from preserved tissues (Pääbo 1989) than nuclear markers. These characteristics make mtDNA a valuable tool in conservation genetics if results are interpreted cautiously and compared with other markers (Cronin 1993; Moritz 1994).

Other carnivores show a variety of phylogeographical patterns of mtDNA sequence variation. Pine martens (*Martes martes*) and polecats (*Mustela putorius*) in Europe (Davison *et al*. 2001) show low sequence variation and little geographical partitioning of haplotypes. American martens (*M. americana*, Carr & Hicks 1997) and North American brown bears (*Ursus arctos*, Waits *et al*. 1998) have structured mtDNA phylogenies that correlate with geography. Finally, grey wolves (*Canis lupus*) show a highly structured mtDNA phylogeny with little geographical partitioning (Vilà *et al*. 1999). These patterns are largely shaped by species differences in the degree of range fragmentation during the last ice age, the level of dispersal following the withdrawal of the glaciers, and the extent of gender-bias in dispersal. Fossil evidence suggests that the fisher expanded from a single refugium (Anderson 1994) and is therefore likely to have little phylogeographical structure. Once genetic patterns have been determined for the fisher, management units (Moritz 1994) can be inferred. For retrospective studies of translocations, structure among populations need not be so deep as to be detectable in phylogenetic analysis, but populations must be distinct enough genetically that individuals can be assigned to their source populations.

Our objectives were to: (i) test for structuring of populations using control-region phylogeography and hierarchical population analysis; (ii) determine the consequences of past translocations by comparing haplotype distributions between native populations and those that received translocations; and (iii) use population structure and history to recommend future translocation strategies.

Methods

Tissue samples

We obtained tissue samples from 141 fishers, each of which we assigned to one of three subspecies based on geographical location (Fig. 1), and to one of two population categories based on translocation history. We defined 'native' populations as those that had not received translocations or that had received translocations of animals from within the same state or province (< 300 km) and of the same subspecies. 'Translocated' populations had received animals from more distant source populations (> 600 km). Translocated populations included both augmented and reintroduced populations (Table 1).

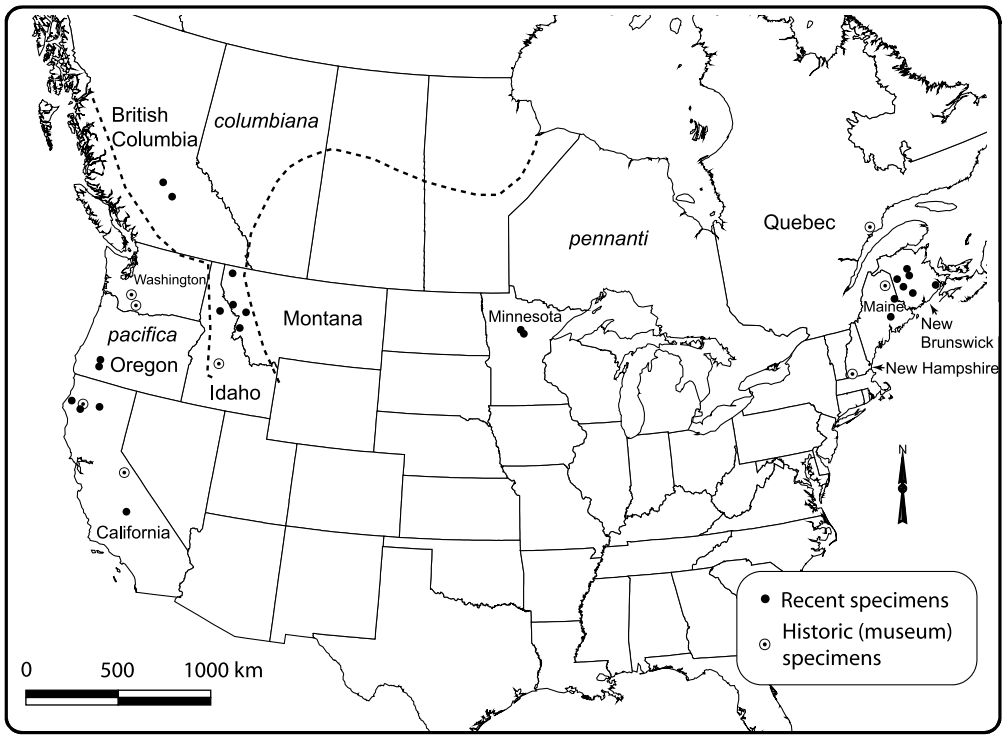

Fig. 1 Sampling localities for recent and historical (museum) specimens of the fisher in North America. Subspecies boundaries are drawn following Hall (1981).

Table 1 Translocation history of sample populations from the United States of America and Canada

