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ANCIENT DNA CONFIRMS NATIVE ROCKY MOUNTAIN FISHER (*MARTES PENNANTI*) AVOIDED EARLY 20TH CENTURY EXTINCTION

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Until recently it was assumed that fishers (*Martes pennanti*) in the Rocky Mountains all were descended from reintroduced stocks. However, a recent study reported that mitochondrial DNA (cytochrome-*b* and control region) haplotypes of fishers found only in west-central Montana are likely derived from a relic population of fishers that escaped harvests conducted in the early 20th century. I compared fishers in west-central Montana to samples from north-central Idaho and found no evidence for substructure between these groups. A museum specimen, collected in 1896 in north-central Idaho before any known translocation, also was the same haplotype as the ''native Montana haplotype'' discovered in the recent study. Thus, fishers in north-central Idaho and west-central Montana are the only confirmed native fishers in the Rocky Mountains, and 1 of a few populations in the West that have maintained native genes. Fishers from Idaho and Montana are not all descendants of translocated individuals, but also are the descendants of fishers that persisted despite early 20th century trapping.

Key words: ancient DNA, control region, cytochrome *b*, fishers, genetics, *Martes pennanti*, mitochondrial DNA, translocation

As the human ecological footprint widens, wildlife populations increasingly require human intervention to persist. One form of intervention is the translocation of animals between areas. Translocations are considered ''reintroductions'' when the historical populations have been extirpated and ''augmentations'' when remnants of historical populations persist (Griffith et al. 1989). Augmentation benefits remnant populations by reducing demographic and genetic stochasticity (Chapron et al. 2003, Moritz 1999) and minimizing inbreeding depression (Hedrick and Kalinowski 2000, Vilà et al. 2003). However, such benefits may be outweighed by potential negative consequences, such as outbreeding depression (reviewed in Tallmon et al. 2004).

In the past 2 decades, conservationists and wildlife managers have refined their approach to wildlife translocation to be more scientifically based (Breitenmoser et al. 2001). For instance, it is now common to use morphological, ecological, or genetic data to find the most appropriate source populations for translocations (Aubry and Lewis 2003; Earnhardt 1999; Maudet et al. 2002; Miller et al. 1999). This is in sharp contrast to past translocations, which often were conducted without a rigorous scientific assessment of the consequences of these translocations on existing populations or ecological communities.

Fishers (*Martes pennanti*) are 2.5- to 7-kg mustelids endemic to North America. Fisher populations were extirpated in many locations throughout the United States through a combination of overtrapping and habitat destruction (Powell 1993; Powell and Zielinski 1994). In some locations where fishers were protected, they declined due to high mortality rates from incidental killing by trappers seeking other furbearers (Lewis and Zielinski 1996).

Historically, the recovery of fisher populations was a priority because fisher pelts were valuable and fisher predation on tree-damaging porcupines (*Erethizon dorsatum*) was considered beneficial by foresters. More recently, fisher recovery has been justified under the Federal Endangered Species Act or similar state legislation (Greenwald et al. 2000; United States Fish and Wildlife Service 2004). One of the most commonly used tools for fisher recovery has been translocations, which have occurred in many areas throughout the fisher's geographic range (Lewis and Hayes 2004; Powell 1993; Powell and Zielinksi 1994; Williams et al. 2000). For example, in the eastern United States, populations in Tennessee, West Virginia, Pennsylvania, Connecticut, and Vermont reportedly comprise only reintroduced individuals (Lewis and Hayes 2004; Williams et al. 2000). In the Rocky Mountains of Montana and Idaho, fishers were translocated to at least 12 different locations between 1959 and 1991 (Vinkey 2003; Vinkey et al. 2006).

