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APPLICATIONS OF GENETIC METHODS IN FURBEARER MANAGEMENT AND ECOLOGY: CASE STUDIES OF FISHERS, AMERICAN MARTENS AND BOBCATS

presented by

BRONWYN WALLER WILLIAMS

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of the requirements for the

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Major Professor's Signature

5-10-06

Date

APPLICATIONS OF GENETIC METHODS IN FURBEARER MANAGEMENT AND ECOLOGY: CASE STUDIES OF FISHERS, AMERICAN MARTENS AND BOBCATS

By

Bronwyn Waller Williams

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ABSTRACT

APPLICATIONS OF GENETIC METHODS IN FURBEARER MANAGEMENT AND ECOLOGY: CASE STUDIES OF FISHERS, AMERICAN MARTENS AND BOBCATS

By

Bronwyn Waller Williams

Furbearer species are an important component of managed wildlife in the Midwestern United States. However, the species' solitary and elusive habits have made collection of ecological and demographic information upon which management decisions are made difficult. The objective of this research was to provide additional methodologies that managers can employ to gather ecological and demographic data on furbearers. Molecular methods were used to estimate levels of spatial genetic structure for martens and fishers that developed from reintroductions to Michigan and Wisconsin, data were used to develop methods to monitor population abundance of martens and fishers, and to determine sources and direction of error in bobcat harvest records. Molecular methods were very useful for describing spatial genetic structure that could be tied to reintroduction events. In addition, aspects of marten and fisher ecology were identified that help explain current spatial genetic structure. Simultaneous estimates of population abundance were determined for martens and fishers. Error was identified in harvest location reporting and sex identification of bobcats. Genetics was proven to be a useful application in furbearer management and ecology.

ACKNOWLEDGEMENTS

I would like to thank all of my committee members: Kim Scribner, Kelly Millenbah, Scott Winterstein, David Foran, and Dwayne Etter. I greatly appreciate the assistance each provided along the way. A special thanks to Kim, my advisor, for his patience and support (in his own way), for making these projects possible, and for giving me the opportunity to attempt to answer it all. Additional special thanks to Damien Lunning. It was his knowledge and generosity that made the field project both possible and successful. Thank you to Mike VanLoan, who was an invaluable asset in the field and in the kitchen. Thank you to my parents, Judy and Jim, for their unwavering support, and for unquestionably helping me through the rough patches.

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I would like to thank the many people involved in my field research for their interest, ideas, and assistance. First, thanks to Steve Babler, Joanne Thurber, and Bob Evans, from the U.S. Forest Service, Ottawa National Forest, all of whom provided helpful input along the way. Also thank you to the folks from the MDNR for assisting with field needs: Craig Albright, Dan Moran, Doug Wagner, Rob Aho, and Brad Johnson.

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TABLE OF CONTENTS

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APPE

LIST OF TABLES

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LIST OF APPENDICES

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CHAPTER 1: GENERAL INTRODUCTION AND OVERVIEW

History of furbearers in the Midwest

Furbearers have played an important role in the history of the Midwestern United States. As white settlers traveled westward in the early 19th century, fur was a resource that could be traded for gold and goods. Furbearers such as bobcats (*Lynx rufus*), American martens (*Martes americana*), and fishers (*Martes pennanti*) were abundant in the heavily forested areas of the Upper Midwest. However, unchecked harvest of these species and deforestation led to sharp declines in abundance and distribution (Hubert 1982). Local extirpations were documented as early as the mid-1800s. Fur traders responded by shifting emphasis towards remaining furbearer species. At the same time, the influx of settlers to the Midwest increased rapidly. Harvest rates for many species remained high through the turn of the 20th century for economic reasons as well as for sport. As a result, furbearer populations steadily declined into the early 1900s (Hubert 1982).

Furbearer management in Michigan

Furbearer "management" in the Midwest began as a way to protect early white settlers from the "wild beasts" of the forest. As farming became more prevalent, there was a need to protect livestock from predatory animals. Bounties were often offered for those species considered most threatening to human interests, including bobcats. The bounty system in Michigan continued until 1965, with the exception of coyotes (*Canis latrans*), which were bountied until 1980 (Baker 1983). Concern over the issue of bounty payments and a recognized need for better understanding of the role predators play in the

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ecosystem initiated research of wildlife populations (Hubert 1982). The bounty system had proven ineffective at either increasing game species or reducing losses to livestock farmers through control of predator populations (Hubert 1982).

In 1909, the Michigan Legislature created the Public Domain Commission to manage the state's natural resources. In 1921, the Commission was replaced by the Department of Conservation. State lands and tax-reverted properties became initial sites of resource management and protection. With protection, mature forests regenerated and populations of several wildlife species began to increase (Earle et al. 2001). Unfortunately, by the time these protective measures had taken effect, certain furbearers, including American martens and fishers, were considered extinct in Michigan.

Currently, the goal of the Michigan Department of Natural Resources (MDNR) is to maintain healthy wildlife populations that provide Michigan residents with a diversity of recreational opportunities (MDNR Vision and Mission). Conservation and management of viable populations necessitates an understanding of the physical habitat and specific environmental parameters affecting population abundance and distribution. Unfortunately, predicting population status and viability is frequently complicated by life histories and habits of individual species. Often little is known of contemporary or historical demographic characteristics such as breeding population size or the potential for dispersal. Mandatory registration of bobcats, fishers and martens allows the MDNR to collect data including date and location of harvest, sex, harvest method, and age. In addition, relative harvest effort can be calculated using surveys of furtakers who received mandatory bobcat or marten tags. The accurate estimation of population size and aspects of species movements related to land-use and habitat is critical to the maintenance of

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populations of Michigan's furbearing animals. Presently, much information on distribution, recruitment, demography, and abundance of furbearers is based on annual track count surveys and inferences from annual trapper and hunter harvest data. While population distribution and trend information are important for the management of specific furbearers, cost-effective means for determining furbearer population size are needed for regional management (Douglas and Strickland 1987).

Study objectives

This study focused on the American marten, fisher, and bobcat, three furbearer species currently harvested in Michigan. Specific objectives were to: 1) develop spatially explicit baseline data for each species for use in state wildlife forensic cases; 2) investigate management implications due to biases in sex and location reported for harvested animals; 3) relate spatial genetic structure of martens and fishers in Michigan and Wisconsin to reintroduction history as a means of evaluation of the success of relocation efforts, and; 4) develop and evaluate population census techniques that can be used for multiple co-distributed species.

Objective 1: Molecular markers have become increasingly important in wildlife forensics (Randi 2003). In Michigan, bobcats were of concern to MDNR Law Enforcement Division because different harvest regulations were enforced in different locations. A goal of this research component was to use allele frequency data from bobcats obtained from the Upper and Lower Peninsulas of Michigan to determine whether individuals can be assigned to either region with sufficient accuracy to be used in law enforcement.

Objective 2: Misrepresentation of sex and location of trapped and hunted individuals can have negative implications for management. Several indices used for tracking stability of furbearer populations rely on harvest-based demographic information. Biases in harvest data can affect projections of future population abundance. The objective of this project component was to use genetic sexing markers to identify sources of error in the collection of demographic data through harvest and determine effects of sexing error on the magnitude and direction of trends in population abundance.

Objective 3: Few attempts have been made to evaluate success of reintroductions of fishers and martens in Michigan. It is not known if population increases were realized through successful reproduction from few or many of the initial translocated adults of either species. Genetic methods and statistical tools can provide resolution to speed and direction of dispersal following introductions and to any factors in the landscape that could be correlated with introduction success or failure and rate of dispersal. Knowledge gained will aid in the design of future restoration efforts. An objective of this research component was to use allele frequency data to determine if current genetic structure of fishers and martens in the Upper Peninsula of Michigan and in Wisconsin was a direct result of reintroduction events, and examine spatial genetic structure within the reintroduced populations of the Upper Peninsula of Michigan to gain additional insight into important aspects of the species' ecology.

Objective 4: Marten, fisher, and bobcat populations in the Upper Peninsula of Michigan are currently monitored using track count indices, harvest data, and survey information (e.g., Cooley et al. 2000, Cooley et al. 2001, Earle 2002, Frawley 2002).

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While winter track counts on standard transects show promise as a population index for furbearers, the ability to detect even relatively large changes in population size (e.g., 25%) requires excessively large sample sizes to achieve sufficient statistical power (Deifenbach et al. 1994). Furthermore, trends in annual harvest reflect changes in population levels only if funding (fur prices) and trapping pressure are relatively constant (Rolley 1987). The development of mark-recapture population estimators based upon genetic tagging would provide the MDNR with an additional tool to use in managing furbearer populations. A population estimate would allow the MDNR to more precisely set and justify trapping and hunting season regulations and to monitor population trends in response to changes in land use, environmental variation, or rates of harvest. The objective of this project component was to develop a non-invasive method to collect genetic data that can be used simultaneously estimate population abundance of martens and fishers.

Thesis organization

This thesis is organized into seven chapters, each dealing with specific management issues concerning furbearing species. Chapter two is a historical overview of fisher and marten in Michigan and Wisconsin, detailing the decline and eventual extirpation, reintroduction, and harvest decisions unique to each species. Much of the information in this chapter has been compiled from inter-office communications and unpublished materials from the various agencies involved in reintroductions and management of these species. Chapter three covers spatial genetic structure following marten reintroductions in Michigan and Wisconsin. Individuals collected from putative

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source populations are compared to genetically distinct clusters in the two states found in the proximity of the reintroduction sites. In addition, patterns of individual movements were identified within each cluster, allowing inference of dispersal patterns and potential barriers to dispersal. Chapter four describes current spatial genetic structure of fishers in Michigan and Wisconsin resulting from human-mediated relocations (reintroductions, translocations), natural dispersal, and genetic drift. As a forest generalist, the agile fisher has colonized much of Wisconsin and the Upper Peninsula of Michigan following a limited number of relocation events. In contrast, the marten is considered an old-growth habitat specialist, and dispersal from reintroduction sites may have been limited. Although martens are trapped in the Upper Peninsula of Michigan, the marten remains a State endangered species in Wisconsin.

Following reestablishment of an extirpated or severely depleted population, it might be necessary to monitor population size and trends to prevent future decline. Chapter five describes a non-invasive technique developed to estimate abundance for two co-distributed species (marten and fisher) in the Upper Peninsula of Michigan. Population estimation models were constructed to account for various biases, including the use of genetic information from harvested individuals.

Harvest surveys provide an important source of data and are often used as an index of population trends. Chapter six describes how biases introduced in harvest summaries through mis-assignment of location and sex mis-identification can affect estimates of population trends based on harvest data. Chapter seven summarizes the conclusions based on information described throughout these research projects.

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References

Baker, R.H. 1983. Michigan Mammals. Michigan State University Press ; Detroit, Michigan.

Cooley, T.M., Schmitt, S.M., Friedrich, P.D., and T.F. Reis. 2000. Bobcat survey 1999-2000. Michigan Department of Natural Resources Wildlife Division Report No. 3325. 19 pp.

Cooley, T.M., Schmitt, S.M., Friedrich, P.D., and T.F. Reis. 2001. Fisher survey 1999. Michigan Department of Natural Resources Wildlife Division Report No. 3329. 12 pp.

Diefenbach, D.R., Conroy, M.J., Warren, R.J., James, W.E., Baker, L.A., and T. Hon. 1994. A test of the scent-station survey technique for bobcats. Journal of Wildlife Management. 58: 10-17.

Douglas, R. and S. Strickland. 1987. pp 267-279 in M. Novak (ed.). Wild furbearer management and Conservation in North America. North Bay, Ontario.

Earle, R.D. 2002. Furbearer winter track count survey of 2001. Michigan Department of Natural Resources Wildlife Report No. 3366. 10 pp.

Earle, R.D., Mastenbrook, L.H., and T.F. Reis. 2001. Distribution and abundance of the American marten in northern Michigan. Michigan Department of Natural Resources Wildlife Report No. 3321: 1-26.

Frawley, B.J. 2002. 2001 marten harvest survey. Michigan Department of Natural Resources Wildlife Division Report No. 3369: 1-4.

Hubert, G.F., Jr. 1982. History of Midwestern furbearer management and a look to the future. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 174-191.

Randi, E. 2003. Using DNA markers and population genetic principles in wildlife forensics: an overview. Forensic Science International. 136, Supp. 1: 378.

Rolley, M. 1987. The furbearer harvest in Canada. Pp 123-145 in M. Novak (ed.), Wild furbearer management and Conservation in North America, North Bay, Ontario.

Vision and Mission [Internet]. Lansing (MI): Michigan Department of Natural Resources. [Updated 2002 May 3; cited 2004 March 17]. Available from: http://www.michigan.gov/dnr/.

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CHAPTER 2: A HISTORICAL PERSPECTIVE ON THE REINTRODUCTION OF THE FISHER AND THE AMERICAN MARTEN IN MICHIGAN AND WISCONSIN

Introduction

The 19th and early 20th centuries brought about extensive degradation of old growth forest in the Midwest as European settlement pressed into wilderness lands. Unchecked harvest of furbearer species, including the American marten (*Martes americana*) and the fisher (*Martes pennanti*), in addition to widespread logging and repeated fires, led to severe declines in population numbers of these species (Hubert 1982). American martens and fishers were extirpated from much of their southern range, including Michigan and Wisconsin (Hagmeier 1956). Following multiple reintroduction and translocation events in the Midwest, both species have recovered numerically to varying degrees and in terms of distribution (e.g., Kohn 2002, Cooley et al. 2001).

History of the fisher in Michigan and Wisconsin

Historical background and extirpation

The fisher is found exclusively in North America. The historic range of the fisher paralleled the distribution of northern coniferous forest from the Atlantic to the Pacific coasts. The species was found south along the Cascade and Sierra-Nevada Mountains, into the Northern Rocky Mountains, the southern Great Lakes states, and the mountains of Tennessee and Virginia (Brander and Books 1973). The northern limit of the fisher's distribution was approximately 10° south of the American marten, most likely due to differing habitat requirements (Figure 2.1; Hagmeier 1956, Gibilisco 1994). Snow depth may also limit the range of the larger, heavier fisher (Raine 1983, Aubry and Houston

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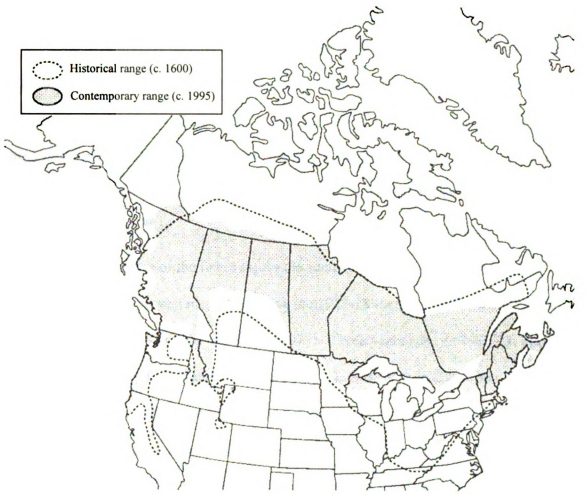

Figure 2.1. Approximation of historic and recent distribution of fishers in North America. Adapted from Hagmeier (1956), Gibilisco (1994), and unpublished MDNR and Wisconsin Department of Natural Resources reports.

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Extensive logging and multiple fires throughout the 19th and early 20th centuries fragmented fisher populations in the Midwest. In addition, high fisher pelt prices increased harvest pressure. In the early 1900s, the pelt of a prime female fisher could cost $150 in pre-war dollars (Cook and Hamilton 1957). The result of economic pressure on fisher populations was further compounded by the ease by which individuals were trapped.

A severe decline of the fisher across the southern portion of its range was documented through harvest records. During the 1917-18 trapping season in Wisconsin, 559 fishers were taken (Brander and Books 1973). Three years later, in 1920-21, only three fishers were trapped (Brander and Books 1973). Similarly, in California 102 fishers were recorded taken in 1920; two fishers were trapped in 1931 (Brander and Books 1973). The fisher trapping season was closed in 1922 in Wisconsin, 1924 in Michigan, 1929 in Minnesota, 1935 in New Hampshire, 1936 in New York and Wyoming, 1937 in Maine and Oregon, and 1946 in California (Brander and Books 1973).

For some of these states, the trapping ban came too late to prevent extirpation of the fisher. In Michigan, where the fisher had formerly been found as far south as Gratiot, Ingham, Washtenaw, Wayne, and Wexford Counties, the last recorded sighting was in 1936, in Marquette County, Upper Peninsula (Hagmeier 1956). Fishers reportedly occurred throughout the entire state of Wisconsin, but the last known sighting was in 1932 (Hagmeier 1956, Petersen et al. 1977).

The timing of the trapping ban allowed the fisher population to recover in other states. By the mid-1930s, the fisher had been nearly trapped to extinction in New York.

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A closed season from 1936 to 1949, in addition to recovery of habitat, was sufficient to promote a population increase (Bradle 1957, Irvine et al. 1964). By 1949, the number of fishers was sufficient to allow a limited trapping season. In 1957, the bag limit was increased to three fishers per year (Bradle 1957). From 1976 to 1979, 43 fishers (19 males and 24 females) were relocated from the Adirondacks to the more southern Catskill Mountains in New York. Northeastern Minnesota retained a remnant fisher population following protection to the species in 1928. Population growth allowed translocation of 15 animals to the northwestern portion of the state (Itasca State Park) in 1968 (Berg 1982). A trapping season was initiated in 1977-78, with a bag limit of three fishers per person (Berg 1982).

The fisher and the porcupine

The decline and eventual extirpation of the fisher from much of its southern range was followed by an apparent increase in the number of porcupines (*Erethizon dorsatum*) in those areas. In the Ottawa National Forest, Upper Peninsula, Michigan, porcupine densities were estimated to be as high as 60 animals per 1.6 km^2 by the late 1950s (Brander and Brooks 1973). A total of 1,799 porcupines were shot on the Ottawa National Forest in 1961. Road hunts that same year yielded an average of one porcupine shot every 2.9 km (Irvine 1961). Porcupines have been associated with timber loss due to their feeding habits. A study performed in 1948-49 on the Argonne Experimental Forest, Wisconsin showed that hardwood-hemlock forests would sustain serious damage with 64 or more porcupines per 1.6 km^2. An intensive porcupine harvest undertaken during this study resulted in 96 porcupines killed per 1.6 km^2 (Irvine and Brander 1971). There was

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major concern in the 1950s that unchecked population growth of porcupines was resulting in substantial timber losses (Olson 1966).

Fishers are efficient predators of porcupines (Schoonmaker 1938, Earle 1978). Reports of increasing fisher populations in the Adirondack Mountains of New York and in northeast Minnesota paralleled reports of declining porcupine populations. This interrelationship was mostly speculation, as no studies had been undertaken to determine if the fisher alone is a major factor in the decline of the porcupine (Irvine and Brander 1971). However, indication that fishers could control the species creating apparent economic turmoil in the timber industry sparked interest in restocking the mustelid to Michigan and Wisconsin (USFS interoffice communication 1959, Olson 1966).

Reintroduction of the fisher in the Midwest

In 1955 the USFS first proposed reintroducing the fisher as a biological control of porcupines. An aesthetic rationale was concurrently proposed by former Wisconsin Conservation Commissioner A.W. Schorger, who was interested in restoring extirpated wildlife species to the State. As a result, Dr. Antoon de Vos of the Ontario Department of Lands and Forests, known for his work with American martens and fishers in Canada, was invited to assess the quality of the habitat in northern Wisconsin for the possibility of future reintroduction of the fisher (Olson 1966).

By the 1950s, the price of fisher pelts had dropped to $5 - $15, making trapping economically unfeasible for many trappers, especially given restricted bag limits (Cook and Hamilton 1957). The main reason for the drop in price was a shift in women's fur fashions to spotted cats. The fashion industry had initially created much of the high demand for fishers (Brander and Books 1973). Due to low pelt prices, illegal trapping of

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fishers was not expected to be a limiting factor in the success of a restocking program implemented at that time.

Shortly following announcement of a "favorable" assessment by Dr. de Vos, the Wisconsin Conservation Department began negotiations with New York to acquire fishers for reintroduction (Irvine et al. 1964). The steady population growth of the fisher in the Adirondack Mountains of New York presented the opportunity for the New York Conservation Department to trade fishers for bobwhite quail from Wisconsin (Bradle 1957, Irvine et al. 1964). During the winter of 1955-56 seven fishers from the southern fringe of the Adirondack Mountains were shipped to Wisconsin. These animals were released in the Argonne District of the Nicolet National Forest, Wisconsin, on 16,187 hectares set aside as a special fisher management area (Bradle 1957, Irvine et al. 1964). Seven additional fishers in three separate shipments from New York were released in the same area during the winter of 1956-1957 (Bradle 1957). A final four animals from New York were released in the fisher management area in 1958. The total released from 1956 to 1958 was 18, including 6 males and 12 females (Table 2.1, Figure 2.2; Petersen et al. 1977).

The fisher management area on the Nicolet National Forest in the Pine River watershed consists mainly of dense hardwoods and large coniferous swamps (Bradle 1957, Irvine et al. 1964). Following the reintroduction of the first fishers, the management area was closed to dry-set trapping because fishers are often incidentally caught in baited traps set to target fox and coyotes (Olson 1966). Wet-set trapping for otter and beaver was still permitted (Petersen et al. 1977). In 1962, the fisher

Table 2.1. Reintroduction events of the fisher in Michigan and Wisconsin.

Relocation	Date	Release type	N (females)	Source Population
1. Nicolet National Forest, WI, Fisher	1956-1958	reintroduction	18 (12)	Adirondack Mountains, New York
Management Unit, Forest County	1958-1959	reintroduction	12 (3)	Superior National Forest, Minnesota
	1962-1963	reintroduction	30 (9)	Superior National Forest, Minnesota
Total:	***1956-1963***	-	***60 (24)***	-
Reference: Bradle 1957; Berg 1982, Petersen et al. 1977				
2. Ottawa National Forest, Michigan	1961	reintroduction	31 (8)	Superior National Forest, Minnesota
Tomlin Hill: T48N R37W Section 20 (1962)	1962	reintroduction	16 (5)	Superior National Forest, Minnesota
Ottawa National Forest, Michigan	1963	reintroduction	14 (6)	Superior National Forest, Minnesota
Total:	***1961-1963***	-	***61 (19)***	-
Reference: Berg 1982				
3. Chequamegon National Forest, WI, Fisher	-	-	-	-
Management Unit, Bayfield and Ashland Cos.	***1966-1967***	reintroduction	***60 (30)***	Superior National Forest, Minnesota
Reference: Berg 1982; Petersen et al. 1977				

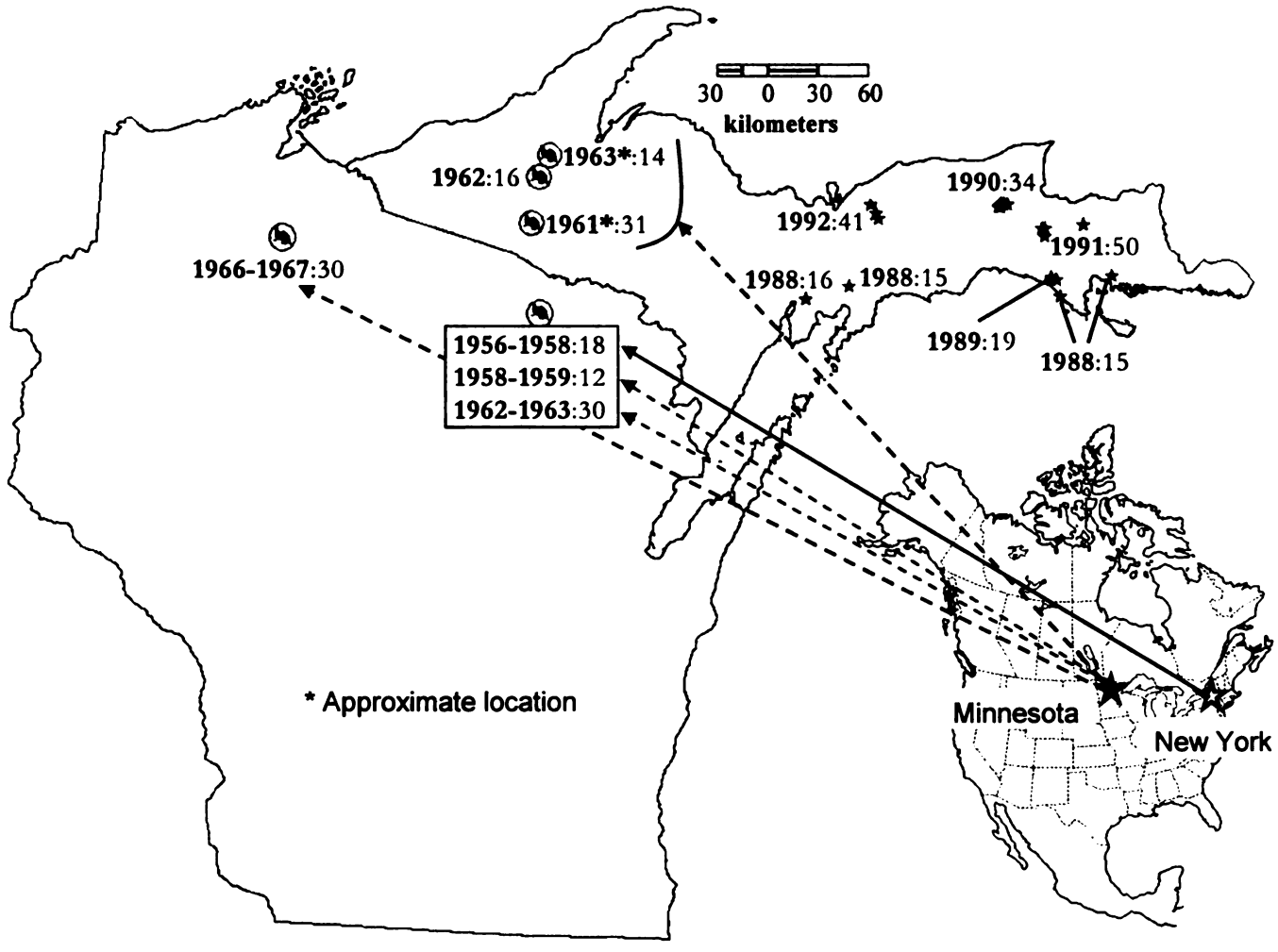

Figure 2.2. Locations of fisher reintroductions (circled fishers) and subsequent translocations (small stars) in Wisconsin and Michigan, 1956-1992. Sources are identified for reintroduced populations. Total numbers of individuals released are given for all releases.

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management area on the Nicolet National Forest was enlarged to 48,562 hectares. All bounty payments in Wisconsin were discontinued to discourage incidental taking of dispersing fishers in dry-set traps (Olson 1966).

Following initiation of fisher restocking in Wisconsin, the USFS became active in the program by arranging with the Minnesota Department of Conservation live-trapping of fisher from the Superior National Forest. These animals would continue the reintroduction onto the Nicolet National Forest as well as stock individuals in the Ottawa National Forest, Michigan, immediately to the north of the Wisconsin border (Irvine et al. 1964). In 1958, three fishers trapped on the Superior National Forest were released onto the Nicolet National Forest fisher management area. In 1959, a further nine individuals were released in the same area. Those 12 fishers included nine males and three females. In 1962, 26 fishers (17 males, 9 females) from the Superior National Forest were released in the Nicolet National Forest. The final release on the Nicolet National Forest was in 1963, when four males were liberated. From 1956 to 1963, a total of 60 fishers (36 males, 24 females) were released on the fisher management area in the Nicolet National Forest, Wisconsin (Table 2.1, Figure 2.2; Petersen et al. 1977).

In 1961, 31 fishers (23 males and 8 females) trapped in the Superior National Forest were released on the Ottawa National Forest, Michigan. In January and February of 1962 an additional 16 fishers (11 males and 5 females) were released north of Kenton, Michigan at Tomlin Hill (T48N, R37W, Section 20). This area is approximately 29 kilometers north of the 1961 release site. One male and one female of the total 18 fishers trapped in the Superior National Forest for the second stocking died prior to release (Irvine 1962). The third and final release of 14 fishers (8 males and 6 females) occurred

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in 1963. From 1961 to 1963, a total of 61 fishers (42 males and 19 females) were released in the Ottawa National Forest (Table 2.1, Figure 2.2; Berg 1982, Irvine 1962). Similar to Wisconsin, all bounty payments were eliminated in Michigan, except on coyotes, to discourage trapping methods that would incidentally capture fishers (Olson 1966).

The release of fishers on the Nicolet and Ottawa National Forests appeared to result in a decrease in the numbers of porcupines in those areas. In 1971, porcupine populations in two localized areas in the Ottawa National Forest were 25% and 55% of what had been recorded in 1962 (Irvine and Brander 1971). Although forest successional changes might have decreased abundance of preferred porcupine habitat and thus species abundance, the speculated relationship between fishers and porcupines prompted the release of 60 fishers (30 males and 30 females) in the Chequamegon National Forest, Wisconsin in 1966 and 1967. In 1966, 31 fishers (18 males and 13 females) from the Superior National Forest, Minnesota, were released onto a second Wisconsin fisher management area of 48,562 hectares in the Chequamegon National Forest, Bayfield and Ashland Counties. In 1967, 29 additional fishers (12 males and 17 females) were released (Table 2.1, Figure 2.2). As in the fisher management area in the Nicolet National Forest, dry-set trapping was banned in the fisher management area in the Chequamegon National Forest (Petersen et al. 1977).

Fishers quickly spread from reintroduction sites. In Wisconsin, the average rate of expansion was estimated to be approximately 3 km per year (Gilbert 2000). However, it was noted that the population initiated in the Chequamegon National Forest was expanding at a greater rate than the population originating in the Nicolet National Forest

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(Petersen et al. 1977). It was speculated that although the numbers released at each area were identical, the period of time over which those releases occurred and the sex ratios could result in differences in overall population expansion. All fishers were stocked in the Chequamegon National Forest over an 11 month period with a balanced sex ratio (30 males, 30 females). Fishers were stocked in the Nicolet National Forest sporadically over a period of seven years and with a male-biased sex ratio (36 males, 24 females) (Petersen et al. 1977). However, the recorded expansion of Wisconsin fishers could be directionally biased through State Department of Natural Resource reports. Fishers from the Nicolet National Forest reintroduction undoubtedly spread into the Upper Peninsula and contributed to the expansion north and east.

In Michigan, the fisher quickly colonized the majority of the western Upper Peninsula. However, it appeared that natural dispersal eastward was halted by a band of agricultural land, and therefore lack of canopy cover, bisecting the Upper Peninsula (MDNR 1990). The boundary of this dispersal barrier follows a north-south line from 16 kilometers east of Marquette to 16 kilometers west of Escanaba (Wagner 1988).

A goal of the fisher reintroduction in Michigan was to restore the extirpated animal to its former range given the existence of suitable habitat. However, the long-term goal was to allow people in Michigan the opportunity to enjoy the fisher aesthetically, ecologically, recreationally, and economically (Wagner 1988). To accomplish both goals given the perceived barrier to natural dispersal to the eastern Upper Peninsula, a five year fisher translocation plan was developed (Table 2.2).

In February and March of 1988, 46 fishers (27 females and 19 males) were trapped in portions of Iron, Gogebic, Ontonagon, Baraga, and Houghton Counties,

Table 2.2. Translocation events of the fisher in Michigan.

Relocation	*Date*	*Release type*	*N (females)*	*Source Population*
4. Rapid River Dist., Hiawatha NF, West Unit	*1988*	translocation	16 (11)	-

Table 2.2. Translocation events of the fisher in Michigan.

Relocation	Date	Release type	N (females)	Source Population
4. Rapid River Dist., Hiawatha NF, West Unit	1988	translocation	16 (11)	-
Manistique Dist., Hiawatha NF, West Unit	1988	translocation	15 (10)	-
St. Ignace Dist., Hiawatha NF, East Unit	1988	translocation	15 (6)	-
Total:	***1988***	-	***46 (27)***	Iron, Gogebic, Ontonagon, Baraga, Houghton Cos., MI
Reference: Steck 1988				
5. Mackinac County, MI	***1989***	translocation	***19 (8)***	Iron, Baraga Counties, MI
Reference: Steck 1989				
6. Luce County, MI	***1990***	translocation	***34 (19)***	Iron, Baraga Counties, MI
Reference: Steck 1990				
7. Chippewa County, MI	***1991***	translocation	***50 (25)***	Ontonagon, Houghton, Baraga Counties, MI
Reference: MDNR unpublished report 1992				
8. Schoolcraft and Mackinac Counties, MI	***1992***	translocation	***41 (22)***	unknown, western Upper Peninsula, MI
Reference: MDNR interoffice communication 1992				

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Michigan. Fifteen of those fishers (9 males and 6 females) were released on the east unit of the Hiawatha National Forest (St. Ignace District) in Mackinac County. Thirty-one animals were released on the west unit of the Hiawatha National Forest in Delta County (Rapid River District: 5 males and 11 females; Manistique District: 5 males and 10 females) (Steck 1988; Figure 2.3).

In February and March 1989, 19 fishers (8 females and 11 males) were trapped in portions of Iron and Baraga Counties, Michigan, and released in Mackinac County (Steck 1989; Figure 2.4). During January and February of 1990, 34 fishers trapped in portions of Iron and Baraga Counties were released in Luce County (Steck 1990; Figure 2.5). In February 1991, 52 fishers were trapped in portions of Ontonagon, Houghton, and Baraga Counties and 50 (25 females and 25 males) were released in Chippewa County (MDNR unpublished report 1992; Figure 2.6). One trapped animal was an albino and was released at the trapping site after much publicity. Another individual escaped while being transferred to the release site (MDNR unpublished report 1992).

The final translocation occurred in 1992, when a total of 41 fishers (22 females and 19 males) were captured. Forty of those animals were relocated. Thirty-seven individuals were released in Schoolcraft County and three fishers were released in Mackinac County (MDNR interoffice communication 1992; Figure 2.7).

Assessment of success of fisher reintroductions and translocations

Fishers appear to have been successful at colonizing most areas across Wisconsin and the Upper Peninsula of Michigan. The first modern trapping season was established in Wisconsin in 1985 (Dhuey et al. 2000). Kohn et al. (2002) estimated the current fisher

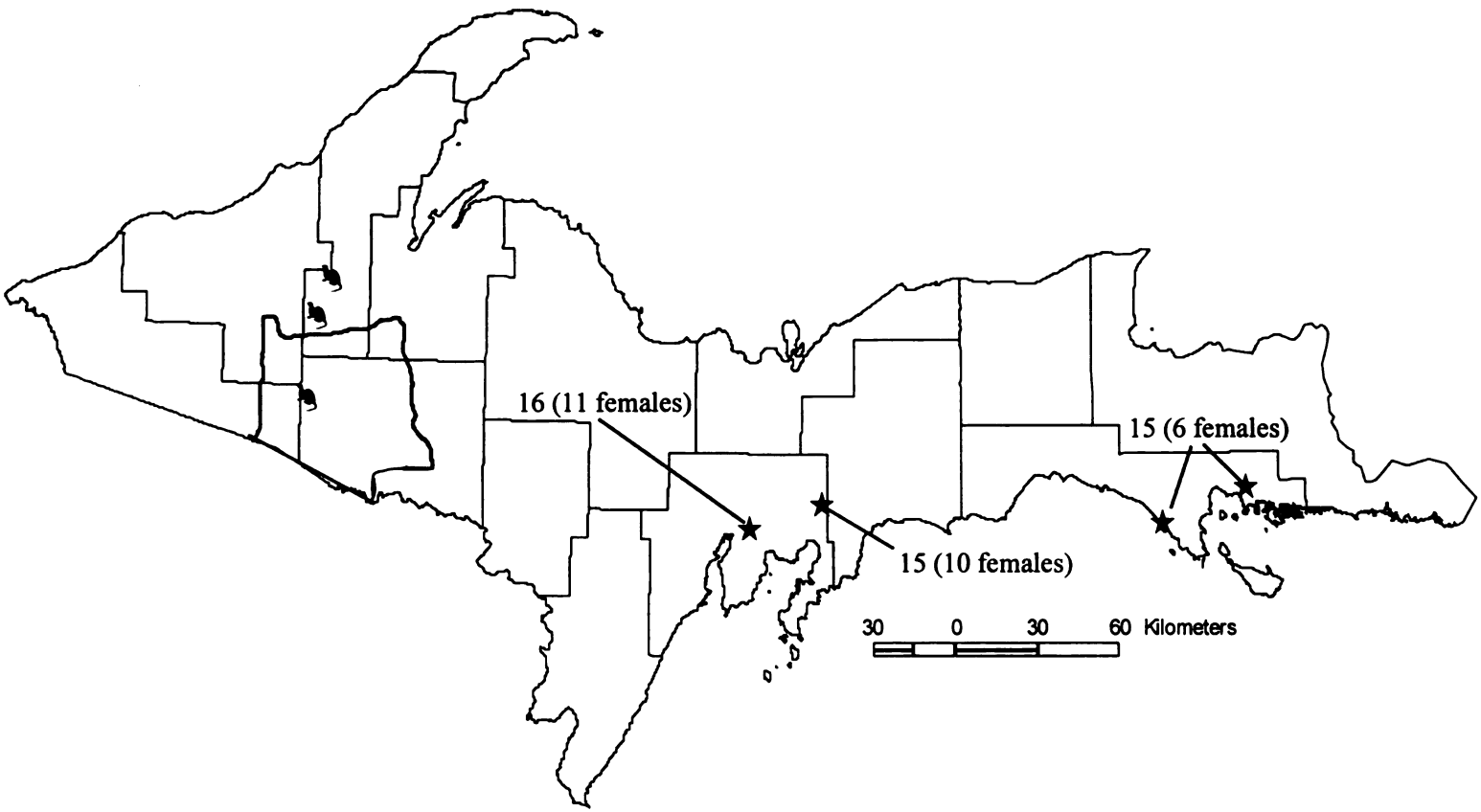

Figure 2.3. Border of the approximate area in the Western Upper Peninsula of Michigan from which 1988 translocated fishers were trapped and release locations (stars). Original sites of fisher reintroductions (1961-1963) are shown as fisher icons.