Subspecies	Sample location	Category	Dates	History
Martes p. pennanti	Southern New Brunswick, Canada	Native	1966–1968	Several translocations from northern to southern New Brunswick at a distance of about 200 km (n = 25 animals, Dilworth 1974).
	North-eastern Maine, USA	Native		No record of translocations.
	North-central Minnesota, USA	Native	1968	One translocation from northwestern to northeastern Minnesota at a distance of about 300 km (n = 15 animals, Berg 1982; William Berg, Minnesota Department of Natural Resources, pers. commun.).
Martes p. columbiana	South-central British Columbia, Canada	Native		No record of translocations.
	North-western Montana and northern Idaho, USA	Augmented	1959–1963	Several translocations from central British Columbia to northwestern Montana (n = 36 animals, Weckwerth & Wright 1968) and Idaho (n = 39 animals, Williams 1963).
			1988–1991	Several translocations from northwestern Minnesota (n = 32 animals, Roy 1991) and northeastern Wisconsin (n = 78 animals, Heinemeyer 1993) to northwestern Montana.
Martes p. pacifica	North-western California, USA	Native		No record of translocations.
	South-central California, USA	Native		No record of translocations.
	South-western Oregon, USA	Reintroduced		Believed extirpated in the Cascade Range of Oregon by the 1940s (Aubry & Lewis 2003).
			1961–1980	One translocation from south-central British Columbia to northeastern Oregon (n = 13 animals, Kebbe 1961), and several translocations from south-central British Columbia to south-western Oregon (n = 28 animals, Kebbe 1961; Aubry & Lewis 2003).
			1981	One translocation from north-central Minnesota to south-western Oregon (n = 13 animals, Berg 1982; Aubry & Lewis 2003).

Five of our study populations fit the criteria for native populations and two had received translocations (Table 1, Fig. 1). Samples from native fisher populations within the described range of *Martes pennanti pennanti* were taken from animals harvested in late 1994 in New Brunswick (n = 28), captive fishers live-trapped in Maine (included with New Brunswick in all analyses, n = 6; see Appendix I for location data), and animals harvested during winter 1996–97 in northern Minnesota (n = 17). Native samples from *M. p. columbiana* included 31 from British Columbia: 11 harvested during winter 1994–95 near 100 Mile House, and 20 live-trapped in 1997 near Williams Lake for relocation within British Columbia. Native samples from *M. p. pacifica* included 38 obtained from 1992 to 1995 during ecological studies in California: 18 from the north-western population and 20 from the south-central population. Samples from translocated populations within the range of *M. p. columbiana* included 12 harvested from 1992 to 1995 in Idaho and Montana in the northern Rocky Mountains, several of which were obtained near the release sites. Translocated samples within the range of *M. p. pacifica* included nine obtained from 1995 to 1997 during ecological studies in south-western Oregon.

Tissue samples from live animals were taken from the centre of the ear with a punch used for ear-tagging. If this did not produce sufficient material, 3 × 3 mm of tissue was cut from the margin of the ear. Tissue samples collected from harvested fishers included ear clips or skeletal muscle. Samples were stored in 95% ethanol or dehydrated in separate microcentrifuge tubes filled with silica gel desiccant and stored at –20 °C.

To evaluate historical patterns of genetic structure, we obtained tissue samples from nine study skins in the collection of the National Museum of Natural History in Washington, DC (Appendix I). Sampled specimens were collected between 54 and 112 years ago, prior to any translocations, and included three *M. p. pennanti* (New Hampshire, n = 1; Maine, n = 1; Quebec, n = 1), one *M. p. columbiana* (Idaho),

and five *M. p. pacifica* (California, $n = 2$; Washington, $n = 3$). A thin strip of tissue (2×15 mm) was removed from the dorsal incision of each specimen using sterile technique to avoid contamination. Samples were stored individually in sterile, airtight plastic bags at room temperature until processed.

Polymerase chain reaction and DNA sequencing

Modern tissues were digested using proteinase-K and extracted using phenol/chloroform. Total genomic DNA was precipitated using ethanol and then resuspended in sterile water or TE buffer. Historical DNA was extracted from museum study-skin samples using the QIAmp Tissue Kit following the mouse-tail protocol (QIAGEN Inc.). All extractions of historical DNA were accompanied by blank extractions to aid in the detection of contamination.

The mtDNA control region was amplified using species-specific primers (MP-F′ and MP-R′, Table 2) designed from preliminary polymerase chain reaction (PCR) and sequencing using universal primers from Kocher *et al*. (1989). Because DNA extracted from preserved tissues is generally highly degraded (Pääbo 1989), primers for PCR amplifications of historical DNA were designed from the sequences obtained from the fresh tissues and selected to produce products 71–107 bp in size (Table 2). These primers encompass all polymorphic sites except one (site 286), which only differentiated two haplotypes.

PCR was conducted in 100-μl total volumes with the following recipe adapted from Masuda and Yoshida (1994): 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 2.0 mM $MgCl_2$, 0.001% bovine serum albumin, 0.4 μM each dNTP, 2–50 nM each of primers MP-F and MP-R, 2.5 units of *Taq* DNA polymerase (Gibco BRL), and 10–1000 ng genomic DNA. PCR conditions for historical DNA were identical except that they were optimized at 3.5 mM $MgCl_2$. PCR was performed with an Amplitron II thermocycler (Barnstead/Thermolyne) with the following profile: predwell at 94 °C for 3 min followed by 35–50 cycles consisting of 94 °C for 45 s, 50 °C for 30 s, and 72 °C for 90 s. Cycling was followed by a postdwell at 72 °C for 10 min. Resulting products were purified and then resuspended in 5–25 μl of sterile water.

To avoid contamination, PCR mixtures with historical DNA were prepared using equipment in a laboratory where fisher DNA extractions had never occurred. Aerosol-resistant tips were used whenever possible, particularly when handling DNA samples. To avoid contamination, blank extractions were tested for amplification and a negative control, containing no template, was added to each PCR run.