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Vinkey et al. (2006) examined the genetic legacy of the Montana translocations using the control region (*CR*) and cytochrome-*b* (*cytb*) gene of the mitochondrial genome. They demonstrated that extant animals in northwestern Montana show the genetic signatures of fishers from either the Midwest (Minnesota and Wisconsin) or British Columbia. However, many fishers from west-central Montana, where at least 4 translocations of British Columbia fishers occurred between 1959 and 1963, could not be maternally linked to fishers from either British Columbia or the Midwest. These fishers have a unique control region haplotype (*CR-12*) that differs by 1 transition from a common British Columbia haplotype, and a unique *Cytb* haplotype (*Cytb-B*) that differs by 1 transversion from all other fisher haplotypes. Because *CR-12* and *Cytb-B* have been found nowhere else in the United States, Vinkey et al. (2006) hypothesized that these fishers were a remnant of a native Montana population that persisted in the Selway–Bitterroot Mountains near the Montana–Idaho border. Thus, the 1959–1963 translocations were not reintroductions, but were an augmentation of a previously unknown fisher population (Vinkey et al. 2006).

If correct, the interpretation of Vinkey et al. (2006) has significant conservation implications, because the west-central Montana population would be the only confirmed population with native genes in the Rocky Mountains of the United States and would demonstrate the survival of fishers through heavy trapping in the early 20th century. However, this is an untested hypothesis. The study of Vinkey et al. (2006) contained no specimens that predated the translocations. A search of major museums in the United States revealed only 2 samples from the Rocky Mountains that were collected before translocations conducted in 1959. One sample (24112; National Museum of Natural History, Washington, D.C.) from Sawtooth Lake, Idaho (now known as Alturas Lake, Blaine County, Idaho), was previously analyzed by Drew et al. (2003); the DNA was too poor in quality to provide definitive results. A 2nd sample was discovered at the Harvard Museum of Comparative Zoology in Cambridge, Massachusetts (R. Vinkey, pers. comm.). This specimen (MCZ B 6964; hereafter, the Harvard fisher), which includes the skull and skin, was collected in December 1896 by the Herbison and Bergamin expedition in Idaho County, Idaho. Idaho County is west of the Bitterroot Divide, which separates Idaho County, Idaho (hereafter called north-central Idaho) from west-central Montana, where Vinkey et al. (2006) collected samples. Thus, for the Harvard fisher to lend support to the hypothesis of Vinkey et al. (2006) that *CR-12* and *Cytb-B* represent a native Montana haplotype, 2 predictions must be met. First, the distribution of fisher haplotypes in west-central Montana must be comparable to the distribution of haplotypes in north-central Idaho. If so, then fishers in these 2 areas belong to a single, interbreeding population. Second, the Harvard fisher must be haplotype *CR-12* and *Cytb-B*. The goal of this study is to use contemporary and historical DNA to investigate the validity of this hypothesis.

Materials and Methods

Sample collection.—During an ongoing study of habitat use by fishers and wolverines (*Gulo gulo*) in the Clearwater River subbasin of north-central Idaho from 2002 to 2005, 33 fishers were captured and ear punches were collected (Fig. 1) following guidelines of the Animal Care and Use Committee (1998) for the capture, handling, and care of mammals. Samples were stored in 10–18-mesh silica gel desiccant (Fisher Scientific, Fair Lawn, New Jersey) until delivery to the genetics laboratory. Samples from west-central Montana, which is separated from north-central Idaho by the Bitterroot Divide, are the same as those reported in Vinkey et al. (2006). The Harvard fisher was sampled at the Harvard Museum of Comparative Zoology (loan 2181). I collected hair from the hide and a portion of the maxilloturbinate bones from the skull following established ancient DNA protocols (see below). The maxilloturbinates are thin bones attached anteriorly to ridges inside the nasal cavity and have been shown to provide high-quality ancient DNA, while minimizing the damage to museum collections (Schwartz et al., in press; Wisely et al. 2004).