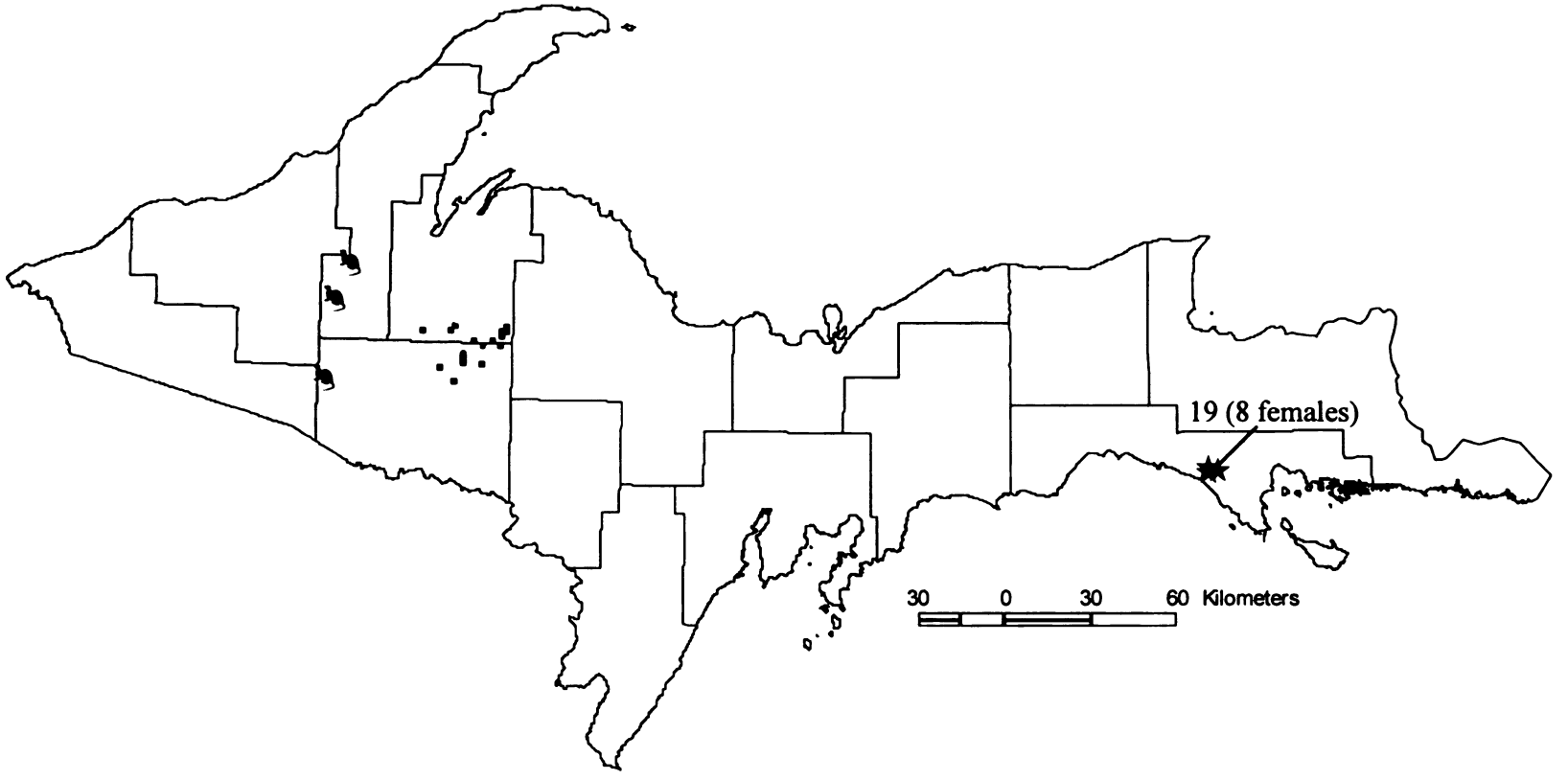

Figure 2.4. Trapping locations (section of capture) in the Western Upper Peninsula of Michigan from which 1989 translocated fishers were collected. Release locations are represented by stars and original fisher reintroduction sites (1961-1963) are shown as fisher icons.

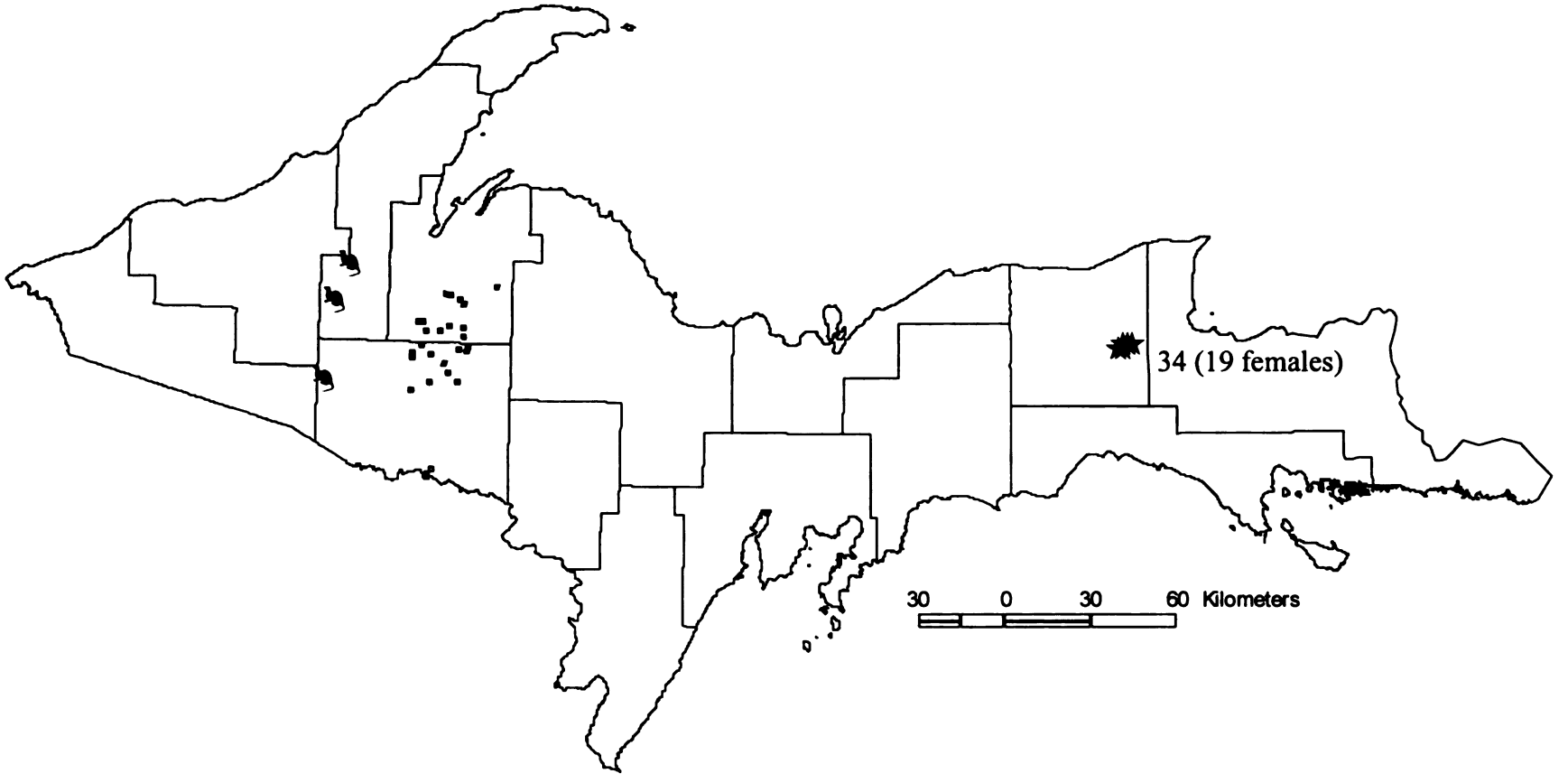

Figure 2.5. Trapping locations (section of capture) in the Western Upper Peninsula of Michigan from which 1990 translocated fishers were collected. Release locations are represented by stars and original fisher reintroduction sites (1961-1963) are shown as fisher icons.

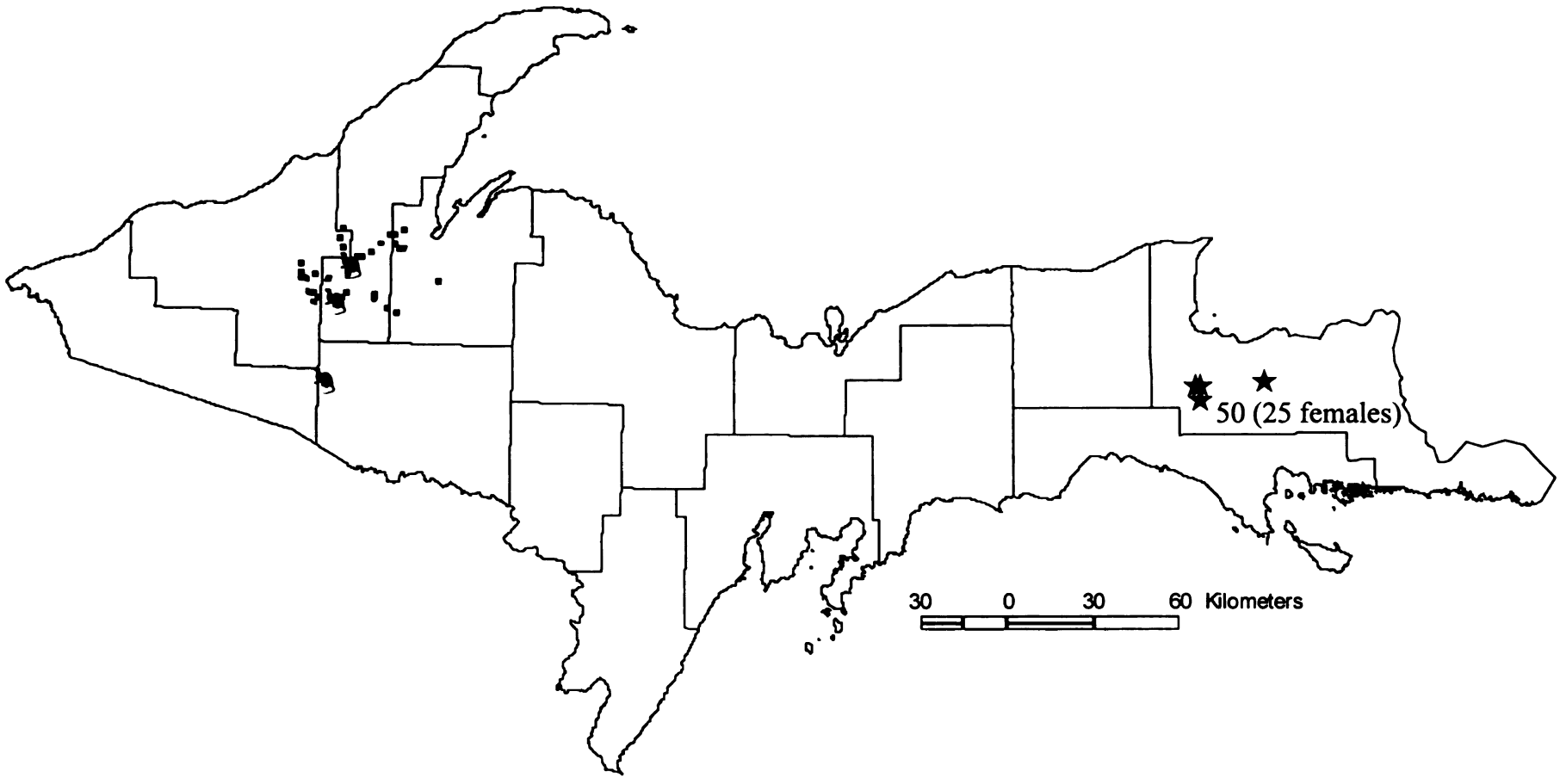

Figure 2.6. Trapping locations (section of capture) in the western Upper Peninsula of Michigan from which 1991 translocated fishers were collected. Release locations are represented by stars and original fisher reintroduction sites (1961-1963) are shown as fisher icons.

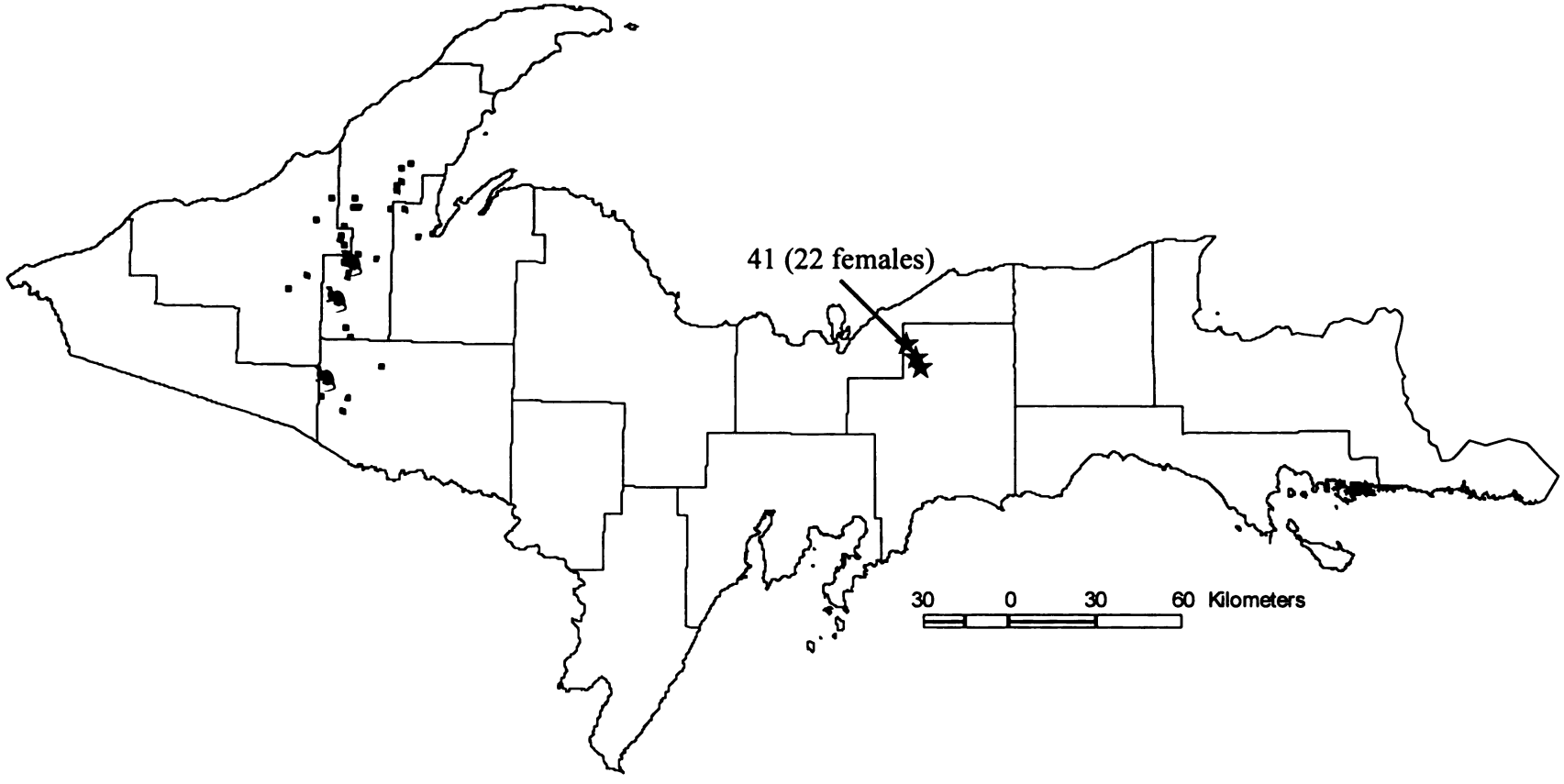

Figure 2.7. Trapping locations (section of capture) in the western Upper Peninsula of Michigan from which 1992 translocated fishers were collected. Release locations are represented by stars and original fisher reintroduction sites (1961-1963) are shown as fisher icons.

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population in Wisconsin to be greater than 9,000 individuals. Fishers have been reported at least as far south as Manitowoc County, Wisconsin. Individuals are also found in Door County, a peninsula that projects into Lake Michigan from east-central Wisconsin. It has been suggested that these fishers crossed the ice from neighboring Marinette County, WI or from the Upper Peninsula of Michigan to the north (Davis 1997). The present range of fishers across northern Wisconsin suggests natural dispersal from Minnesota in addition to dispersal from the release points in the Chequamegon and Nicolet National Forests.

The MDNR began a formal survey of accidentally trapped or road killed fishers in 1981 (Cooley et al. 1982). Eight carcasses were examined during the winter of 1981. These individuals had been collected from Gogebic, Ontonagon, Houghton, Baraga, Iron, and Marquette Counties in the western Upper Peninsula (Cooley et al. 1982). A marked increase in the number of fishers accidentally killed occurred in the following years, peaking at 50 during 1987 and 1988 (Cooley et al. 1986, Cooley et al. 1987, Cooley et al. 1988).

In 1989, fishers were considered abundant in much of the western Upper Peninsula of Michigan (Cooley et al. 1990). As a result, limited harvest by trapping was allowed in Baraga, Gogebic, Houghton, Iron, Marquette, and Ontonagon Counties, on 12,276.5 square kilometers in the western Upper Peninsula known as Fisher Management Unit A (Sodders 1999, Cooley et al. 2001). The original harvest season was designed to be conservative and was limited to 11 days in December with a one fisher per trapper bag limit. Additionally, registration of all captured fishers was mandatory. In 1993, the bag limit was increased to three per trapper. In 1994, fisher trapping was expanded to include Fisher Management Unit B (west-central Upper Peninsula), which included Alger, Delta,

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Dickinson, Houghton, Keweenaw, Marquette, and Menominee Counties. This increased the trapping area to a total of 26,231.4 square kilometers (Sodders 1999). The bag limit in Unit B was one fisher per trapper. The remaining 15,920.7 square kilometers of the eastern Upper Peninsula (except Drummond Island) was opened to fisher trapping in 1996 and added to Unit B (Cooley et al. 2001). The bag limit remained at one fisher per trapper in Unit B (Cooley et al. 2001). Current regulations continue to allow three fishers per trapper, one of which can be taken in Unit B (MDNR 2005).

Fisher harvest trends from 1989 to present in Michigan are shown in Figure 2.8. There has been a general increase in the number of fishers taken since the opening of the first legal harvest season in sixty years. The spatial dispersion of reported harvested fishers is displayed in Figure 2.9.

History of the marten in Michigan and Wisconsin

Historical background and extirpation

The historic range of the American marten was coincident with the distribution of the northern coniferous forests (Figure 2.10; Hagmeier 1956, Gibilisco 1994). The northern boundary of the range was the tree line in Manitoba, Ontario, and Quebec (Hall and Kelson 1959). Martens can be found in hardwoods, especially mixed deciduous-coniferous forests that sustain substantial populations of prey species (de Vos 1951, Wright 1999, Earle et al. 2001). Much of the marten's distribution has been sympatric with that of the larger fisher, potentially resulting in interference competition. However, in areas with substantial elevation differences, such as in the West or Northeast, martens tend to occur at higher elevations than fishers (Hagmeier 1956). Snow depth also appears

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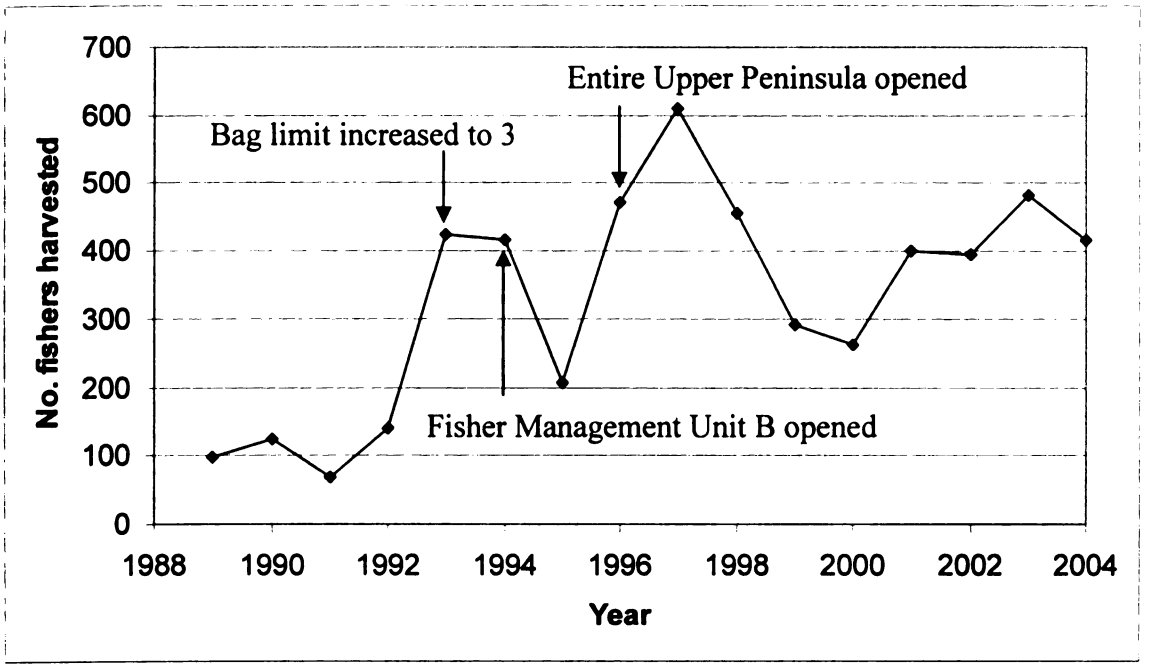

Figure 2.8. Total harvest of fisher from 1989 to 2004 in Michigan, detailing years in which management decisions were made regarding quotas and areas trapped. (Data from Cooley et al. 1990, 1991, 1992, 1993, 1994, 1995, 1997a, 1997b, 1998, 2001, M. Cosgrove, MDNR, personal communication.)

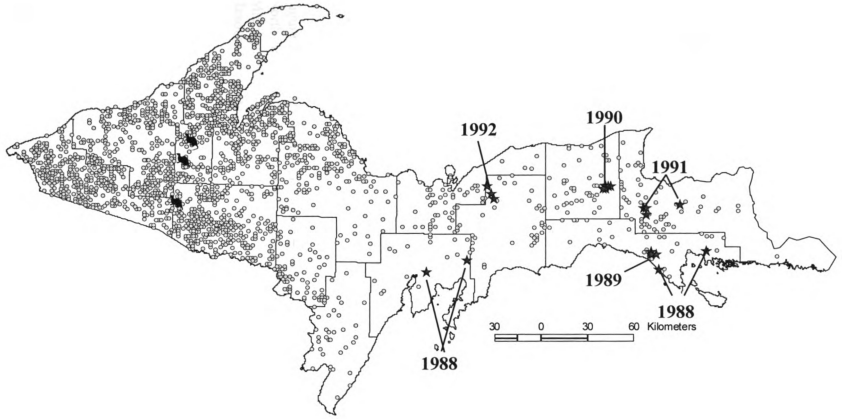

Figure 2.9. Location of reported harvested fishers in the Upper Peninsula of Michigan from 1989 to 2004. Original 1961-63 release sites in the western UP (fisher symbols) and translocations (stars) are provided for reference.

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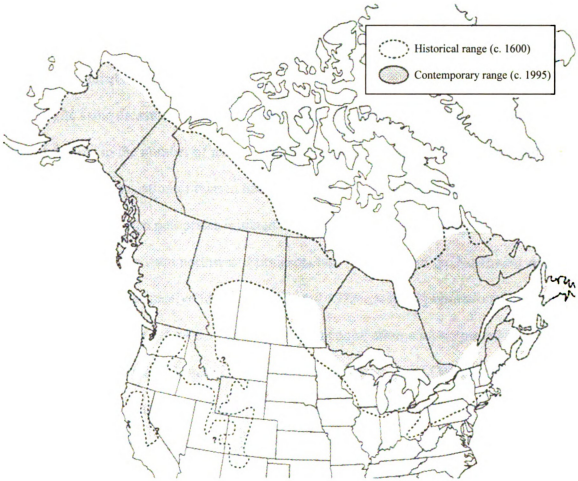

Figure 2.10. Approximation of historic and current distribution of American martens in North America. Adapted from Hagmeier (1956), Gibilisco (1994), and unpublished Michigan Department of Natural Resources and Wisconsin Department of Natural Resources reports.

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to limit the co-distribution of the two species, with the larger, heavier fishers unable to maneuver efficiently through deep snow (Raine 1983, Aubry and Houston 1992, Krohn et al. 1995, Krohn et al. 1997).

The same pressures affecting fishers in the 19th and early 20th centuries also led to a rapid decline in the number of martens across the southern portion of its range and in the Midwest. Degradation of marten habitat (old growth forest) occurred through logging and fires. High marten pelt prices initiated unregulated levels of trapping and poisoning. The last marten taken from northwest Minnesota was in 1920 from the Northwest Angle, but the species did not receive full protection until 1933. A small population remained in the northeast corner of the state. The protection in conjunction with suspected migration of martens into northeast Minnesota from the Thunder Bay area of Ontario resulted in a gradual increase in numbers during the 1950s and 1960s (Mech and Rogers 1977).

Protection of martens came too late to prevent extirpation in Michigan and Wisconsin. Formerly found as far south in Michigan as Allegan County, the last confirmed report of a marten in the northern Lower Peninsula was in 1911, from near Lewiston, Montmorency County (Wood and Dice 1924, Hagmeier 1956). The more remote Upper Peninsula provided slightly better refuge for the species: the last confirmed report was from the Huron Mountains, Marquette County, in 1939 (Manville 1948). In Wisconsin, the marten was formerly found at least as far south as Brown, Jackson, Juneau, La Crosse, and St. Croix Counties, following riparian habitats along major rivers. The trapping season was closed in 1921. However, the last confirmed report of a marten in Wisconsin was an individual trapped in Douglas County in 1925 (Jackson 1961). By the early 1940s, marten abundance across North America reached its lowest level and the

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Michigan marten reintroductions

The pelt value of the marten has historically been lower than that of other furbearer species. However, the species has been recognized as a "unique and desirable component of wilderness forest ecosystems" (Berg 1982: 165). Reintroduction of the marten would fill the "niche in nature" vacated with the species' extirpation (Michigan Department of Conservation, MDOC, unpublished report 1953). By the 1950s, enough continuous habitat in the Upper Peninsula of Michigan was deemed suitable for the survival of the marten and discussions regarding the restoration of the species began (MDOC unpublished report 1953).

On February 24, 1955, four martens (two females and two males) were released in the Porcupine Mountain State Forest, Ontonagon County (T51N R42W Section 18; MDOC unpublished report 1955, Switzenberg 1955; Table 2.3, Figure 2.11). This area was predominated by mature hemlock (*Tsuga canadensis*) and other conifers interspersed with openings of sapling- and pole- sized hardwoods on rough, broken terrain (MDOC unpublished report 1956, Harger and Switzenberg 1958). The release included one male and one female purchased from a fur farm run by Mr. E. Salamder near Perkins, Michigan. These martens were originally from British Columbia and had been held in captivity for approximately five years (MDOC 1957). On March 29, 1955, two martens (two males) were released in the same area of the Porcupine Mountains. A single male was released on July 21, 1955 and on April 11, 1956, one male was released. With the exception of the two fur-farmed individuals, all martens released in the Porcupine

Table 2.3. Relocation events of the American marten in Michigan.

Relocation	Date	Release type	N (females)	Source Population
1. Porcupine Mountains State Park, MI	3-24/1966			

Table 2.3. Relocation events of the American marten in Michigan.

Relocation	Date	Release type	N (females)	Source Population
1. Porcupine Mountains State Park, MI	2/24/1955	reintroduction	4 (2)	White River Country, Algoma District, Ontario,
Ontonagon County, T51N R42W Sec 18	-	-	-	Canada (2); local MI fur farm (2), originally
	-	-	-	from British Columbia, Canada
	3/29/1955	reintroduction	2 (1)	White River Country, Algoma District, Ontario,
	-	-	-	Canada
	7/21/1955	reintroduction	1 (0)	CCGP°, Ontario, Canada
	4/11/1956	reintroduction	1 (0)	CCGP°, Ontario, Canada
	2/6/1957	reintroduction	4 (2)	CCGP°, Ontario, Canada
	2/14/1957	reintroduction	8 (4)	CCGP°, Ontario, Canada
	2/19/1957	reintroduction	4 (1)	CCGP°, Ontario, Canada
	2/28/1957	reintroduction	4 (0)	CCGP°, Ontario, Canada
	4/12/1957	reintroduction	1 (1)	CCGP°, Ontario, Canada
Total:	***1955-1957***	-	***29 (11)***	-
Reference: Harger and Switzenberg 1958, MDOC unpublished report				
2. Whitefish River Valley, Rapid River Dist.,	4/15/1969	reintroduction	4 (1)	Near Nipigon, Ontario, Canada*
Hiawatha National Forest, West Unit, MI	4/17/1969	reintroduction	16 (3)	Near Nipigon, Ontario, Canada*
T43N R20W Sec 29;	4/23/1969	reintroduction	9 (3)	Near Nipigon, Ontario, Canada*
T42N R20W Secs 7 and 19	5/21/1969	reintroduction	8 (3)	Near Nipigon, Ontario, Canada*
	6/9/1969	reintroduction	7 (2)	Near Nipigon, Ontario, Canada*
	10/28/1969	reintroduction	16 (5)	Near Nipigon, Ontario, Canada*
	11/7/1969	reintroduction	11 (8)	Near Nipigon, Ontario, Canada*
	12/5/1969	reintroduction	20 (10)	Near Nipigon, Ontario, Canada*
	3/16/1970	reintroduction	8 (2)	Near Nipigon, Ontario, Canada*
Total:	***1969-1970***	-	***99 (37)***	-
Reference: MDNR interoffice communication; Schupbach 1977				

Table 2.3 (cont'd)

Relocation	Date	Release type	N (females)	Source Population

Table 2.3 (cont'd)

Relocation	Date	Release type	N (females)	Source Population
3.a. Huron Mountain Club, Marquette Co., MI	1979	reintroduction	39 (8)	Algonquin Provincial Park, Ontario, Canada
b.Huron Mountain Club, Marquette Co.;	-	-	-	-
Carrol-Paul Forest, Marquette Co., MI	1980	reintroduction	40 (23)	Algonquin Provincial Park, Ontario, Canada
c.McCormick Experimental Forest, Ottawa	-	-	-	-
National Forest (ONF), MI	1980	reintroduction	22 (13)	Algonquin Provincial Park, Ontario, Canada
d.Webb Lake, Iron County, MI (ONF)	1981	reintroduction	10 (6)	Algonquin Provincial Park, Ontario, Canada
e.Perch Lake area, Iron County, MI (ONF)	1981	reintroduction	38 (21)	Algonquin Provincial Park, Ontario, Canada
Total:	***1979-1981***	-	***148 (71)***	-
Reference: Churchill et al. 1981				
4. Pigeon River Country State Forest, MI,	11/7/1985	reintroduction	10 (2)	CCGP°, Ontario, Canada
Cheboygan County T33N R1W, Otsego	11/13/1985	reintroduction	10 (3)	CCGP°, Ontario, Canada
County T31N R1W	11/21/1985	reintroduction	10 (8)	CCGP°, Ontario, Canada
	11/27/1985	reintroduction	12 (7)	CCGP°, Ontario, Canada
	12/6/1985	reintroduction	6 (4)	CCGP°, Ontario, Canada
	3/19/1986	reintroduction	1 (0)	CCGP°, Ontario, Canada
Total:	***1985-1986***	-	***50 (24)***	-
Reference: Ludwig 1986				
5. Manistee National Forest, MI	3/5/1986	reintroduction	15 (9)	CCGP°, Ontario, Canada
Pere-Marquette State Forest, MI	3/12/1986	reintroduction	15 (7)	CCGP°, Ontario, Canada
Manistee National Forest, MI	3/18/1986	reintroduction	6 (0)	CCGP°, Ontario, Canada
Total:	***1986***	-	***36 (16)***	-
Reference: Ludwig 1986				
6. Tahquamenon Bay, Hiawatha National	-	-	-	-
Forest, East Unit	***1989***	translocation	***20***	Hiawatha National Forest, West Unit, MI
Reference: MDNR unpublished report				

Table 2.3 (cont'd)

Table 2.3 (cont'd)

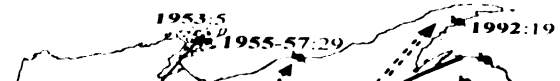
Relocation	Date	Release type	N (females)	Source Population
7. Tahquamenon Bay, Hiawatha National Forest, East Unit *Reference: MDNR unpublished report*	- ***1989-1990***	- translocation	- ***27***	- Iron County, MI
8. Keweenaw County, MI *Reference: MDNR unpublished report 1992*	***1992***	translocation	***19 (5)***	Houghton County, MI

° CCGP: Crown Chapleau Game Preserve, Ontario, Canada

* The source location is not known for certain. An unidentified newspaper article stated the martens for the 1969-1970 reintroduction were flown to Marquette from Port Arthur, Ontario, Canada, which is in the Thunder Bay area. MDNR interoffice communication from November 1968 identified the Ontario contact for martens as T. Galarneau who ran a trapline 25 miles north of the town of Nipigon. A handwritten message below stated MI should contact Ontario for the martens. Thus, the inference is that the martens were trapped along Mr. Galarneau's line.

A trade was negotiated between Ontario and Michigan to procure martens for one of the early Michigan reintroduction events in exchange for fox squirrels (*Sciurus niger*; Berg 1982). It is not known when this trade occurred.

Figure 2.11. Release locations of reintroductions and translocations of American martens in Michigan and Wisconsin are labeled with year(s) and total number of animals released. Source areas for each reintroduction are represented by stars and are linked to relocation points using arrows of differing patterns. Double dashed arrows show the approximate areas from which martens were trapped for two translocation events within the Upper Peninsula of Michigan. For further reference see Table 2.3 and Table 2.4.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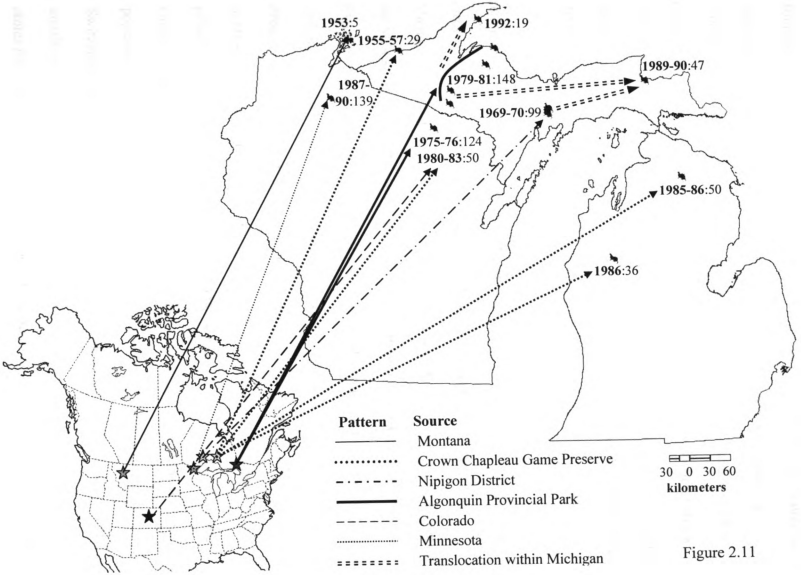
1953:5
1955-57:29
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1989-90:47
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1969-70:99
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Mountains in 1955 and 1956 were purchased from Ontario trappers through arrangements made with the Ontario Department of Lands and Forests. Locations of capture are recorded as from the White River Country, Algoma District, Ontario, Canada (MDOC unpublished report 1955). Payment in 1955 went to a tribal trapper in Mobert (MDOC interoffice communication 1956). Further payment was coordinated through the Ontario Department of Lands and Forests in Franz, Ontario (MDOC interoffice communication 1955).

The results were disappointing to those in the MDOC involved in the reintroductions. Alternate employment opportunities, primarily uranium prospecting, made it difficult to interest the Ontario Indian trappers in capturing martens for Michigan's restoration effort (MDOC 1957). In the winter of 1957, three employees of the MDOC, Elsworth "Al" Harger, Game Biologist from the Houghton Lake Wildlife Experimental Station, Conservation Officer Bruce "Sid" Andrews of Newberry and John Arduin, Predatory Control Officer from Newberry were sent the Crown Chapleau Game Preserve, Ontario to trap the remainder of the martens for the Porcupine Mountains reintroduction (Harger and Switzenberg 1958). The 7,381.5 square kilometer wildlife preserve has been closed to trapping since 1925. Marten trapping was banned in the Ontario 1948 due to severely depleted numbers province-wide. One of the few remaining populations at that time was in the Crown Chapleau Game Preserve (Harger and Switzenberg 1958). The Crown Chapleau Game Preserve consists of large areas of coniferous forest (jack pine *Pinus banksiana*, black spruce *Picea mariana* and balsam fir *Abies balsamea*) interspersed with ridges of deciduous forest, including aspen *Populus* spp. and white birch *Betula papyrifera* (Ludwig 1986).

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The Michigan trappers were based out of Camp 6 run by the Newaygo Timber Company, Ltd., a subsidiary of Consolidated Waterpower and Paper Company of Wisconsin. It was located approximately 40 kilometers east of Mosher and 354 km north of Sault Ste. Marie (Harger and Switzenberg 1958). Twenty-two martens were trapped during the winter of 1957 and sent to the Cusino Wildlife Research Station, Shingleton, Michigan for release. Twenty-one martens were trapped. Two escaped and one died during transport to Michigan. Three martens were obtained from a fisher-trapping project in Ontario. In total, 21 (13 males and 8 females) were shipped to Michigan (Table 2.3, Figure 2.11; Harger and Switzenberg 1958). On February 6, 1957, 2 males and 2 females were released into the Porcupine Mountains. On February 14, 1957, 4 males and 4 females were released in the same area. On February 19, 1957, 3 males and 1 female were released and four males were released on February 28, 1957. A final release of a single pregnant female that had been held at Cusino was made on April 12, 1957 (MDOC unpublished report 1957). Some of the martens obtained from Ontario were traded for other species such as sharptailed grouse and wild turkey (MDOC interoffice communication 1958).

Between April 15, 1969 and March 16, 1970, 99 (62 males and 37 females) martens were released by the MDNR in cooperation with the USFS in the Whitefish River Valley on the Rapid River District of the Hiawatha National Forest, West Unit. The three release locations were T43N R20W Section 29, and T42N R20W Sections 7 and 19 (USFS interoffice communication 1972; Table 2.3, Figure 2.11). These animals had been flown into Marquette, Michigan from Port Arthur, Ontario. The contact for securing these martens was Mr. T. Galarneau, of Nipigon, Ontario and it can be inferred

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that the 99 marten were captured along his trapline 40 kilometers north of the town of Nipigon (MDNR interoffice communication 1968). To protect the newly released martens from incidental trapping, dryland sets were banned from the 12 townships surrounding the release sites for five years following reintroduction (Schupbach 1977, Doran 1984).

In 1979, with funding from the U.S. Endangered Species Program, the MDNR contracted trapping and release of a third marten reintroduction event with Michigan Technological University, Houghton, Michigan. The goal was a release of 150 martens. Under the supervision of Dr. Norman Sloan, 39 martens (31 males and 8 females) were live-trapped in Algonquin Provincial Park, Ontario, Canada. Algonquin Provincial Park is a 7,571 km^2 reserve in Central Ontario between Georgian Bay and the Ottawa River. One male died during shipment to Michigan, but the remaining 38 animals were released on the Huron Mountain Club near Lake Superior in northern Marquette County. The Huron Mountain Club is privately owned with restricted access, offering protection from trapping to released animals.

In July of 1980, Ecological Research Services, Inc., a consulting firm based out of Iron River, Michigan, continued the marten relocation efforts supported by the U.S. Endangered Species Program, the MDNR, and the Ontario Ministry of Natural Resources (OMNR). Forty martens (17 males and 23 females) were released on the Huron Mountain Club and the adjacent Carrol-Paul Forest in 1980 and 1981. During December 1980, 22 martens (9 males and 13 females) were released on the Cyrus H. McCormick Experimental Forest Tract, a 70 km^2 primitive area in Marquette and Baraga Counties (Churchill et al. 1981). The McCormick Tract is a satellite of the Ottawa National Forest,

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located to the west of the main body of the forest, and is closed to all motorized vehicles. The McCormick Tract consists of mature mixed hardwoods- conifer (hemlock, yellow birch *Betula lutea*, balsam fir, and white pine *Pinus strobus*) as well as a few cedar swamps, open areas, and stands of birch *Betula* spp. and aspen. This release site is approximately 16 km south of the primary release locations in the Huron Mountains, and was thus chosen to encourage migration between the two populations (Churchill et al. 1981).