PCR products were sequenced using the Big-Dye™ sequencing chemistry (Applied Biosystems) following a protocol adapted from the manufacturer. Products generated from fresh tissue were sequenced in both directions using primers MP-F′ and MP-R4; products from historical DNA were sequenced using the same primers used in PCR (Table 2). Sequences were run on an ABI Prism® 377 DNA Sequencer (Applied Biosystems) and edited with SEQUENCHER Version 3.0 (GeneCode, Inc.) to generate a consensus sequence. The resulting sequences were aligned by eye or by using the program CLUSTAL W (Thompson *et al*. 1994).

Data analysis

A minimum spanning network was constructed using the program MINSPNET (L. Excoffier, University of Geneva, Switzerland) to provide a visual representation of the relationships among haplotypes. Population differentiation and relationships were evaluated in two ways. First, differences in haplotype frequencies among subspecies and between populations within subspecies for native fishers were examined using a χ^2-randomization test calculated using the program CHIRXC (Zaykin & Pudovkin 1993). Second, haplotype frequencies and distributions were further analysed using analysis of molecular variance (AMOVA, Excoffier *et al*. 1992). AMOVA analyses molecular frequencies in a nested ANOVA format and incorporates relatedness of haplotypes in the form of squared Euclidean distances. The program also generates Φ-statistics (Φ_{ST}) that measure population subdivision analogous to Wright's *F*-statistics

Primer name	Sequence	Polymorphic site
MP-F′	5′-TCAAGGAAAGAACAATAGCC-3′	
MP-R′	5′-TCGTGAACTCTTCTAGGCAT-3′	
MP-R4	5′-GCATGACACCACAGTTATGTGTGATCATGGGCTG-3′	
MP-F73	5′-CATCTCATGTACTTCCCCAG-3′	83, 92, 93, 120, 121
MP-R158	5′-GCTTATATGCATGGGGCAAACC-3′	
MP-F113	5′-CCCCTATGTATATCGTGCAT-3′	120, 121, 154, 156, 163
MP-R199	5′-GTGAAGTGCACGTATGTCA-3′	
MP-F137	5′-GGTTTGCCCCATGCATATAAGC-3′	154, 156, 163, 179, 190
MP-R243	5′-GGGTTGATGGTTTCTCGAGGCTTG-3′	
MP-F207	5′-CGAGCTTAATCACCAAGCCTCGAG-3′	234
MP-R277	5′-GGGCCCGGAGCGAGAAGAGG-3′	

Table 2 Polymerase chain reaction and sequencing primers for the control region of the fisher, *Martes pennanti*. For recent material, MP-F′ and MP-R′ were used for PCR and MP-F′ and MP-R4 were used for sequencing. The remaining four primer pairs were used for PCR and sequencing of museum specimens to screen specific polymorphic sites. Forward (F) and reverse (R) are in reference to the light strand sequence

(F_{ST}) (Wright 1992). The significance of these statistics was tested using permutation tests of 5000 replications.

Initially, AMOVA was used to test for differentiation between all pairs of populations. Visual representations of population relationships measured by pairwise Φ_{ST} values were generated using the neighbour-joining option of MEGA Version 2.1 (Kumar *et al*. 2001). To examine natural patterns of population differentiation, a tree was generated only for native populations. To investigate the potential effects of past translocations on the genetic structure of extant populations, a second tree was then generated that included both native and translocated populations. Using these results, several hierarchical AMOVA models were constructed to test relationships among populations based on subspecific designations and geographical affinities for native populations alone and for native and translocated populations combined.

Table 3 Polymorphic sites found in 12 fisher haplotypes in reference to the complete sequence found in GenBank (Accession nos: AY143663–AY143674)

	Polymorphic site											
Haplotype	83	92	93	120	121	154	156	163	179	190	234	286
1	A	T	–	A	T	C	A	G	C	T	A	C
2	A	T	–	A	T	C	G	G	C	T	A	C
3	G	T	–	A	T	C	A	G	C	T	A	C
4	A	T	–	A	T	C	A	G	C	T	G	C
5	A	–	–	A	C	T	A	A	C	T	A	C
6	A	T	–	A	T	C	G	G	C	T	G	C
7	A	T	–	A	T	C	A	A	C	T	A	C
8	A	–	–	G	T	T	A	A	C	T	A	C
9	A	T	–	A	T	C	A	A	C	T	G	C
10	G	T	–	A	T	C	A	G	C	T	A	A
11	A	T	T	A	C	C	A	G	C	C	A	C
12	A	T	–	A	T	C	A	A	T	T	A	C

Results

From the modern samples, we sequenced and aligned 299–301 bp of the control region nearest the *tRNA*^{Pro} gene consisting of the left domain and the conserved region. Twelve haplotypes were identified, 0.09 haplotypes for every individual sampled (Table 3). These haplotypes were characterized by 10 polymorphic sites: 9 transitions and 1 transversion. In addition, a repeat region of thymine bases showed variation in length, ranging from 4 bp in haplotypes 5 and 8 to 6 bp in haplotype 11. Haplotypes differed by only 1–7 base changes resulting in sequence divergences ranging from 0.33 to 2.33%. One animal from British Columbia and one from New Brunswick were excluded from the analyses because they produced sequences that were ambiguous at the polymorphic sites.

A minimum-spanning network shows that most transitions between adjacent haplotypes are due to single base changes (Fig. 2A). The actual relationships between some haplotypes were difficult to determine due to instances of homoplasy and to alternative links between pairs of haplotypes at two locations (Fig. 2A). The occurrence of homoplasy in such a small number of polymorphic sites suggests that haplotypes could have arisen independently, but we were unable to determine this. In the following analyses, we assume that each haplotype represents a single evolutionary lineage.