DNA extraction, sequencing, and data analysis.—DNA was extracted from the ear punches using standard protocols (DNeasy Tissue Kit, QIAGEN Incorporated, Hilder, Germany). DNA was extracted from the maxilloturbinates using the QIAmp DNA Micro Kit (Qiagen Incorporated) with a protocol developed for bones. DNA was extracted from the hide using the QIAmp DNA Micro Kit and the standard protocol for tissues with low amounts of DNA. The ancient DNA protocols of Fleischer et al. (2000) and Gilbert et al. (2005) were followed to avoid contamination. Briefly, this involved the following precautions: extracting DNA in a physically separate laboratory at the Rocky Mountain Research Station in Missoula, Montana, used only to extract DNA from museum specimens; the extensive use of negative controls, including exposing a negative control (sterile water) to ambient conditions at the Harvard Museum of Comparative Zoology and another negative control to the ambient conditions in the ancient DNA laboratory; amplifying small fragments; and reproducing the results multiple times (see below), with 2 sample types (hair and bone). These precautions were deemed sufficient because, in general, this type of ancient DNA analysis is considered ''low risk'' for contamination (see criteria in Gilbert et al. 2005), as opposed to ancient DNA studies on humans, microorganisms, paleopathogens, or domestic plants and animals.

Once the DNA was extracted, a 301-base-pair (bp) segment of the control region (*CR*) was amplified using the polymerase chain reaction with species-specific primers MP-F′ and MP-R′ (see Vinkey et al. 2006). In addition, a 428-bp segment of the cytochrome-*b* region (*Cytb*) was amplified using primers *CanidL1* and *H15149* (Kocher et al. 1989; Paxinos et al. 1997; Riddle et al. 2003). For both regions, polymerase chain reactions were executed under the same conditions as reported in Vinkey et al. (2006). The control region polymerase chain reaction product was analyzed as in Vinkey et al. (2006), whereas the *Cytb* products were analyzed using the *Hinf*I digestion detailed in Riddle et al. (2003). This restriction enzyme distinguishes between the 2 *Cytb* haplotypes observed in fishers.

Haplotype frequencies from north-central Idaho were compared to frequencies from west-central Montana using contingency tables and Yates-corrected chi-square values. Furthermore,