A third set of marten release sites were made in the Iron River District of the Ottawa National Forest during the spring of 1981. The general release location was chosen due to its close proximity to a reintroduced population on the fisher management unit of the Nicolet National Forest, Forest County, Wisconsin (see below). A goal of the Iron River releases was to form a link between the restricted gene pools of the two reintroductions via migration. Ten marten (4 males and 6 females) were released near Webb Lake, approximately 6.5 km northwest of the City of Iron River (Iron River Township, Section 9). This was considered a high density release (10 martens per 2.59 km^2). Thirty-eight additional animals (17 males and 21 females) were released north of Webb Lake in 28 sections to comprise a lower density release (approximately 1.5 martens per 2.59 km^2). Releases were made approximately 1 km apart along Ottawa National Forest roads 137, 144-145, and 146. Releases were also made along FR 347 towards Blockhouse Campground, FR 144-145 on the south side of Perch Lake, and along the north side of Perch Lake near the campground. With this final release, a total of 148 martens (77 males and 71 females) had been reintroduced in the west-central Upper Peninsula of Michigan from 1979 to 1981, two animals shy of the initial goal of 150

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In 1984, the MDNR requested permission from the OMNR to live-trap martens from Algonquin Provincial Park for a series of reintroductions in the Northern Lower Peninsula. This restoration effort was planned to occur over 2-3 years and involved 5 or 6 releases spaced roughly 32-64 km apart. Each release was to include approximately 40 martens for a total of 220-240 animals, which was anticipated to maintain genetic diversity through natural dispersal between sites. Further, it was anticipated that 10-15 fishers would be incidentally trapped during the process and would also be released in the Northern Lower Peninsula to begin the restoration of that species to its former range in Michigan. The OMNR declined permission to trap in Algonquin Provincial Park, in part due to criticism the agency had fielded in regards to removing wildlife from the reserve for the Michigan Moose Reintroduction Project. However, in 1985 the OMNR consented to allow live-trapping of up to 100 marten in the Crown Chapleau Game Preserve (Ludwig 1986).

Ecological Research Services, Inc. had again been contracted by the MDNR to undertake the trapping effort in Ontario. The releases were cooperative efforts between the MDNR and the USFS. Funding was provided by the Michigan Non-game Wildlife Fund, the MDNR Forest Wildlife Fund, the USFS Wildlife Fund, and the Harger Foundation of Michigan (Ludwig 1986). Live-trapping began in late October, 1985. On November 6, 1985, 10 martens (8 males and 2 females) were shipped to Michigan for release on the Pigeon River Country State Forest (Cheboygan, Otsego, and Montmorency Counties). This area is predominantly forested, consisting of aspen, red pine (*Pinus resinosa*), jack pine, white pine, northern hardwoods, and white cedar (*Thuja*

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occidentalis)-mixed swamp conifer. The Pigeon River Country State Forest was chosen as a reintroduction site because it includes "preferred" marten habitat and is a large tract of public land (Earle 1996). The wide range of habitat types also allowed for a better understanding of habitat preference and avoidance in landcover types not found in the source location.

The animals transported in the first group were released on November 7 in Cheboygan County along Fisherman Road., east on Webb Road., and north on Osmun Road. Ten additional martens (7 males and 3 females) were transported to Michigan on November 12, 1985, and released In Otsego County on November 13 along Tin Bridge Shanty Road, north on House's Lost Cabin Road. On November 21, ten animals (2 males and 8 females), shipped from Ontario the previous day, were released in the Cheboygan and Otsego Counties along Fisherman Road, east on Webb Road, north on Osmun Road, and along Tin Shanty Bridge Road. A shipment of 12 martens (5 males and 7 females) on November 26, 1985, was released the following day in Cheboygan and Otsego Counties along Osmun Road, Webb-Clark, and House's Lost Cabin Road. A final group of six martens (2 males and 4 females) was transported from Ontario on December 5, 1985 and released in Otsego County on December 6 along Hardwood Lake Road, north on Osmun Road to Hemlock Lake (Ludwig 1985).

The reintroduction of marten into the Northern Lower Peninsula continued in March 1986. On March 4, 1986, 15 martens (6 males, 9 females) were transported by Ecological Research Services, Inc. from the Crown Chapleau Game Preserve to Michigan. These animals were released on the Manistee National Forest in Lake and Wexford Counties on March 5, 1986 (T20N R11W Sections 1 and 2, T21N R11W

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Section 36). On March 12, 1986, 15 martens (8 males and 7 females) were shipped to Michigan and released on the Pere-Marquette State Forest in Lake County on March 18 (T20N R12W Sections 16, 21, and 29). The Manistee National Forest and the Pere-Marquette State Forest were chosen as release sites by the USFS due to the availability of acceptable marten habitat in addition to the proximity of the two sites meeting the qualifications within the Opportunity Area Analysis Plan. On March 17, six males, three of which were fitted with radio collars, were captured in Ontario and released the following day on the Manistee National Forest in Lake County (T20W R11W Sections 12 and 31). A single juvenile male was transported from the Crown Chapleau Game Preserve on March 18, 1986, and was released in Cheboygan County, on the Pigeon River Country State Forest on March 19, 1986 (Earle 1996).

The MDNR sought to continue the reintroduction effort through the winter of 1986-1987 in an attempt to release an additional 200 marten into the northern Lower Peninsula. However, public sentiment in Ontario had sparked a government review of activities in provincial parks and Crown game preserves, including hunting, trapping, or any similar removal of animals from the wild. The OMNR was concerned that removal of 200 martens from any one area of the province could potentially negatively affect the remaining population in Ontario. In addition, it was doubtful that the number of beaver carcasses needed for bait could be obtained in time. In the event that live-trapping was allowed and able to proceed given resources, Ontario trappers would have to be included in the effort (Ecological Research Services, Inc. – MDNR intraoffice communication 1986). Ecological Research Services, Inc. reported that the live-trapping effort in 1985 and 1986 resulted in controversy with the Chapleau local trappers' council. The council

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felt that Ontario trappers should have been given the opportunity to place a bid on the marten relocation project. The OMNR had initially held a public position that local trappers were not qualified for the project. The disagreement was taken all the way to the Ontario House of Commons. A major concern was in the handling and anesthesia techniques used on the animals and an understanding was finally reached by which through proper training and initiative, local trappers could be involved in future release efforts (Ecological Research Services, Inc. – MDNR intraoffice communication 1986).

As a result of the above issues, no further translocations of martens occurred into the Northern Lower Peninsula. The total number of martens reintroduced onto the Pigeon River Country State Forest, the Manistee National Forest, and the Pere-Marquette State Forest was 85 (40 females and 45 males). Forty-nine animals (25 males and 24 females) were released on the Pigeon River Country State Forest. Thirty-six martens (16 females and 20 males) were released on the Manistee National Forest and Pere-Marquette State Forest (Ludwig 1986).

Translocations were performed to assist in the dispersal of the marten across its former range in Michigan's Upper Peninsula. In the fall of 1989, 20 martens were moved by the USFS from the west unit of the Hiawatha National Forest, Alger County, to the Tahquamenon Bay area in the east unit of the Hiawatha National Forest, Chippewa County. During the winter of 1989 and 1990, 27 individuals were relocated from Iron County to the Tahquamenon Bay area by the MDNR. In 1992, 19 martens (14 males and 5 females) were moved from southern Houghton County to southeastern Keweenaw County (Table 2.3, Figure 2.11; MDNR unpublished report 1992). This last translocation was made in conjunction with fisher translocations already in progress.

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Wisconsin marten reintroductions

The marten reintroduction effort in Wisconsin had begun in 1953 with the release of five individuals from near Kalispell, Montana onto Stockton Island, Apostle Islands National Lakeshore, Ashland County (Kohn and Eckstein 1987; Table 2.4, Figure 2.11). However, no further stocking was undertaken in this area, and this stocking effort was largely reported as a failure (Kohn and Eckstein 1987).

The first large-scale reintroduction was initiated in on January 28, 1975 with the release of eight martens (5 males and 3 females) from the Crown Chapleau Game Preserve onto the fisher management area in the Nicolet National Forest (Davis 1983). Between February and October 1975, a further 25 martens (21 males and 4 females) from the Crown Chapleau Game Preserve were liberated in the Nicolet National Forest. From December 1975 to April 1976, 91 individuals (71 males and 20 females) trapped in Algonquin Provincial Park continued the restoration effort (Davis 1983). A trade was subsequently negotiated to acquire martens from Colorado in exchange for Wisconsin river otter (*Lutra canadensis*; Berg 1982). Between December 1980 and March 1981, 19 martens (9 males and 10 females) trapped near Berthoud Pass, Guanella Pass, or Loveland Pass, Colorado were released on the Nicolet National Forest (J. George, Colorado Division of Wildlife, personal communication). During March of 1981, a further 18 individuals (9 males and 9 females) were trapped in Algonquin Provincial Park and released on the Nicolet National Forest (WDNR 1986). The captures in the Algonquin Provincial Park were part of the larger trapping effort by Ecological Research Services, Inc. to release a substantial number of martens in the west-central Upper Peninsula of Michigan. During the winter of 1981 and 1982, four more martens trapped

Table 2.4. Relocation events of the American marten in Wisconsin

Table 2.4. Relocation events of the American marten in Wisconsin

Relocation	Date	Release type	N (females)	Source Population
1. Apostle Island National Lakeshore	-	-	-	-
Stockton Island, Ashland Co., WI	***1953***	reintroduction	***5 (unk)***	near Kalispell, Montana
Reference: Schupbach 1977, Kohn and Eckstein 1987, J. Wright, USFS, personal communication				
2. Nicolet National Forest, WI	1/28/1975	reintroduction	8 (3)	CCGP, Ontario, Canada
Fisher Management Unit, Forest Co.	2/27/1975	reintroduction	11 (2)	CCGP, Ontario, Canada
	4/3/1975	reintroduction	7 (1)	CCGP, Ontario, Canada
	10/20/1975	reintroduction	7 (1)	CCGP, Ontario, Canada
	12/16/1975	reintroduction	22 (5)	Algonquin Provincial Park, Ontario, Canada
	1/16/1976	reintroduction	3 (0)	Algonquin Provincial Park, Ontario, Canada
	2/12/1976	reintroduction	9 (1)	Algonquin Provincial Park, Ontario, Canada
	3/14/1976	reintroduction	21 (6)	Algonquin Provincial Park, Ontario, Canada
	3/25/1976	reintroduction	19 (5)	Algonquin Provincial Park, Ontario, Canada
	4/2/1976	reintroduction	17 (3)	Algonquin Provincial Park, Ontario, Canada
	12/80-3/81	reintroduction	19(10)	Berthoud, Guanella, or Loveland Pass, CO
	3/81	reintroduction	18 (9)	CCGP, Ontario, Canada
	12/81-1/82	reintroduction	4 (2)	Berthoud, Guanella, or Loveland Pass, CO
	8/82-3/83	reintroduction	9 (3)	Berthoud, Guanella, or Loveland Pass, CO
Total:	***1975-1983***	-	***172 (51)***	-
Reference: Davis 1983, WDNR 1986; J. George, Colorado Division of Wildlife, personal communication				
3. Chequamegon National Forest, WI	-	-	-	-
Fisher Management Unit, Bayfield	-	-	-	-
and Ashland Counties	***1987-1990***	reintroduction	***139 (45)***	Minnesota (135); fur farm (4)
Reference: J. Wright, USFS, personal communication; Slough 1994				

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in Colorado were relocated to the Nicolet National Forest. A final release in this area was made in 1982-83 when nine individuals from Colorado were released (4 males, 3 females, and 2 unknown). One of the males from this last release escaped in Minocqua, Wisconsin on March 14, 1983 while being held at the Northwoods Wildlife Center. One of the individuals of unknown sex died during shipment from the source. One-hundred seventy-two martens (120 males, 51 females, and one unknown) were stocked on the fisher management area of the Nicolet National Forest between 1975 and 1983 (Table 2.4, Figure 2.11). Thirty-three martens (26 males and 7 females) originated in the Crown Chapleau Game Preserve, Ontario. One-hundred nine animals (80 males and 29 females) had been translocated from Algonquin Provincial Park, Ontario. Thirty-two martens originated in Colorado.

A final marten reintroduction into Wisconsin was undertaken from 1987 to 1990. During that final period, 139 martens (94 males and 45 females) were released onto the fisher management area of the Chequamegon National Forest (Table 2.4, Figure 2.11). The majority of these animals originated in Minnesota. However, four of the 139 martens had come from a fur farm (Slough 1994).

Assessment of marten reintroductions in Michigan

Martens are unlikely prolific colonizers, and are often slow to expand their range (Bostick 2003). However, the homing instinct of the American marten has been shown to be very strong (Harger and Switzenberg 1958). On July 28, 1955, one of the fur farm animals released in the Porcupine Mountains State Park five months previously was found dead on Route 2 in Masonville, 225 km from the release site, and approximately

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10 km from the fur farm where it had been raised (Harger and Switzenberg 1958). Ludwig (1986) found evidence for homing instincts in males that were captured and released in Algonquin Provincial Park during the live-trapping program in 1985 and 1986. These marten were trapped up to 56 km from their release point, often within a time span of days. For example, one male (M-138), captured in Algonquin Provincial Park on March 15, 1986 was released 55 km from the trap site. The following day, on March 16, that marten was trapped in the same initial trap (Ludwig 1986). The tendency towards a strong homing instinct could be problematic for marten reintroduction efforts as individuals may not remain in the release area (MDOC interoffice communication 1957).

At the time of the first reintroduction in Michigan, the MDOC was aware of this issue and believed that release of a sufficient number of animals in a given area should result in at least a few martens establishing territories. However, it was also believed that success of any reintroduction would ultimately lie with the females. There was hope that some of the females released in the Porcupine Mountains State Park were pregnant. Additionally, a single female was held in captivity at the Cusino Wildlife Experiment Station for release in April, near the time when she would give birth. The resulting maternal instinct might overcome the homing instinct and the female with her kits may remain near the release area (Harger and Switzenberg 1958). However, it was unknown if that female was indeed pregnant (MDOC interoffice communication 1957).

In the years immediately following the marten releases in the Porcupine Mountains State Park, many sightings of martens were reported, however few were considered valid. In 1958, the MDOC began running systematic survival checks in the

area surrounding the release site. Routes were traveled via foot, truck, and Sno-cat in search of marten tracks. Checks within the first year resulted in documentation of very few fresh tracks (MDOC unpublished report 1958, Schupbach 1977). Surveys conducted in December through January 1958-1959 and February through March 1960 included an attempt to live-trap existing martens. No martens were trapped during either period (Switzenberg and Laycock 1961). A minimum of two fresh marten tracks were recorded during the 1958-1959 checks. No tracks were discovered during the 1960 effort. An additional survey was undertaken in 1965. There was again no sign of the species in the area surrounding the release site. In 1966, the MDOC concluded that the reintroduction attempt had failed (Shupbach 1977). However, winter track surveys and harvest records are currently used to gauge population status and distribution (Earle 2002, Frawley 2002). Both indicate martens inhabit areas in proximity to the original release site (e.g., Figure 2.12). It is unknown whether the animals in these areas are products of the original reintroduction or dispersal from later relocation efforts from either Michigan or Wisconsin.

Following the marten reintroduction of 1969 and 1970, the MDNR, formerly the MDOC, compiled records of sightings of animals, their tracks and any carcasses. By 1977, 59 records suggested an apparent wide dispersal of martens from their release locations on the Hiawatha National Forest west unit. Martens were sighted along the Lake Superior shore north of the Hiawatha National Forest, along the Lake Michigan shore to the south, Ontonagon County to the northwest, and Luce and Mackinac Counties to the east (Figure 2.13). The average distance reported from the reintroduction sites was 40 km. However, individuals were observed up to 180 km away (Schupbach 1977).

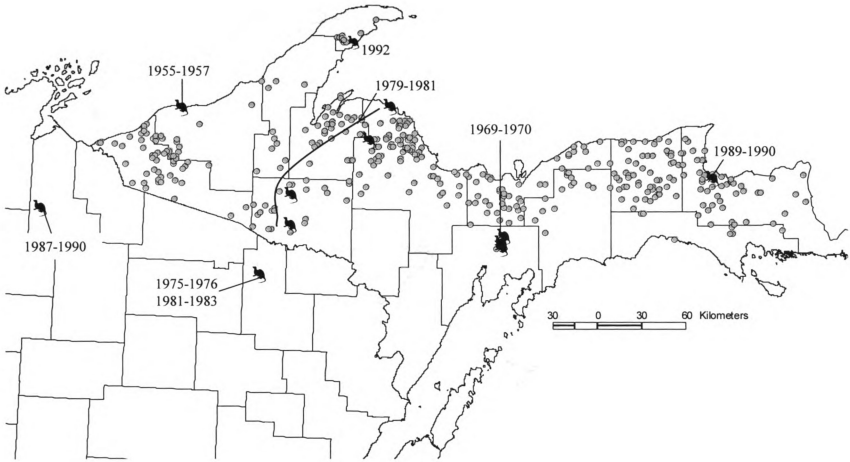

Figure 2.12. Distribution of reported marten harvests in the Upper Peninsula of Michigan from 2000 to 2004. Reintroduction sites and dates are added for reference.

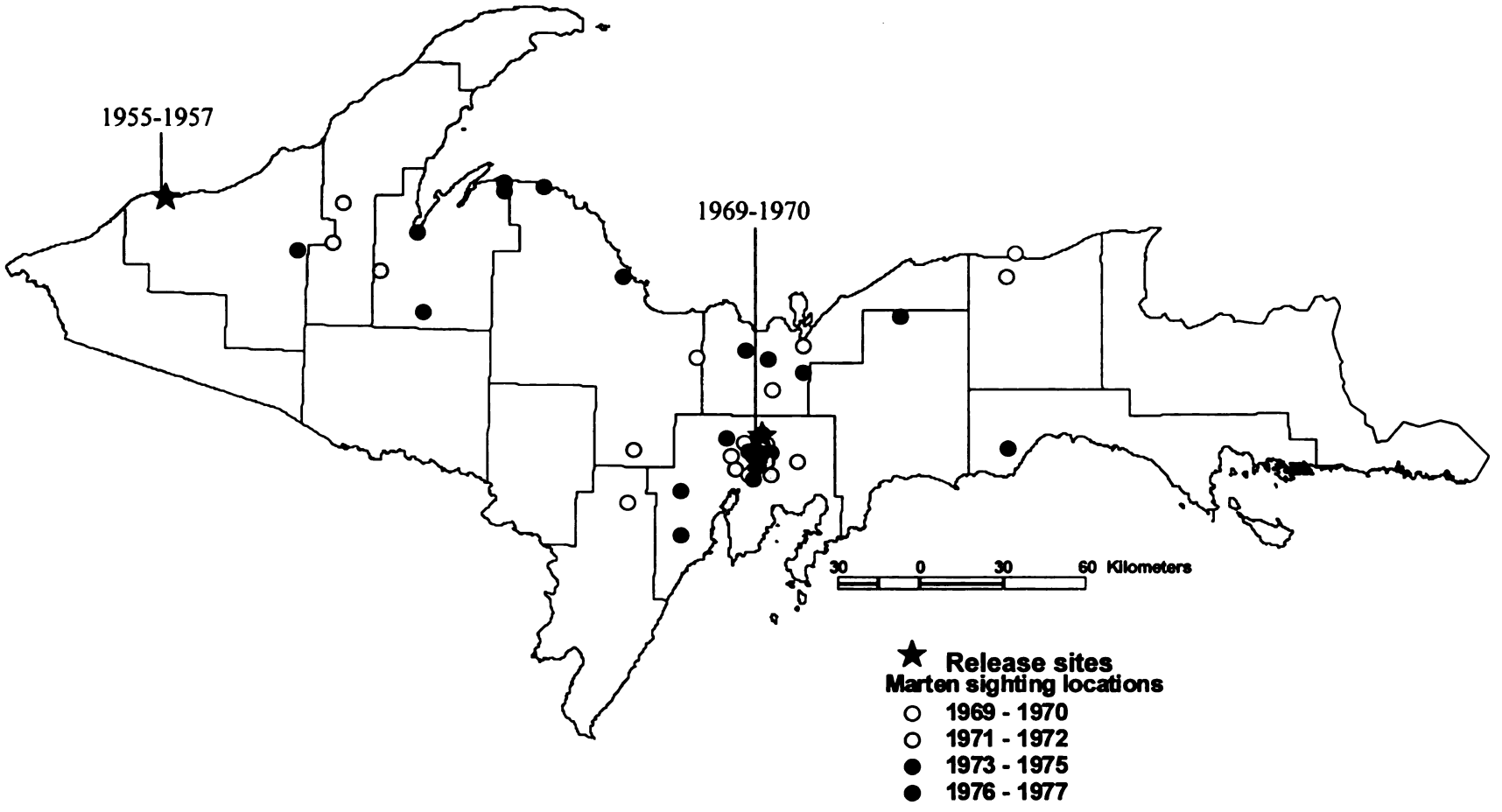

Figure 2.13. Reports of marten sightings in the Upper Peninsula of Michigan from April 1969 to October 1977. Adapted from Schupbach (1977).

During January and February of 1976 and 1977, Schupbach (1977) surveyed an area of 673 km^2 surrounding the Delta County reintroduction site for sign of martens. No tracks were discovered. Local trappers and residents also provided information of very few sightings. Schupbach (1977) estimated that fewer than 50 martens inhabited the 673 km^2 surveyed. Illegal dry-set trapping, incidental takes in wet-set traps, and random shooting of martens were targeted as reasons inhibiting the establishment of a stable population in the area surrounding the 1969-1970 reintroduction. The scattered nature of reports and lack of juveniles (untagged individuals) away from this area suggested it was highly unlikely that a breeding nucleus could exist elsewhere in the Upper Peninsula of Michigan (Schupbach 1977). Current MDNR winter track count surveys and harvest records indicate limited presence of martens in this area of the Hiawatha National Forest. However, a number of individuals have been detected approximately 20 to 40 kilometers to the north, suggesting martens reintroduced in 1969 and 1970 may have dispersed and established territories towards Lake Superior (Figure 2.13). If this is the case, the reintroduction was successful at founding a persisting population.

The martens reported in Luce and Mackinac Counties, if valid sightings, could only have been products of the 1969-1970 reintroduction due to the distance and direction from all previous release locations. However, those individuals reported in Ontonagon and Baraga Counties may have resulted from either the 1955-1957 release in the Porcupine Mountains State Forest or the 1969-1970 release in the Hiawatha National Forest.

During the winter of 1981 and 1982, Ecological Research Services, Inc. enacted a live-trapping program around the reintroduction sites of 1979 through 1981 as a means to

assess survival and dispersal of released martens. Five martens that had been part of the original release were trapped on the Huron Mountain Club. They were all trapped close to their initial release site and were deemed to be in good or excellent condition (Churchill et al. 1982). Seven martens were captured in Iron County during the live-trapping effort. Five of the seven animals, including an unmarked juvenile, were trapped near the Perch Lake and Winslow Lake areas where the majority of martens were released. One female was captured approximately 24 km from her original release site. One male was captured approximately 23 km from his Nicolet National Forest, Wisconsin, release site (Churchill et al. 1982).

The relocations into the Northern Lower Peninsula of Michigan in 1985 and 1986 were not intended as stand-alone events. The two populations are separated by over 160 km, resulting in potential geographic isolation. Some limited exchange has been suggested between the two populations. However, martens are considered habitat specialists, and especially given limited options for preferred habitat, are not known for rapid range expansion or colonization (Bostick 2003). Fragmentation of habitat is negatively correlated with marten numbers (Hargis and Bisonnette 1997). The area between the two marten populations in the Northern Lower Peninsula is fragmented by agricultural land, highways, and urban areas (Bostick 2003).

The American marten had been protected from legal harvest in Michigan since 1955. In 1978, the marten was listed as a "State Threatened Species" (Earle et al. 2001). An increase in the number of incidentally trapped marten and field sign resulted in pressure from fur taker organizations to open a trapping season. Martens were removed from Michigan's threatened species list in March 1999. In 2000, a limited marten

trapping season was opened in the Upper Peninsula for the first time since 1924 (Frawley 2002). Legal harvests have been consistently greater than 100 individuals per year, with a bag limit of one per trapper. Trapping for marten is prohibited in the Lower Peninsula.

The number of martens taken has doubled since 2002. In addition, one hundred ninety-one people acquired a marten trapping permit in 2000 compared to 156 people in 2001.

Assessment of marten reintroductions in Wisconsin

A single marten was observed on Stockton Island, Wisconsin during the winter of 1971-1972, almost 20 years following reintroduction (Schupbach 1977, Davis 1978). No further reports were made, and the reintroduction had been considered a failure (Kohn and Eckstein 1987).

Davis (1978) conducted a study from 1975 to 1976 to evaluate the reintroduction of martens onto the Nicolet National Forest. Several martens were radio-tracked and in combination with winter track counts and other observations, it was determined that the species populated the area surrounding the releases immediately following the effort. However, relatively few females had been included in the release (27 females of a total of 124) and no reproduction had been reported (Davis 1978, Davis 1983). Without further releases, the final outcome of the reintroduction was uncertain (Davis 1978).

By 1986, the marten population in the Nicolet National Forest was considered to be between 150 and 200 individuals. The population was projected to reach 300 individuals by 1990 (WDNR 1986). The spread of martens from the release point had been limited. A greater part of the population appears to have remained within 20 km of

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the reintroduction sites (Wright 1999). The current population estimate of martens in the Nicolet National Forest is 221 +/- 61 (Woodford et al. 2006).

Martens have remained in and around the area of the reintroduction onto the Chequamegon National Forest, and breeding has occurred (Wright 1999). However, it is unknown if the population is presently increasing. Research on the Chequamegon National Forest by the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) and the USFS seek to answer questions regarding habitat use and selection of martens as well as determine population size and range. A current population of forty martens has been estimated on the Chequamegon National Forest (J. Gilbert, GLIFWC, personal communication).

The American marten is a state endangered species in Wisconsin. Migration between marten populations in the Upper Peninsula of Michigan and the Nicolet National Forest population in Wisconsin has been documented (Churchill 1982). It may be likely that movement of individuals also occurs between the Chequamegon National Forest population and populations in Michigan due to close proximity and dispersion of harvested martens in the Upper Peninsula. It is unclear, however, why the species has struggled to expand its range and increase in numbers in Wisconsin while recovering to a level of sustainable harvest mere kilometers away in Michigan.

Additional considerations from past marten reintroductions

Each reintroduction effort into Michigan and Wisconsin was characterized by different numbers, sex ratios, and sources of martens released, release techniques, and time spans over which the releases occurred. It was generally advised that the greater the number of martens released, the greater the chance of a successful reintroduction

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(Schupbach 1977, Davis 1978). Releases consisting of equal or female-biased sex ratios were deemed more likely to promote a viable population. Davis (1983) suggested female-biased sex ratios should be used for short-term releases, however, a restoration effort spanning several years should consist of a release of equal numbers of males and females. Reintroduction occurring near time of parturition may decrease homing instinct of pregnant females (i.e., long-range dispersal patterns) and increase probability of establishing local territories due to strong maternal instinct (de Vos and Guenther 1952, Harger and Switzenberg 1958).

Davis (1983) described a difference in the health of martens trapped from the Crown Chapleau Game Preserve and the Algonquin Provincial Park and released onto the Nicolet National Forest, Wisconsin. Individuals shipped from the Crown Chapleau Game Preserve were all deemed to be of good health. Animals from Algonquin Provincial Park were of variable physical condition. Two-thirds (66 of 92) of the Algonquin marten displayed broken or excessively worn canines, believed to have resulted from gnawing behavior promoted by the welded-wire cages used for transportation (Davis 1978, Davis 1983).

Ludwig (1985) reported differences in the size and health of martens trapped in Algonquin Provincial Park from 1979 to 1981 and animals trapped in the Crown Chapleau Game Preserve in 1985 and 1986. No direct comparison can be made due to differences in time periods and potential environmental conditions. However, Ludwig (1985) described Crown Chapleau marten as weighing substantially less than those previously trapped in Algonquin. Initial reactivity to anesthesia was faster with Crown Chapleau Game Preserve animals, while recovery was slower. Two deaths of Crown

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Chapleau Game Preserve animals occurred during the anesthesia process in contrast to no deaths with Algonquin martens (Ludwig 1985).

Five different source populations were used for the marten reintroductions in Michigan and Wisconsin. With the exception of Colorado, all source populations had historically been part of a contiguous range of martens across Ontario and into the Midwestern United States. Human disturbance, including habitat fragmentation and exploitation, created a number of smaller disjunct refugia where martens remained in existence into turn of the 20th century. Selection may have acted upon individuals in these remaining populations causing local adaptation to environmental conditions. Similarly, habitat preference would be determined by forest types found in these refugia as well as forest types containing prey items. Any local adaptation, even subtle, may result in an unfavorable response of individuals to relocation. Prey should drive habitat preference and selection. However, relocated martens may disperse in search of forest types similar to their source area. Research presented as part of this Master's thesis has determined the genetic disjointedness of all source populations except Colorado. In addition, reintroduced populations in Michigan and Wisconsin are assigned to sources to examine relative success of each event and dispersal patterns from the release locations.

Two techniques were used during the 1975-1976 marten release onto the Nicolet National Forest. Individuals that were "quick-released" were liberated within 24 hours of arrival on site. "Gentle-released" animals were held at relocation sites in pens for approximately seven days prior to liberation. Five quick-released males, five quick-released females, four gentle-released males, and six gentle-released females were radio-collared to examine post-release movement patterns. Dispersal from the release site

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appeared to be limited by the gentle-release technique (Davis 1983). However, similar results might be obtained if release of pregnant females occurs close to parturition, or if food items, such as deer carcasses, are placed at the release site (Davis 1983). A similar examination into release techniques was performed for martens released on the Huron Mountain Club in 1979 and 1980 (Churchill 1982). In this case no difference was found in the post-release movements and establishment of territories as a result of release technique.

The 1955-1957 Porcupine Mountains State Park release, the 1969-1970 Whitefish River Valley reintroduction, and the 1975-1983 Nicolet National Forest reintroduction each consisted of small releases of often 10 or less individuals over a period of two or more years (Table 2.3 and Table 2.4). The total number of martens released is deceptive: the number released at a specific time, or even during a single season, was too low to expect establishment of a viable breeding population, especially given high dispersal rates due to strong homing instinct.

The above issues often require consideration in reintroduction scenarios. In the following chapters, implications for making species restoration management decisions will be investigated.

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References

Aubry, K.B. and D.B. Houston. 1992. Distribution and status of the fisher in Washington. Northwestern Naturalist. 73: 69-79.

Berg, W. E. 1982. Reintroduction of fisher, pine marten, and river otter. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 158-173.

Bostick, D. 2003. 2002-2003 American marten winter track count monitoring project. Huron-Manistee National Forests Final Report, Draft.

Bradle, B.J. 1957. The fisher returns to Wisconsin. Wisconsin Conservation Bulletin. 22: 9-11.

Brander, R.B. and D.J. Books. 1973. Return of the fisher. Natural History. 82: 52-57.

Churchill, S.J., L.A. Herman, M.F. Herman, and J.P. Ludwig. 1981. Final report on the completion of the Michigan marten reintroduction program. Ecological Research Services, Inc., Environmental Report. Iron River, MI.

Churchill, S.J., L.A. Herman, and J.P. Ludwig. 1982. A report on the winter 1981-1982 Michigan marten livetrapping program. Ecological Research Services, Inc., Environmental Report. Iron River, MI.

Cook, D.B. and W.J. Hamilton, Jr. 1957. The forest, the fisher, and the porcupine. Journal of Forestry. 55: 719-722.

Cooley, T.M., S.M. Schmitt, and P.D. Friedrich. 1982. Fisher survey – 1981-1982. Michigan Department of Natural Resources Wildlife Division Report No. 2918. 3 pp.

Cooley, T.M., S.M. Schmitt, and P.D. Friedrich. 1986. Fisher survey – 1984, 1985, 1986. Michigan Department of Natural Resources Wildlife Division Report No. 3034. 3 pp.

Cooley, T.M., S.M. Schmitt, and P.D. Friedrich. 1987. Fisher survey – 1985, 1986, 1987. Michigan Department of Natural Resources Wildlife Division Report No. 3062. 3 pp.

Cooley, T.M., S.M. Schmitt, and P.D. Friedrich. 1988. Fisher survey – 1987, 1988. Michigan Department of Natural Resources Wildlife Division Report No. 3081. 4 pp.

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Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and J.N. Stuht. 1990. Fisher survey – 1989. Michigan Department of Natural Resources Wildlife Division Report No. 3126. 8 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and J.N. Stuht. 1991. Fisher survey – 1990. Michigan Department of Natural Resources Wildlife Division Report No. 3140. 8 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1992. Fisher survey – 1991. Michigan Department of Natural Resources Wildlife Division Report No. 3172. 6 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1993. Fisher survey – 1992. Michigan Department of Natural Resources Wildlife Division Report No. 3189. 9 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1994. Fisher survey – 1993. Michigan Department of Natural Resources Wildlife Division Report No. 3215. 17 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1995. Fisher survey – 1994. Michigan Department of Natural Resources Wildlife Division Report No. 3239. 16 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1997a. Fisher survey – 1995. Michigan Department of Natural Resources Wildlife Division Report No. 3258. 11 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1997b. Fisher survey – 1996. Michigan Department of Natural Resources Wildlife Division Report No. 3264. 19 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 1998. Fisher survey – 1997. Michigan Department of Natural Resources Wildlife Division Report No. 3286. 24 pp.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 2001. Fisher survey 1999. Michigan Department of Natural Resources Wildlife Division Report No. 3329. 12 pp.

Davis, M.H. 1978. Reintroduction of the pine marten into the Nicolet National Forest, Forest County, Wisconsin. M.S. Thesis. University of Wisconsin, Stevens Point: 64.

Davis, M.H. 1983. Post-release movements of introduced marten. Journal of Wildlife Management. 47: 59-66.

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de Vos, A. 1951. Recent findings in fisher and marten ecology and management. Transactions of the Sixteenth North American Wildlife Conference. 16: 498-507.

de Vos, A. and S.E. Guenther. 1952. Preliminary live-trapping studies of martens. Journal of Wildlife Management. 16: 207-214.

Dhuey, B., B. Kohn, and J. Olson. 2000. Fisher Harvest 2000. Wisconsin Department of Natural Resources. 6 pp.

Doran, K.R. 1984. Winter habitat selection of the pine marten on the Hiawatha National Forest. Unpublished USFS report. 26 pp.

Earle, R.D. 1978. The fisher-porcupine relationship in Upper Michigan. Dept. of Biological Sciences. M.S. Thesis. Department of Biology. Michigan Technological University: 126.

Earle, R.D. 1996. Winter habitat preferences of marten. MDNR job final report. Project W-127-R-14, Study 127-20.

Earle, R.D. 2002. Furbearer winter track count survey of 2001. Michigan Department of Natural Resources Wildlife Report No. 3366. 10 pp.

Earle, R.D., L.H. Mastenbrook, and T.F. Reis. 2001. Distribution and abundance of the American marten in northern Michigan. Michigan Department of Natural Resources Wildlife Report No. 3321: 1-26.

Frawley, B.J. 2002. 2001 marten harvest survey. Michigan Department of Natural Resources Wildlife Division Report No. 3369: 1-4.

Gibilisco, C.J. 1994. Distributional dynamics of modern *Martes* in North America. Pp. 59-71 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Gilbert, J. H. 2000. Impacts of reestablished fisher on Wisconsin's bobcat population. Ph.D. dissertation. University of Wisconsin Madison, Madison, WI.

Gilbert, J.R. 1979. Techniques and problems of population modeling and analysis. Pp. 130-133 *in* Bobcat Research Conference Proceedings: Current Research on Biology and Management of Lynx Rufus : October 16-18. Scientific & Technical Series, No. 6. National Wildlife Federation, Washington, D.C.

Hagmeier, E.M. 1956. Distribution of marten and fisher in North America. The Canadian Field-Naturalist. 70: 101-148.

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Hall, E. and K. Kelson. 1959. The Mammals of North America, volume 2. New York: The Ronald Press Company.

Harger, E.M. and D.F. Switzenberg. 1958. Returning the pine marten to Michigan. Michigan Department of Conservation, Game Division Report 2199.

Hubert, G.F., Jr. 1982. History of Midwestern furbearer management and a look to the future. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 174-191.

Irvine, G.W. 1961. Progress report on the porcupine problem on the Ottawa National Forest. USFS, Ottawa National Forest. 3 pp.

Irvine, G.W. 1962. Fisher restoration project progress report. USFS, Ottawa National Forest 4 pp.

Irvine, G.W. and R.B. Brander. 1971. Progress report on a fisher-porcupine study on the Ottawa National Forest. U.S.D.A. Forest Service. 12 pp.

Irvine, G.W., L.T. Magnus, and B.J. Bradle. 1964. The restocking of fisher in Lake States forests. Transactions of the North American Wildlife and Resource Conference. 29: 307-315.

Jackson, H.H.T. 1961. Mammals of Wisconsin. University of Wisconsin Press, Madison. 504 pp.

Kohn, B.E. and R.G. Eckstein. 1987. Status of Marten in Wisconsin, 1985. Research Report No. 143. Bureau of Research, Wisconsin DNR, Madison, WI. 18 pp.

Kohn, B.E., Rolley, R.E., and J.E. Woodford. 2002. Fisher Population Analyses 2001, Wisconsin Department of Natural Resources. 4 pp.

Krohn, W.B., S.M. Arthur, and T.F. Paragi. 1994. Mortality and vulnerability of a heavily trapped fisher population. Pp. 137-145 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Krohn, W.B., K.D. Elowe, and R.B. Boone. 1995. Relations among fishers, snow, and martens: Development and evaluation of two hypotheses. The Forestry Chronicle. 71: 97-105.

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Krohn, W.B., W.J. Zielinkski, and R.B. Boone. 1997. Relations among fishers, snow, and martens in California: results from small-scale spatial comparisons. Pp. 211-232 *in* Martes: taxonomy, ecology, techniques, and management. G. Proulx, H.N. Bryant, and P.M. Woodard, eds. Provincial Museum of Alberta, Edmonton, Alberta, Canada.

Ludwig, J.P. 1985. Internal progress report on fall 1985 marten trapping project in the Chapleau Provincial Game Preserve. Ecological Research Services, Inc., Environmental Report. Boyne City, MI.

Ludwig, J.P. 1986. A short report on the Michigan marten reintroduction program Lower Peninsula in 1985-86: Comparison to trapping success with the Algonquin Park efforts of 1980-81. Ecological Research Services, Inc., Environmental Report. Boyne City, MI.

Manville, R.H. 1948. The vertebrate fauna of the Huron Mountains, Michigan. American Midland Naturalist. 39: 615-640.

Mech, L.D. and L.L. Rogers. 1977. Status, distribution, and movements of martens in northeastern Minnesota. U.S.D.A. For. Serv. Res. Pap. NC-14-3. 7 pp.

Michigan Department of Conservation. 1957. Current status of pine marten releases in Upper Peninsula. Michigan Department of Conservation, Cusino Wildlife Experiment Station. Special Report No. 14.

Michigan Department of Natural Resources. 1990. Fisher management plan. MDNR, Wildlife Division.

Michigan Department of Natural Resources. 2005. 2005 Michigan Hunting and Trapping Guide. Lansing, MI. 46 pp.

Olson, H.F. 1966. Return of a native. Wisconsin Conservation Bulletin. 31: 22-23.

Petersen, L.R., M.A. Martin, and C.M. Pils. 1977. Status of fishers in Wisconsin, 1975. Department of Natural Resources, Research report 92.

Raine, R.M. 1983. Winter habitat use and responses to snow of fisher (*Martes pennanti*) and marten (*Martes americana*) in southeastern Manitoba. Canadian Journal of Zoology. 61: 25-34.

Schoonmaker, W.J. 1938. The fisher as a foe of the porcupine in New York State. Journal of Mammalogy. 19: 373-374.

Schupbach, T.A. 1977. History, status, and management of the pine marten in the Upper Peninsula of Michigan. M.S. Thesis. Department of Forestry. Michigan Technological University: 79.

Slough, B.G. 1994. Translocations of American martens. Pp. 165-178 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Sodders, B. 1999. "Comeback Carnivore." Michigan Out-of-Doors. February. Pp. 8, 39.

Steck, M. 1988. A translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 21 pp.

Steck, M. 1989. The 1989 translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 16 pp.

Steck, M. 1990. The 1990 translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 20 pp.

Switzenberg, D.F. 1955. Report on first release of Canadian pine martens (*Martes americana*) in Upper Peninsula. Michigan Department of Conservation, Game Division Report 2040.

Switzenberg, D.F. and W.E. Laycock. 1961. Marten and fisher introductions. Michigan Department of Conservation, Game Policy No. 29.