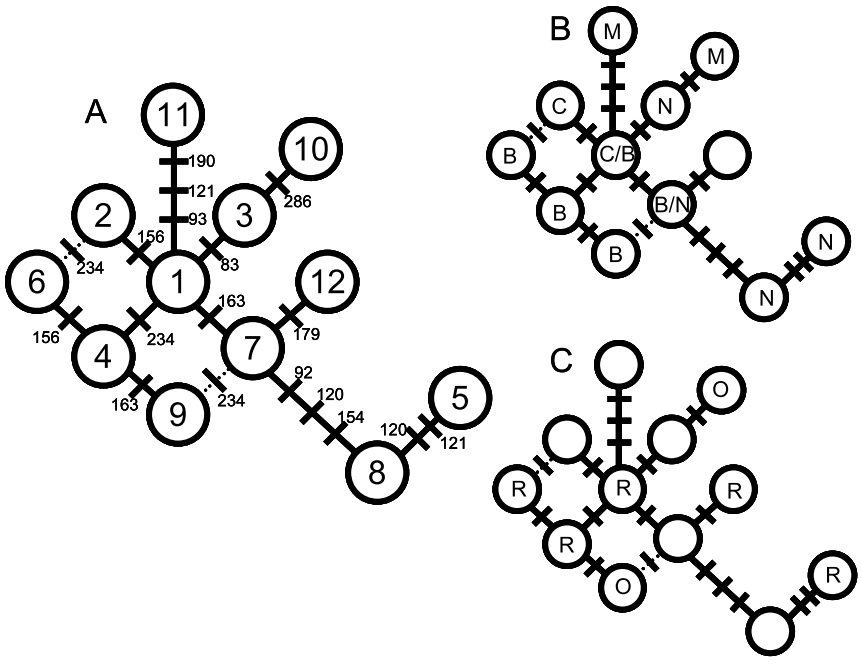

Fig. 2 (A) Possible minimum spanning network for the 12 haplotypes observed for the fisher. Base changes are shown for each transition between haplotypes. Alternative pathways are indicated by dotted lines. Geographic locations of (B) native and (C) translocated populations mapped onto the network. Open circles indicate the absence of the respective haplotype for respective groups. B = British Columbia, C = California, M = Minnesota, N = New Brunswick and Maine, O = Oregon, R = Rocky Mountains.

Table 4 Distribution of haplotypes of *Martes pennanti* for each sample population within a subspecies and for each subspecies both excluding and including populations that had translocations. Two specimens had ambiguous sequences and were deleted from the table. BC = British Columbia, CA-N = north-western California, CA-S = south-central California, MN = Minnesota, NB = New Brunswick and Maine, OR = Oregon, RM = Rocky Mountains (Montana and Idaho)

	Martes p. pennanti		*Martes p. columbiana*		*Martes p. pacifica*			Subspecies excluding translocated populations			Subspecies including translocated populations		
Haplotype	NB	MN	RM*	BC	OR*	CA-N	CA-S	*pen.*	*col.*	*pac.*	*col.*	*pac.*	Totals
3	17							17					17
8	7							7					7
10		16			1			16				1	17
11		1						1					1
5	4		1					4			1		5
7	5			5				5	5		5		10
4			2	3					3		5		5
6			3	13					13		16		16
12			5								5		5
9				8	8				8		8	8	16
1			1	1		12	20		1	32	2	32	34
2						6				6		6	6
N	33	17	12	30	9	18	20	50	30	38	42	47	139
No. haplotypes	4	2	5	5	2	2	1	6	5	2	6	4	

*Populations that received translocations.

Table 5 AMOVA pairwise analysis of sample populations. Pairwise Φ_{ST} values are below the diagonal. *P*-Values calculated from permutation tests with 5000 replications are above the diagonal. Tests were considered significant at $P < 0.05$. BC = British Columbia, CA-N = north-western California, CA-S = south-central California, MN = Minnesota, NB = New Brunswick and Maine, OR = Oregon, RM = Rocky Mountains (Montana and Idaho)

	Martes p. pennanti		*Martes p. columbiana*		*Martes p. pacifica*		
	NB	MN	RM*	BC	OR*	CA-N	CA-S
NB		0.000	0.000	0.000	0.000	0.000	0.000
MN	0.478		0.000	0.000	0.000	0.000	0.000
RM*	0.238	0.656		0.024	0.005	0.000	0.000
BC	0.414	0.726	0.146		0.011	0.000	0.000
OR*	0.392	0.827	0.217	0.213		0.000	0.000
CA-N	0.323	0.798	0.307	0.436	0.734		0.000
CA-S	0.325	0.892	0.417	0.547	0.857	0.309	

*Populations that received translocations.

Haplotype frequencies were significantly different among subspecies for native samples (Table 4; $\chi^2 = 213.4$, $P < 0.001$). Five haplotypes appeared to be unique to native *Martes p. pennanti* populations, three to *M. p. columbiana* populations, and one to *M. p. pacifica* populations. Pairs of adjacent subspecies had only one haplotype in common (Table 4) and haplotype frequencies were significantly different in each case (*pennanti* and *columbiana* $\chi^2 = 69.3$, $P < 0.001$; *columbiana* and *pacifica* $\chi^2 = 64.1$, $P < 0.001$). No haplotypes were shared between *pennanti* and *pacifica* populations.