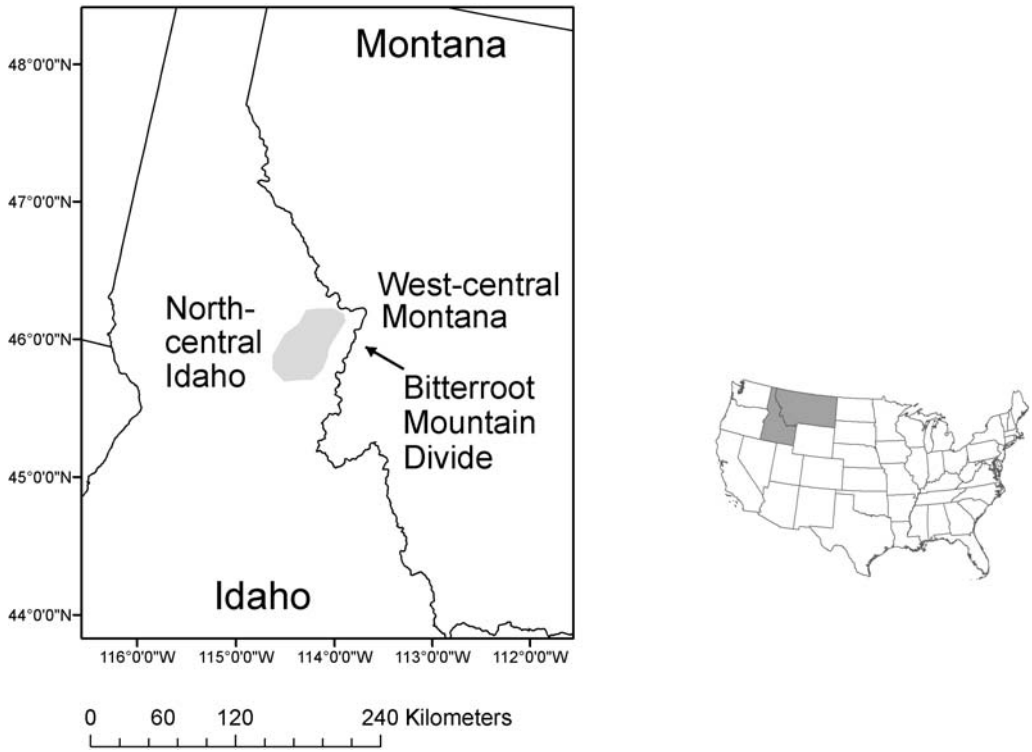

FIG. 1.—The 2 areas where fishers (*Martes pennanti*) were sampled in this study: north-central Idaho and west-central Montana. These areas are separated by the Bitterroot Divide—a steep, high-elevation mountain chain that runs north–south. The historical sample was collected in 1896 in Idaho County, which is located in north-central Idaho. The shaded block in Idaho represents the approximate sampling area of the contemporary north-central Idaho samples.

gene frequencies and an index of substructure between the areas (Φ_{ST}) were estimated using program Arlequin version 2.00 (Excoffier et al. 1992; Michalakis and Excoffier 1996).

RESULTS

Haplotype *CR-12* was found in the contemporary north-central Idaho sample of fishers ($n = 33$) and, similar to the west-central Montana sample ($n = 67$), was the most common haplotype (Fig. 2). The haplotype frequencies were not significantly different ($\chi^2 = 0.71$, $P = 0.87$; Fig. 2) between north-central Idaho and west-central Montana. Gene diversity was also similar in both areas: 0.66 ± 0.03 *SD* in north-central Idaho and 0.67 ± 0.05 in west-central Montana. Φ_{ST} was 0.0 ($P = 0.7$), suggesting no substructure between north-central Idaho and west-central Montana.

The Harvard fisher was *CR-12*, the most common haplotype in both west-central Montana and north-central Idaho samples. This fisher also was analyzed at the *Cytb* region and was haplotype *Cytb-B*. In Vinkey et al. (2006) all *CR-12*s were *Cytb-B* and all other control region haplotypes were *Cytb-A*; that pattern also was found in this study.

DISCUSSION

To confirm the augmentation hypothesis of Vinkey et al. (2006) required finding a well-documented west-central Montana fisher skull or mount that predated 1959, the year fishers were 1st translocated from British Columbia to the United States Rocky Mountains, and showing that this sample had the control region haplotype *CR-12*. Although a pre-1959 specimen from Montana was not discovered, a skull and corresponding skin were found from a nearby area in north-central Idaho. This skull and skin are haplotype *CR-12,* the native Montana type, and *Cytb-B*, demonstrating that Idaho had extant fishers with the ''native Montana'' haplotype before the translocations in 1959. To claim that the Harvard fisher supports

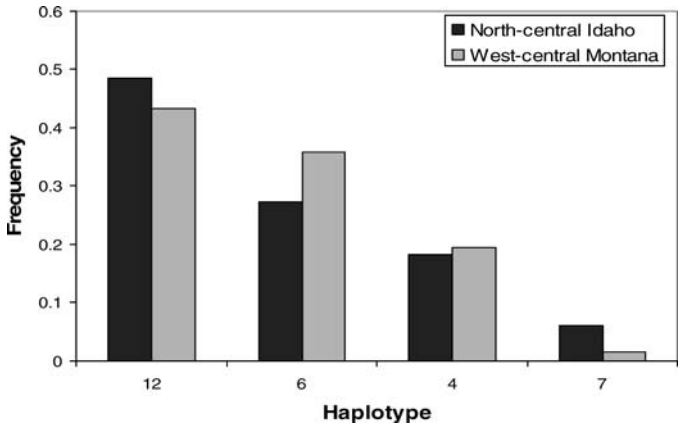

FIG. 2.—The frequency of control region haplotypes in west-central Montana (light gray bars) and north-central Idaho (dark gray bars). Four haplotypes of fishers (*Martes pennanti*) are found in both regions, with the native Montana haplotype 12 common to both.

the hypothesis of Vinkey et al. (2006), which was specific to Montana, it was important to also demonstrate that fishers in west-central Montana are connected to fishers in north-central Idaho, and represent a single population. Haplotype frequency data from fishers from north-central Idaho show that the Bitterroot Mountain Divide is not a barrier to fisher movements because the 2 samples appear to have been drawn from 1 population (Fig. 2).