Wagner, D. 1988. Fisher Plan Rough Draft. Michigan Department of Natural Resources, Wildlife Division.

Wisconsin Department of Natural Resources. 1986. Pine marten recovery plan.

Wood, N.A. and L.R. Dice. 1924. Records of the distribution of Michigan mammals. Michigan Academy of Sciences, Arts and Letters. 3: 425-469.

Woodford, J., C. Eloranta, T. Rinaldi, and B. Kohn. Inventory, status, and management needs of American marten in northeast Wisconsin. 2005 Summary Report. Bureau of Endangered Resources Ecological Inventory and Monitoring Section, Wisconsin Department of Natural Resources, Rhinelander, WI. 10 pp.

Wright, J.L. 1999. Winter home range and habitat use by sympatric fishers (*Martes pennanti*) and American martens (*Martes americana*) in northern Wisconsin. Department of Natural Resources. University of Wisconsin, Stevens Point: 73.

CHAPTER 3: GENETIC PATTERNS OF RECOLONIZATION OF REINTRODUCED AMERICAN MARTEN POPULATIONS IN MICHIGAN AND WISCONSIN.

Introduction

Many wildlife species have experienced widespread declines during the 20th century primarily due to anthropogenic factors such as habitat fragmentation and overharvest (e.g., Maudet et al. 2002, Williams et al. 2002, Vernesi et al. 2003). In some cases these declines resulted in extirpation. Reintroductions or translocations have been a means by which species are restored. The goal of any reintroduction is establishment of a self-sustaining population. Because most translocated species are game species, an additional goal is often to restore abundance to a level that can sustain harvest (Griffith et al. 1989, Slough 1994). It is particularly important for these species that demographic and genetic results of reintroduction are evaluated to ensure that success is not only short term (Griffith et al. 1989, Williams et al. 2002).

Recent studies have investigated the effects of reintroductions on genetic diversity and population structure in species including elk (*Cervus elaphus*, Williams et al. 2002), sea otters (*Enhydra lutris*, Bodkin et al. 1999, Larson et al. 2002), and ibex (*Capra ibex*, Maudet et al. 2002). Many reintroductions have involved release of a small number of animals (Griffith et al. 1989). Theory predicts that a small number of founding individuals will result in a bottleneck and concomitant loss of genetic diversity (Chakroborty and Nei 1977). If the bottleneck is sustained, continued reductions in levels of genetic diversity would be expected, due to genetic drift and inbreeding. Loss of genetic diversity would lead to concomitant decreases in a population's evolutionary potential (Frankam 1995).

Few studies have assessed how reintroductions affect fine-scale spatial genetic structure within reintroduced populations (Leberg and Ellsworth 1999). Alternatively, studies of invasive species have provided evidence for the utility of genetic methods to detect patterns of colonization and gene flow following introduction (e.g., Colautti et al. 2005, Spencer and Hampton 2005). These same principles can be applied to restored populations to determine the role reintroduction on shaping patterns of genetic spatial structure at different spatial scales. Further, new advances in molecular and statistical techniques in the field of landscape genetics can be used determine how patterns of gene flow and resulting genetic structure can be affected by habitat and other landscape features (Manel et al. 2003).

The American marten (*Martes americana*) in Michigan and Wisconsin provided a unique opportunity to examine the effects of multiple reintroductions into an area from which the species was formerly extirpated. Stocking records that included source, numbers and sex ratios released, and location of release, in conjunction with extensive genetic data, could be used to empirically test theoretical predictions relating to founder events. In addition, these genetic data could be used to elucidate important aspects of the ecology of the species over known time periods.

A rapid decline in the number of American martens occurred in the 19th and 20th centuries across the southern portion of its range. Degradation of old growth forest occurred through logging and fires. High marten pelt prices precipitated unregulated levels of trapping and poisoning (Berg 1982). By the early 1940s, marten abundance across North America reached its lowest level and the species' range was restricted to a fraction of its historical distribution (Hagmeier 1956).

Since 1934, extensive efforts have been made to restore the marten to its former range (Slough 1994). Early reintroductions involved little or no assessment of success. Starting in the 1970s, short-term monitoring techniques were used (Davis 1978, Churchill et al. 1981). Only two studies have assessed the genetic variability of reintroduced martens. McGowan et al. (1999) used randomly amplified polymorphic DNA (RAPD) markers to estimate genetic diversity among marten populations from three native and one reintroduced population. Heterozyosity ranged from 0.026 to 0.226. Swanson et al. (2006) attempted to assess genetic success of reintroduction of martens in Michigan.

Restoration of martens to Michigan and Wisconsin involved multiple releases over a period of forty-nine years. The reintroduction history is described in chapter 2 of this thesis and summarized in Figure 2.11, Table 2.4, and Table 2.5.

Assessments of marten reintroduction success performed in Michigan and Wisconsin suggested that outcomes have varied greatly. The releases on Stockton Island, in the Porcupine Mountains State Park, and in the Whitefish River Valley were considered failures due to the lack of marten sightings or lack of evidence of a breeding population following release (Schupbach 1977, Davis 1978). The status of martens released onto the Nicolet National Forest was initially uncertain (Davis 1978). It was later determined that a small number of martens (150 – 200) remained near the site of reintroduction, but dispersal had been limited (Wright 1999). The two populations in the Lower Peninsula of Michigan remain small and fragmented. A small population ($N < 50$) remains near the release site on the Chequamegon National Forest (J. Gilbert, GLIFWC, personal communication).

Winter track surveys and harvest records are currently used to determine

population status and distribution (Earle 2002, Frawley 2002). Survey data indicate species presence in areas near release sites and along much of the Michigan coast of Lake Superior. Marten presence-absence data suggests at least one of the reintroduction events in Michigan's Upper Peninsula was successful. Martens presently occur across much of the Upper Peninsula, although the sources or history of colonization remain unknown.

The objectives of this study were to (1) evaluate the success of reintroductions of martens in the Upper Peninsula of Michigan and Wisconsin using measures of genetic diversity; (2) determine if current genetic structure of martens in the Upper Peninsula of Michigan and Wisconsin was a direct result of multiple reintroductions; (3) examine spatial genetic structure within the reintroduced populations of the Upper Peninsula of Michigan to gain additional insight into processes of dispersal across a contiguous landscape.

Methods

Study area and sample collection

Assessment of differential genetic contribution of source populations used for reintroduction required the sources to be genetically differentiated themselves. Contemporary marten samples from Ontario and Minnesota were used to characterize genetic diversity in the populations used as the sources of the reintroductions into Michigan and Wisconsin. Samples were not obtained from Colorado. Algonquin Provincial Park and the Crown Chapleau Game Preserve have been closed to trapping since prior to the reintroductions into Michigan and Wisconsin. Consequently marten tongues were acquired from individual trappers through Ontario Ministry of Natural

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Resources (OMNR) Districts surrounding those areas during the 2003-2004 harvest seasons. Samples from Parry Sound, Pembroke, and North Bay Districts represented Algonquin Provincial Park ($N = 115$). Samples from Hearst and Chapleau Districts represented Crown Chapleau Game Preserve ($N = 61$). Additional samples were collected from the Nipigon District ($N = 17$). Marten tissue samples from northeastern Minnesota were obtained through the Minnesota Department of Natural Resources ($N = 60$). Each tongue and slice of tissue was placed in 1.5 mL tube filled with preservative tissue buffer (0.1 M Tris/HCL, 10 mM EDTA, 0.2 M NaCl, 4 M urea, and 0.5% sarcosine). Tubes were pre-labeled with unique identification numbers corresponding to information provided by each trapper. All samples were stored frozen at -70°C.

Heads or carcasses of American martens were collected from hunters and trappers by the Michigan Department of Natural Resources (MDNR) as a mandatory requirement for harvest registration. American marten registration in Michigan also required reporting of harvest location to a section (1 section = 1.6 km^2). Each individual was assigned a coordinate for the center of the section in which it was trapped. Muscle tissue was removed from the skull by MDNR employees and placed in 1.5 mL tubes labeled with sequential laboratory numbers.

To estimate measures of genetic diversity of reintroduced populations in the Upper Peninsula of Michigan, a series of twenty-two sample groups were created near release sites and in areas between release sites in which occurred relatively high densities of marten harvests (Figure 3.1). Individuals were grouped based on contiguity and to maximize sample size and the number of replicates within each reintroduced population while maintaining sensitivity to potentially sharp boundaries between genetically

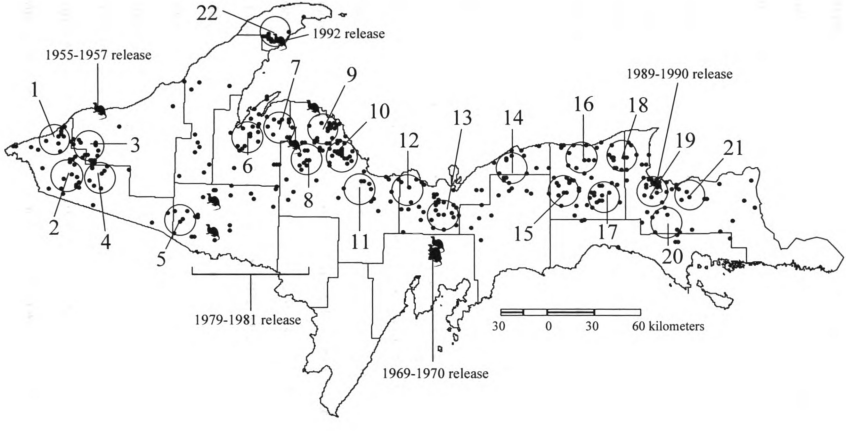

Figure 3.1. The locations of reported marten harvests from 2000 to 2004 and the 22 sample groups used in this study.

divergent reintroduced populations. Each group covered of an area approximately 250 km^2. All martens obtained from the 2000, 2001, 2002, 2003 and 2004 trapping seasons ($N = 576$) were used for spatial analysis.

Samples from Wisconsin were obtained through the USDA Forest Service, North Central Research Station, GLIFWC, and the Wisconsin Department of Natural Resources. Tissue and blood samples were collected during the course of ongoing research were sub-sampled for the research presented here. Twenty-five samples represented martens from the Chequamegon National Forest in the immediate vicinity of the release site.

Microsatellite analysis

DNA was extracted from each sample using Quiagen DNeasy® Tissue Kits. DNA was quantified by spectrophotometry and diluted to a concentration of 20 ng/μL. Ten microsatellite primers selected from Davis and Strobeck (1998) and six microsatellite primers selected from Fleming et al. (1999) were screened. Twelve variable microsatellite primers were identified for use in this study: Ma-2, Ma-5, Ma-8, Ma-14, Ma-19, Gg-3, Gg-7, Tt-1, Tt-4, Mvis072, Mer022, and Mer041 (Appendix 3.A). Marten DNA was amplified with each primer using polymerase chain reaction (PCR). DNA was amplified using each primer in a 10μL reaction with the following conditions: 2 pmol reverse primer, 2 pmol forward primer, dNTPs at 200 μM each, 200 μM 10x PCR2 buffer (1 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) or LGL buffer (1 mM Tris-HCl pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl, 10 μg/mL BSA, 0.0025% Tween 20), variable amounts of 25 mM

$MgCl_2$, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, primer specific annealing temperature for 1 minute (Appendix 3.A), and 72°C for 1 minute. The amplified product was separated on a 6% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™), Lincoln, NB. Fragments were viewed using Saga Generation 2 software, LiCor, Lincoln, NB.

A series of quality control protocols were used to minimize genotyping errors that could result in biased estimates of genetic diversity or structure. All genotypes were scored independently by two experienced laboratory personnel. Samples that could not be genotyped at a locus were re-amplified. Following re-amplification, samples lacking genotypes at more than two of eleven loci were culled from analysis.

Analysis of genetic diversity

Reintroductions have often been carried out with a limited number of founding individuals (Williams et al. 2002). Small numbers of founding animals would be expected to cause a demographic and genetic bottleneck and concomitant loss of genetic diversity (Frankam 1995, Lacy 1987). These founders would not bring with them either the same levels of diversity or be representative of allele frequencies of the founding populations. Further, not all individuals will survive and reproduce. Losses should be more evident in allelic diversity than heterozygosity, because rare alleles have the highest probability of loss, but do not significantly affect overall levels of heterozygosity (Cornuet and Luikart 1996).

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Measures of gene diversity

Tests of linkage disequilibrium for each pair of loci in each population and tests for departure from Hardy-Weinberg equilibrium (HWE) using the exact test of Guo and Thompson (1992) were performed using the Markov chain Monte Carlo (MCMC) approach of GENEPOP version 3.4 (Raymond and Rousset 1995). Bonferroni tests (Rice 1989) were used to correct for multiple tests. Means were weighted for differences in sample size.

A series of measures of genetic variation were calculated to describe differences between groups surrounding different release locations and all sampled source populations. Allele counts, allele frequencies and expected and observed heterozygosities of source and reintroduced populations were calculated using MICROSATELLITE ANALYSER version 3.12 (Dieringer and Schlötterer 2003). Allelic richness, F_{ST}, and F_{IS}, the inbreeding coefficient, were calculated using FSTAT 2.9.3 (Goudet 2000). The number of private alleles in each source and reintroduced population was determined using GenAlEx version 6.0 (Peakall and Smouse 2006). Genetic distance based on the proportion of shared alleles (Bowcock et al. 1994) was calculated using a Microsoft Excel macro (dist.xla; A. Topchy, Michigan State University, personal communication) as an additional measure of inter-individual relatedness.

Bottleneck detection

The occurrence of a bottleneck in a population and corresponding reduction in effective population size (N_e) results in reduction in the number of alleles and heterozygosity. Because allelic diversity is reduced more rapidly than heterozygosity,

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populations having experienced a recent bottleneck would exhibit significant heterozygosity excess (Cornuet and Luikart 1996). Program BOTTLENECK v 1.2.02 (Piry et al. 1999) was used to test differences between observed heterozygosity (H_O) and heterozygosity expected (if a population were in drift-mutation equilibrium) from the number of alleles observed at each locus.

We tested for bottlenecks that may have occurred as the result of reintroduction events. For this analysis, marten sample groups were clustered together based on proximity to release site. Groups 1 – 4 were located closest to the 1955 – 1957 release site in the Porcupine Mountains. Groups 5 – 10 were closest in proximity to the 1979 – 1981 reintroduction sites. Groups 11 – 14 were located nearest to the 1969 – 1970 Whitefish River Valley release location. Groups 15 – 21 were closest to the 1989-1990 release site and group 22 was in close proximity to the 1992 location of release.

Two extreme mutation models can be implemented in BOTTLENECK. According to the infinite allele model (IAM), all mutations result in novel alleles (Kimura and Crow 1964). In contrast, the stepwise mutation model (SMM) assumes an equal probability of an allele to change through mutation one step forward or backward (Ohta and Kimura 1973). Mutations in microsatellite markers more closely follow the SMM (Valdes et al. 1993) than the IAM. However, diallelic microsatellites, such as most loci used in this study, might be best described by intermediate mutation models.

Marten clusters were evaluated for occurrence of a recent bottleneck under the two-phase model (TPM) in which 90% of mutations occurred as single steps (SMM) and the remaining 10% were multi-step mutations (Piry et al. 1999, Garza and Williamson 2001). This model is intermediate to the more conservative SMM and the IAM. The

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Wilcoxon sign rank test was used to determine significance due the relatively small number of loci used in this study (Piry et al. 1999).

Based on theoretical models, a genetic bottleneck can be detected up to time $4N_e$ (Cornuet and Luikart 1996). Because the initial founding number and sex ratio was known, N_e was calculated as:

$$N_e = \frac{4N_F N_M}{(N_M + N_F)}$$

where N_e is the effective size of the founding population, N_F is the number of founding females, and N_M is the number of founding males. N_e ranged from 27 in the 1955 – 1957 release to 147 in the 1979-1981 release. Thus, the period over which a bottleneck could be detected would range from 54 to 294 years (27 to 147 generations). However, the detection window narrows significantly with highly variable genetic markers (heterozygosity > 0.3) under SMM because of high mutation rates that can rapidly return the markers to mutation-drift equilibrium following a bottleneck (Cornuet and Luikart 1996).

Spatial genetic analyses

A marked decline in marten populations during the 19th and early 20th century resulted in population fragmentation across the southern portion of the species' range (Hagmeier 1956). The level of genetic divergence between the source populations would reflect the degree of geographic partitioning resulting from fragmentation. In turn, the genetic patterns in the source population might be reflected in the genetic structure of the

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reintroduced populations in Michigan and Wisconsin. Different features of each marten reintroduction event (e.g., number released, N_e, and source used) and different habitat characteristics of the release area might result in different patterns of dispersal and colonization. Spatial patterns in the marten genetic data could be manifested differently. Genetic structure might be random, or lack significant spatial patterns. However, genetic structure could be based on source population and distance from release site. Alternatively, different genetic patterns might be found in the periphery versus the center of a population. Rapid evolution of genetic structure on a micro-geographic scale might also arise as a function of familial associations. Spatial analyses were used to describe the effects of reintroduction on genetic structure of martens in Michigan and Wisconsin.

Population structure of source populations

To examine genetic contributions of the source populations to the reintroduced marten populations in Michigan and Wisconsin, it first had to be determined if martens from the four putative source groups were genetically differentiated, and if descendents from these sources would be tractable on the basis of genotype. The software STRUCTURE 2.0 (Pritchard et al. 2000, Falush et al. 2003) was used to assign individuals from Minnesota, the Nipigon District, Crown Chapleau Game Preserve, and Algonquin Provincial Park to populations. To estimate the number of populations (K), ten independent iterations of $K = 1 - 10$ were completed using 100,000 MCMC replicates and a 100,000 iteration burnin period assuming admixture and correlated allele frequencies. Samples were grouped into populations that minimize Hardy-Weinberg and linkage disequilibrium (Pritchard et al. 2000, Manel et al. 2003). This Bayesian

assignment method does not require prior spatial information as is needed for many frequency-based assignment methods. STRUCTURE calculates posterior probabilities of individual assignment to each population for each *K*. The optimal number of populations was chosen as the value of *K* with the maximal log-likelihood value (Pritchard et al. 2000).

To estimate the magnitude of genetic differentiation between the putative source populations, F_{ST} (Weir and Cockerham 1984) was calculated using FSTAT based on the multilocus genotypes of each marten within each population. Pairwise F_{ST} values were calculated using exact tests.

As an additional approach to investigating degree of differentiation in allele frequency among source populations, a neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance (D_C) was constructed. Bootstrapping was conducted to determine statistical support of branches (population clusters) using PHYLIP 3.5c (Felsenstein 1993). Results were visualized in TreeView version 1.6.6. (Page 1998).

Population structure of reintroduced populations

Martens from four different source populations were used for four different reintroductions into Michigan and Wisconsin. If the source populations were genetically differentiated, and if dispersal and random mating had not admixed genes from different source populations, current spatial genetic structure of martens in the reintroduced populations should reflect a pattern relating to the stocking events. Only one reintroduction involved martens from more than one source. Animals from Algonquin Provincial Park, the Crown Chapleau Game Preserve, and an unsampled source,

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Colorado, were released onto the Nicolet National Forest over a period of eight years. The resulting admixture would be expected to affect martens in surrounding release sites and in adjoining areas of Michigan's Upper Peninsula north of the release sites.

The number of genetic populations most supported by genotypic data for all Michigan martens was estimated using STRUCTURE. Ten independent iterations of $K = 1 - 10$ were completed using 100,000 MCMC replicates and a 100,000 iteration burnin period assuming admixture and correlated allele frequencies. Individual posterior probabilities of genetic cluster association were imported into ArcView3.2 (ESRI) to visualize the spatial distribution of genetic clusters and inferentially regions colonized by descendents from different source populations. The martens sampled from Wisconsin were not included in this analysis due to lack of information on individual locations.

The complex reintroduction history in Michigan and Wisconsin was not fully apparent in the distribution of marten harvests (Figure 3.1). Program GENELAND (Guillot et al. 2005b) was used to identify geographic distinctions between spatial genetic clusters in Michigan that may reflect release events. The Wisconsin martens could not be used as individual coordinates were not known. A main assumption of GENELAND is that individuals are often spatially dependent. Thus, the program uses a Bayesian model in which a priori information on spatial distribution is needed, but without prior knowledge of specific population units or boundaries. Individual multilocus genotypes are used to infer the number of populations in Hardy-Weinberg equilibrium. GENELAND uses a MCMC technique to determine distinct genetic groups across a landscape. Posterior probabilities of assignment are then mapped to the landscape (Guillot et al. 2005a).

GENELAND offers a means to spatially detect divergent genetic clusters without a

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priori knowledge of population structure. This is an advantage over other similar Bayesian methods such as STRUCTURE, which often require postprocessing methods to visualize, but genetic discontinuities are not objectively identified as they occur on the landscape (Guillot et al. 2005a).

The number of populations of martens in the Upper Peninsula was estimated using GENELAND for the purpose of post-processing the data for visual representation of genetically identified populations. To both determine the number of populations and post-process the data, 100,000 MCMC iterations were used, saving only each 5^{th} iteration. This resulted in 20,000 MCMC iterations used for inference. No error in individual coordinates was assumed (delta.coord = 0).

An output option of GENELAND displayed posterior probabilities of assignment to each genetic population as contour lines on a landscape. The steepness of the contours was related to the spatial dispersion of samples and the abruptness of genetic discontinuity between populations.

The use of four different source populations for neighboring release events might result in genetic differentiation among the reintroduced populations if the source populations were differentiated from each other, and if gene flow and admixture among members or descendants from different source populations had been minimal. Pairwise F_{ST} values were calculated using exact tests in FSTAT to characterize genetic structure between sample groups in Michigan.

Sample groups 1 – 22 were assigned to populations based on results of STRUCTURE, GENELAND, and FSTAT. Individuals in groups within a specified population were pooled. The Chequamegon National Forest, Wisconsin population was also added

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to this analysis. Measures of gene diversity were averaged across sample groups within a population for comparison to gene diversity of the putative source population. Pairwise F_{ST} values were again calculated to describe genetic differentiation between marten populations in Michigan and Wisconsin.

An additional method was used to illustrate the relationships between and among source and reintroduced populations. A neighbor-joining tree was estimated from Cavalli-Sforza and Edwards (1967) chord distance (D_C) for all putative source and reintroduced populations and bootstrapped over all loci using PHYLIP. Results were visualized in TreeView.

Spatial autocorrelation of martens in Michigan

An objective of this study was to determine patterns of spatial structure within marten populations in Michigan. To decrease the scale at which we could make inferences and increase sample size, all harvested individuals were used for these analyses. Martens were grouped into populations defined by the results of the STRUCTURE, GENELAND, and FSTAT analyses as described above.

The role of isolation by distance in creating observed genetic differentiation in marten populations in Michigan was investigated using spatial autocorrelation. Pairwise Euclidian geographic and linear genetic distances were calculated for martens within each population using GenAlEx. The Mantel test (1967), implemented in GenAlEx, was used to estimate the relationship between geographic distance and genetic similarity between individuals.

The Mantel test describes correlative patterns across all samples. If these patterns

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are weak or if non-linear micro-geographic structuring is present, correlation may not be detected using a simple Mantel analysis. Fine-scale spatial structure within populations may be evaluated using alternate individual-based spatial autocorrelation analyses. Spatial autocorrelation across the Upper Peninsula was determined using a multivariate multilocus approach (Peakall et al. 2003) which differs from the allele-by-allele, locus-by-locus analysis of traditional spatial autocorrelation methods (see Epperson 2003 for review). Pairwise geographic and genetic distances were generated using GenAlEx. Spatial autocorrelation of martens was evaluated over increasing distance class sizes in each population. Different distance class sizes (range: 1 km – 20 km) were used to determine the scale at which spatial genetic structure was best detected. Intervals spaced greater than the scale at which genetic structure exists would lead to failure to detect structure. Intervals smaller than the scale of genetic structure would increase the amount of inter-individual variance within a distance class that would decrease the probability of detection of spatial structure.

Significance of degree of autocorrelation (r) was determined using 1000 permutations to estimate r about the null hypothesis of no spatial genetic structure. In addition, bootstrap estimates of r were determined with replacement. The r value for the bootstrap was calculated for individual distance classes over 1000 bootstraps. Significance of the bootstrap test was determined if the 95% confidence interval at a distance class did not include zero. The bootstrap test was considered more conservative than the permutation tests. For this study, significant spatial genetic structure was detected if the results of both the permutation test and bootstrap test rejected the null hypothesis.

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The output described the effect of increasing distance on genetic autocorrelation. Estimated *r* values were shown with 95% confidence interval error bars estimated by bootstrapping, where U and L represented the upper and lower bounds for the 95% CI about the null hypothesis ($r = 0$; Peakall et al. 2003).

Multilocus genotypes of individuals can be used to examine genetic structure at the scale of individuals. Spatial genetic autocorrelation can be examined across a two-dimensional landscape to assess fine-scale nonrandom genetic patterns among specific groupings of individuals. Barriers to dispersal and kin structure are factors that might create such nonrandom patterns. Because martens are habitat specialists (Mech and Rogers 1977), it is expected that spatial genetic autocorrelation will exist as a result of limited dispersal distance and direction.

A two-dimensional local spatial analysis was implemented in GenAlEx as described by Double et al. (2005). Focal groups are defined by a contiguous group of individuals (a focus individual and its *n* nearest neighbors). The parameter *n* is based on an estimate of the number of individuals found in locations within a distance class immediately adjacent to any individual. To determine *n* for martens in this study, reported home range sizes averaged across males and females from Minnesota and Ontario (Mech and Rogers 1977, Thompson and Colgan 1987) were used. These two populations were selected because they approximated the habitat in which martens are located in Michigan's Upper Peninsula. The diameter of the average home range size was calculated to be 1.67 km. Most neighboring martens of any given individual were expected to be included within that distance. The Nearest Neighbors Distance function in GenAlEx was used to estimate the number of nearest neighbors within a distance of

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1.67 km for each reintroduced population.

A local autocorrelation coefficient (*lr*) was calculated using pairwise comparisons between each individual and it's specified nearest neighbors. This analysis was repeated for all individuals within the data set. The analysis is similar to standard autocorrelation analysis except distance classes are redefined as number of nearest neighbors (Double et al. 2005). The results of the local autocorrelation analysis were converted into bubble plots showing the spatial orientation of martens, identifying those individuals that were significantly positively or negatively spatially genetically autocorrelated ($lr < 0.05$) in relation to their *n* nearest neighbors.

Results

Genetic variation

Following Bonferroni correction of *P*-values within all groups and all populations, only one locus – group pair deviated from HWE (Ma-14 in sample group 7). Linkage disequilibrium was only detected in two inter-locus comparisons (Ma-14 – Gg-7 in Algonquin Provincial Park and Ma-14 – Ma-19 in Minnesota). Accordingly, all loci were considered independent and were retained for analysis.

Measures of genetic diversity were similar for all putative source populations sampled for this study (Table 3.1). The Algonquin Provincial Park population displayed the greatest observed heterozygosity. Allelic richness and F_{IS} were highest in the Nipigon District population. The lowest values for allelic richness and observed heterozygosity were exhibited by the Minnesota population.

Genetic diversity differed among sample groups and the Chequamegon National

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Table 3.1. Summary of measures of genetic diversity for putative marten source populations.

		Summary means of genetic diversity				
Population	*N*	alleles	A^A	H_O	H_e	F_{IS}
Algonquin Provincial Park	115	5.9	4.16	0.647	0.656	0.026
Crown Chapleau Game Preserve	61	6.0	4.00	0.626	0.643	0.009
Nipigon District	17	5.5	4.22	0.608	0.663	0.159
Minnesota	60	5.8	3.85	0.597	0.618	0.028

[A] Allelic richness

Forest population (Table 3.2). In general, sample groups 1 – 10, which were in close proximity to the release sites of the two reintroduction events in the western Upper Peninsula, displayed greater heterozygosity and higher values of inter-individual relatedness than sample groups 11 – 22. F_{IS} in the Chequamegon National Forest population was negative, indicating a low level of allelic correlations among individuals relative to the population as a whole. In addition, inter-individual relatedness in the Chequamegon National Forest population was lower than sample groups in Michigan.

Martens in sample groups in close proximity to each release site were initially assumed to be descendants of original founders. Therefore, average measures of genetic diversity across sample groups based on proximity to each release site provided a preliminary means to compare genetic diversity in source and corresponding reintroduced populations.

Allelic diversity was lower in all comparisons of averaged sample groups to putative source population (Table 3.3). Levels of heterozygosity were similar except a comparison between sample groups 11 – 14 near the Whitefish River Valley release sites and the putative source, Nipigon District, where average heterozygosity was lower across the Michigan sample groups.

Genetic bottlenecks were not detected in any of the sample groups in Michigan using the TPM model. Likewise, the Chequamegon National Forest population did not display evidence of a recent genetic bottleneck.

Source population genetic structure

Martens from Minnesota and the Nipigon District, Crown Chapleau Game

Table 3.2. Summary measures of genetic diversity for marten sample groups as shown in Figure 3.2. The release

Table 3.2. Summary measures of genetic diversity for marten sample groups as shown in Figure 3.2. The release site closest in proximity to each sample group is identified.

			Summary means of genetic diversity					
Sample group	**Proximal release location**	***N***	**alleles**	**A[A]**	**H_O**	**H_e**	**F_{IS}**	**shared alleles[B]**
1	Porcupine Mt. State Park	12	4.1	3.58	0.644	0.632	-0.015	0.536
2	Porcupine Mt. State Park	14	5.0	4.06	0.626	0.668	0.087	0.585
3	Porcupine Mt. State Park	21	5.1	3.64	0.648	0.613	-0.064	0.496
4	Porcupine Mt. State Park	13	4.3	3.63	0.664	0.622	-0.043	0.493
5	Iron River Dist., Webb Lake	10	4.1	3.61	0.623	0.605	-0.039	0.510
6	Huron Mt., McCormick Tract	11	4.6	4.07	0.651	0.662	0.017	0.569
7	Huron Mt., McCormick Tract	13	4.5	3.78	0.621	0.647	0.087	0.537
8	Huron Mt., McCormick Tract	17	4.7	3.77	0.647	0.638	-0.019	0.526
9	Huron Mt., McCormick Tract	26	5.1	3.89	0.614	0.646	0.058	0.556
10	Huron Mt., McCormick Tract	27	5.3	3.85	0.642	0.650	0.011	0.549
11	Whitefish River Valley	8	4.2	3.77	0.589	0.589	0.029	0.500
12	Whitefish River Valley	8	3.6	3.40	0.544	0.565	0.023	0.490
13	Whitefish River Valley	20	3.8	3.16	0.549	0.540	-0.017	0.445
14	Whitefish River Valley	11	4.0	3.35	0.579	0.545	-0.057	0.440
15	Tahquamenon Bay	19	4.3	3.45	0.568	0.586	0.033	0.495
16	Tahquamenon Bay	11	4.1	3.54	0.537	0.581	0.111	0.504
17	Tahquamenon Bay	16	3.7	3.05	0.545	0.541	-0.021	0.441
18	Tahquamenon Bay	17	4.1	3.42	0.579	0.592	0.031	0.501
19	Tahquamenon Bay	15	4.0	3.38	0.525	0.565	0.066	0.486
20	Tahquamenon Bay	7	3.9	3.71	0.556	0.577	0.021	0.509
21	Tahquamenon Bay	9	4.1	3.56	0.545	0.566	0.033	0.481
22	Keweenaw	11	3.8	3.82	0.576	0.609	0.057	0.493
-	Chaquamegon National Forest	25	4.5	4.43	0.604	0.564	-0.071	0.476

[A] Allelic richness

[B] Average genetic distance based on the proportion of shared alleles (Bowcock et al. 1994)

Table 3.3. Average measures of diversity for sample groups in close proximity to release sites, and putative source populations. Also provided are field data relating to release events.

		Field data			Summary means of genetic diversity				
Sample groups/ population	**Proximal release site**	**Relocation year**	**Putative source**	**# released**	**alleles**	**A^A**	**H_0**	**H_e**	**F_{IS}**
1,2,3,4	Porcupine Mt. State Park	1955-1957	CCGP	29 (11 females)	4.7	3.73	0.646	0.632	-0.014
				Crown Chapleau Game Preserve:	6.0	4.00	0.626	0.643	0.009
5,6,7,8,9,10	Iron River Dist., Webb Lake	1980	APP	48 (27 females)	4.9	3.83	0.632	0.644	0.023
	Huron Mt., McCormick Tract	1979-1980	APP	101 (44 females)					
				Algonquin Provincial Park:	5.9	4.16	0.647	0.656	0.026
11,12,13,14	Whitefish River Valley	1969-1970	ND	99 (37 females)	3.8	3.42	0.559	0.555	-0.007
				Nipigon District:	5.5	4.22	0.608	0.663	0.159
15,16,17,18,19,20,21	Tahquamenon Bay	1989-1990	ONF, HNF	47	4.0	3.44	0.552	0.573	0.037
Chaquamegon National Forest		1987-1990	MN	139 (45)	4.5	4.43	0.604	0.564	-0.071
				Minnesota:	5.8	3.85	0.597	0.618	0.028

[A] Allelic richness

Preserve, and Algonquin Provincial Park in Ontario were sampled as four known source populations used to reintroduce the American marten into Michigan and Wisconsin. Using program STRUCTURE, the maximal log-likelihood value was $K = 2$, indicating the presence of two different genetic populations across marten source populations. Review of the posterior probabilities at $K = 2$ shows a population that is comprised predominantly of samples from the Crown Chapleau Game Preserve and a second population comprised predominantly of martens from Algonquin Provincial Park. Posterior probabilities of assignment for individuals from Minnesota and the Nipigon District were apportioned between clusters of individuals representing the Crown Chapleau Game Preserve and Algonquin Provincial Park source populations.

Genetic associations among source populations were visualized based on a neighbor-joining tree generated using Cavalli-Sforza and Edwards chord distance (Figure 3.2). A bootstrap percentage of 100 indicated clear genetic differentiation of the Crown Chapleau Game Preserve and Nipigon District populations from the Algonquin Provincial Park and Minnesota populations. Branch lengths supported genetic divergence between all populations. However, the associations presented in the neighbor-joining tree did not appear to be entirely consistent with geographic proximity of the populations. The Algonquin Provincial Park and Minnesota populations were the farthest distance apart geographically, but group together genetically based on the tree.

Mean F_{ST} estimated across the source populations was 0.044, indicating a moderate but highly significant variance in allele frequency. All inter-population pairwise comparisons were significant ($P < 0.05$, ranging from 0.040 between the Crown Chapleau Game Preserve – Nipigon District and 0.050 between the Crown Chapleau

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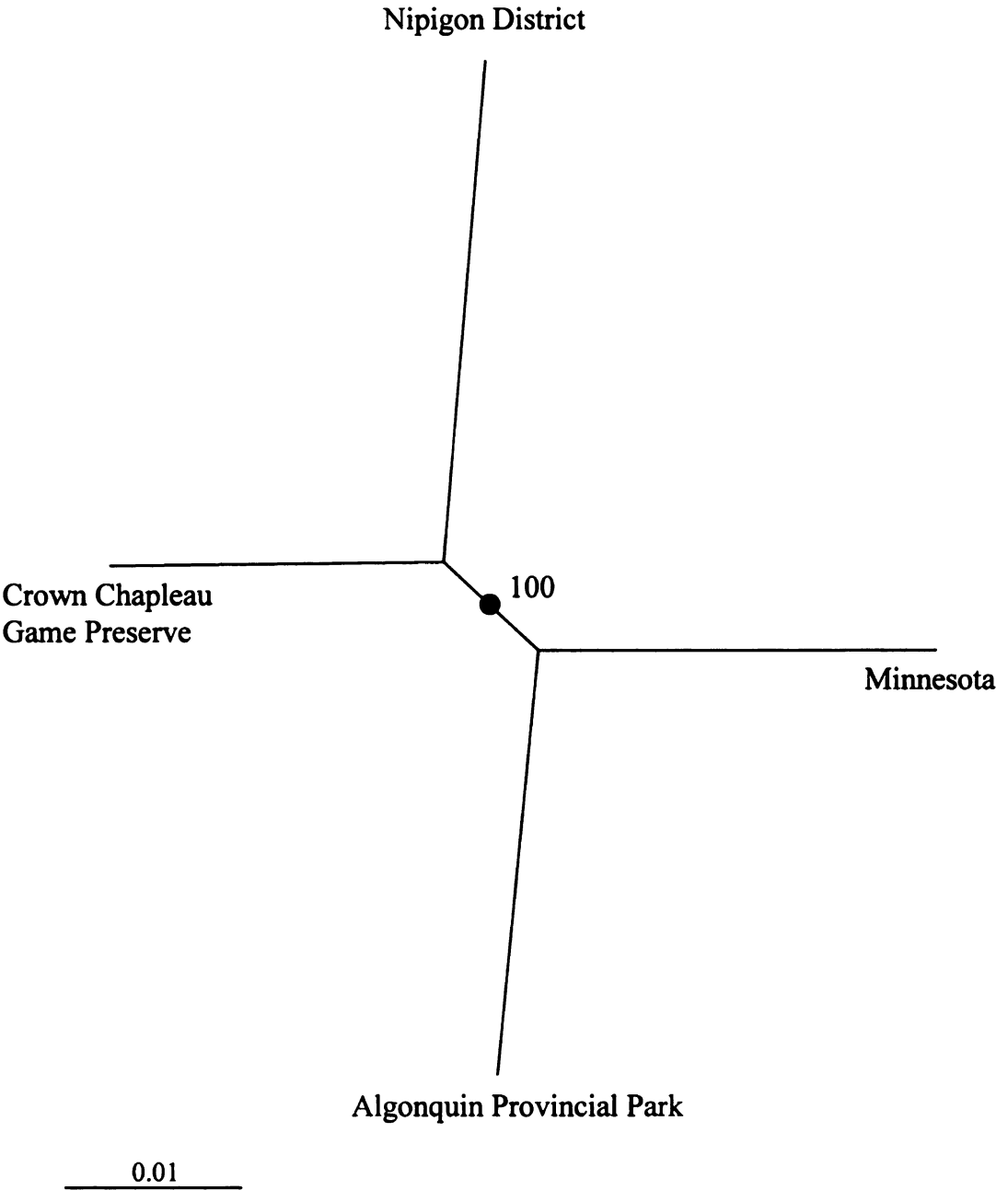

Figure 3.2. A Neighbor-joining tree representing the relationships between putative marten source populations. A bootstrap value shows the percentage of replicates over which the Crown Chapleau Game Preserve and Nipigon District populations were separated from the Algonquin Provincial Park and Minnesota populations.

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Game Preserve – Minnesota). Data were consistent with genetic differentiation between all populations used to establish martens into Michigan.

Genetic structure of reintroduced populations

Three genetically distinct populations might be expected in Michigan representing three stocking events, each from a different genetically distinct putative source. However, given the generally continuous distribution of marten harvests in the Upper Peninsula, the boundaries of marten populations with ancestral background tied to one or multiple source populations was unclear.

Program STRUCTURE was used to infer the number of genetic clusters in the Upper Peninsula and individual membership to each cluster. The maximal log-likelihood value was $K = 3$, indicating the presence of three distinct genetic populations. The three populations were geographically distinct (Figure 3.3). One was located in the western tip of the Upper Peninsula, south of the 1955 – 1957 release site in the Porcupine Mountains State Park. A second genetic population was located east of the first population, from the border of Michigan – Wisconsin north of the Nicolet National Forest, north to the shore of Lake Superior, and east to Marquette. This area included all of the release sites of the 1979 – 1981 reintroduction. Also included in the second population were martens located in the Keweenaw Peninsula. A third genetic population consisted of martens harvested from locations east of Marquette, and included most of the eastern half of the Upper Peninsula.