Native populations within subspecies also differed significantly in haplotype frequencies (Table 4, Fig. 2B; *pennanti* $\chi^2 = 39.1$, $P < 0.001$; *pacifica*, Fisher's exact test, $P < 0.007$). Translocated populations included some haplotypes not found in nearby native populations (Table 4, Fig. 2C). Oregon fishers shared no haplotypes with native *pacifica* samples from California, but shared one haplotype each with samples from British Columbia (*columbiana*) and Minnesota (*pennanti*). Haplotype 5 occurred in the Rocky Mountain population and in *pennanti* samples from New Brunswick, but not in *columbiana* samples from British Columbia. When relationships among haplotypes were also considered by using AMOVA, differences between all populations were significant (overall $\Phi_{ST} = 0.509$, $P < 0.0002$; Table 5).

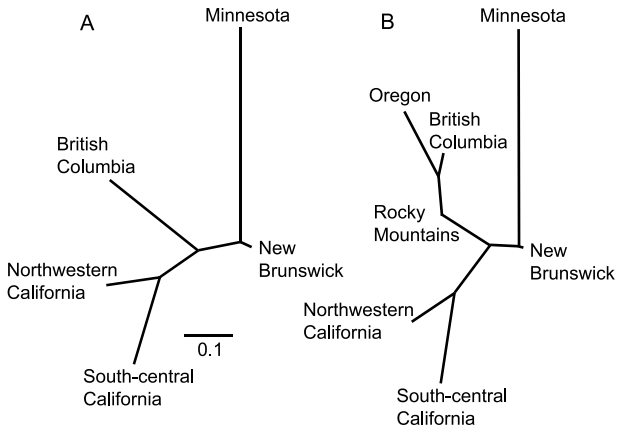

Fig. 3 Neighbour-joining trees of fisher populations generated using Φ_{ST} values from Table 5 for (A) native populations and (B) native and translocated populations. Rocky Mountains includes sampling localities in Montana and Idaho.

Neighbour-joining trees for native and for native and translocated populations combined (Fig. 3A,B, respectively) reflect a pattern that is largely concordant with geographical distribution. The Minnesota population is more distant than anticipated from New Brunswick (Table 4), even though the most common haplotype in the Minnesota sample (haplotype 10) can be derived by a single mutation from the most common haplotype in New Brunswick (haplotype 3; Fig. 2A). Translocated populations in Oregon and the Rocky Mountains align closely with British Columbia (Fig. 3B). Based on geography, populations in Oregon would be expected to be more similar to those in northern California.

To further examine genetic relationships between translocated and native populations, we conducted several hierarchical AMOVA analyses by assigning populations to different groupings. We first grouped native populations by subspecies. This model was nonsignificant ($P = 0.15$) and explained little of the variance (20.0%). The failure of this model appears to be due to variation between *pennanti* populations: a model that included Minnesota and New Brunswick populations as separate groups was highly significant ($\Phi_{ST} = 0.518$, $P < 0.0002$) and explained 51.8% of the variance. We then grouped each translocated population with nearby native populations. When Oregon and California populations were grouped together, the model was nonsignificant ($P = 0.08$). Grouping Oregon populations with those in British Columbia, however, produced a significant model ($\Phi_{ST} = 0.425$, $P < 0.0002$) that explained 42.5% of the variance. The Rocky Mountain population grouped best with British Columbia for all models (data not shown).

Analysis of the nine historical DNA samples revealed five to six haplotypes. Because of problems associated with sequencing highly degraded DNA extracted from preserved tissues, some individuals could only be resolved to two haplotypes. Also, because polymorphic site 286 differentiated only two closely related haplotypes (3 and 10) that were associated with the *pennanti* samples, this site was not screened. The *pennanti* samples included haplotypes 3 or 10 (New Hampshire), 7 (Maine), and 9 (Quebec). The *columbiana* sample (Idaho) was either haplotype 1 or 2. The *pacifica* samples included haplotype 1 (California, $n = 1$) and haplotype 4 (California, $n = 1$; Washington, $n = 2$), and one sample from Washington that was either haplotype 1 or 4. The occurrence of haplotype 4 only in historical specimens from Washington and California and modern specimens from British Columbia, indicates that there was greater connectivity among populations along the Pacific coast in the past than at present.

Discussion

Genetic structure

Genetic structuring in the fisher was detectable on a population level, but, as anticipated, phylogenetic analysis was precluded due to the low level of sequence divergence (0.33–2.33%). These sequences diverged ≈ 33 000–233 000 years ago, assuming 10% sequence divergence per Myr (Aquadro & Greenberg 1982). This estimate agrees with the earliest appearance of the modern fisher in fossil fauna deposits from the Pleistocene, dated at 29 870 years BP (Anderson 1994), and may correspond to the rapid geographical expansion of the fisher following the last glaciation (Graham & Graham 1994). The low sequence variation in fisher is similar to that observed for *Martes martes* and *Mustela putorius* in continental Europe (Davison *et al*. 2001), each of which is presumed to have originated from a single refugium. The fisher, however, exhibits greater hierarchical population structure than these two species.

Examination of native populations of fisher using AMOVA and neighbour-joining analyses (Fig. 3A) revealed patterns that were similar to subspecies designations. Additional support for linkage within *Martes pennanti pennanti* comes from analysis of allozyme data from populations from Maine, New Hampshire, New York, and Minnesota that showed no significant population differentiation (Williams *et al*. 2000). However, our results are extremely similar to an isolation-by-distance pattern detected by Kyle *et al*. (2001) for microsatellite loci among predominantly Canadian fisher populations, but within a geographical area comparable in size to ours. We anticipate that inclusion of additional sequence data, particularly from populations between Minnesota and New Brunswick, will further support this pattern. This consistency between both types of markers suggests that the genetic subdivision and isolation-by-distance pattern are not simply due

to sex-biased dispersal, which may affect fine-scale patterns (Williams *et al*. 2000), but to deeper historical mechanisms. Based on these patterns, however, we cannot conclude that our results provide a genetic validation of previously described subspecies for the fisher (Goldman 1935).