My study provides support for the assertion of Vinkey et al. (2006) that *CR-12* was present in Montana before translocations. It does not support an alternative hypothesis presented in Vinkey et al. (2006) that *CR-12* was present at low frequency in British Columbia (the source of several west-central Montana translocations), introduced into Montana in the 1950s and 1960s, and subsequently drifted to represent the lineage of nearly half the contemporary population.

In terms of conservation of western fishers, haplotype analyses suggest that California and southern Oregon have native fisher populations, whereas central Oregon has fishers that are descendants of previous translocations (Drew et al. 2003). In the Rocky Mountains, British Columbia has 5 control region haplotypes that are common (4, 6, 7, 9, and 11) and 1 haplotype that is rare (1), all of which are *Cytb-A*. Northwestern Montana mostly has control region haplotypes that are from the midwestern translocations, although it also has *CR-4* and *CR-6* that could be from a translocation in 1959, remnants of a historical population, or from immigration from nearby British Columbia where these haplotypes are common (Vinkey et al. 2006). West-central Montana, where fishers are legally harvested, and Idaho, where fishers are listed as critically imperiled by the state, have both descendants of fishers that are either from translocations or are native (represented by *CR-4, CR-6,* and *CR-7*) and descendants of fishers that are from native stocks (represented by *CR-12*).

There are several conservation lessons to be learned from translocations of fishers to the Rocky Mountains. First, comprehensive surveys to determine the presence or absence of remnant populations should be conducted before translocations are initiated. This can be done using traditional detection–nondetection devices such as track-plate boxes (e.g., Foresman and Pearson 1998; Zielinski and Truex 1995) or with noninvasive genetic sampling (e.g., Fernandez et al. 2006; Pires and Fernandes 2003; Schwartz et al. 2007; Zielinski et al. 2007).

Translocations of fishers have been proposed for both the Olympic National Park and Cascade Range in Washington State (Lewis and Hayes 2004). Given that the biogeography of the Olympic Peninsula has generated endemic species (e.g., Olympic Marmot [*Marmota olympus*] and Olympic torrent salamander [*Rhyacotriton olympicus*]) and subspecies (e.g., Olympic ermine [*Mustela erminea olympica*]), and remains the only location in the United States where the vagile cougar (*Puma concolor*) has a unique haplotype (Culver et al. 2000), it is possible that fishers from this region are, or were, genetically unique. Thus, to avoid repeating the legacy of translocations of fishers in the Rocky Mountains, I recommend extensive searches be conducted for native fishers in the Olympic Mountains.

Second, when choosing source populations for translocation it makes the most sense to obtain animals of the same subspecies or management unit (Moritz 1999), especially given the potential for outbreeding depression (Tallmon et al. 2004). Traditionally, determining the taxonomic status of historical populations would have relied solely on morphological data. As this study shows, DNA from museum specimens provides an alternative data source to use for identifying appropriate source populations. Future translocations of fishers, such as those proposed in Washington State, should use these types of data to guide their management actions.

This study demonstrates that both north-central Idaho and west-central Montana contained a unique haplotype that represents the native fisher population. Signals of this haplotype persist today, although contemporary populations may represent a mix of native fishers and individuals translocated from British Columbia. Regardless, these data show that fishers in north-central Idaho and west-central Montana are not simply descendants of translocated individuals, but are also the descendants of fishers that persisted despite early 20th century trapping.

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