The three populations were not absolute in their geographic distinction. Figure 3.3 shows individuals that displayed high posterior probabilities of assignment to a

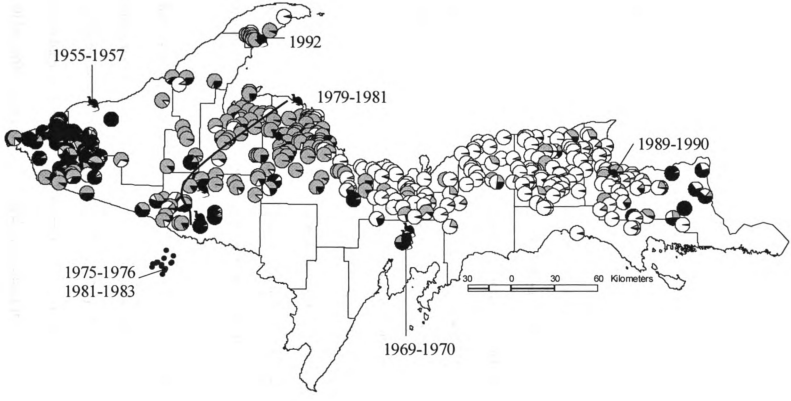

Figure 3.3. Results of STRUCTURE analysis for three genetic clusters ($K = 3$). Each pie chart represents the location and posterior probabilities of assignment to the three clusters for every harvested marten. The three groups appear representative of reintroduction events (labeled by year(s) released).

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genetic population other than the one predominant at that location. The genetic data indicated potential dispersal and admixture between populations. Clusters of individuals that shared genetically discontinuous posterior probabilities of assignment relative to geographic location were also apparent. For example, a small group of martens in the eastern end of the Upper Peninsula assigned more closely to the genetic population in the western tip than to the genetic population spread across the eastern Upper Peninsula.

Program GENELAND was used to help objectively define the geographic and genetic discontinuities of marten populations in the Upper Peninsula. Five spatial genetic clusters, or populations, were identified in the Upper Peninsula using GENELAND (Figure 3.4), including three large populations similar to the genetic populations displayed by the results of the STRUCTURE analysis. However, two additional, but small genetic populations were also detected. One was comprised of three martens in southern Houghton County in addition to 4 martens on the eastern coast of the Keweenaw Peninsula (Figure 3.5). The second included three martens in harvested in the far eastern Upper Peninsula (Figure 3.6). Two of these three martens were located on islands near to the coast of Michigan (Sugar and Neebish Islands), suggesting presence of either remnant populations that survived extirpation, or more likely, dispersers from Canada.

The three major genetic populations were generally concordant with release areas. Martens in the western tip of the Upper Peninsula comprised a genetically and geographically distinct group, located near to, but south of the 1955-1957 release site in the Porcupine Mountains State Park (Figure 3.7). Based on proximity to the location of reintroduction, it is likely the Porcupine Mountains population (as this genetic population will be further termed) was initiated by founders from the Crown Chapleau Game

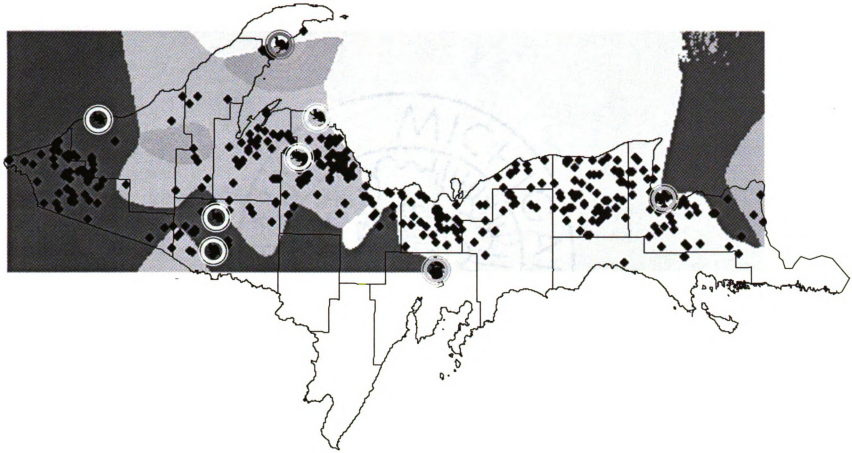

Figure 3.4. Map showing locations of the five genetically distinct populations determined using GENELAND. Release locations are circled.

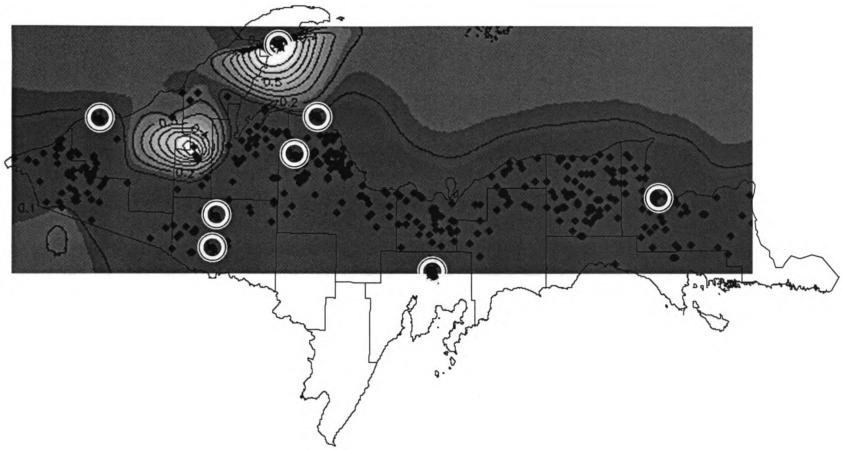

Figure 3.5. GENELAND-based posterior probabilities of assignment of martens to a small disjunct genetic population in the western Upper Peninsula. Light areas represent individuals with high posterior probabilities of assignment. Dark areas represent very low posterior probabilities of assignment. Release sites are circled.

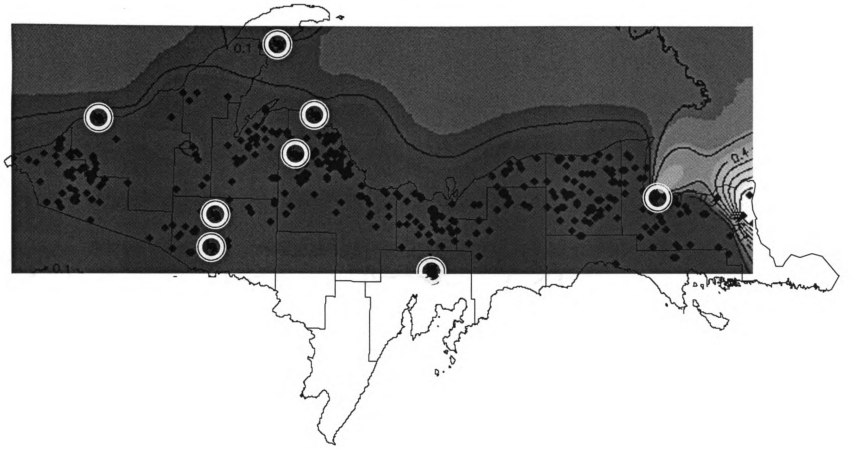

Figure 3.6. GENELAND-based posterior probabilities of assignment of martens to a small disjunct genetic population in the western Upper Peninsula. Light areas represent individuals with high posterior probabilities of assignment. Dark areas represent very low posterior probabilities of assignment. Release sites are circled.

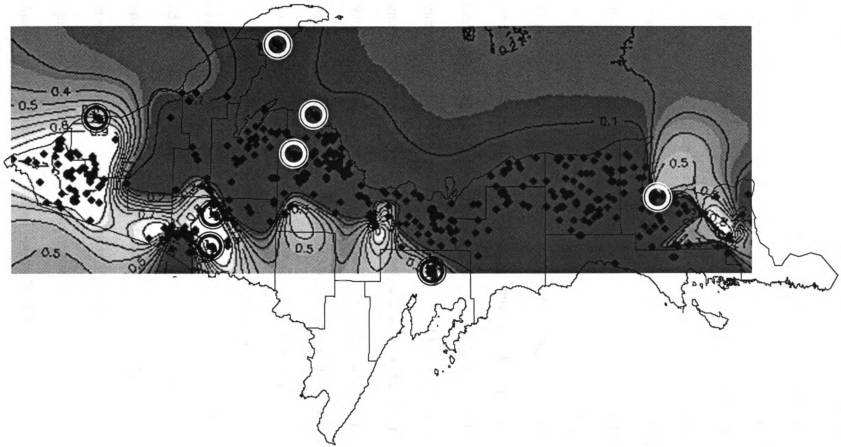

Figure 3.7. GENELAND-based posterior probabilities of assignment of martens to a major genetic population in the western tip of the Upper Peninsula. Light areas represent individuals with high posterior probabilities of assignment. Dark areas represent very low posterior probabilities of assignment. Release sites are circled.

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Preserve. A second, smaller group of martens with high probabilities of assignment to the Porcupine Mountains population was located near two of the 1979 – 1981 release sites and northeast of the Nicolet National Forest release sites. A third group was located in the eastern Upper Peninsula, east of the 1989 – 1990 translocation site.

To the east of the Porcupine Mountains population, martens corresponded to a second major genetic population, hereby termed the Huron Mountain population, concordant with the northernmost two releases on the Huron Mountain Club and McCormick Tract during 1979-1981 (Figure 3.8). Proximity to the release sites indicates this population was likely founded by martens from Algonquin Provincial Park. The Huron Mountain population was genetically separated from martens to the east by a very steep series of contour lines, indicating a narrow zone of genetic intergradation between genetically distinct populations. The eastern edge of the Huron Mountain population generally followed the Lake Superior coastline to an area in close proximity to the 1969 – 1970 release sites. An additional small group of martens displaying high posterior probabilities of assignment to the Huron Mountain population was located near the Michigan – Wisconsin border, north of the Nicolet National Forest.

Martens east of the Huron Mountain population formed the third major genetic population (Figure 3.9). The 1969 – 1970 release sites were on the south-western edge and the 1989 – 1990 translocation site was located on the north-eastern edge of this population. The large area across which this population spans and the consistency of the genetic signature suggests the population (further termed the Whitefish River Valley population) was likely a result of the reintroduction of martens from the Nipigon District, Ontario, into the Hiawatha National Forest, West Unit.

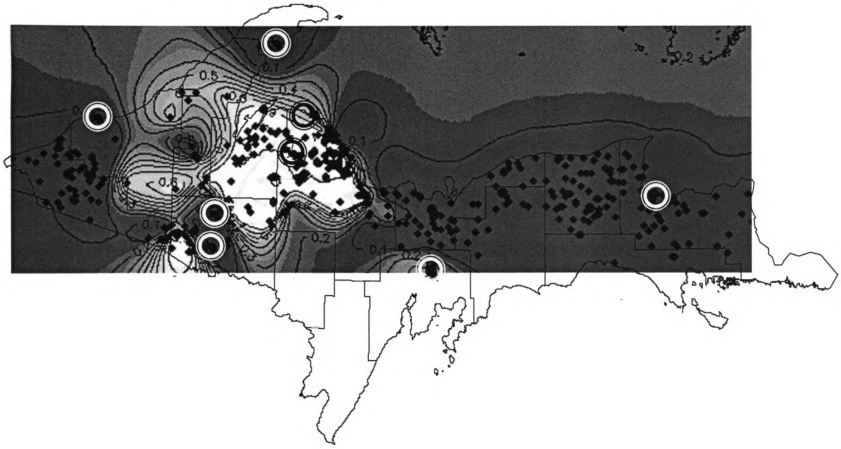

Figure 3.8. GENELAND-based posterior probabilities of assignment of martens to a second major genetic population in the west-central Upper Peninsula. Light areas represent individuals with high posterior probabilities of assignment. Dark areas represent very low posterior probabilities of assignment. Release sites are circled.

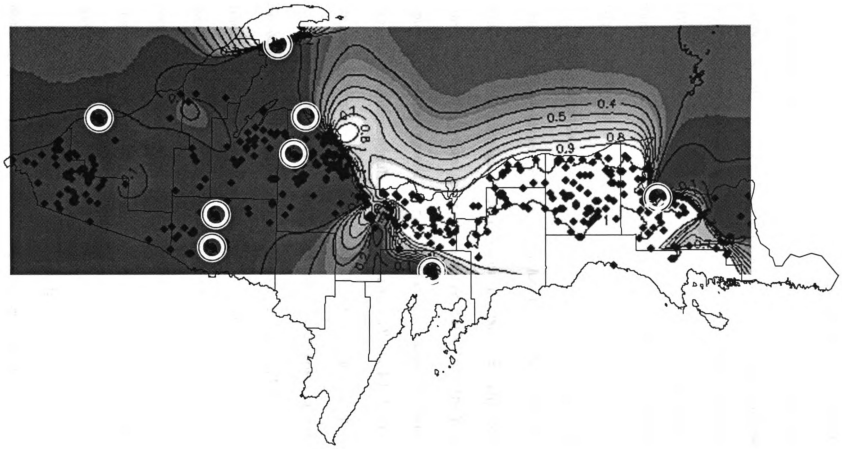

Figure 3.9. GENELAND-based posterior probabilities of assignment of martens to a third major genetic population spread across the eastern Upper Peninsula. Light areas represent individuals with high posterior probabilities of assignment. Dark areas represent very low posterior probabilities of assignment. Release sites are circled.

A pairwise F_{ST} matrix of the twenty-two sample groups resulted in the identification of three genetic clusters, corroborating with the results of the two Bayesian methods (results not shown). The three populations were geographically distinct, except for sample groups 11 and 12 (Figure 3.1) which were nonsignificant in relation to genetic clusters two and three as defined by GENELAND. Further observation showed that sample groups 11 and 12 were located along the steep gradient of contour lines delineating the two genetic clusters in GENELAND.

The 22 sample groups were pooled within the three major genetic populations, or reintroduced populations. Pairwise F_{ST} values between reintroduced populations, including the Chequamegon National Forest, indicated generally greater genetic differentiation with greater geographic distance (Table 3.4). The Whitefish River Valley population was most genetic divergent from the Chequamegon National Forest and Porcupine Mountains populations. Conversely, the Whitefish River Valley population was genetically most similar to its neighbor, the Huron Mountain population.

The Algonquin Provincial Park population was most similar to its corresponding reintroduced populations, the Huron Mountain population (Table 3.4). Likewise, the Minnesota population was most similar to its corresponding reintroduction, the Chequamegon National Forest. The Crown Chapleau Game Preserve population was not strongly aligned with its corresponding population, the Porcupine Mountains. Likewise, none of the sampled source populations was strongly associated with the Whitefish River Valley population.

A neighbor-joining tree estimated using Cavalli-Sforza and Edwards chord distance graphically depicted relationships between and among source and reintroduced

Table 3.4. Pair-wise estimates of inter-sample variance in allele frequency (F_{ST}) between all reintroduced genetic clusters and source marten populations. All values were significant ($P < 0.05$), shown in the upper half matrix. Reintroduced populations: PM = Porcupine Mountains; HM = Huron Mountain; WR = Whitefish River Valley; WI = Chequamegon National Forest, Wisconsin. Source populations: CCGP = Crown Chapleau Game Preserve; APP = Algonquin Provincial Park; ND = Nipigon District; MN = Minnesota.

	Recipient populations				Source populations			
	PM	HM	WR	WI	CCGP	APP	ND	MN
PM	-	*	*	*	*	*	*	*
HM	0.0275	-	*	*	*	*	*	*
WR	0.0797	0.0467	-	*	*	*	*	*
WI	0.0567	0.0471	0.0776	-	*	*	*	*
CCGP	0.0514	0.0479	0.0619	0.0373	-	*	*	*
APP	0.0273	0.0189	0.0739	0.0532	0.0448	-	*	*
ND	0.0445	0.0318	0.0717	0.0587	0.0395	0.0406	-	*
MN	0.0570	0.0419	0.0641	0.0336	0.0500	0.0424	0.0479	-

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populations similar to those described using analysis of pairwise F_{ST} values (Figure 3.10). Minnesota and the Chequamegon National Forest populations clustered together as did Algonquin Provincial Park and the Huron Mountain population. The remaining source populations did not cluster with their corresponding reintroduced populations, suggesting the ability to reconcile relationships between source and reintroduced populations was time dependent. The effects of genetic drift on a small, newly introduced population would likely distort genetic signatures of historical relationships.

Spatial autocorrelation of martens in Michigan

Based on population-level analyses (above), individual martens were assigned to three populations in the Upper Peninsula of Michigan (Figure 3.11) within which all spatial autocorrelation analyses were performed.

Genetic distance between individuals was positively correlated with geographic distance in the Porcupine Mountains population (Mantel test, $R^2 = 0.0082$, $P = 0.03$) and in the Whitefish River Valley population (Mantel test, $R^2 = 0.0073$, $P = 0.002$), indicating an overall pattern of isolation by distance. However, no correlation between genetic and geographic distance existed in the Huron Mountain population ($P > 0.05$).

Genetic patch size, determined by calculating the spatial autocorrelation coefficient, r, over increasing distance classes, is the distance over which effective gene flow occurs in a population. A distance class size of 10 km was determined to be the scale at which spatial genetic structure was best detected in the three reintroduced populations.

The estimated value of r was significantly positive to a distance of 100 km in the

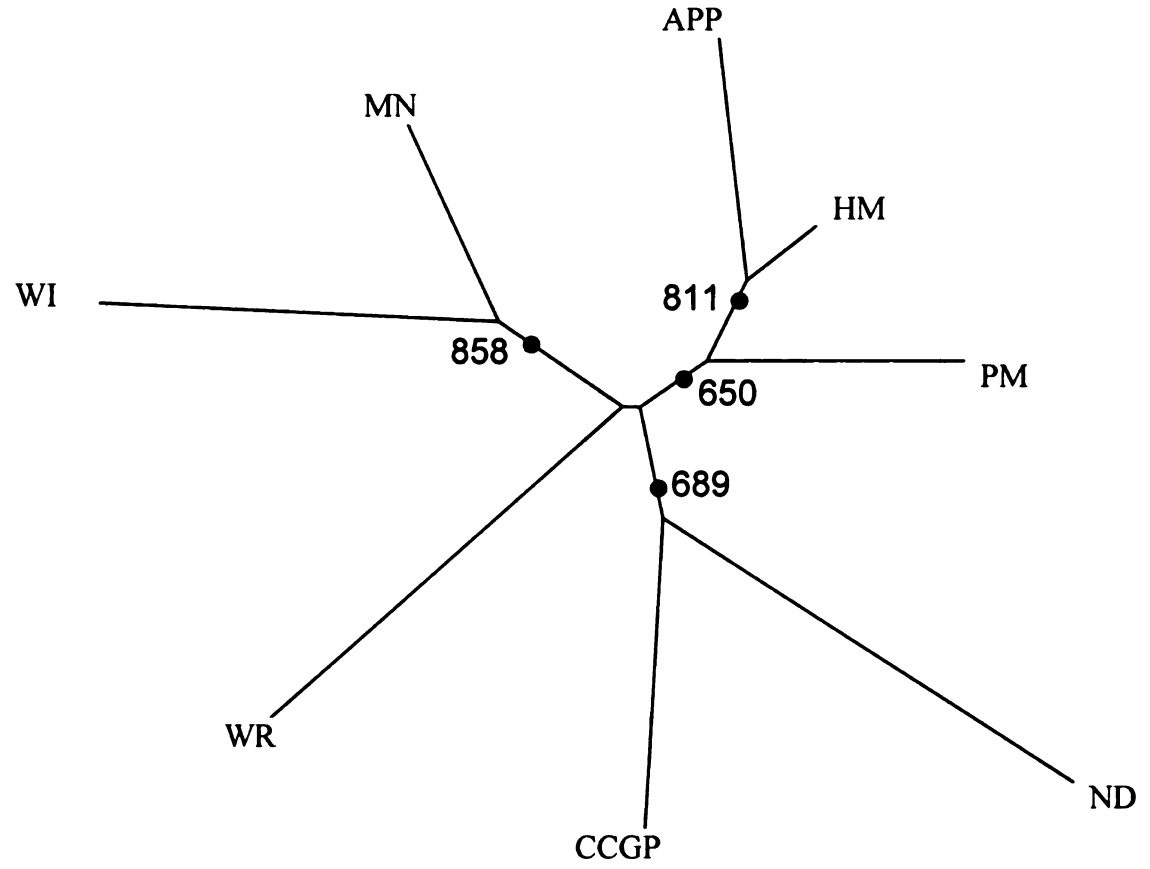

Figure 3.10. Neighbor joining tree showing the relationship of reintroduced marten populations to each other and to putative source populations. Bootstrap values greater than 50% are provided for corresponding nodes.

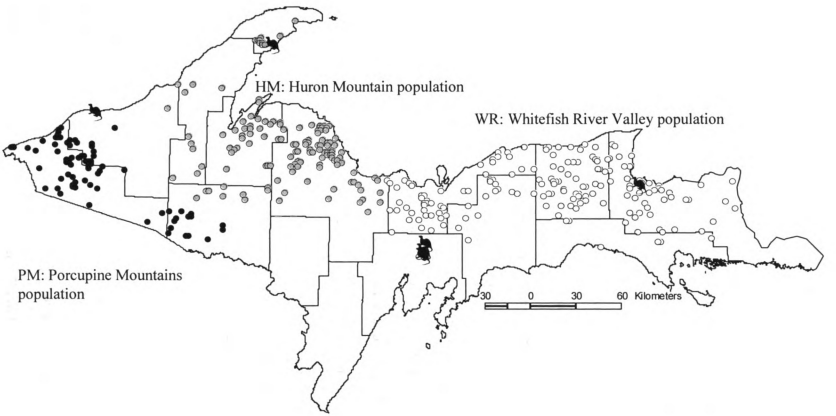

Figure 3.11. Assignment of American martens to genetic clusters formed from reintroduction events based on analysis of F_{ST} values of the groups shown in figure 3.1 as well as GENELAND and STRUCTURE results. The cluster associated with the reintroduction from the Crown Chapleau Game Preserve, Ontario (PM), is shaded black. The cluster associated with the reintroduction from Algonquin Provincial Park, Ontario (HM), is shaded grey. Finally, the cluster formed from the reintroduction from near Lake Nipigon, Ontario (WR), is shown in white.

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Porcupine Mountains population (Figure 3.12). However, the bootstrap test resulted in significance of r at distances from 0 – 30 km. Therefore, patch size was determined to be 30 km for martens in this population.

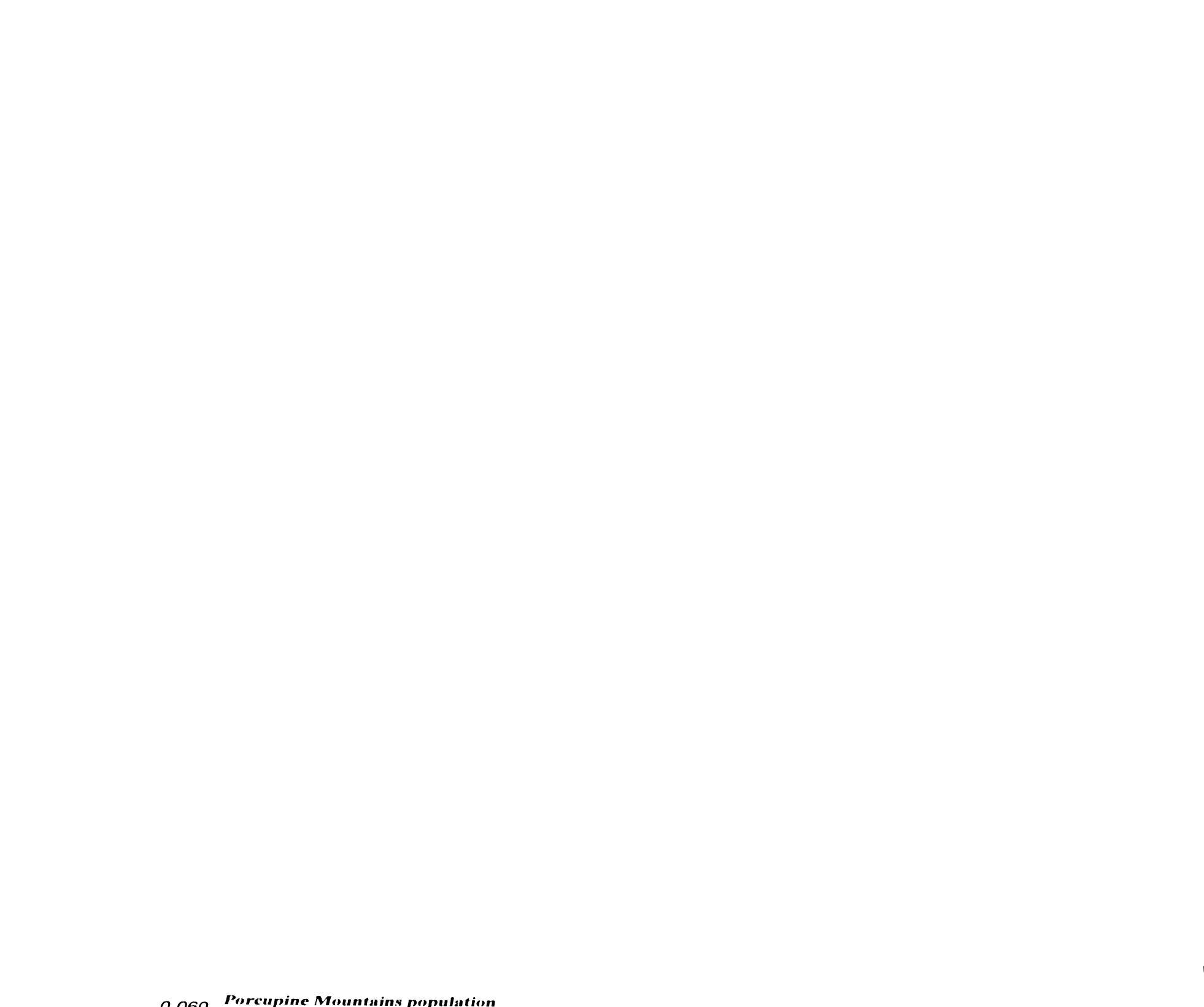
In the Huron Mountain population, r remained significantly positive to a distance of 60 km (Figure 3.12). The bootstrap test displayed significance of r to a distance of 40 km. Accordingly, patch size for martens in this population was considered 40 km.

Estimated values of r were significant to a distance of 160 km in the Whitefish River Valley population (Figure 3.12). The bootstrap test indicated a patch size of 120 km, four times the distance over which positive genetic spatial autocorrelation was detected in the Porcupine Mountains population.

Overall trends in autocorrelation did not reveal the spatial juxtaposition of significant groups of individuals within a population. Two-dimensional ordination methods can be used to determine spatially explicit patterns of genetic autocorrelation among neighboring individuals. Based on an average home range diameter of 1.67 km, the number of nearest neighbors was estimated as 3, 7, and 8 for the Porcupine Mountains, Huron Mountain, and Whitefish River Valley populations, respectively.

For the 112 martens in the Porcupine Mountains population, 14% of the autocorrelation values (lr) were significantly positive (one-tailed test $P \leq 0.05$, P ranging from 0.002 to 0.046). The positively spatially autocorrelated martens were clustered into two main groups (Figure 3.13). One group was located on the northern edge of the population and immediately south of the 1955 – 1957 release site. The second cluster was located in the satellite group of the Porcupine Mountains population, north of the Nicolet National Forest release site. Only 3% of the autocorrelation values (lr) were

Figure 3.12. Results of spatial autocorrelation analyses for each of 3 genetic clusters identified in Michigan's Upper Peninsula. The estimated value of *r* is shown with the 95% CI error bars. U and L represent the upper and lower bounds of the 95% CI of r around the null hypothesis of $r = 0$. Spatial autocorrelation was significant if the value of r was greater than the 95% CI of *r* (U and L) and if the 95% CI error bars did not contain zero. Patch size was 30 km, 40 km, and 120 km for the Porcupine Mountains, Huron Mountain, and Whitefish River Valley populations, respectively.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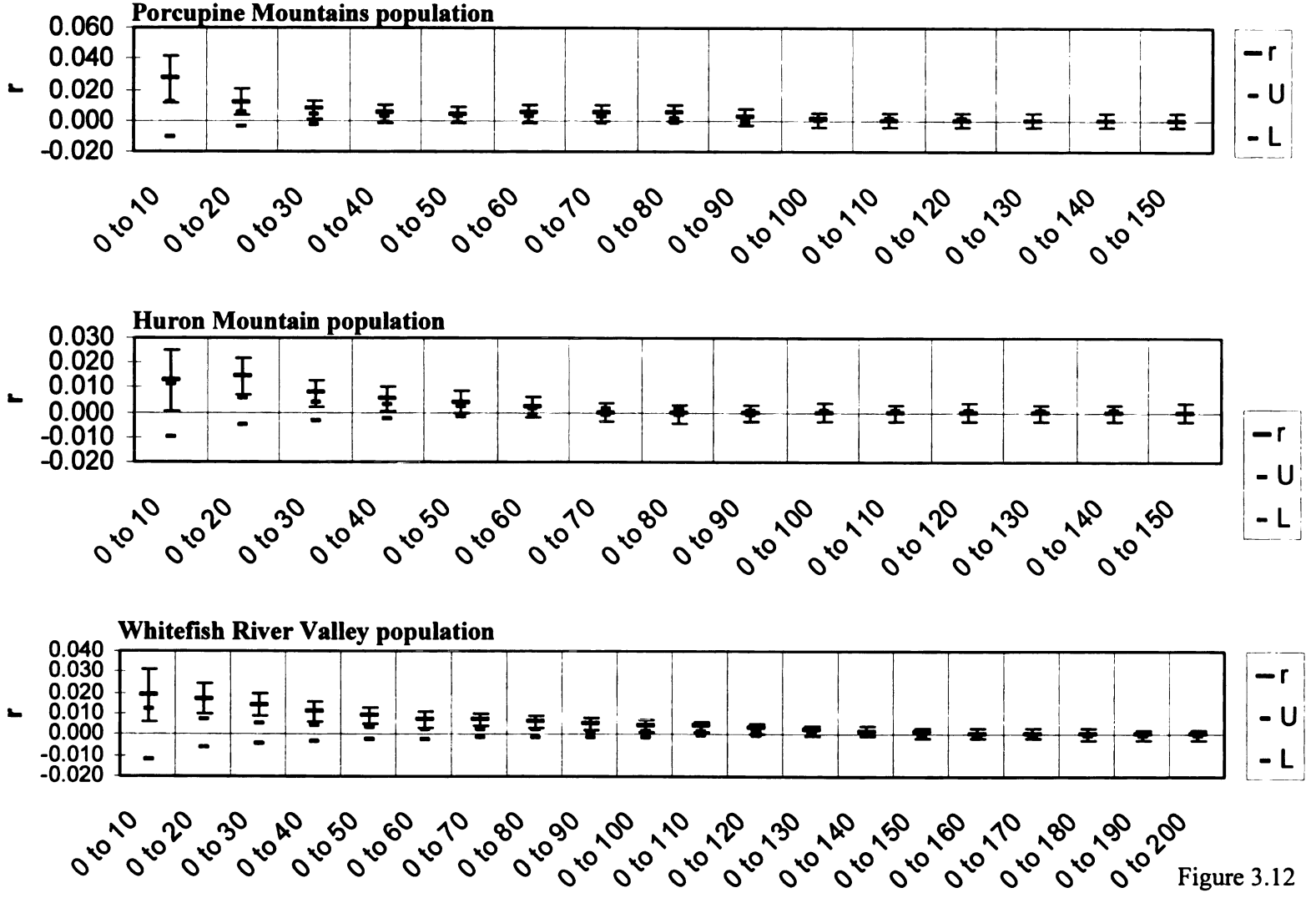
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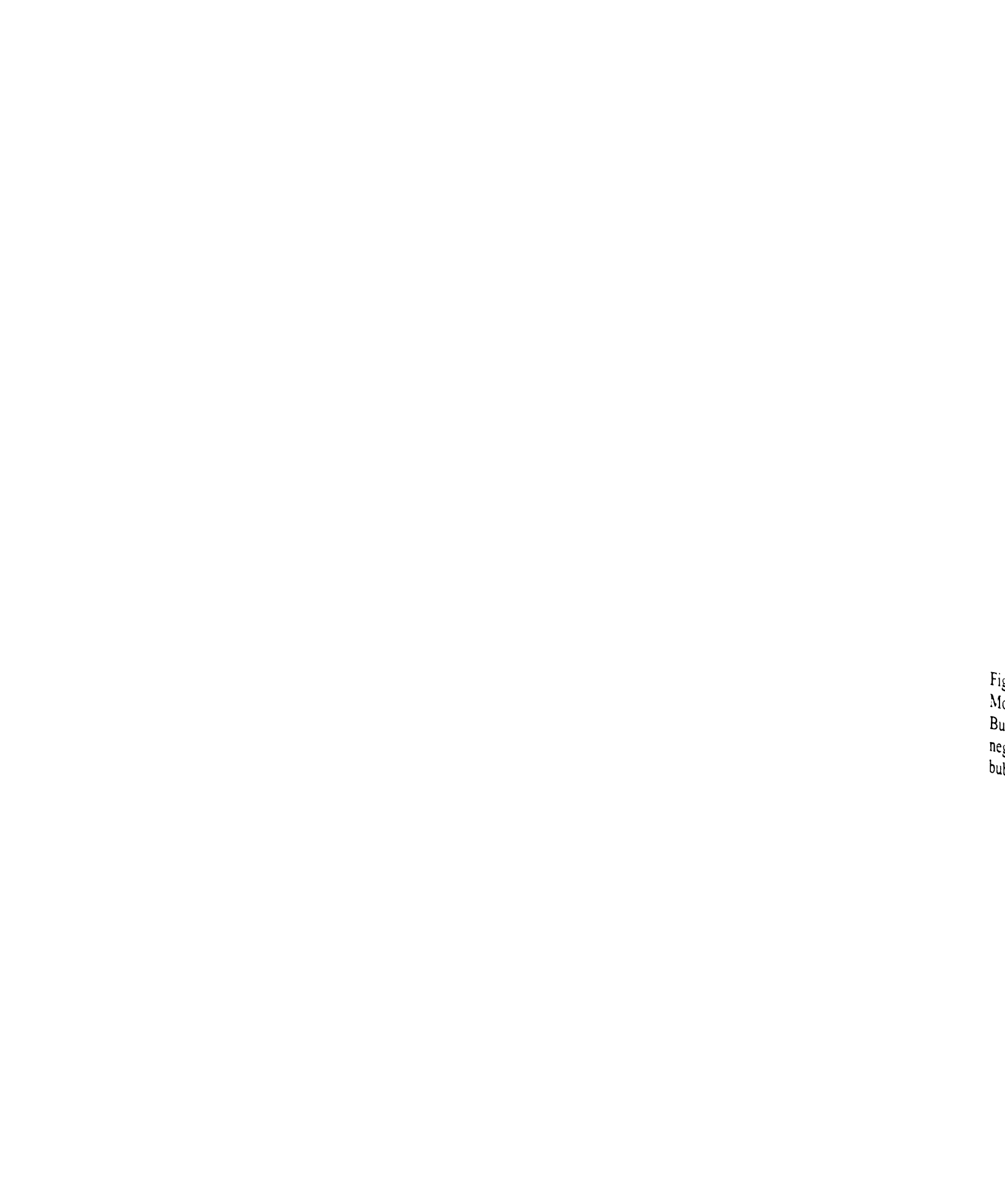

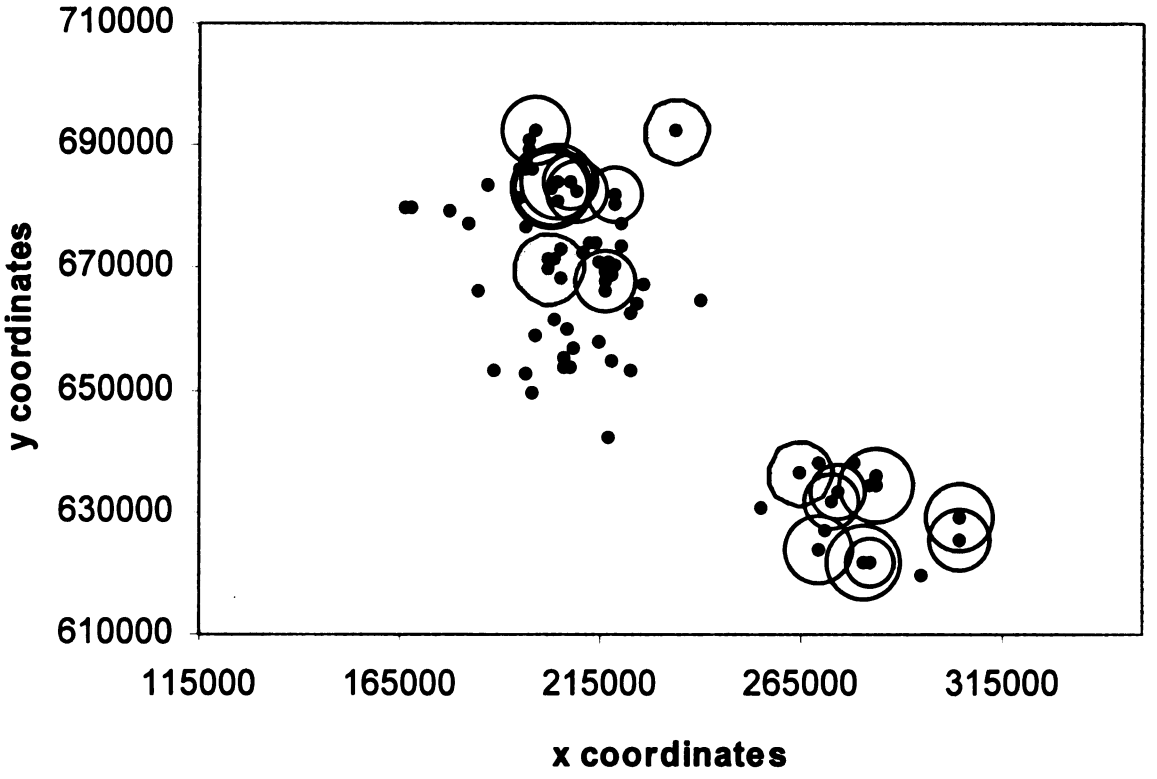

Figure 3.13. Plot of two-dimensional spatial autocorrelation of martens in the Porcupine Mountains population. Locations of all individuals are represented by black dots. Bubbles surround individuals with significant ($P \leq 0.05$) positive (open circles) or negative (grey circles) autocorrelation values relative to 3 nearest neighbors. Size of the bubble indicates magnitude of the correlation.

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Fifteen percent of the 152 autocorrelation values (*lr*) were significantly positive in the Huron Mountain population ($P \leq 0.05$, P ranging from 0.001 to 0.05). These individuals were clustered into one main group in the south-eastern corner of the population and three smaller clusters in the central and northern part of the population (Figure 3.14). Four percent of the autocorrelation values were significantly negative. All but one of these individuals clustered along the eastern boundary of the population.

Of the 232 martens in the Whitefish River Valley population, 14% of the autocorrelation values were significantly positive ($P \leq 0.05$, P ranging from 0.001 to 0.04). Clusters of positive autocorrelation were present on the far eastern and western boundaries of the population, as well as scattered in the middle (Figure 3.15). Four percent of the local autocorrelation values were significantly negative. These individuals were found in between clusters of positively autocorrelated martens.