Whether or not Goldman's (1935) subspecific designations are valid taxonomically, however, it is clear that population subdivision is occurring within the species, especially among populations in the western USA and Canada. The California populations, in particular, differ strongly in haplotype frequencies from each other and from all other populations. These results are consistent with the conclusions of Aubry & Lewis (2003) that native populations in California and the reintroduced population in south-western Oregon have become isolated from the main body of the species' range due to the extirpation of fishers in Washington and northern Oregon. This loss of connectivity with northern fisher populations is most likely the result of a combination of human impacts during the early 20th century that included overtrapping, loss of habitat from extensive clearcut logging, and predator control activities. Consequently, because extant populations of fishers in California and Oregon are both discrete and biologically significant as defined by the US Fish and Wildlife Service, we conclude that they are 'distinct population segments' (US Fish & Wildlife Service & National Marine Fisheries Service 1996).

Effects of translocations

Our data track the effects of human manipulations on populations that received translocations. Both the Rocky Mountains and Oregon populations show evidence of the movement of genetic material. Both translocated populations cluster with British Columbia (Fig. 3B), which was the source for many of the fishers translocated to these areas. The Rocky Mountains population has one haplotype (5) that probably originated from translocations of fishers from within the range of *M. p. pennanti* (Table 4). The specimen containing this haplotype was collected in Lincoln County, Montana, near the site of the 1990–91 introductions from Wisconsin. The Rocky Mountains population also shares several haplotypes with the British Columbia population that may have resulted from translocations, but which also may represent native haplotypes. The Oregon population contains only haplotypes that occur in the source populations of reported translocations (haplotype 9, British Columbia and haplotype 10, Minnesota), and shares no haplotypes with populations in California (Table 4). These patterns indicate that either the native Oregon population has been replaced by transplanted animals from other portions of its range or that native mtDNA has been replaced by introduced haplotypes through genetic drift. The apparent success of fisher translocations from Minnesota and Wisconsin to the Rocky Mountains and Oregon argues against the hypothesis that western fishers have special adaptations to local conditions that eastern fishers lack (Roy 1991). However, we cannot exclude the possibility that these translocations have been successful only because habitat changes in the western states from timber harvest and human development have created conditions that are more suitable for midwestern fishers than for native animals.

Historical gene flow

Our findings suggest that gene flow once occurred between fisher populations in British Columbia and those in the Pacific states, but extant populations in these regions are now genetically isolated. The fisher was once distributed throughout dense coniferous forests in British Columbia, Washington, Oregon and California. Gene flow between British Columbia and California populations must have occurred historically, as evidenced by the detection of haplotype 4 in museum specimens from California (2 of 3) and Washington (1 of 2), and in modern populations from British Columbia. Furthermore, modern populations in British Columbia and California share haplotype 1. Because fisher populations have declined to extreme rarity or extirpation in Washington and Oregon (Aubry & Houston 1992; Aubry & Lewis 2003), and to isolation and population fragmentation in California (Zielinski *et al*. 1995), haplotype 4 was probably lost from the California populations because of genetic drift and a lack of gene flow.

Future translocations

The similarity between Oregon and British Columbia populations and our limited assessment of historical gene flow through the region suggest that future translocations of fishers into Oregon should be carried out with animals from British Columbia. A translocation programme is in the initial planning stages for Washington (Lewis 2002). Our historical data suggest that British Columbia would also be an appropriate source population for Washington. The necessity for translocating animals into California will increase as further habitat loss, population reduction and isolation continues. Our data show that historical gene flow along the Pacific coast has been disrupted and that haplotypes have apparently been lost from the California populations. The relatively recent isolation of the California populations would act to prevent the maintenance of genetic diversity through gene flow, but it may also have allowed populations to adapt to local selective forces (Storfer 1999). Such local adaptation could be disrupted by translocations. We believe, however, that the need to use translocations to counter continued threats of increasing

isolation, greater susceptibility to catastrophic events and population reduction far outweighs such considerations and may be essential for the persistence of fisher populations in the Pacific states.

Acknowledgements

We thank K. Adams, D. Call, T. Hardig, R. Kuzoff, S. Mech, M. Mort, C. Small and W. Young for providing technical advice and theoretical discussion. S. Hoang and M. Durkin provided laboratory assistance and C. Orrego provided assistance at the beginning of the project. D. Soltis and G. Thorgaard provided access to their laboratories and reviewed an early manuscript. T. Glenn (National Museum of Natural History) provided advice about DNA extraction and PCR protocols for preserved tissues. N. Anderson, B. Berg, P. Blackwell, E. Burkett, J. Casson, A. Clevenger, S. Dark, N. Duncan, K. Eagle, S. Fain, E. Farmer, R. Fierro, B. Frost, P. Gauthier, R. Golightly, J. Hunter, W. Krohn, J. Lewis, J. Mulholland, C. Ogan, B. Olsen, W. Philpott, G. Schmidt, K. Schmidt, R. Schlexer, A. Seglund, S. Self, T. Sabo, I. Teske, R. Truex and G. Will supplied tissue samples. R. Fisher (National Museum of Natural History) made arrangements for D. Houston to obtain the study skin tissues. The manuscript benefited from the comments of two anonymous reviewers and the Subject Editor, R. Wayne. Our work was funded by the Center for Wildlife Conservation, USDA Forest Service, Northwest Scientific Association, and the College of Sciences and Department of Zoology at Washington State University.