Discussion

Genetic diversity in marten populations

The four source populations examined in this study were historically part of one contiguous population of martens north of the Great Lakes. Historical levels of spatial genetic structure are unknown. Gradients in allele frequency might have existed across formerly contiguous habitat throughout the species' range. Marten distribution was fragmented in the late 19th and early 20th centuries creating small and isolated populations. It is possible that genetic diversity was reduced in these remnant populations as a result of population fragmentation and declines in abundance. However,

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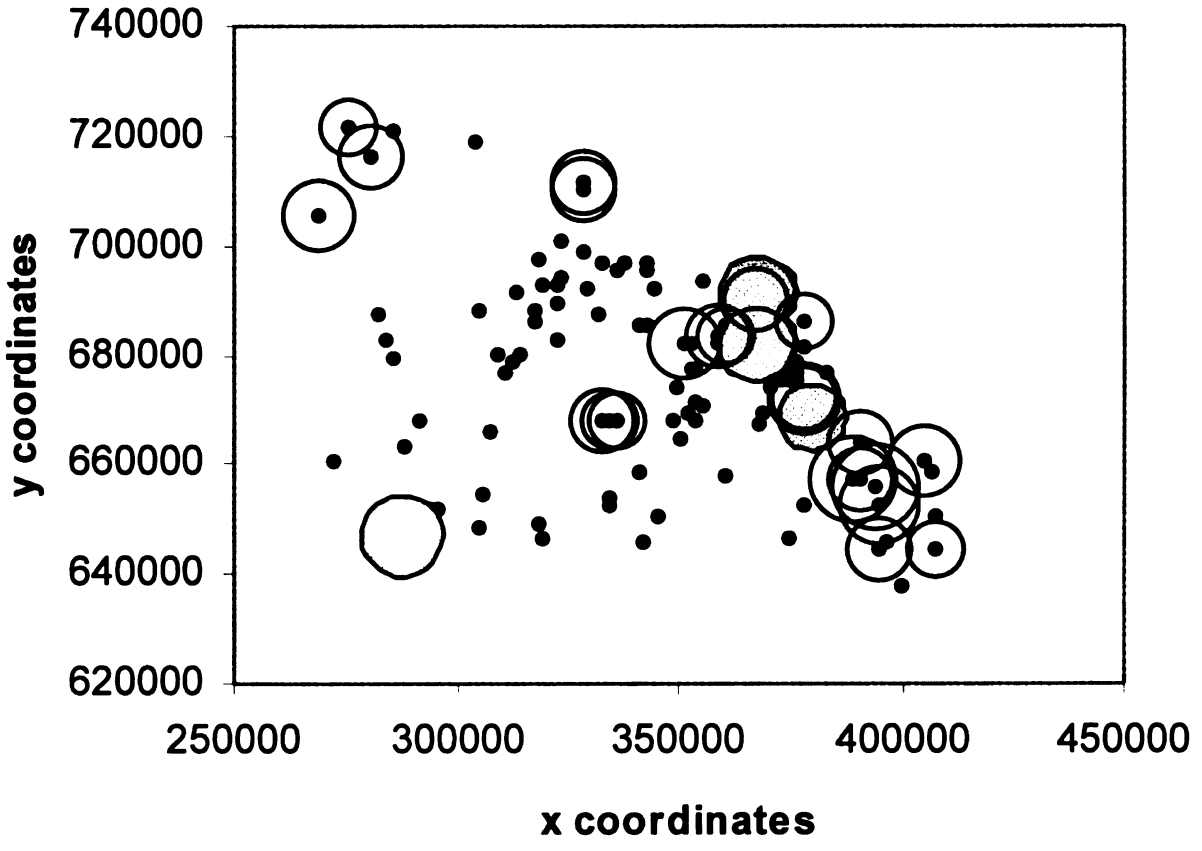

Figure 3.14. Plot of two-dimensional spatial autocorrelation of martens in the Huron Mountain population. Locations of all individuals are represented by black dots. Bubbles surround individuals with significant ($P \leq 0.05$) positive (open circles) or negative (grey circles) autocorrelation values relative to 7 nearest neighbors. Size of the bubble indicates magnitude of the correlation.

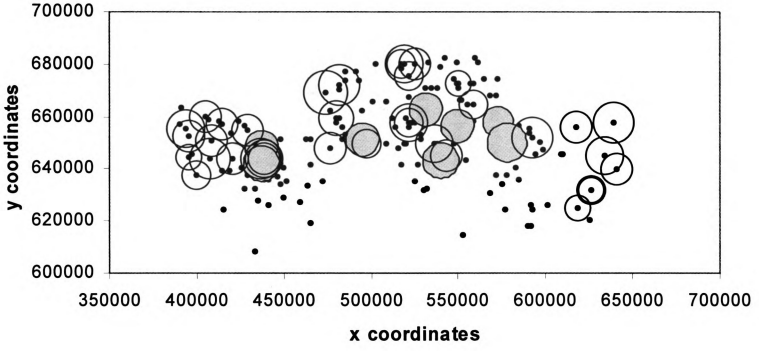

Figure 3.15. Plot of two-dimensional spatial autocorrelation of martens in the Whitefish River Valley population. Locations of all individuals are represented by black dots. Bubbles surround individuals with significant ($P \leq 0.05$) positive (open circles) or negative (grey circles) autocorrelation values relative to 8 nearest neighbors. Size of the bubble indicates magnitude of the correlation.

levels of diversity estimated in the putative source populations (average H_E = 64%) were consistent with levels of diversity found in indigenous populations of North American mustelid species (martens H_E = 66%, Kyle et al. 2000; fishers, *Martes pennanti*, H_E = 63%, Kyle et al. 2001; wolverines, *Gulo gulo*, H_E = 63%, Kyle and Strobeck 2001). Population fragmentation and decline did not appear to have greatly affected genetic variation in these marten populations.

Populations that have experienced severe genetic bottlenecks due to reduction in size often show loss of genetic diversity coupled with an increase in level of inbreeding (Lacy 1987). If the bottleneck was sustained, high levels of homozyosity could result in low viability and fecundity and increase potential for extinction (Lacy 1987, Schultz and Lynch 1997). It is important to identify bottlenecked populations to determine appropriate management strategies.

No significant bottleneck effects were detected in any of the reintroduced populations. Cornuet and Luikart (1996) suggested recent immigration from genetically divergent populations can mask the effects of bottlenecks. A similar bias can occur if the sampled populations included either individuals from more than one population or hybrids between populations (Cornuet and Luikart 1996). Potential immigrants and hybrids from neighboring reintroduced populations in Michigan were detected using program STRUCTURE.

Levels of genetic diversity in the reintroduced populations were generally not lower compared to the source populations, indicating demographic bottlenecks, if had occurred, were of short duration. Similar findings were described in translocated populations of the wild boar (*Sus scrofa*, Vernesi et al. 2003) and sea otter (*Enhydra*

lutris, Larson et al. 2002).

The high level of genetic diversity estimated for the Wisconsin population was contradictory to its small predicted population size ($N < 50$). This suggested although the current population is currently small, the genetic diversity of a large number of founders ($N = 139$) is still represented by remnant individuals. However, if the population were to remain small, loss of genetic diversity would be expected.

The reintroduced population with the lowest overall genetic variation was the Whitefish River Valley population. A relatively large number of martens were released during the 1969 – 1970 reintroduction ($N = 99$). The release event resulted in recolonization of a very large area across the eastern Upper Peninsula (Figure3.3, Figure 3.4, Figure 3.9). Patterns of colonization away from the release site would have involved movements of limited numbers of animals, decreasing local genetic diversity in a manner similar to a linear series of small bottlenecks.

Effects of reintroductions on genetic structure

The current level of genetic divergence between source populations indicated historical fragmentation and decline in abundance resulted in genetic differentiation. The role of genetic drift in creating this differentiation would have been proportional to the size of the remnant population, as genetic diversity in small populations tends to be affected by random genetic drift to a greater degree than larger populations (Lacy 1987). Genetic divergence between source populations provided the opportunity to relate genetic structure in Michigan to multiple reintroduction events from these different sources.

Three genetically distinct populations were identified in the Upper Peninsula that

geographically reflected the three main reintroduction events. The level of genetic differentiation within the reintroduced populations was higher than among the source populations. Low numbers of founding individuals would have increased the effects of genetic drift that would randomly cause divergence from an original genetic signature.

Analysis of the populations using program STRUCTURE provided evidence for a low level of admixture (Figure 3.3). Due to the lack of geographic discontinuity of martens across the central and eastern Upper Peninsula, admixed martens were expected in the contact zone between the Huron Mountain and Whitefish River Valley populations. However, many genetically mixed individuals were in the southern portion of the geographically distinct Porcupine Mountains population, and displayed a strong affiliation to the genetic signature of the Huron Mountain population. Marten dispersal may have occurred westward from the Huron Mountain population, founded by individuals from Algonquin Provincial Park. However, the southern location of the admixed individuals in the Porcupine Mountains population indicated potential dispersal of martens from the southeast following release of founders from Algonquin Provincial Park onto the Nicolet National Forest in 1975 and 1976.

Dispersal of martens following release onto the Nicolet National Forest might also explain a small area near the southernmost 1979 – 1981 release sites that displayed a strong affiliation to the Porcupine Mountains population (Figure 3.7). In 1975, the initial martens released onto the Nicolet National Forest were from the Crown Chapleau Game Preserve. A second release of Crown Chapleau Game Preserve animals was made in 1981. Either release may have resulted in dispersal north across the border into Michigan and established a small, but genetically discrete population in relation to the neighboring

Huron Mountain population to the west and north.

Dispersal across the contact zone between the Huron Mountain and Whitefish River Valley populations was evident, although appeared rare (Figure 3.8, Figure 3.9). The abrupt nature of genetic distinction indicated a boundary between individuals of neighboring reintroduced populations. U.S. Route 41 runs through the area of the apparent border between the Huron Mountain and Whitefish River Valley populations, and may have created a barrier to dispersal. However, no additional high-use roads appear to have affected genetic structure of martens in the Upper Peninsula, indicating U.S. 41 may not be a singular explanation for lack of dispersal between the Huron Mountain and Whitefish River Valley populations.

A small cluster of individuals east of the Tahquamenon Bay release site assigned very highly to the Porcupine Mountains population. The two sources used for the 1989 – 1990 translocation were from Iron County, Michigan and the Hiawatha National Forest, West Unit. Martens from the Hiawatha National Forest, West Unit would be expected to be genetically similar to the Whitefish River Valley population. Conversely, martens harvested from Iron County displayed posterior probabilities of assignment to both the Porcupine Mountains and Huron Mountain populations. Therefore, it was likely that martens trapped in Iron County in 1989 and 1990 would have included individuals from both genetic populations. However, the lack of a signature of the Huron Mountain population in the eastern Upper Peninsula suggested that only martens from the Porcupine Mountains population present in Iron County were translocated, were the only survivors of the translocation, or dispersed eastward from release into a potentially unoccupied area, allowing for maintenance of a historical genetic signature over a small

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The genetic signature of each source population was expected to be represented by reintroduced populations in Wisconsin and the Upper Peninsula. Assignment methods were used to understand genetic affiliation of reintroduced populations to sources (Maudet et al. 2002). The degree of representation would be dependent upon time since release. The Huron Mountain population and the Chequamegon National Forest population were both more genetically similar to their putative source populations than any other source or reintroduced population (Figure 3.2). In contrast, the Porcupine Mountains and Whitefish River Valley populations did not show genetic affinity to their putative source populations. The Whitefish River Valley population was highly differentiated from all populations (source and reintroduced). This could be due to lack of sampling of the true source population, a large effect of stochastic genetic processes on the population at or shortly after time of release, or a combination of the two explanations.

Spatial analysis of reintroduced populations

Overall spatial genetic structure of martens in the Upper Peninsula of Michigan reflected reintroduction history. However, differences in measures of genetic diversity and spatial patterns of genetic relationships differed between reintroduced populations.

The Porcupine Mountains and Whitefish River Valley populations displayed positive correlations of isolation by distance, whereby individuals that are more distant geographically are more distant genetically. This indicated one of two extremes. Positive correlation indicates a 'long-distance cline' and has been suggested to be

indicative of either a lack of distinct subpopulations or highly structured subpopulations with increasing differentiation with increasing distance (Diniz-Filho and Telles 2002). The former pattern is the most likely explanation for spatial structure in these populations as dispersal from a single release area would be relatively linear. In contrast, an overall pattern of isolation by distance was not detected in the Huron Mountain population based on the Mantel test. The Huron Mountain population was colonized through a series of four releases using martens from the same source population. Dispersal from all four release sites would create a non-linear pattern of overall genetic spatial autocorrelation.

Martens in each reintroduced population displayed different genetic patch sizes, or distances over effective gene flow occurs. Patch size in the Porcupine Mountains and Huron Mountain populations was roughly similar, indicating positive correlation to a distance of 30-40 km. In contrast, the Whitefish River Valley population displayed positive genetic spatial autocorrelation to a distance of 120 km (Figure 3.12). The 3- to 4-fold difference in genetic patch size between martens in the eastern and western Upper Peninsula suggests differences in dispersal ability, either due to genetic or environmental factors. For example, low prey abundance or limited habitat might be expected to increase average dispersal distances (Thompson and Colgan 1987). Evaluation of correlation between inter-individual genetic relationships and landscape features, including habitat, would help elucidate the causes of differential distances of effective gene flow. Correlation of genetic relatedness and landscape could provide a means to investigate not only historic movement patterns, but also predict future dispersal between genetic populations or into currently unoccupied areas of high quality habitat, or regions of low-density marten abundance.

Patterns of directional spatial autocorrelation can be used to identify areas of positive or negative autocorrelation indicative of dispersal patterns or presence of kin groups. Clusters of individuals that were significantly positively spatially autocorrelated with nearest neighbors likely represented kin groups. Patterns of kin structure could be elucidated through identification of sex and age of significantly positively spatially autocorrelated martens. Conversely, individuals negatively autocorrelated with nearest neighbors likely represented dispersers or migrants from neighboring populations.

Plots representing the two-dimensional local spatial analyses for each population showed localized areas of positive and negative autocorrelation generally consistent with the analyses of genetic structure as a function of historical reintroductions (Figure 3.13, Figure 3.14, Figure 3.15). Individuals negatively autocorrelated with nearest neighbors were located in areas where admixed individuals were detected using program STRUCTURE. Positive autocorrelation may represent kin groups, but in areas of genetic congruity within a genetic population. Further analyses should be performed to determine if patterns of autocorrelation are a function of different age and/or sex classes, and the effect of habitat on shaping spatial genetic patterns.

References

Berg, W. E. 1982. Reintroduction of fisher, pine marten, and river otter. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 158-173.

Bodkin, J.L., B.E. Ballachey, M.A. Cronin, and K.T. Scribner. 1999. Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conservation Biology. 13: 1378-1385.

Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd, and L.L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature. 368: 455-457.

Cavalli-Sforza, L.L. and A.W.F. Edwards. (1967) Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics. 19: 233-257.

Churchill, S.J., L.A. Herman, M.F. Herman, and J.P. Ludwig. 1981. Final report on the completion of the Michigan marten reintroduction program. Ecological Research Services, Inc., Environmental Report. Iron River, MI.

Colautti, R.I., M. Manca, M. Viljanen, H.A.M. Ketelaars, H. Bürgi, H.J. Macisaac, and D.D. Heath. 2005. Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and gene flow using microsatellites. Molecular Ecology. 14: 1869-1879.

Cornuet, J.M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014.

Chakraborty, R., and M. Nei. 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. Evolution 31: 347-356.

Davis, C.S. and C. Strobeck. 1998. Isolation, variability and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7: 1771-1788.

Davis, M.H. 1978. Reintroduction of the pine marten into the Nicolet National Forest, Forest County, Wisconsin. M.S. Thesis. University of Wisconsin, Stevens Point: 64.

Dieringer, D. and C. Schlötterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes. 3: 167-169.

Diniz-Filho, J.A.F. and M.P.C. Telles. 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. Conservation Biology. 16: 924-935.

Double, M.C., R. Peakall, N.R. Beck, and A. Cockburn. 2005. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). Evolution. 59: 625-635.

Earle, R.D. 2002. Furbearer winter track count survey of 2001. Michigan Department of Natural Resources Wildlife Report No. 3366. 10 pp.

Epperson, B. 2003. Geographic Genetics. Princeton Univ. Press, Princeton, NJ.

Falush, D., M. Stephens, and J. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 164: 1567-1587.

Felsenstein, J. 1993. PHYLIP (*Phylogeny inference package*), Version 2.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Fleming, M.A., E.A. Ostrander, and J.A. Cook. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology. 8: 1351-1362.

Frankam, R. 1995. Conservation genetics. Annual Review of Genetics. 29: 305-327.

Frawley, B.J. 2002. 2001 marten harvest survey. Michigan Department of Natural Resources Wildlife Division Report No. 3369: 1-4.

Garza, J.C. and E.G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology. 10: 305-318.

Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. Journal of Heredity. 86: 485-486.

Griffth, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science. 477-480.

Guillot, G., A. Estoup, F. Mortier, and J.F. Cosson. 2005a. A spatial statistical model for landscape genetics. Genetics. 170: 1261-1280.

Guillot, G., F. Mortier, and A. Estoup. 2005b. GENELAND: a computer package for landscape genetics. Molecular Ecology Notes. 5: 712-715.

Guo S.W. and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics. 48: 361-372.

Hagmeier, E.M. 1956. Distribution of marten and fisher in North America. The Canadian Field-Naturalist. 70: 101-148.

Kimura, M. and J.F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics. 49: 725-738.

Kyle, C.J., C.S. Davis, and C. Strobeck. 2000. Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. Canadian Journal of Zoology. 78: 1150-1157.

Kyle, C.J., J.F. Robitaille, and C. Strobeck. 2001. Genetic variation and structure of fisher (*Martes pennanti*) populations across North America. Molecular Ecology. 10: 2341-2347.

Kyle, C.J. and C. Strobeck. 2001. Genetic structure of North American wolverine (*Gulo gulo*) populations. Molecular Ecology. 10: 337-347.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology. 1: 143-157.

Larson, S., R. Jameson, J. Bodkin, M. Staedler, and P. Bentzen. 2002. Microsatellite DNA and mitochondrial variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy. 83: 893-906.

Leberg, P.L. and D.L. Ellsworth. 1999. Further evaluation of the genetic consequences of translocations on southeastern white-tailed deer populations. Journal of Wildlife Management. 63: 327-334.

Manel, S., M.K. Schwarz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution. 18: 189-197.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research. 27: 209-220.

Maudet, C., C. Miller, B. Bassano, C. Breitenmoser-würsten, D. Gauthier, G. Obexer-Ruff, J. Michallet, P. Taberlet, and G. Luikart. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex (ibex)*]. Molecular Ecology. 11: 421-436.

McGowan, C., L.A. Howes, and W.S. Davidson. 1999. Genetic analysis of an endangered pine marten (*Martes americana*) population from Newfoundland using randomly amplified polymorphic DNA markers. Canadian Journal of Zoology. 77: 661-666.

Mech, L.D. and L.L. Rogers. 1977. Status, distribution, and movements of martens in northeastern Minnesota. USDA Forest Service Research Paper. NC-143. 7 pp.

Ohta, T. and M. Kimura. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genetical Research Camb. 22: 22: 201-204.

Page, R.D.M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences. 12: 357-358. Available at: http://taxonomy.zoology.gla.ac.uk/rod/treeview.html

Peakall, R., M. Ruibal, and D.B. Lindenmayer. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat (*Rattus fuscipes*). Evolution. 57: 1182-1195.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288-295.

Piry, S., G. Luikart, and J.M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity. 90: 502-503.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86: 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225.

Schultz, S.T. and M. Lynch. 1997. Mutation and extinction: The role of variable mutational effects, synergistic epistasis, beneficial mutations, and degree of outcrossing. Evolution. 51: 1363-1371.

Schupbach, T.A. 1977. History, status, and management of the pine marten in the Upper Peninsula of Michigan. M.S. Thesis. Department of Forestry. Michigan Technological University: 79.

Slough, B.G. 1994. Translocations of American martens. Pp. 165-178 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Spencer, P.B.S. and J.O. Hampton. 2005. Illegal translocation and genetic structure of feral pigs in western Australia. Journal of Wildlife Management. 69: 377-384.

Swanson, B.J., L.R. Peters, and C.J. Kyle. 2006. Demographic and genetic evaluation of an American marten reintroduction. Journal of Mammalogy. 87: 272-280.

Thompson, I.D. and P.W. Colgan. 1987. Numerical responses of martens to a food shortage in northcentral Ontario. Journal of Wildlife Management. 51: 824-835.

Valdes, A.M., M. Slatkin, and N.B. Freiner. 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics. 133: 737-749.

Vernesi, C., B. Crestanello, E. Pecchioli, D. Tartari, D. Caramelli, H. Hauffe, and G. Bertorelle. 2003. The genetic impact of demographic decline and reintroduction in the wild boar (*Sus scrofa*): a microsatellite analysis. Molecular Ecology. 12: 585-595.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38: 1358-1370.

Williams, C.L., T.L. Serfass, R. Cogan, and O.E. Rhodes, Jr. 2002. Microsatellite variation in the reintroduced Pennsylvania elk herd. Molecular Ecology. 11: 1299-1310.

Wright, J.L. 1999. Winter home range and habitat use by sympatric fishers (*Martes pennanti*) and American martens (*Martes americana*) in northern Wisconsin. Department of Natural Resources. University of Wisconsin, Stevens Point: 73.

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CHAPTER 4. THE EFFECTS OF REINTRODUCTION ON GENETIC STRUCTURE OF FISHER POPULATIONS IN MICHIGAN AND WISCONSIN

Introduction

Throughout the late 19th and early 20th centuries, fisher (*Martes pennanti*) populations across North America experienced sharp declines in abundance and distribution (de Vos 1951, Brander and Books 1973). Habitat loss due to extensive logging and frequent fires fragmented fisher populations. High fisher pelt prices during this period coupled with the ease by which the species is trapped resulted in overharvest (Cook and Hamilton 1957, Powell 1979). By the 1930s, the fisher had been extirpated from much of its southern range. Only a limited number of refugia for remnant fisher populations existed in the United States, including northeastern Minnesota and the Adirondack Mountains in New York (Brander and Books 1973). A trapping ban coincident with cessation of intensive logging activities and subsequent reforestation allowed remnant populations to recover (Bradle 1957, Irvine et al. 1964).

The decline and eventual extirpation of fishers from locations across its southern range was followed by an apparent increase in the number of porcupines (*Erethizon dorsatum*) in those areas. By the 1950s, concern had arisen for the potential of substantial timber loss resulting from the porcupine's feeding habits (Olson 1966). Fishers are efficient predators of porcupines (Schoonmaker 1938, Earle 1978). Reports of increasing fisher populations in the Adirondack Mountains of New York and in northeast Minnesota paralleled reports of declining porcupine populations (Olson 1966, Brander and Brooks 1973). The potential of the fisher as a biological control and its economic and aesthetic values as a furbearer species sparked interest in species

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reintroduction to formerly occupied areas across North America. By the middle of the 20th century, fisher abundance in remnant populations had reached levels to be considered sources for restocking events in areas in which the species had been extirpated (Bradle 1957, Irvine et al. 1964).

From 1955 to 1967, 181 fishers were released onto the Nicolet and Chequamegon National Forests in Wisconsin and the Ottawa National Forest in the western Upper Peninsula of Michigan (Figure 2.2). The first eighteen fishers released were from the Adirondack Mountains in New York. The remaining individuals originated in the Superior National Forest in Minnesota (Bradle 1957, Irvine et al. 1964).

Fishers spread rapidly from the reintroduction sites. However, dispersal eastward appeared to be halted by a band of agricultural land in the central Upper Peninsula of Michigan. A series of translocations moved fishers from the western Upper Peninsula to the eastern Upper Peninsula between 1988 and 1992 (Steck 1988, Steck 1989, Steck 1990, MDNR unpublished report 1992, MDNR interoffice communication 1992; Figure 2.2).

Reintroductions and translocations have been used as a management tool to restore wildlife populations that have been extirpated (e.g., Leburg et al. 1994, Scribner and Stüwe 1994, Maudet et al. 2002, Williams et al. 2002). Theory predicts that a small number of founding individuals, as is often used in reintroduction efforts, will result in a bottleneck and concomitant loss of genetic diversity (Lacy 1987). Loss of genetic diversity has been linked to an increase in genetic drift and inbreeding. Increase in homozygosity has also been theorized to cause a decrease in overall fitness and evolutionary potential (Mills and Smouse 1994, Frankam 1995). Molecular methods in

combination with recent statistical methods have proven useful for assessing effects of reintroduction efforts on genetic diversity and population structure in a number of species, including elk (*Cervus elaphus*, Williams et al. 2002), sea otters (*Enhydra lutris*, Bodkin et al. 1999, Larson et al. 2002), and ibex (*Capra ibex*, Scribner and Stüwe 1994, Maudet et al. 2002).

The fisher provided a unique opportunity to examine the effects of reintroductions into an area from which the species was formerly extirpated. Using stocking records that include source, numbers and sex ratios released, and location of release, in conjunction with an extensive genetic dataset, we could empirically test theoretical predictions relating to founder events and infer ecological processes such as dispersal (Sarrazin and Barbault 1996).

The objectives of this study were fourfold. First, we wanted to assess the levels of genetic diversity of fisher populations of Minnesota and New York due to their history as refugia for remnant populations in the early 1900s. These remnant populations were expected to be small and may have resulted in significant declines in genetic variation. In addition, we needed to assess levels of genetic differentiation between the two populations to determine if populations were differentiated to a degree that would allow statistical analyses of contributions as source populations for Michigan reintroductions. Second, we wanted to compare genetic diversity of reintroduced fisher populations in Wisconsin and Michigan to contemporary populations in New York and Minnesota. Reintroductions of limited numbers of individuals could have resulted in founder events, decreasing genetic variation in relation to source populations. Third, we wanted to evaluate overall genetic structure of reintroduced populations as a result of releases from

two different sources. In other words, we wished to determine if genetic signatures from each source population remained in and around the regions into which fishers were reintroduced, or if all founding groups had admixed. Fourth, we wanted to use estimates of spatial genetic structure in the reintroduced and translocated populations in the Upper Peninsula of Michigan to infer patterns of colonization (i.e., distance and direction) from release sites.

Methods

Source population sample collection

Assessment of differential genetic contribution of source populations used for reintroduction requires the sources to be genetically differentiated themselves. In 1968, an internal translocation was made in Minnesota, moving fishers from the northeastern portion of the state (Superior National Forest) to northwestern Minnesota (Itasca State Park; Berg 1982). Because of widespread translocations, it is likely that fishers across northern Minnesota are genetically homogeneous. Samples collected from this area should provide adequate representation of contemporary fishers in the proximity of the source population in the Superior National Forest. Fisher tongues ($N = 45$) were acquired from individual trappers and Minnesota Department of Natural Resources personnel in northeastern and north-central Minnesota during the 2004 and 2005 trapping seasons. Each tongue was placed in 1.5 mL tube filled with preservative tissue buffer (0.1 M Tris/HCL, 10 mM EDTA, 0.2 M NaCl, 4 M urea, and 0.5% sarcosine). Tubes were pre-labeled with unique identification numbers corresponding to information provided by each trapper.

Tissue samples were obtained from across much of the Adirondack Mountain region of northern New York during the 2003 trapping season through the New York State Museum of Natural History ($N = 15$) and during the 2004 trapping season through the New York State Department of Environmental Conservation ($N = 48$). Tissue samples were stored in 1.5 mL tubes in either preservative tissue buffer or 95% ethanol. Each tube for tissue collection during 2004 was assigned a unique identification number prior to sampling corresponding to information provided.

Reintroduced population sample collection and sampling strategy

A large number of fishers were harvested each year from Michigan ($N > 400$) and Wisconsin ($N > 1{,}000$), allowing sampling of individuals across a broad area within the reintroduced populations. In Wisconsin fisher pelts are required to be sealed and tagged each year, however, the Wisconsin Department of Natural Resources requires the submission of whole carcasses every three years to collect additional demographic information. Carcasses were submitted during the 2004 harvest and tissue was collected from samples selected for genetic representation across much of Wisconsin. Additional tissue and hide samples were collected directly from trappers during the 2003 trapping season. A total of 161 samples were collected from Wisconsin. Tissue samples were placed in pre-labeled 1.5 mL tubes in preservative tissue buffer. Small squares of hide were placed in pre-labeled paper envelopes. All fisher samples were stored frozen at -70°C.

Heads or carcasses of fishers were collected from trappers by the MDNR as a mandatory requirement for harvest registration. Muscle tissue was removed from the

skull by MDNR employees and placed in 1.5 mL tubes. Individuals were obtained from the 2001, 2002, 2003, and 2004 Michigan fisher harvests ($N = 555$).

The scale at which fisher harvest locations are reported differed in Wisconsin and Michigan, determining the scale of resolution for analyses. Fisher trappers in Wisconsin were encouraged to report harvest location as deer management unit (DMU) and county. Resulting locations were often hundreds of square kilometers in size. Counties and DMUs were intersected using ArcView GIS 3.2 (ESRI). All fishers located within an intersected area were assigned to a set of coordinates located in the center of each area (Figure 4.1). The large scale of harvest reporting in Wisconsin allows characterization of general spatial genetic structure following reintroduction. In contrast, fisher registration in Michigan requires reporting of harvest location to a section (one section = 1.6 km^2). Each individual was assigned a coordinate for the center of the section in which it was trapped. Although exact trap locations are not provided, the scale of reporting in Michigan was sufficiently small to allow characterization of genetic structure a) between source and reintroduced populations, b) between populations colonized by primary reintroductions and secondary translocations, and c) within reintroduced populations. Two regions were identified in the Upper Peninsula of Michigan to examine genetic diversity and population structure within and between reintroduced populations. Region one, Michigan's western Upper Peninsula was colonized based on reintroductions made during 1961-1963 onto the Ottawa National Forest followed by natural dispersal from the release areas. Fishers were translocated into region two, the eastern Upper Peninsula, through releases of individuals from the western Upper Peninsula from 1988-1992. To facilitate spatial reconstruction of dispersal patterns and of potential founder events

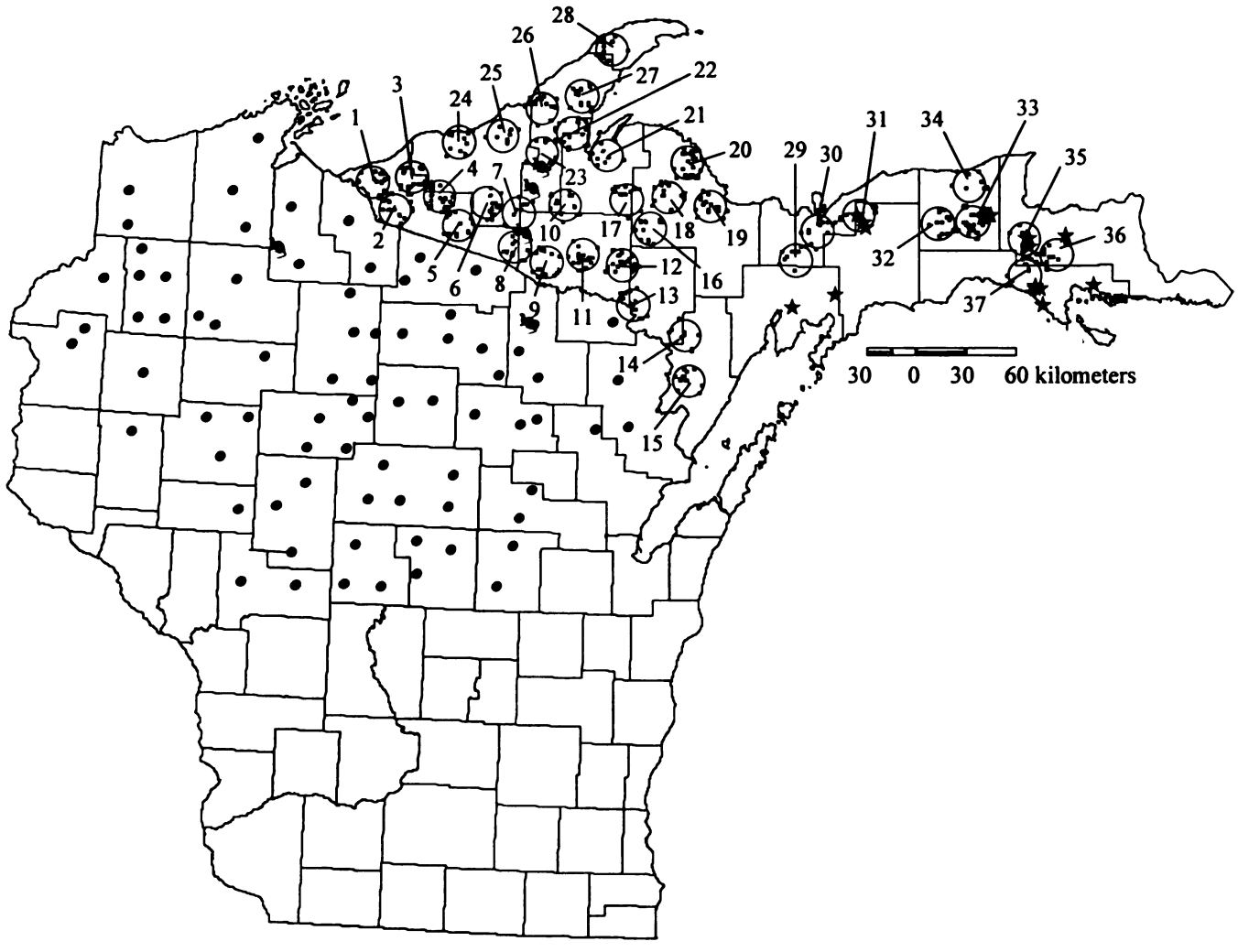

Figure 4.1. Locations of fishers grouped by harvest sampling location in Michigan and individual location of harvest in Wisconsin.

associated with reintroduction events, individuals were assigned to sampling groups in both regions. These groups represented a wide dispersion of locales across the current range of the fisher in Michigan. Sample groups were approximately 250 km^2 based on spatial contiguity and maximum area of kin group, or the home range of one male and several adjacent females. The locations of 28 groups in the western Upper Peninsula were defined as a means to maximize sample size ($N = 8 - 30$, mean = 15) with which to estimate allele frequency, and to maximize the number of inter-area comparisons for each of several distance classes and ordinations used in spatial analyses. Nine additional sample groups were identified surrounding each translocation site in the eastern Upper Peninsula (Figure 4.2) to examine potential differences in levels of genetic diversity potentially related to the number of founding individuals.

DNA isolation and microsatellite analysis

DNA was extracted from each sample using Quiagen DNeasy® Tissue Kits. DNA was quantified by spectrophotometry and diluted to a concentration of 20 ng/μL. Ten microsatellite primers selected from Davis and Strobeck (1998) and six microsatellite primers selected from Fleming et al. (1999) were screened. Ten variable microsatellite primers were identified for use in this study: Ma-1, Ma-2, Ma-19, Gg-7, Tt-1, Tt-4, Mvis002, Mvis072, Mer022, and Mer041 (Appendix 4.A). Fisher DNA was amplified with each primer using polymerase chain reaction (PCR). DNA was amplified using each primer in a 10μL reaction with the following conditions: 2 pmol reverse primer, 2 pmol forward primer, dNTPs at 200 μM each, 200 μM 10x PCR2 buffer (1 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01%

Triton X-100) or LGL buffer (1 mM Tris-HCl pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl, 10 μg/mL BSA, 0.0025% Tween 20), variable amounts of 25mM $MgCl_2$, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, primer specific annealing temperature for 1 minute (Appendix 4.A), and 72°C for 1 minute. The amplified product was separated on a 6% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™), Lincoln, NB. Fragments were viewed using Saga Generation 2 software, LiCor, Lincoln, NB. All genotypes were scored independently by two experienced laboratory personnel.

Analysis of genetic diversity

Reintroductions often involved small number of founding individuals (Slough 1994, Williams et al. 2002). Theory predicts that small founding population size would result in a bottleneck and subsequent loss of genetic diversity (Lacy 1987, Frankam 1995). Founder individuals would not be representative of the levels of genetic diversity nor allele frequencies of the source population. Additionally, not all founders will survive and reproduce. Reduction in genetic diversity results in a high probability of loss of rare alleles that do not greatly affect overall levels of heterozygosity. As a result, losses should be more evident in allelic diversity than in heterozygosity (Cornuet and Luikart 1996).

Genetic variation in source and reintroduced populations

Lower levels of genetic diversity might be expected in populations resulting from reintroduction or translocation events due to a small number of colonizers taken from a

localized area. Allele counts, allele frequencies and expected and observed heterozygosities of source and reintroduced populations were calculated using MICROSATELLITE ANALYSER version 3.12 (Dieringer and Schlötterer 2003). Allelic richness and F_{IS}, the inbreeding coefficient, were calculated using FSTAT 2.9.3 (Goudet 2000). Genetic distance based on the proportion of shared alleles (Bowcock et al. 1994) was calculated using a Microsoft Excel macro as an additional measure of mean relatedness. All of the above measures of genetic diversity were calculated across different scales to assess differences between the two sources, between source and reintroduced populations, between regions within Michigan, and between groups within regions.

Tests of linkage disequilibrium for each pair of loci in each population and tests for departure from Hardy-Weinberg equilibrium (HWE) using the exact test of Guo and Thompson (1992) were performed using the Markov chain Monte Carlo (MCMC) approach of GENEPOP version 3.4 (Raymond and Rousset 1995). Bonferroni tests (Rice 1989) were used to correct for multiple tests. Means of measures of diversity were weighted for differences in sample size.

Detection of bottlenecks

As refuges for fishers during the early 20th century, it was likely that populations in New York and Minnesota experienced a genetic bottleneck as a result of severely depleted numbers. Additionally, reintroductions into Michigan and Wisconsin represent demographic founder events with a limited number of released individuals ($N = 60$). Subsequent translocations to the eastern Upper Peninsula represent potential secondary

bottleneck events. We can also view natural dispersal from a localized release event as a series of potential bottlenecks. A limited number of dispersers would be the "founders" of previously unoccupied habitat. The occurrence of a bottleneck in a population and concomitant reduction in effective population size (N_e) results in reduction in the number of alleles and heterozygosity. Populations having experienced a recent bottleneck would exhibit significant heterozygosity excess due to a more rapid reduction in allelic diversity compared to heterozygosity (Cornuet and Luikart 1996). Program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996) tests differences between observed heterozygosity (H_O) and heterozygosity expected from the number of alleles observed at each locus.

Our objective was to test for bottlenecks that occurred as a result of a demographic decline (source populations), reintroduction events (Michigan sample groups and Wisconsin), and subsequent natural colonization (Michigan sample groups). Each population and sample groups were independently evaluated for occurrence of a recent bottleneck under the two-phase model (TPM) in which 90% of mutations occurred as single steps and the remaining 10% were multi-step mutations (Piry et al. 1999, Garza and Williamson 2001). The Wilcoxon sign rank test was used to determine significance due the relatively small number of loci used in this study (Piry et al. 1999).

Theoretical models predict detection of a bottleneck to time $4N_e$ (Cornuet and Luikart 1996). N_e was calculated as:

$$N_e = \frac{4N_F N_M}{(N_M + N_F)}$$

where N_e is the effective size of the founding population, N_F is the number of founding

females, and N_M is the number of founding males. Reintroductions into Wisconsin and Michigan occurred over a period between 1956 and 1967, or approximately 17 to 22 generations before sampling. Calculations of $4N_e$ ranged from 208 to 240 generations, indicating time since reintroduction is within the window of detection. Similarly, the translocations of fishers into the eastern Upper Peninsula of Michigan occurred between 1988 and 1992, or approximately 10 generations prior to sampling. Calculations of $4N_e$ range from 72 to 200, again well within the theoretical window of detection.

Spatial genetic analyses

We used spatial genetic autocorrelation analysis within the reintroduced Michigan fisher population to infer patterns of colonization and dispersal. The degree of geographic partitioning was expected to be reflected in the level of genetic structuring in the source populations of New York and Minnesota. Consequently, the pattern of genetic structuring in the reintroduced populations would be based on the level of genetic contribution from each source and subsequent dispersal from reintroduction sites.

Population differentiation in source populations

To examine genetic contributions of the two source populations to the reintroduced fisher populations in Michigan and Wisconsin, fishers from Minnesota and New York must be genetically differentiated. The software STRUCTURE (Pritchard et al. 2000) was used to assign individuals from Minnesota and New York to subpopulations. To estimate the number of subpopulations (K), ten independent iterations of $K = 1 - 8$ were completed at 100,000 MCMC replicates and a 100,000 iteration burnin period

assuming admixture and correlated allele frequencies. This Bayesian assignment method does not require prior spatial information as is needed for many frequency-based assignment methods. STRUCTURE calculates posterior probabilities of individual assignment to each subpopulation for each K. The optimal number of subpopulations was chosen as the value of K with the maximal log-likelihood value. The optimal number of subpopulations was confirmed using a graphical method developed by Evanno et al. (2005). A test statistic (ΔK) was calculated from the second order rate of change in the log-likelihood function between successive K values. The modal value of the distribution of ΔK was shown to accurately estimate the "true" number of subpopulations across multiple simulations (Evanno et al. 2005).

To estimate the magnitude of genetic differentiation between the Minnesota and New York populations, F_{ST} (Weir and Cockerham 1984) was calculated using FSTAT 2.9.3 (Goudet 2000) based on the multilocus genotypes of each fisher within each population.

Overall population structure in reintroduced Michigan populations

Successful and wide-spread dispersal and colonization of formerly unoccupied habitat by fishers from both source populations in Wisconsin and Michigan could result either in panmixia (i.e., inter-breeding among members of both source populations), geographic segregation of individuals from each source population, or a gradient between these two extremes. We employed program STRUCTURE to estimate the number of subpopulations for all fishers, source and reintroduced to determine overall level of structure in the reintroduced populations. Ten independent iterations of $K = 1 - 8$ were

completed at 100,000 MCMC replicates and a 100,000 iteration burnin period assuming admixture and correlated allele frequencies. Posterior probabilities of individual membership to a particular subpopulation were averaged across each source population, sample group in the western Upper Peninsula of Michigan, and location in Wisconsin. FSTAT 2.9.3 was used to further evaluate genetic differentiation between each reintroduced population (Wisconsin and Michigan region 1) and between each source and reintroduced population.

Spatial genetic structure of Michigan fishers

The role of geographic distance among sites as a predictive measure of the degree of genetic differentiation among fisher populations in Michigan was investigated using spatial autocorrelation. Mantel tests (Mantel 1967) were performed using program PASSAGE version 1.1 (Rosenberg 2001) to examine the correlation between geographic distance and genetic distance between sample groups. Each sample group was assigned a pair of coordinates representing the geographic center of the distribution of all individuals within the group. Geographic distance was defined as Euclidian distance between coordinates representing the center of each sample group. Mantel tests were conducted separately for the two regions of the Upper Peninsula to evaluate correlative patterns resulting from different release strategies over different time periods. Relationships between geographic and genetic distance were further characterized by standardized variance in allele frequency [$F_{ST}/(1 - F_{ST})$] plotted as a function of geographic distance between population samples. A linear relationship is expected if inter-location variance accrues as a linear function of geographic proximity between groups (i.e., isolation by

distance; Rousset 1997). The ratio of $F_{ST}/(1 - F_{ST})$ was calculated using pairwise F_{ST} values generated using FSTAT.

Measures of isolation by distance describe genetic spatial patterns across entire populations. However, structure can occur within a population due to other factors such as dispersal or social structure. To investigate fine-scale spatial structure, we utilized spatial autocorrelation methods. Spatial autocorrelation analyses for fishers sample groups in region 1 were performed using the Moran's *I* product-moment coefficient (Moran 1950, Cliff and Ord 1973, Sokal and Oden 1978a, b) and program PASSAGE.

Moran's *I* is a measure of genetic correlation, quantifying the spatial inter-dependence in allele frequency between all pairs of populations within a specified distance class (Epperson et al. 1999). We used a series of different distance classes to determine the interval distance which maximized replicates and the resolution of autocorrelation. Based on these criteria, we chose ten equal interval distance classes. Based on the range of inter-population distances, ten distance classes were selected at intervals of 22.43 km.

Moran's *I* values range from -1 and 1. A positive value signified greater genetic similarity between populations within the specified distance class compared to populations randomly chosen from the entire data set. A composite correlogram was constructed showing mean Moran's *I* across all alleles at all loci and mean Moran's *I* across all loci plotted as a function of distance. The significance of each correlogram was adjusted using a Bonferroni procedure (Oden 1984) due to lack of independence of distance classes. The distance over which positive spatial autocorrelation occurred represented genetic patch size, or the distance over which effective gene flow occurred.

Our goal was to obtain spatial autocorrelation estimates averaged over all alleles and loci, especially since the values for any one allele are not likely to be meaningful if spatial structure is weak. As long as the number of loci is greater than three or four, depending on the array of allele frequencies, the spatial autocorrelations for different alleles are nearly independent. In other words, the spatial patterns of frequencies of alleles are nearly independent. In our study, there were more alleles in total than are typical in other studies of spatial genetic structure (review by Epperson 2003).

Barriers to movements including habitat discontinuities, differences in land use practices, or other physical impediments could have affected fisher dispersal from stocking sources both in terms of distance and ordination. Two dimensional spatial autocorrelation analyses have been used in a number of studies of genetic structure in human populations (e.g. Sokal et al. 1986, Sokal et al. 1989, Sokal and Thomson 1998) and in insects (Sokal et al. 1987). A bearing correlogram is a means of displaying spatial autocorrelation using the Moran's *I* statistic, by grouping individuals into contiguous spatial associations with a fixed directional compass bearing. Applied across a set of fixed bearings, the scale and direction of spatial autocorrelation can be determined (Rosenberg 2000).

Distance classes were represented by successive half circles, or arcs. Autocorrelation coefficients were plotted above or below each distance class. The magnitude of each coefficient was provided as its distance from its assigned arc. For each correlation coefficient the angle of the radius from the origin (distance 0) to any correlation coefficient represented the fixed bearing, or direction (Rosenberg 2000).

The Nicolet National Forest in Wisconsin was the release site used as the origin for autocorrelation analyses within region 1. This origin was selected to describe dispersal patterns into the Upper Peninsula of Michigan by fishers from descendents from both New York and Minnesota source populations. Because the bearing correlogram determined autocorrelation between groups within a distance class as well as direction, we reduced the number of distance classes to ensure an adequate number of comparisons. Based on the maximum distance in our data, seven distance classes resulted in 32 km intervals. Six fixed bearings were set at 30° intervals to maximize sample size. A bearing correlogram was constructed for each locus. We also used the posterior probabilities of assignment the two genetic clusters defined by program STRUCTURE averaged across all individuals within a sample group. Posterior probabilities were used in this analysis to infer directional patterns of dispersal from the Nicolet National Forest, the only site where individuals from both source populations were released.

Significance of individual coefficients was calculated using a Bonferroni method (Oden 1984) to account for multi-directional tests. The Bonferroni-corrected *p*-value was α/b where α is the standard level of significance (e.g., 0.05) and b was the number of fixed bearings used in the analysis. For this analysis, individual coefficients were tested against the critical value of 0.05/6 = 0.0083. Multiple distance classes must be accounted for when determining significance of the entire bearing correlogram (Rosenberg 2000). The critical value was α/bd, where d represented the total number of distance classes. The Bonferroni-corrected *P*-value for each correlogram constructed in this analysis was 0.05/(6*7) = 0.0012.

Results

Genetic diversity of source and reintroduced populations

Four population – locus comparisons exhibited significant departures from HWE following Bonferroni correction (Ma-2 and Tt-1 in New York, Gg-7 in Minnesota, and Mer022 in Michigan sample group 16). All deviations were due to heterozygote deficiencies. Linkage disequilibrium was detected in two locus-locus comparisons (Ma-2 – Mer041 in New York and Ma-2 – Mer041 in Michigan sample group 4). Due to the large number of tests performed in this data set, it is likely these departures occurred by chance alone. Analyses were performed using all loci.

Allelic richness (*A*) was highest in the Minnesota population (Table 4.1). Locus Tt-4 was monomorphic in the Minnesota population. Heterozygosity was similar in the two populations. Three unique alleles were found in each population. The F_{ST} value estimated between the New York and Minnesota populations was 0.128, consistent with values previously reported for geographically widely separated fisher populations across North America (Kyle et al. 2001). The high degree of genetic heterogeneity between sources made it possible to ask questions about success of descendants from either founding population in Michigan and Wisconsin.

Mean allelic richness was lower in the two regions of Michigan relative to the source populations (Table 4.1). In contrast, mean levels of heterozygosity (H_E) were higher in the Michigan regions than the New York, Minnesota or Wisconsin populations, indicating a lack of overall effect of reintroduction and translocation on genetic diversity. Heterozygosity was slightly lower in the Wisconsin population, also suggesting that founder events did not appreciably affect genetic diversity. Mean F_{IS} is greatly reduced

Table 4.1. Summary of measures of genetic diversity and demographic background for reintroduced and translocated populations of fishers. Genetic diversity measures are also provided for individuals sampled from putative source populations. Population numbers correspond to locations in the Upper Peninsula of Michigan in Figure 4.1.

		Field data				Summary means of genetic diversity								
Category	Pop.	Reloc year	No. of indiv. released	N_e[A]	*N*	alleles	*A*[B]	H_0	H_e	PA[C]	F_{IS}	shared alleles[D]	Post. prob[E]	Dist NNF[F]
Source	MN	-	-	-	*45*	5.0	5.0	0.553	0.605	*3*	0.086	0.517	0.287	-
	NY	-	-	-	*63*	4.6	4.5	0.519	0.608	*3*	0.147	0.529	0.933	-
Reintroduction	WI	*1956-1967*	*120 (54 females)*	118	*161*	5.1	4.7	0.557	0.583	1	0.046	-	-	-
Reintroduction	1	*1961-1963*	*61 (19 females)*	52	*19*	4.4	3.9	0.600	0.610	0	0.017	0.507	0.307	123.0
	2				*15*	4.3	3.9	0.593	0.615	0	0.036	0.515	0.268	101.8
	3				*28*	5.0	4.0	0.587	0.623	2	0.058	0.532	0.368	109.8
	4				*30*	4.7	3.9	0.577	0.626	0	0.080	0.539	0.414	90.7
	5				*12*	4.2	3.8	0.600	0.588	0	-0.021	0.485	0.410	69.6
	6				*13*	4.3	3.9	0.531	0.625	0	0.156	0.554	0.469	75.6
	7				*10*	3.9	3.7	0.590	0.579	0	-0.020	0.478	0.545	66.1
	8				*12*	3.8	3.5	0.575	0.597	0	0.038	0.495	0.399	45.7
	9				*20*	4.3	3.8	0.650	0.602	0	-0.083	0.476	0.583	39.5
	10				*12*	4.0	3.8	0.636	0.613	0	-0.040	0.516	0.327	75.6
	11				*18*	4.2	3.7	0.561	0.606	0	0.077	0.521	0.493	55.2
	12				*26*	4.3	3.6	0.580	0.596	0	0.028	0.517	0.515	69.8
	13				*15*	4.3	3.9	0.547	0.611	0	0.108	0.533	0.596	67.9
	14				*12*	3.6	3.4	0.531	0.548	0	0.032	0.472	0.472	98.3
	15				*10*	3.8	3.6	0.627	0.572	1	-0.102	0.460	0.383	106.2
	16				*10*	4.3	4.1	0.600	0.665	0	0.103	0.579	0.484	96.0
	17				*16*	4.7	4.0	0.639	0.641	0	0.004	0.543	0.507	97.5
	18				*15*	3.8	3.6	0.667	0.642	0	-0.041	0.512	0.408	116.2
	19				*16*	4.1	3.7	0.669	0.619	0	-0.084	0.489	0.507	133.4
	20				*26*	4.1	3.6	0.612	0.605	0	-0.010	0.490	0.382	138.7

Table 4.1 (cont'd).

		Field data			Summary means of genetic diversity									
Category	Pop.	Reloc year	No. of individuals released	N_e[A]	*N*	alleles	*A*[B]	H_0	H_e	PA[C]	F_{IS}	shared alleles[D]	Post. prob[E]	Dist NNF[F]
Reintroduction	21				*11*	4.3	4.0	0.627	0.617	0	-0.018	0.505	0.324	113.2
	22				*21*	4.3	3.7	0.581	0.611	1	0.051	0.516	0.238	118.4
	23	*1961-1963*	*61 (19 females)*	52	*11*	3.8	3.7	0.599	0.621	0	0.036	0.531	0.344	103.0
	24				*13*	4.1	3.8	0.554	0.637	0	0.135	0.556	0.375	115.1
	25				*9*	3.7	3.6	0.522	0.575	0	0.097	0.503	0.227	113.3
	26				*20*	4.4	3.7	0.586	0.611	1	0.043	0.523	0.318	129.7
	27				*17*	3.9	3.5	0.576	0.613	0	0.062	0.511	0.246	141.0
	28				*16*	4.2	3.6	0.631	0.606	0	-0.042	0.485	0.322	173.3
			Mean:		*453*	4.2	3.8	0.595	0.611	-	0.026	0.513	-	-
Translocation.	29	*1988*	*46 (27 females)*	44	*9*	3.9	3.8	0.696	0.635	0	-0.103	0.506	-	-
	30	*1988*			*11*	4.1	3.8	0.636	0.640	0	0.006	0.522	-	-
	31	*1992*	*44 (22 females)*	44	*8*	3.9	3.9	0.575	0.627	0	0.088	0.539	-	
	-													
	32	*1990*	*34 (19 females)*	33	*15*	4.0	3.6	0.547	0.621	0	0.123	0.540	-	-
	33	*1990*			*20*	4.0	3.5	0.635	0.604	0	-0.053	0.479	-	-
	34	*1990*			*8*	3.8	3.8	0.588	0.635	0	0.080	0.545	-	-
	35	*1991*	*50 (25 females)*	50	*10*	4.1	4.0	0.620	0.638	0	0.030	0.526	-	-
	36	*1991*			*11*	3.7	3.4	0.464	0.545	0	0.156	0.484	-	-
	37	*1989*	*19 (8 females)*	18	*10*	3.7	3.6	0.640	0.594	0	-0.083	0.461	-	-
			Mean:	-	*102*	3.9	3.7	0.600	0.613	-	0.024	0.508	-	-

[A] Estimated effective size of founding population as $[4N_MN_F/(N_F + N_M)]$ where N_M is the number of founding males and N_F is the number of founding females.

[B] Allelic richness.

[C] Number of unique alleles per population.

[D] Average pairwise estimate of inter-individual relatedness based on the proportion of shared alleles (Bowcock et al. 1994).

[E] Posterior probability of assignment to New York generated using program STRUCTURE.

[F] Distance (km) from the central release location in the Nicolet National Forest, Wisconsin.

in all reintroduced populations relative to the sources, indicating low inter-individual variance. Five unique alleles were found in region 1 and one unique allele was observed in Wisconsin.

Detection of bottlenecks

The Minnesota population did not show evidence of a bottleneck. However, the New York population exhibited significant heterozygosity excess under the TPM model (Wilcoxon sign rank test, one tail test $P = 0.01$), providing evidence of a recent bottleneck in this source population. Sample groups 18, 22, 23, 24 and 27 in the upper peninsula of Michigan also showed evidence for recent population bottlenecks under the TPM model ($P < 0.05$). These groups are generally located on the northern edge of region one in Michigan, indicating that bottlenecks likely arose through recent natural colonization, likely by few initial founders (Figure 4.1). No sample groups in region 2 showed evidence of population bottlenecks.

Population structure of source populations

The mean log likelihood value generated by program STRUCTURE suggested the existence of three subpopulations ($K = 3$). The modal value of ΔK based on the results of the Evanno et al. (2005) statistic was also 3. Forty-two of 45 individuals trapped in Minnesota were assigned to a single subpopulation with a high degree of probability ($q \geq 0.90$ when q is the highest posterior probability of membership). This q value has been suggested a critical value in previous studies (Manel et al. 2002, Cegelski et al. 2003). The q value of the remaining three Minnesota fishers also group in the Minnesota-based

subpopulation, but at a threshold below 0.90).

In contrast, for New York fishers, data were consistent with the existence of two genetic populations, both different from the subpopulation from Minnesota. The two New York populations appeared to be geographically structured. The majority of fishers trapped across northern New York were clustered in one subpopulation. However, fishers harvested near the border of Massachusetts clustered highly into a different subpopulation. The presence of two genetically distinct groups was concordant with the deviations from HWE discovered in the New York samples. It is likely this eastern genetic cluster was a result of migration from an expanding fisher population in western Massachusetts. However, without evidence eliminating these animals as contemporary representatives of the source population used in Wisconsin, all New York fishers remained for further analysis.

Genetic population structure of reintroduced populations

The maximal log-likelihood value for all populations, source and reintroduced generated by program STRUCTURE was at $K = 5$. However, the modal value of ΔK based on the results of the Evanno et al. (2005) statistic was 1, suggesting a single panmictic population. Our objective was to evaluate patterns of colonization by the source populations of New York and Minnesota. Since fishers from the two source populations were genetically differentiated, there was an expectation of two genetic signatures in the reintroduced populations if individuals from both sources were successful colonizers. The level of admixture was unknown. Figure three shows individual posterior probabilities resulting from $K = 2$ mapped across Wisconsin and Michigan. New York

fishers clustered very highly to a single subpopulation (mean posterior probability q = 0.993). In contrast, Minnesota fishers were assigned to the alternate subpopulation with a mean posterior probability q = 0.713 (Figure 4.2).

Estimates of F_{ST} averaged over all populations were 0.028. Very few statistical comparisons between sample groups in Michigan were significant ($P > 0.05$). However, New York was genetically differentiated from all reintroduced populations ($P < 0.05$; average pair-wise F_{ST} 0.114; range 0.081 to 0.150). The lower pair-wise F_{ST} values within this range were found in comparisons with sample groups located on the central and north-eastern portions of region 1. Likewise, the Minnesota population was significantly differentiated from the Wisconsin population and 28 of the 37 Michigan sample groups (average pairwise F_{ST} = 0.041, range 0.016 to 0.075). The Michigan sample groups that were not genetically differentiated from the Minnesota population were generally located in the western, northern, and eastern periphery of region one. Two of these nonsignificant comparisons occurred with sample groups in region 2. No significant differentiation was detected between sample groups in region 2 ($P > 0.05$). Likewise, 350 of 378 pairwise comparisons of sample groups in region 1 were nonsignificant. However, the 28 significant pairwise F_{ST} values appeared to occur in a spatially nonrandom pattern. Sample groups 9, 12, 13 and 14 located to the north and east of the Nicolet National Forest release site were significantly genetically differentiated to sample groups (22, 27 and 28) in the Keweenaw Peninsula of Michigan (the northernmost point). Significant F_{ST} values also occurred between sample groups on the eastern side of region 1 relative to groups along the western edge of the study site.

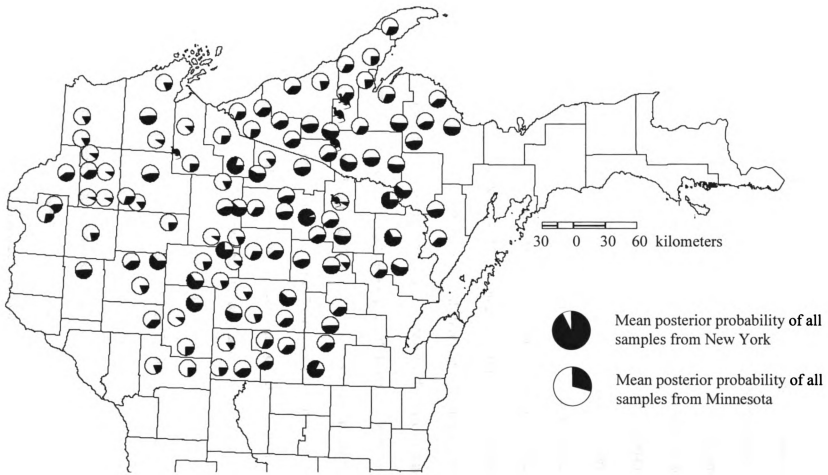

Figure 4.2. Mean posterior probabilities obtained using program STRUCTURE for the source populations and sample groups in Michigan and Wisconsin. Analyses were conducted using a prediction of two genetic groups ($K = 2$) based on two source populations. The five reintroduction locations are indicated by fisher icons.

Spatial genetic structure in Michigan

Analyses were performed on sample groups in the Upper Peninsula of Michigan to assess the role of isolation by distance in creating genetic differentiation at the regional scale (within regions 1 and 2). Mantel tests revealed significant correlations between geographic and genetic distance in both regions, however the correlation was stronger in region 2, the eastern Upper Peninsula (region 1: $R^2 = 0.1055$, $P = 0.00045$; region 2: $R^2 = 0.2470$, $P = 0.00284$). Analysis of standardized variance in allele frequency within region plotted against geographic distance revealed overall patterns (Figure 4.3). Least squares regression lines of fit to pairwise values showed a stronger correlation between groups in region 2 (region 1: $R^2 = 0.246$; region 2: $R^2 = 0.102$). The slope of the least squares regression line was steeper in region 2 than region 1, indicating a stronger correlation of decreasing genetic similarity with increasing distance in region 2.

The correlogram shown in Figure 4.4 represents the mean Moran's *I* values in each distance class across all loci and all alleles of fisher sample groups in Region 1. Error bars were added from each overall mean Moran's *I* value to designate the maximum and minimum Moran's *I* value of individual loci at each distance class. Spatial autocorrelation in allele frequency existed between fisher sample groups but declined with increasing geographic distance. Mean estimates of autocorrelation declined to zero at inter-location distanced of approximately 68 km. Positive genetic correlation among groups located less than 68 km apart provided an estimate of genetic patch size or the distance over which effective gene flow occurred.

Dispersal often occurs in a non-linear pattern. Thus, we would expect that non-random spatial genetic associations (spatial autocorrelation) occur directionally based on

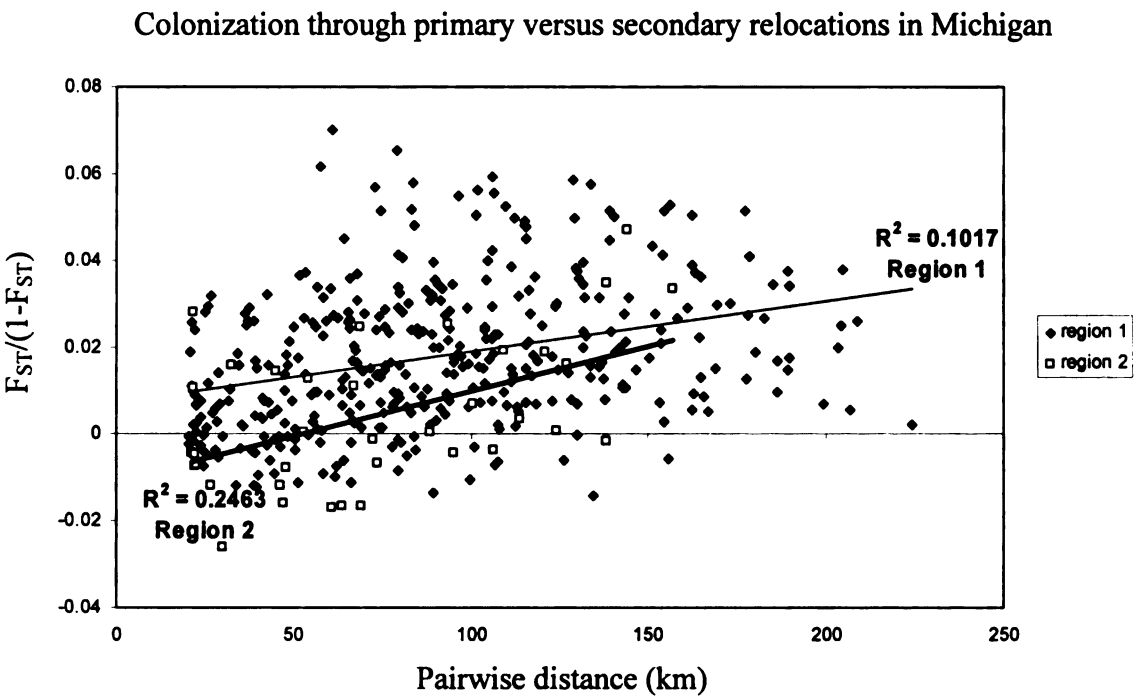

Figure 4.3. Relationships of inter-location genetic differentiation (F_{ST}) and geographic distance within region 1 (western Upper Peninsula) and region 2 (eastern Upper Peninsula) exhibited by the least squares regression lines of best fit (R^2). The bold line represents the regression of region 2.

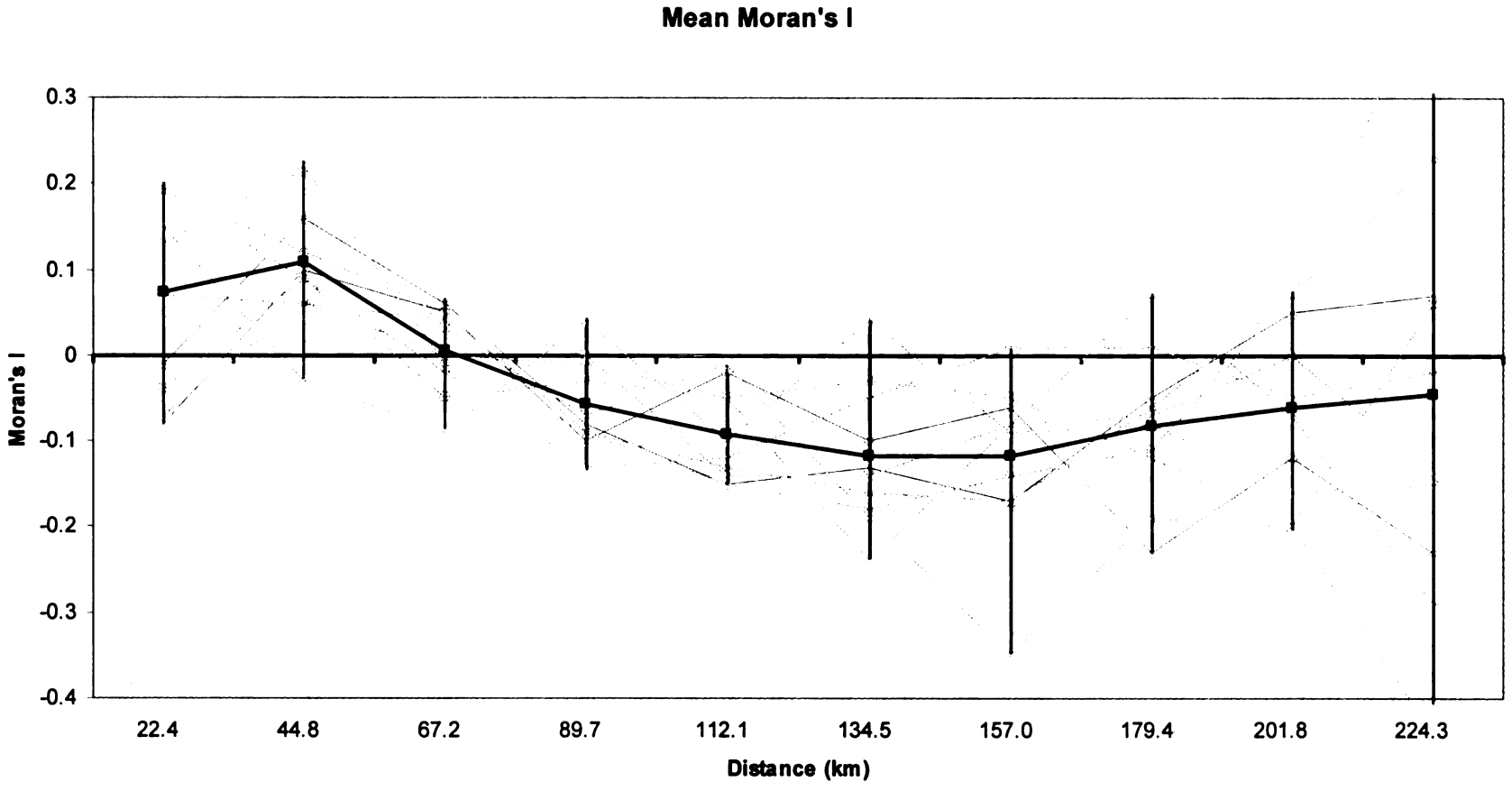

Figure 4.4. Correlogram of mean Moran's *I* statistic across all alleles and all loci (bold trendline) and of each locus (grey trendlines) as a function of distance for fishers in the western Upper Peninsula of Michigan. Error bars show deviation from the overall mean to the high and low means of individual loci at each distance class. The upper bound of the distance class is represented by each point and is labeled below the graph. The correlogram crosses the x-axis at approximately 68 km.

factors such as heterogeneous habitat or kin structure. Bearing correlograms were constructed to support the autocorrelation exhibited by the Moran's *I* statistic and add a second ordinal dimension to inter-location genetic relationships within Michigan's region 1. Figure 4.5 shows the bearing correlogram for each locus and for the posterior probabilities of assignment to one of two genetic clusters identified using program STRUCTURE. Bearing correlograms were visualized as radially symmetric half circles. Most correlograms indicated positive autocorrelation in the first and second distance classes (to a distance of approximately 64 km), a value consistent with the analysis of the Moran's *I* statistic over distance. Although direction of positive correlation was not consistent across all loci, a pattern appeared in a south-west to north-east direction beyond the second distance class. As was generally consistent with other correlograms, the outermost distance class may be unreliable and was omitted from analysis (Rosenberg 2000).

Discussion

Population structure within source populations

A limited number of remnant populations survived a severe decline in numbers across much of the fisher's southern range during the early 20th century. Northeastern Minnesota and the Adirondack Mountains of New York were two refuges. The lowest numbers experienced in these areas is unknown, although it is likely both populations experienced reductions in levels of genetic diversity. However, only the New York population showed evidence of a recent genetic bottleneck. We do not know the level of depletion sustained in these areas during the early 1900s. However, based on the number

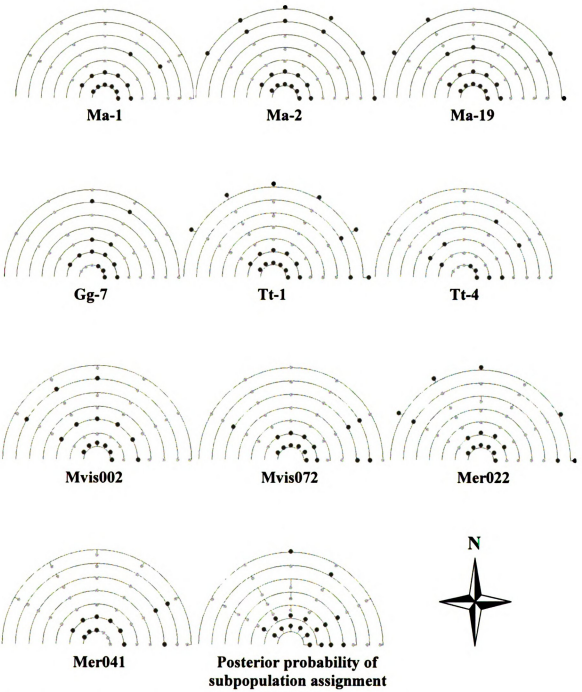

Figure 4.5. Two-dimensional correlograms (Rosenberg 2000) for Michigan fisher populations at 10 microsatellite loci and posterior probabilities of assignment to the New York source population. Each point is located at 30° intervals around the compass points, with compass provided. Each arc represents distance classes of 32 km from a pairwise distance of zero. Black points represent positive correlation coefficients. Grey points represent negative correlation coefficients. The magnitude of each coefficient is denoted by its distance above or below the arc representing its distance class.

of generations that have passed since the decline (> 30 generations), the bottleneck identified in the New York population most likely was severe to still be so strongly detected.

Results from analyses using program STRUCTURE indicated fishers sampled from northern Minnesota represented a single subpopulation. This is consistent with range expansion from a single founding group as would have been located in the northeastern corner of the state. In contrast, samples from New York State represented two genetic groups. There was overlap in the spatial distribution of the two subpopulations. However, one cluster was found in samples collected across northern New York, from Lake Ontario to Lake Champlain on the northern New York – Vermont border. The second cluster was predominantly located near the eastern border of New York with Massachusetts. The fisher population in Massachusetts has recently and significantly expanded. It is likely that individuals are migrating into contiguous areas of New York during range expansion. Therefore, the genetic signature most similar to that of fishers from the New York source population is that represented by the first genetic subpopulation in northern New York. However, neither genetic cluster can be eliminated as a representative of the putative source without evidence of an alternative source.

Recolonization of fishers in Massachusetts is a likely result of dispersal from regional reintroduction events. One hundred twenty-one fishers were reintroduced into Vermont that occurred from 1959-1967 (Wood 1977, Williams et al. 2000). In 1989 and 1990, 30 fishers were relocated from New Hampshire to western Connecticut. Vermont was the source for a release of an additional four fishers into western Connecticut in 1989 (Williams et al. 2000). Genetic comparison of contemporary fishers from the

Northeastern United States with the eastern cluster in New York would provide evidence of fisher dispersal from Massachusetts westward.

Fisher stocking events in Wisconsin and Michigan occurred after a substantial increase in population numbers in New York and Minnesota. There have been no records of significant declines in the source areas since the reintroductions. The effects of genetic drift would be minimized by a large panmictically breeding population. Therefore, contemporary genetic diversity was expected to closely represent that exhibited by the populations from which founding individuals were removed.

Genetic diversity of reintroductions

Mean allelic richness was lower in Michigan than in Wisconsin or the two source populations, suggesting a decrease in genetic diversity at a local scale. However, levels of heterozygosity (H_E) of fishers sampled from New York (0.608) and Minnesota (0.605) and reintroduced populations within Wisconsin (0.583) and Michigan (region 1: 0.611, region 2: 0.613) were comparable to heterozygosity reported in other mustelid studies (e.g. fishers, 0.623 Kyle et al. 2001; American martens, 0.660 Kyle et al. 2000, 0.580 Small et al. 2003; wolverines, *Gulo gulo*, 0.630 Kyle and Strobeck 2001), indicating that significant population declines in abundance did not appreciably reduce levels of genetic diversity or the bottleneck was of short duration. Alternatively, fishers are a high gene flow species and extensive movements enable the species to maintain genetic contiguity over large areas and thus maintain diversity even at low densities.

Previous studies have examined the effect of stocking events on genetic variation in fisher populations. Williams et al. (1999, 2000) found little genetic structure between

source and reintroduced populations in the northeastern United States using allozymes. Kyle et al. (2001) used microsatellite markers and described a slight decrease in genetic diversity in reintroduced populations relative to adjacent indigenous fisher populations.

In contrast, bottlenecks from reintroduction of small numbers of individuals have resulted in significant decline in genetic diversity in a number of species, including elk (Williams et al. 2002) and ibex (Maudet et al. 2002), suggesting lower rates of recovery due to low fecundity or habitat discontinuity.

Bottlenecks, reintroduction, and natural colonization

No evidence of a recent bottleneck was detected in samples from Wisconsin, or in Michigan sample groups closest to release locations. However, we discovered evidence of bottleneck events in sample groups located on the northern portion of Michigan region one. Following release, founding individuals would have dispersed to establish new territories. A limited number of dispersers colonizing and breeding in given area might result in a genetic bottleneck. Because areas furthest from release sites were likely the last to be colonized, we would expect evidence of bottlenecks to be stronger in peripheral groups than in sample groups located near the release sites. The general pattern displayed by the bottleneck tests suggests that colonization occurred with few individuals, but high rates of gene flow quickly supplemented recruitment for reproduction within local areas. However, straight-line, or Euclidian distance from release sites may not fully explain the patterns of bottlenecks in sample groups. Fishers are habitat specialists. The species subsists in areas of cover where prey is found, and the fisher's diet consists of a variety of items, in particular snowshoe hares and porcupines

(Powell 1993). The species is limited by deep snow and areas with little or no canopy cover (Raine 1983, Buskirk and Powell 1994). Open habitat or other dispersal barriers would have resulted in gene flow occurring asymmetrically based on direction and distance. In addition, frozen lakes or rivers may present a dispersal barrier due to the risk of exposure to predators (Wisely et al. 2004). Analysis of spatial distribution of bottleneck events using habitat parameters could further elucidate patterns of dispersal.

Population structure of reintroduced populations

The results from analysis using program STRUCTURE adjusted using the ΔK statistic of Evanno et al. (2005) indicated fishers in Michigan and Wisconsin were panmictic. However, we were interested in differential genetic contribution of each source population. We observed evidence for wide-spread gene flow from founders of both source populations based on the strong level of genetic differentiation between source populations ($F_{ST} = 0.128$) and a high posterior probability of cluster assignment for representatives of either source population. Genetic contributions to the recipient populations by founders from the New York source appear to be greatest in and around the Nicolet National Forest release site and into western Michigan along a northeast to southwest ordination (Figure 4.5). New York was used as a source only for the Nicolet National Forest reintroduction, making it a numerical minority genetic contributor.

This pattern was further supported by analysis of pairwise F_{ST} values between all source and reintroduced populations and sample groups. In general, fishers in Michigan and Wisconsin are differentiated from both New York and Minnesota. However, the magnitude of differentiation is stronger relative to the New York population. Variation

existed within the range of pairwise F_{ST} values for each Michigan sample group and New York that showed lower levels of genetic differentiation in areas to the north and northeast of the Nicolet National Forest release site. In contrast, the Michigan sample groups most similar to the Minnesota population were generally located at the periphery of region one. These results indicate strong spatial and temporal components of dispersal following reintroduction that are supported by additional analyses presented in this study.

Spatial autocorrelation

Significant patterns of isolation by distance were identified in both regions of the Upper Peninsula of Michigan, where genetic divergence increases with increasing geographic distance. The stronger correlation exhibited by fishers in region 2 was likely due to colonization through multiple releases from a similar genetic source (region 1) across a large area. Patterns of isolation by distance were established over short time intervals suggesting high rates of gene flow. Eighteen years have passed since the first translocation into region 2. Less than ten generations would be expected during this time period resulting in genetic structure likely a direct result of each release. The pattern of isolation by distance exhibited by fishers in both regions of Michigan is a 'long-distance cline' and might be suggestive of either a lack of distinct subpopulations or highly structured subpopulations with increasing differentiation with increasing distance (Diniz-Filho and Telles 2002). Results of STRUCTURE analysis and the magnitude of pairwise F_{ST} across Michigan supported the lack of discrete subpopulations.

In region one of Michigan, there is little evidence of F_{ST} or pairwise F_{ST} spatial variance, but there was an effective distance over which allele frequencies were

autocorrelated. Genetic patch size of fishers in Michigan was estimated to be approximately 68 km, suggesting high levels of gene flow and potential to colonize new areas. Fishers located in locales separated by distances less than 70 km were more genetically similar in allele frequencies than random. The estimate of patch size or effective distance of gene flow corresponded to maximum dispersal distances from natal home ranges reported in Manitoba, Canada (Raine 1987). Average natal dispersal distance in Maine was reported to be much shorter (10 km, Arthur et al. 1993).

Dispersal rarely occurs linearly. For habitat specialist species, such as the fisher, forest types limit the direction of dispersal. Two dimensional spatial autocorrelation analyses evaluate relationships based on distance and ordination. The bearing correlogram was concordant with the Moran's *I* result of genetic patch size. However, ordination was apparent beyond that distance in a northeast to southwest direction. This suggested dispersal occurred beyond 68 km, but only in a limited direction. This pattern of autocorrelation as it existed in a localized direction was too weak to be made evident using Moran's *I* statistic which used all comparisons within a distance class.

The correlogram representing posterior probabilities of assignment to each of two genetic clusters generated using STRUCTURE showed the strongest directional pattern of autocorrelation to a distance of 128 km. One hundred twenty-eight kilometers reaches the eastern sample groups within region 1. Posterior probabilities were substituted for allele frequencies to generate a correlogram to assess colonization and dispersal from the Nicolet National Forest based on differential genetic contributions of each source. The results of the correlogram concurred with evaluation of pairwise F_{ST} values of sample groups in Michigan region one and New York that show less genetic partitioning in

groups north or northeast of the Nicolet National Forest.