References

Amos B, Hoelzel AR (1992) Applications of molecular genetic techniques to the conservation of small populations. *Biological Conservation*, **6**, 133–144.

Anderson E (1994) Evolution, prehistoric distribution, and systematics of *Martes*. In: *Martens, Sables, and Fishers: Biology and Conservation* (eds Buskirk SW, Harestad AS, Raphael MG, Powell RA), pp. 13–25. Cornell University Press, Ithaca, NY.

Aquadro CF, Greenberg BD (1982) Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals. *Genetics*, **103**, 287–312.

Aubry KB, Houston DB (1992) Distribution and status of the fisher (*Martes pennanti*) in Washington. *Northwestern Naturalist*, **73**, 69–79.

Aubry KB, Lewis JC (2003) Extirpation and reintroduction of fishers (*Martes pennanti*) in Oregon: implications for their conservation in the Pacific states. *Biological Conservation*, in press.

Berg WE (1982) Reintroduction of fisher, pine marten, and river otter. In: *Midwest Furbearer Management* (ed. Sanderson GC), pp. 159–173. The Wildlife Society, Bloomington.

Buskirk SW, Powell RA (1994) Habitat ecology of fishers and American martens. In: *Martens, Sables, and Fishers: Biology and Conservation* (eds Buskirk SW, Harestad AS, Raphael MG, Powell RA), pp. 283–296. Cornell University Press, Ithaca, NY.

Carr SM, Hicks SA (1997) Are there two species of marten in North America? Genetic and evolutionary relationships with in *Martes*. In: *Martes: Taxonomy, Ecology, Techniques, and Management* (eds Proulx G, Bryant HN, Woodard PM), pp. 15–28. Provincial Museum of Alberta, Edmonton, Alberta, Canada.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, **15**, 290–295.

Cronin MA (1993) Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. *Wildlife Society Bulletin*, **21**, 339–348.

Davison A, Birks JDS, Brookes RC, Messenger JE, Griffiths HI (2001) Mitochondrial phylogeography and population history of pine martens *Martes martes* compared with polecats *Mustela putorius*. *Molecular Ecology*, **10**, 2341–2347.

Dilworth TG (1974) Status and distribution of fisher and marten in New Brunswick. *Canadian Field-Naturalist*, **88**, 495–498.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752.

Gibilisco CJ (1994) Distributional dynamics of modern *Martes* in North America. In: *Martens, Sables, and Fishers: Biology and Conservation* (eds Buskirk SW, Harestad AS, Raphael MG, Powell RA), pp. 59–71. Cornell University Press, Ithaca, NY.

Goldman EA (1935) New American mustelids of the genera *Martes*, *Gulo*, and *Lutra*. *Proceedings of the Biological Society of Washington*, **48**, 175–186.

Graham RW, Graham MA (1994) Late Quaternary distribution of *Martes* in North America. In: *Martens, Sables, and Fishers: Biology and Conservation* (eds Buskirk SW, Harestad AS, Raphael MG, Powell RA), pp. 26–58. Cornell University Press, Ithaca, NY.

Greenwald DN, Carlton J, Schneider B (2000) *Petition to List the Fisher (Martes pennanti) as an Endangered Species in its West Coast Range*, pp. 1–91. Center for Biological Diversity, Tucson, AZ.

Greig JC (1979) Principles of genetic conservation in relation to wildlife management in southern Africa. *South African Journal of Wildlife Research*, **9**, 57–78.

Griffith B, Scott MJ, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: status and strategy. *Science*, **245**, 477–480.

Grinnell J, Dixon JS, Linsdale JM (1937) *Fur-Bearing Mammals of California*. University of California Press, Berkeley.

Hagmeier EM (1959) A re-evaluation of the subspecies of fisher. *Canadian Field-Naturalist*, **73**, 185–197.

Hall ER (1981) *The Mammals of North America*. 2nd edn. John Wiley & Sons, New York.

Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Evolution and Ecology*, **4**, 6–11.

Heinemeyer KS (1993) Temporal dynamics in the movements, habitat use, activity, and spacing of reintroduced fishers in northwestern Montana. MSc Thesis, University of Montana.

Johnson WE, Eizirik E, Roelke-Parker M, O'Brien SJ (2001) Applications of genetic concepts and molecular methods to carnivore conservation. In: *Carnivore Conservation* (eds Gittleman JL, Funk SM, Macdonald D, Wayne RK), pp. 335–358. Cambridge University Press, London.

Jones JL (1991) Habitat use of fisher in northcentral Idaho. MSc Thesis, University of Idaho.

Kebbe CE (1961) Return of the fisher. *Oregon State Game Commission Bulletin*, **16**, 3–7.

Kocher TD, Thomas WK, Meyer A *et al*. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science, USA*, **86**, 6196–6200.

Kumar S, Tamura KIB, Jakobsen Nei M (2001) MEGA2: molecular

evolutionary genetics analysis software. *Bioinformatics*, **17**, 1244–1245.

Kyle CJ, Robitaille JF, Strobeck C (2001) Genetic variation and structure of fisher (*Martes pennanti*) populations across North America. *Molecular Ecology*, **10**, 2341–2347.

Lewis JC (2002) Feasability study underway for fisher reintroduction in Washington. *Martes Working Group Newsletter*, **10**, 11.

Lewis JC, Stinson DW (1998) *Washington State Status Report for the Fisher*, pp. 1–64. Washington Department of Fish and Wildlife, Olympia, WA.

Masuda R, Yoshida MC (1994) A molecular phylogeny of the family Mustelidae (Mammalia, Carnivora), based on comparison of mitochondrial cytochrome *b* nucleotide sequences. *Zoological Science*, **11**, 605–612.

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.

Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proceedings of the National Academy of Sciences, USA*, **86**, 1939–1943.

Powell RA (1993) *The Fisher: Life History, Ecology, and Behavior*, 2nd edn. University of Minnesota Press, Minneapolis.

Powell RA, Zielinski WJ (1994) Fisher. In: *The Scientific Basis for Conserving Forest Carnivores: Marten, Fisher, Lynx and Wolverine in the Western United States* (eds Ruggiero LF, Aubry KB, Buskirk SW, Lyon LJ, Zielinski WJ), pp. 38–73. General Technical Report RM-254. US Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO.

Roy KD (1991) Ecology of reintroduced fishers in the Cabinet Mountains of northwest Montana. MSC Thesis, University of Montana.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution*, **1**, 9–10.

Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. *Biological Conservation*, **87**, 173–180.

Templeton AR (1986) Coadaptation and outbreeding depression. In: *Conservation Biology: the Science of Scarcity and Diversity* (ed. Soule ME), pp. 105–116. Sinauer Associates, Sunderland, MA.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

US Fish and Wildlife Service (1991) Notice of 90-day finding on petition to list the Pacific fisher as endangered. *Federal Register*, **56**, 1159–1161.

US Fish and Wildlife Service (1996) 90-day finding for a petition to list the fisher in the western United States as threatened. *Federal Register*, **61**, 8016–8018.

US Fish and Wildlife Service and National Marine Fisheries Service (1996) Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. *Federal Register*, **61**, 4721–4725.

Vilà A, Amorium IR, Leonard JA *et al*. (1999) Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology*, **8**, 2089–2103.

Waits LP, Talbot S, Ward RH, Shields GF (1998) Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation. *Conservation Biology*, **12**, 408–417.

Weckwerth RP, Wright PL (1968) Results of transplanting fishers in Montana. *Journal of Wildlife Management*, **32**, 977–980.

Williams RM (1963) Trapping and transplanting. Part I — fisher. Final Segment Report, Project W 75-D-10, pp. 391–396. Idaho Fish and Game Department, Boise, ID.

Williams RN, Rhodes OE Jr, Serfass TL (2000) Assessment of genetic variance among source and reintroduced fisher populations. *Journal of Mammalogy*, **81**, 895–907.

Wolf CM, Griffith B, Reed C, Temple SA (1996) Avian and mammalian translocations: update and reanalysis of 1987 survey data. *Conservation Biology*, **10**, 1142–1154.

Wright S (1922) Coefficients of inbreeding and relationship. *American Naturalist*, **56**, 330–338.

Zaykin DV, Pudovkin AI (1993) Two programs to estimate significance of Chi-square values using pseudo-probability test. *Journal of Heredity*, **84**, 152.

Zielinski WJ, Kucera TE, Barrett RH (1995) Current distribution of the fisher, *Martes pennanti*, in California. *California Fish and Game*, **81**, 104–112.

Laboratory research was conducted by Rob Drew and Sandy Koepf while they were completing their MSc degrees under the supervision of Jim Hallett and Ken Cullings, respectively. Jim Hallett is interested in the effects of habitat fragmentation on the population and community structure of vertebrates. Keith Aubry and Bill Zielinski are research scientists with the USDA Forest Service and are actively involved in ecological studies of forest carnivores.

Appendix I

New Brunswick (locations are given by Wildlife Management Zone. Zone maps are available at http://www.gnb.ca/0078/f&w/zones.htm): Zone 7 (n = 9), Zone 10 (2), Zone 12 (8), Zone 16 (2), Zone 21 (1), Zone 24 (6); British Columbia, vicinity of 100 Mile House: 100 Mile House (6), Chimney Lake (2), Helena Lake (2), Lac La Hache (1); British Columbia, vicinity of Williams Lake: Alexis Creek (4), Nimpo Lake (1), Williams Lake (15); California, north-western: Humboldt Co. (3), Shasta Co. (3), Trinity Co. (12); California, south-central: Tulare Co. (20); Idaho: Clearwater Co. (2); Maine: Aroostook Co. (3), Piscataquis Co. (3); Minnesota: Cass Co. (2), Leech Lake Reservation, Chippewa National Forest (5), unknown northern Minnesota (10); Montana: Mineral Co. (2), Ravalli Co. (5), Missoula Co. (1), Lincoln Co. (2); Oregon: Douglas Co. (1), Jackson Co. (8).

Locations and collection dates for study skin samples obtained from the National Museum of Natural History, Washington, DC. Accession nos are in parentheses. Quebec: 1889 (188222); California: Yosemite, 1911 (171002), Trinity Co. 1917 (227118); Idaho: Sawtooth Lake, 1890 (24112); Maine: Aroostook Co. (143151); New Hampshire: Monadnock, 1902 (119724); Washington: Trout Lake, 1900 (99457), 1901 (108213), Iron Creek, 1923 (243790).