In conclusion, we have shown that reintroductions of fishers into Michigan and Wisconsin have had little effect on the genetic diversity as would be expected from bottlenecks resulting from release of a small number of founders (Lacy 1987). Fishers in this area appear to be highly successful colonizers, capable of high levels of gene flow over a large geographic distance. Evidence of recent bottlenecks in locales along the periphery of the reintroduced population in Michigan reflects areas of recent population expansion through dispersal.

References

Arthur, S.M., T.F. Paragi, and W.B. Krohn. 1993. Dispersal of juvenile fishers in Maine. Journal of Wildlife Management. 57: 868-874.

Berg, W. E. 1982. Reintroduction of fisher, pine marten, and river otter. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 158-173.

Bodkin, J.L., B.E. Ballachey, M.A. Cronin, and K.T. Scribner. 1999. Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conservation Biology. 13: 1378-1385.

Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd, and L.L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature. 368: 455-457.

Bradle, B.J. 1957. The fisher returns to Wisconsin. Wisconsin Conservation Bulletin. 22: 9-11.

Brander, R.B. and D.J. Books. 1973. Return of the fisher. Natural History. 82: 52-57.

Buskirk, S.W. and R.A. Powell. 1994. Habitat ecology of fishers and American martens. Pp. 283-296 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Cegelski, C.C., L.P. Waits, and N.J. Anderson. 2003. Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. Molecular Ecology. 12: 2907-2918.

Cliff, A.D. and J.K. Ord. 1973. Spatial Autocorrelation. London: Pion.

Cook, D.B. and W.J. Hamilton, Jr. 1957. The forest, the fisher, and the porcupine. Journal of Forestry. 55: 719-722.

Cornuet, J.M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014.

Davis, C.S. and C. Strobeck. 1998. Isolation, variability and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7: 1771-1788.

de Vos, A. 1951. Recent findings in fisher and marten ecology and management. Transactions of the Sixteenth North American Wildlife Conference. 16: 498-507.

Dieringer, D. and C. Schlötterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes. 3: 167-169.

Diniz-Filho, J.A.F. and M.P.C. Telles. 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. Conservation Biology. 16: 924-935.

Earle, R.D. 1978. The fisher-porcupine relationship in Upper Michigan. Dept. of Biological Sciences. M.S. Thesis. Department of Biology. Michigan Technological University: 126.

Epperson, B. 2003. Geographic Genetics. Princeton Univ. Press, Princeton, NJ.

Epperson, B.K., Z. Huang, and T.-Q. Li. 1999. Measures of spatial structure in samples of genotypes for multiallelic loci. Genetical Research Camb. 73: 251-261.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14: 2611-2620.

Fleming, M.A., E.A. Ostrander, and J.A. Cook. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology. 8: 1351-1362.

Frankam, R. 1995. Conservation genetics. Annual Review of Genetics. 29: 305-327.

Garza, J.C. and E.G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology. 10: 305-318.

Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. Journal of Heredity. 86: 485-486.

Guo S.W. and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics. 48: 361-372

Irvine, G.W., L.T. Magnus, and B.J. Bradle. 1964. The restocking of fisher in Lake States forests. Transactions of the North American Wildlife and Resource Conference. 29: 307-315.

Kyle, C.J., C.S. Davis, and C. Strobeck. 2000. Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. Canadian Journal of Zoology. 78: 1150-1157.

Kyle, C.J., J.F. Robitaille, and C. Strobeck. 2001. Genetic variation and structure of fisher (*Martes pennanti*) populations across North America. Molecular Ecology. 10: 2341-2347.

Kyle, C.J. and C. Strobeck. 2001. Genetic structure of North American wolverine (*Gulo gulo*) populations. Molecular Ecology. 10: 337-347.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology. 1: 143-157.

Larson, S., R. Jameson, J. Bodkin, M. Staedler, and P. Bentzen. 2002. Microsatellite DNA and mitochondrial variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy. 83: 893-906.

Leberg, P.L., P.W. Stangel, H.O. Hillestad, R.L. Marchinton, and M.H. Smith. 1994. Genetic structure of reintroduced wild turkey and white-tailed deer populations. Journal of Wildlife Management. 58: 698-711.

Manel, S., P. Berthier, and G. Luikart. 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conservation Biology. 16: 650-659.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research. 27: 209-220.

Maudet, C., C. Miller, B. Bassano, C. Breitenmoser-würsten, D. Gauthier, G. Obexer-Ruff, J. Michallet, P. Taberlet, and G. Luikart. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex (ibex)*]. Molecular Ecology. 11: 421-436.

Mills, L.S. and P.E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations.

Moran, P.A.P. 1950. Notes on continuous stochastic phenomena. Biometrika. 37: 17-23.

Oden, N.L. 1984. Assessing the significance of a spatial correlogram. Geographical Analysis. 16: 1-16.

Olson, H.F. 1966. Return of a native. Wisconsin Conservation Bulletin. 31: 22-23.

Piry, S., G. Luikart, and J.M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity. 90: 502-503.

Powell, R.A. 1979. Fishers, population models, and trapping. Wildlife Society Bulletin. 7: 149-154.

Powell, R.A. 1993. The Fisher: Life History, Ecology, and Behavior. University of Minnesota Press, MN.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Raine, R.M. 1987. Winter food habits and foraging behaviour of fishers (*Martes pennanti*) and martens (*Martes americana*) in southeastern Manitoba. Canadian Journal of Zoology. 65: 745-747.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86: 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225.

Rosenberg, M.S. 2000. The bearing correlogram: a new method of analyzing directional spatial autocorrelation. Geographical Analysis. 32: 267-278.

Rosenberg, M.S. 2001. PASSAGE. Pattern analysis, spatial statistics, and geographic exegesis. Version 1.1. Department of Biology, Arizona State University, Tempe, AZ.

Rousset, F. 2000. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 145: 1219-1228.

Sarrazin, F. and R. Barbault. 1996. Reintroduction: challenges and lessons for basic ecology. Trends in Ecology and Evolution. 11: 474-478.

Schoonmaker, W.J. 1938. The fisher as a foe of the porcupine in New York State. Journal of Mammalogy. 19: 373-374.

Scribner, K.T. and M. Stüwe. 1994. Genetic relationships among Alpine ibex *Capra ibex* populations re-established from a common ancestral source. Biological Conservation. 69: 137-143.

Slough, B.G. 1994. Translocations of American martens. Pp. 165-178 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Small, M.P., K.D. Stone, and J.A. Cook. 2003. American marten (*Martes americana*) in the Pacific Northwest: population differentiation across a landscape fragmented in time and space. Molecular Ecology. 12: 89-103.

Sokal, R.R., R.M. Harding, and N.L. Oden. 1989. Spatial patterns of human gene frequencies in Europe. American Journal of Physical Anthropology. 80: 267-294.

Sokal, R.R. and N.L. Oden. 1978a. Spatial autocorrelation in biology 1. Methodology. Biological Journal of the Linnean Society. 10: 199-228.

Sokal, R.R. and N.L. Oden. 1978b. Spatial autocorrelatin in biology 2. Some biological implications and four applications of evolutionary and ecological interest. Biological Journal of the Linnean Society. 10: 229-249.

Sokal, R.R., N.L. Oden, and J.S.F. Barker. 1987. Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation. American Naturalist. 129: 122-142.

Sokal, R.R., P.E. Smouse, and J.V. Neel. 1986. The genetic structure of a tribal population, the Yanomama Indians. XV. Patterns inferred by autocorrelation analysis. Genetics. 114: 259-287.

Sokal, R.R. and B.A. Thomson. 1998. Spatial genetic structure of human populations in Japan. Human Biology. 70: 1-22.

Steck, M. 1988. A translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 21 pp.

Steck, M. 1989. The 1989 translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 16 pp.

Steck, M. 1990. The 1990 translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 20 pp.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38: 1358-1370.

Williams, C.L., T.L. Serfass, R. Cogan, and O.E. Rhodes, Jr. 2002. Microsatellite variation in the reintroduced Pennsylvania elk herd. Molecular Ecology. 11: 1299-1310.

Williams, R.N., L.K. Page, T.L. Serfass, and O.E. Rhodes, Jr. 1999. Genetic polymorphisms in fishers (*Martes pennanti*). American Midland Naturalist 141: 406-410.

Williams, R.N., O.E. Rhodes, Jr., and T.L. Serfass. 2000. Assessment of genetic variance among source and reintroduced fisher populations. Journal of Mammalogy. 81: 895-907.

Wisely, S.M., S.W. Buskirk, G.A. Russell, K.B. Aubry, and W.J. Zielinski. 2004. Genetic diversity and structure of the fisher (*Martes pennanti*) in a peninsular and peripheral metapopulation. Journal of Mammalogy. 85: 640-648.

Wood, J. 1977. The fisher. Natural Wildlife. 15: 18-21.

CHAPTER 5: ESTIMATION OF POPULATION SIZE OF CO-DISTRIBUTED FISHERS AND AMERICAN MARTENS USING NON-INVASIVE HAIR SAMPLING AND GENETIC TAGGING

Introduction

Wildlife managers frequently struggle to make management decisions for furbearing species based upon a paucity of ecological and demographic information owing to the species' solitary and elusive habits. Estimation of population abundance is a fundamental requisite or need for effective population management. Harvest surveys and winter track counts are often used as indices of abundance or to provide estimates of population trends for furbearer species. However, indices are often unreliable due to biases such as reporting error and inconsistency in environmental conditions (e.g., Raphael 1994). Mark-recapture is one method for estimating animal abundance. Traditional mark-recapture studies have included use of colored bands, radiotransmitters, ear tags, and other techniques to "mark" individuals in the field (Nietfeld et al. 1994). However, these invasive methods involve capture and handling of animals. Costs associated with use of these techniques are often high and resources rarely allow marking of sufficient sample sizes for accurate population estimation.

Ideally, a mark should be noninvasive, highly visible, unambiguous, cost effective, and permanent (Woods et al. 1999). Recent advances in the field of molecular ecology have enabled the use of molecular markers as "genetic tags" or marks. DNA is usually collected from biopsied tissue samples or blood samples of trapped animals. With the advent of polymerase chain reaction (PCR), small amounts of DNA are sufficient to provide genotypes for multiple loci which constitute a genetic "tag" that can be used to identify individual animals. Noninvasive sampling is designed to obtain DNA

from individuals without direct contact. Source material can originate from hair, feathers, nest materials and scat. For example, DNA has been collected from hair of chimpanzees (*Pan troglodytes*, Morin et al. 1994), northern hairy-nosed wombats (*Lasiorhinus krefftii*, Sloane et al. 2000), American martens (*Martes americana*, Foran et al. 1997b; Mowat and Paetkau 2002), and brown bear (*Ursus arctos*, Taberlet et al. 1997; Mowat and Strobeck 2000). Feathers and nest materials have been used as sources for DNA in birds (Pearce et al. 1997, Segelbacher 2002). DNA was collected from feces in bonobos (*Pan paniscus*, Gerloff et al. 1995), mountain lions (*Puma concolor*, Ernest et al. 2000), Iberian lynx (*Lynx pardinus*, Palomares et al. 2002), and wolverines (*Gulo gulo*, Flagstad et al. 2004). Non-invasive marking techniques can reduce behavioral trap response that is often inherent when animals are captured and handled. Genetic methods have no impact on survival, and marks can be identified and reported correctly upon capture, and are permanent and cannot be lost over time.

Hair and fecal material are the two most commonly used source materials for noninvasive sampling to estimate population size (Waits and Paetkau 2005). Feces are easily collected without interaction with targeted individuals. DNA is collected from epithelial cells shed from the intestinal lining as fecal material passes through the gut (Kohn and Wayne 1997). However, the amount of sloughed epithelial cells is variable and can be difficult to collect without contamination from prey species or other biological contaminants (e.g., plant secondary compounds) in the scat that may increase genotyping errors or inhibit PCR (Kohn and Wayne 1997). In addition, patterns of fecal deposition over time and space are often unknown, requiring collection of an excessive number of samples to confidently estimate abundance (Sloane et al. 2000).

Noninvasive hair collection methods are not without drawbacks. An animal must first be attracted to a location and then induced to deposit a sample of hair from which DNA can be extracted. However, the design of hair snares can be altered to account for the ecology and behavior of the target species. Lures have been constructed to elicit rubbing behavior on hair snares in lynx (*Lynx canadensis,* McDaniel et al. 2000). Double-sided sticky tape placed across burrow entrances was used successfully to snare hair for the development of a population census technique for northern hairy-nosed wombats (Sloane et al. 2000). A number of studies have estimated population size of brown and black bears (*Ursus americanus*) using a method that collects hair as individuals pass beneath barbed wire set around a baited site (Woods et al. 1999, Mowat and Strobeck 2000, Poole et al. 2001, Boerson et al. 2003).

Previous studies have assessed effectiveness of non-invasive techniques for DNA analysis on single target species. Belant (2004) developed a technique using curry combs in cage traps that was effective at collecting hair from multiple species. However, the ability of the technique to collect hair for the purpose of genetic tagging remains untested. A thorough search of the literature revealed no reported studies investigating the use of non-invasive genetic tagging for fishers (*Martes pennanti*).

Fishers and American martens are two furbearer species co-distributed across the Upper Peninsula of Michigan. Both species were extirpated from Upper Midwestern United States by the early 20th century due to habitat loss and exploitation (Berg 1982). Multiple reintroduction efforts were made to restore both species to the forested areas of Michigan and Wisconsin. Three reintroductions of fishers were made from two source populations in Michigan and Wisconsin from 1956 to 1967 (Bradle 1957, Irvine et al.

1964, Petersen et al. 1977, Berg 1982). Fisher colonization eastward across the Upper Peninsula of Michigan was slow. As a result, a five year translocation project was initiated in 1988 to stock areas in the eastern Upper Peninsula with fishers trapped in the area of primary introduction in the Ottawa National Forest (Steck 1988). Translocations were also used to restore the American marten to Michigan and Wisconsin. Eight reintroductions were made from five different source populations from 1953 to 1990 (Davis 1978, Churchill et al. 1981, Ludwig 1986, Slough 1994). Two additional translocations relocated martens from established populations in the Upper Peninsula to areas with few or no martens (Earle et al. 2001).

Few studies have been made assessing the success of the many reintroductions of fishers and martens in Michigan (Schupbach 1977) and few data are available describing species distribution and abundance. Despite limited demographic data, trapping seasons were instituted in the Upper Peninsula beginning in 1989 and 2000 for fishers and martens, respectively. Because fishers and martens were reintroduced and are currently managed through harvest, it is important to monitor population trends. Winter track count surveys and harvest surveys have been used as indices to track population trends (e.g., Earle 2002, Frawley 2002). However, to properly interpret trends, the indices should periodically be validated by estimates of abundance.

Ecological factors determine abundance and distribution. Although fishers and martens are sympatric in the Upper Peninsula, they are often segregated on a microgeographic scale due to habitat preference and prey availability (Buskirk and Powell 1994). Fishers are habitat specialists, found in forested areas with overhead canopy cover (Powell 1993), and open areas are avoided if possible (Thomasma et al.

1991, Jones and Garton 1994). Martens are greater habitat specialists, and remain in coniferous or mixed hardwood stands with understory containing a significant amount of dead woody material (Mech and Rogers 1977, Wright 1999). There is overlap in prey species of fishers and martens. However, studies of marten feeding ecology in Ontario indicate a preference for microtines but also predation on snowshoe hares (*Lepus americanus*) and birds (Raine 1987). Fishers prefer snowshoe hares, but will also feed on porcupines and occasionally martens (Powell 1993). Overlap in prey selected and in habitat suitability could lead to competitive exclusion of martens by fishers in areas of dense fisher populations and low prey numbers.

Competitive exclusion has not been considered a factor in the distribution of fishers and martens in the Upper Peninsula. Harvest distributions of the two species overlap across much of the region (Figure 5.1). However, densities of both species in these areas of overlap are not expected to be high, potentially minimizing contact and competition.

In this study a method was developed to simultaneously enumerate population abundance for two co-distributed species using the same trapping design even though the species differ in abundance, density, dispersal capabilities and habitat use. Little definitive information is available on population abundance of recently reestablished fishers and American martens in Michigan. There is a need to develop additional analytical tools to identify and eliminate non-target species before analyses can be conducted. Several non-target species are congeners (i.e., *Mustela* spp.) and use of heterologous PCR primers will likely produce PCR products and genotypes consistent with those of target species. This study extended previous genetic-based mark-recapture

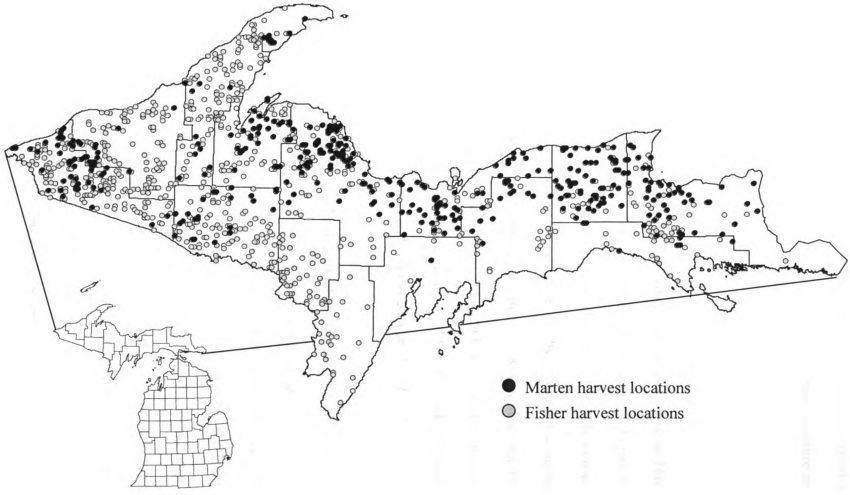

Figure 5.1. Map of the Upper Peninsula of Michigan showing distribution of reported marten and fisher harvest locations from 2000 to 2004.

studies by incorporation of samples from the harvest as a sampling period, to evaluate closure assumptions, and to maximize the potential to increase recapture probabilities.

Methods

Study area

The study area included a 671 km^2 area of the Ottawa National Forest in Gogebic, Iron, Houghton, and Ontonagon Counties in the Upper Peninsula (Figure 5.2). The study area was predominated by hardwoods and mixed hardwoods stands with scattered swamps. An area of conifers and mixed conifer-hardwoods stretched across the site in a northwest to southeast direction. Small areas of conifers were scattered throughout the area (USFS 2004). Large stands of aspen and birch were located in the northwestern portion of the study site. In addition to martens and fishers, mink (*Mustela vison*), short-tailed weasels (*Mustela erminea*) and long-tailed weasels (*Mustela frenata*) inhabit the area.

High-use state routes and a forest highway surrounded the study area and potentially geographically restrict movements by resident martens and fishers. This area included the 1961 fisher reintroduction location and the source locations for the 1988-1992 fisher translocations. The area was also close to the 1981 Webb Lake and Perch Lake marten reintroduction locations as well as the Nicolet National Forest Fisher Management Unit, Wisconsin, where martens and fishers were reintroduced in the 1950s through the 1970s. Fishers and martens have been harvested annually from the study area since 1989 and 2000, respectively (Cooley et al. 2001, Frawley 2002). However, the densities of historical harvests of both species are low in the study area relative to nearby

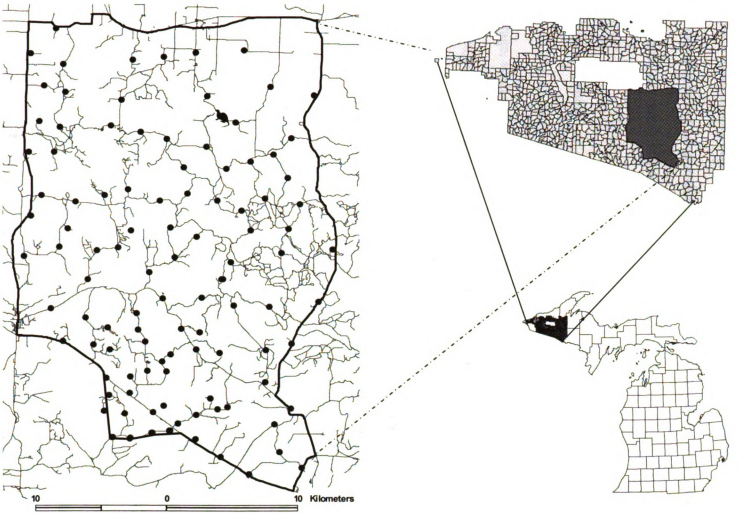

Figure 5.2. Location of the 671 km^2 study area in the Ottawa National Forest, Upper Peninsula, Michigan. All access routes are shown, including roads and two-tracks. The area includes the site of the 1961 fisher reintroduction, shown by the fisher icon. Each of the 133 trap locations is designated by a point.

regions of comparable size (Figure 5.1 and Figure 5.2). Patterns of dispersion for martens and fishers were examined at densities when competitive exclusion is not a likely factor in determining distribution. Additionally, we were able to assess the viability of the trapping design for populations found in low densities or patchy areas similar to many threatened or endangered species.

Trapping methods

The sexually dimorphic fisher is much larger than its congener, the marten. On average, adult males and females often weigh 3.5 to 5.5 kg and 2.0 to 2.5 kg, respectively (Powell 1993). In contrast, average body mass of martens has been reported to range from 0.53 kg in adult females to 1.05 kg in adult males (de Vos 1951, Strickland et al. 1982). The different sizes of the two species results in dissimilar spacing and dispersal patterns. Fisher home ranges have been estimated to be between 35 to 49 km^2 for males and 7.5 and 15 km^2 for females in Michigan and Wisconsin (Powell 1977, Johnson 1984). Mean natal dispersal was reported to be 10 km (Arthur et al. 1993). Average marten home ranges have been estimated across the species range to be 5.6 km^2 for males and 2.9 km^2 for females (Powell 1994). Dispersal distances were expected to be proportionally less than that of fishers, however, maximum dispersal distance for martens have been reported up to a distance of 61 km in British Columbia (Lofroth 1993).

Fishers and martens are agile climbers. This behavior allowed use of a single trap design to collect hair from both species. However, the differences in spatial distribution and dispersal affected the distribution of traps in the study area to maximize capture probabilities for fishers and martens concurrently.

Wooden triangle "squeeze tubes" fashioned after Foran et al. (1997b) were fastened to trees at a height of approximately 1.5 m. Tubes were approximately half a meter in length and were open at the top and bottom. Two sizes of traps were built to target each species. Fisher traps were constructed from 1 x 12 inch lumber and marten traps were constructed from 1 x 10 inch lumber to maximize contact of the animal with the hair snares. Traps were attached to trees of minimum diameter of 30 cm using rubber cord (Rubber Rope Products Co., Inc., Watersmeet, MI). Lumber was boiled with maple bark or dyed with speed dip (Andy Stoe's Speed Dip, Penn Yan, NY) to darken the wood for camouflage and to lessen processed odors.

Hair snares for each species have been designed based on the assumption that animals can be attracted to bait. Castor-based long-range signal lure was placed at each trap site. Beaver (*Castor canadensis*) meat was used as bait for each species. Frozen carcasses were dissected into pieces approximately 10 cm in diameter. Bait was placed in tubular mesh bags fastened to the top of the trap. A nail in the center of the trap was used to hook the mesh to prohibit the animal from easily removing the bait from the trap. Patches of adhesive glue (commercially available mouse/rat traps: Catchmaster®) approximately 3 x 3 cm were tacked to the inside of the tube at both ends. As the animal attempted to remove the bait, contact with the glue patches removed hair for use in DNA analysis.

D. Foran (Michigan State University, personal communication) found that multiple "captures" was not a confounding factor for American martens. Similarly, Mowat and Paetkau (2002) found no evidence of trap visitation by more than a single animal. Once an animal had chewed into the mesh to procure the bait, the meat dropped

out of the trap. The trap design limited the potential for multiple "captures" of hair from different animals.

Glue patches with hair were removed from the trap and placed in small plastic snap-top containers partially filled with silica. Hairs were stored at room temperature until lab analyses could be initiated. If hair was deposited on both top and bottom glue patches, they were considered independent samples and placed in different vials. Hair found on different glue patches that were different in appearance (e.g., color, pattern) were also treated as separate samples. All samples were collected for genetic identification of species.

DNA was extracted within seven days of collection. Glue was removed from the hair using chloroform (Foran et al. 1997b). Hair roots were removed from the hair shafts and placed in a 1.5mL tube.

Hair snare placement

Mark-recapture experiments often use a systematic grid design where female home range size determined grid size (White et al. 1982, Mowat and Strobeck 2000). Four traps per grid is the suggested density to increase probability of an animal encountering a trap site and being captured, subsequently increasing the precision of the population estimate (Otis et al. 1978, White et al. 1982). Based on this grid design, 179 and 926 traps would have needed to be set in our field site to target fishers and martens, respectively. This was not feasible given constraints of available personnel and access.

A systematic grid design was implemented for this study, but was modified to accommodate both target species into a single grid. Likewise, trap density was reduced.

Female home range size was averaged across martens and fishers (8.95 km^2). The diameter of the resulting average home range was calculated (1.7 km) and used as the distance between the centers of each grid. Based on these calculations, 133 traps were spaced approximately 1.5 to 2 km apart across the study site (Figure 5.2). However, traps in the northern portion of the study area were placed at intervals greater than 2 km due to access and logging operations. Conversely, traps in the south-western portion of the study site were spaced at distances of less than 1.5 km to assess probability of multiple captures of individuals over shorter distances.

High-use roads were used as boundaries. However, a small portion of the grid overlapped a main thoroughfare on the south-western portion of the study area to test the effects of such a road on marten and fisher movements and thus assess the assumption of closure in the study.

Traps were set in varied forest stand types, the only limitation being the presence of a minimum of one tree greater than approximately 30 cm in diameter. The 133 traps included 80 fisher-sized traps and 53 marten-sized traps. Trap size did not preclude either species from entering the trap depending on the size of tree upon which the trap was placed. A larger tree would increase the space inside a marten-sized squeeze tube, thereby allowing a fisher access to the bait.

Traps were set during the spring and summer of 2004 without glue traps and bait. The area around each trap was pre-baited with meat scraps during September 2004. Two weeks following pre-baiting, glue traps and bait were affixed to the traps. Traps were checked weekly over four consecutive week-long trapping periods conducted from 26 September to 24 October, 2004. Harvest season for martens and fishers (1-10 December)

was used as a final recapture period in the population estimate. Late fall was chosen for the hair snaring periods to minimize the time between sample collection and harvest without overlapping deer firearm season.

Microsatellite analysis

To establish probabilities of individual identity for multi-locus genotypes characterized from hair samples collected during the capture period, a baseline genetic database was created using 112 martens and 453 fishers harvested in the western Upper Peninsula of Michigan from 2000 to 2004. A suite of 11 (Ma-2, Ma-5, Ma-8, Ma-14, Ma-19, Gg-3, Gg-7, Tt-4, Mvis072, Mer022, and Mer041; Davis and Strobeck 1998, Fleming et al. 1999) and 10 (Ma-1, Ma-2, Ma-19, Gg-3, Gg-7, Tt-1, Tt-4, Mvis002, Mvis072, Mer022, and Mer041; Davis and Strobeck 1998, Fleming et al. 1999) microsatellite markers for martens and fisher, respectively, were screened across all background samples to determine allele frequencies and expected genotype frequencies under Hardy-Weinberg equilibrium and were used to determine probability of identity.

The number of microsatellite loci used in a non-invasive genetic study depends on the goal of the study to resolve individual relationships, population size, degree of consanguinity within the population, and variability of the loci. Mark-recapture population estimate studies should ideally utilize the smallest number of highly variable microsatellite loci needed to discriminate individual genotypes. Too few loci will result in an underestimate of population size due to an increased non-zero probability of two individuals sharing the same genotype. Too many loci will inflate the estimate of population size through the multiplicative effect of genotyping error (Waits et al. 2001).

The trap design for this study was developed to target the tree climbing behavior of fishers and martens. However, congeners such as mink and short-tailed (*Mustela erminea*) or long-tailed weasels (*Mustela frenata*) are present on the study area and would be expected to be attracted to the bait at trap sites. Other non-target species would also be expected to deposit hair, including red squirrels (*Tamiasciurus hudsonicus*) and flying squirrels (*Glaucomys* spp.). Many of the microsatellite markers developed by Davis and Strobeck (1998) and Fleming (1999) cross-amplified in multiple mustelid species. To identify and remove congeners from analysis a genetic baseline dataset across all loci was developed using long-tailed and short-tailed weasel tissue samples and compared to genotypes in the marten and fisher baseline datasets.

All hair samples were genotypes at 2 loci (Gg-3 and Gg-7). Locus Gg-3 (Davis and Strobeck 1998) was discovered to be monomorphic (one allele) in fishers. The allele differed in size from the alleles documented in all sampled martens, but we observed an overlap in allele size between martens and other *Mustela* species. A second locus, Gg-7, was used to separate martens from short-tailed and long-tailed weasels based on differences in allele sizes. In addition, a mammal sexing primer, TET SRY/ZFX (Aasen and Medrano 1990, Taberlet et al. 1993, Woods et al. 1999; Appendix 5.A), was used to determine DNA quality. If an individual did not amplify at the sexing marker, DNA quality and/or quantity was believed to be deficient ant the sample was removed from further analysis. All hair samples were amplified using these three markers. Samples that failed to produce PCR product at the three loci were culled. Samples that resulted in product at the Gg-3 and/or Gg-7 locus were identified as fishers, martens or unknowns, and were subsequently genotyped at the remaining loci. Unknowns were likely non-

target species but possessed alleles both present and absent in background samples of martens and fishers. To confidently rule out unknowns as non-target species, the samples were genotyped at all loci. The remaining loci were determined using estimates of probability of identity derived from estimates of allele frequencies from baseline samples (see below).

DNA was extracted from each hair sample using Quiagen DNeasy® Tissue Kits. DNA was assumed to be of low concentration and was not quantified or diluted. DNA was amplified at each locus for every hair sample using PCR. DNA was amplified using each primer in a 10μL reaction with the following conditions: 5 μL of template, 2 pmol reverse primer, 2 pmol forward primer, dNTPs at 200 μM each, 200 μM 10x PCR2 buffer (1 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) or LGL buffer (1 mM Tris-HCl pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl, 10 μg/mL BSA, 0.0025% Tween 20), and 0.2 units of Taq DNA polymerase. The thermal profile for PCR amplification was 94°C for 2 minutes, followed by 42 cycles of 94°C for 45 seconds, primer specific annealing temperature for 1 minute (Appendix 5.A, Appendix 5.B), and 72°C for 1 minute, followed by a final 5 minute extension at 72°C. The amplified product was separated on a 6% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™), Lincoln, NB. Molecular weight standards and individuals of known genotypes from the background samples were systematically included on each gel. Fragments were viewed using Saga Generation 2 software, LiCor, Lincoln, NB.

Quality control

Errors can be introduced into a genetics study at all stages. Consistency in field

and laboratory methods and timing was critical to reduce contamination or other human-caused errors. Because the samples used in this study were non-invasively collected hairs, DNA quantities were assumed to be very low. In addition, although traps were checked every 7 days, the time from deposition of a sample until DNA extraction was a minimum of 5 days and a maximum of 10. Variable weather conditions (e.g., heat, rain) might have aided in degradation of DNA. All of these factors could result in genotyping errors leading to misidentification of individuals and ultimately overestimation of population. However, a series of quality control protocols based on Paetkau (2003) were used to minimize genotyping errors to a negligible level. These protocols were instituted beginning at the DNA extraction through the analysis process.

Previous studies have shown that the greater the number of hair follicles extracted in a sample, the lower the genotyping error rate. Goossens et al. (1998) estimated error rate was decreased from 14% genotyping error for extraction of a single hair to 4.86% and 0.29% for three and 10 hairs, respectively, in the alpine marmot (*Marmota marmota*, Sciuridae). Similarly, Dreher (2004) estimated a total error rate of 4.21% using five or more hairs per sample. In this study, extraction was performed for samples with a minimum of five follicle-containing hairs. If possible, $\geq$ 10 hairs were used per sample. Due to the assumed low quantity of DNA from the extracted hair, the amount of template was increased to 5 μL in a 10 μL reaction.

Saga Generation 2 software was used to genotype samples at each locus. This software allowed for direct export of allele sizes into a database, eliminating transcription errors. A minimum of two experienced laboratory personnel independently examined genotypes of all individuals. Samples inconsistent in the independently recorded

genotypes were amplified a second time at the locus (loci) in question. Any sample that did not amplify at three or more of the six loci was removed from further analyses. Samples exhibiting three or more alleles at a locus, indicative of visits by more than one animal, were also removed.

In most cases, the probability of genotyping error was low. However, mismatching of individuals in a mark-recapture data set was of concern and most often occurs as a genotyping error at one locus or less likely at two loci. As a result, a genotyped individual that differed from another genotyped individual by one (1-MM, 1-mismatch pair) or two (2-MM, 2 mismatch pairs) markers would be closely inspected (Paetkau 2003). Mismatched samples were identified using the program GENECAP (Wilberg and Dreher 2004). If a mismatch could not be resolved through visual inspection of the alleles on the gels, the samples were reamplified. If the genotype differences were confirmed after reamplification, samples were considered to have come from two different individuals. In addition, matched samples were examined spatially by trapping period to identify unlikely distances traveled for a recapture.

Statistical analysis

Probability of identity and marker selection

The probability of identity, $P_{(ID)}$, an estimator of the probability of matching genotypes in a population (Paetkau and Strobeck 1994) was estimated as:

$$P_{(ID)} = \Sigma p_i^4 + \Sigma\Sigma(2p_ip_j)^2 \quad (1)$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles respectively and $i \neq j$ (Paetkau and Strobeck 1994), or as:

$$P_{(ID)unbiased} = \frac{n^3(2a_2^2 - a_4) - 2n^2(a_3 + 2a_2) + n(9a_2 + 2) - 6}{(n-1)(n-2)(n-3)} \quad (2)$$

correcting for sample size where n is the sample size, $a_i = \Sigma p_j^i$, and p_j is the frequency of the j^{th} allele (Paetkau et al. 1998). However, in populations exhibiting substructure, assumptions underlying Hardy-Weinberg equilibrium and linkage disequilibrium, and consequently assumptions of the theoretical $P_{(ID)}$, are often violated (Waits et al. 2001). The resulting bias can lead to overestimation of actual $P_{(ID)}$ (Waits et al. 2001). A more conservative estimator is $P_{(ID)sib}$ which accounts for the probability of matching genotypes between two full siblings in the population and can be represented by the following equation:

$$P_{(ID)sib} = 0.25 + (0.5\ \Sigma p_i^2) + [0.5(\Sigma p_i^2)^2] - (0.25\ \Sigma p_i^4) \quad (3)$$

where p_i is the frequency of the i^{th} allele (Paetkau and Strobeck 1994, Evett and Weir 1998). The actual probability of identity value of the population is most likely between $P_{(ID)}$ and $P_{(ID)sib}$ (Wilberg and Dreher 2004).

Paetkau (2003) suggested an *ad hoc* guideline for selection of markers based on mean expected heterozygosity (H_E). Six microsatellite markers were deemed sufficient if mean H_E was between 0.7 and 0.8 based on a background sample. Heterozygosity of the

background samples for martens and fishers were calculated using MICROSATELLITE ANALYSER version 3.12 (Dieringer and Schlötterer 2003). Allele frequencies were estimated using GENECAP (Wilberg and Dreher 2004). $P_{(ID)}$ and $P_{(ID)sib}$ were calculated for each locus using allele frequencies. A subset of loci was chosen for each species to maximize $P_{(ID)}$ and $P_{(ID)sib}$ over all loci. The selected loci were tested for Hardy-Weinberg and linkage equilibrium using the exact test of Guo and Thompson (1992) performed using the Markov chain Monte Carlo (MCMC) approach implemented in program GENEPOP version 3.4 (Raymond and Rousset 1995). Bonferroni tests (Rice 1989) were used to correct for multiple tests.

A total of six loci for each species were chosen to characterize individuals for mark-recapture population estimation. In addition to the fixed Gg-3 locus and polymorphic Gg-7 locus, Ma-1, Ma-2, Ma-19, and Tt-1 (Davis and Strobeck 1998; Appendix 5.A) were chosen to develop multilocus genotypes for fishers on the field site. Six polymorphic loci were chosen to genotype martens from the field site: Gg-3, Gg-7, Ma-2, Ma-5, Ma-8, and Ma-19 (Davis and Strobeck 1998; Appendix 5.B).

Population estimation

Previous mark-recapture studies have used closed capture models in program CAPTURE (White et al. 1982, Mowat and Paetkau 2002). We anticipated small sample sizes ($N < 50$) for both fishers and martens based on sparse distribution of historical harvests on the study area. Acknowledging the limitations of small numbers of captures and recaptures, we used different population estimation techniques to test the appropriate method and converge on an estimate of $\hat{N}$. To assess the usefulness of adding harvest as

a final capture period, all population estimate methods were run for data that included harvest within the boundaries of the study area and again for data that did not include harvest.

Program CAPTURE

CAPTURE as implemented in program MARK (White and Burnham 1999) was used in this study to determine fisher and marten population size. Models were chosen based on knowledge of the biology of the species, field methods, and the model selection test in CAPTURE. A time response (model M_t) was expected over the course of the four week-long trapping sessions and harvest recapture period due to differences in environmental factors (e.g., temperature, precipitation). It was also probable that a behavioral response (M_b) may have resulted from the trapping method. A "trap-happy" response could have occurred from the use of the bait as a reward. Conversely, a "trap-shy" response might have resulted from removing hair.

Heterogeneity is described as random individual variation (Otis et al. 1978). Trap density affects heterogeneity. Otis et al. (1978) suggested placement of four traps per home range per occasion to reduce heterogeneity. This density is often unattainable in large-scale studies due to time, manpower, and budgetary constraints. However, lower trap density will tend to increase heterogeneity. Due to the constraints on this study and methods used for two different species, it was likely heterogeneity was a factor in the probabilities of capture. We tested for the effects of heterogeneity (M_h) using program CAPTURE.

All models were tested against the null (M_o) which does not allow for any variation in capturability, thereby considered biologically unrealistic. Models were also tested that combined responses. M_{tb} would account for both a time and behavioral response. Time and heterogeneity would be tested using model M_{th}.

Program MARK

In addition to CAPTURE, closed capture models in MARK were used to account for capture and behavioral responses by capture occasion and incorporating genetic misidentification into closed capture models (Lukacs and Burnham 2005). Assumptions of these models include geographic and demographic closure, use of a number of loci sufficient to distinguish individuals, and genotyping errors that will create unique genotypes not existing in the population. The models were developed by Lukacs and Burnham (2005) and include an additional parameter, α, defined as the probability an individual was correctly genotyped given its first observation. Capture histories are used to derive the value of α, however, a pre-measured estimate of genotyping error can also be used (Lukacs and Burnham 2005). Genotyping error rate was not directly estimated in this study due to the lack of "control" samples, or individuals from which we obtained both tissue and hair samples. Consequently, a derived value of α was used in each model.

Similar to above for CAPTURE, *a priori* models were constructed for fishers and martens based on knowledge of the biology of each species and field methodology. Model parameters as described below are based on Otis et al. (1978) and are noted as follows:

p_i = probability of an individual's initial capture in time period i

c_i = probability of an individual's recapture in time period i

N = population size

The first model accounted for the probability of temporal variation across all capture occasions (model M_t, parameters $p_1, p_2, p_3, p_4, p_5, c_2, c_3, c_4, c_5, N$). Because harvested individuals were not caught using the same mechanism as the hair snares, the behavioral response was tested only during the snaring periods, treating harvest separately (M_b, parameters $p_{1-4}, c_{2-4}, p_5, c_5, N$). However, a 'trap-happy' response may have resulted from the use of bait in both hair snares and techniques used for harvest. This model was also tested (model $M_{b+harvest}$, parameters p_{1-5}, c_{2-5}, N). The three competing models were ranked based on Akaike Information Criterion (AIC_c) to determine which models were best supported by the data (Burnham and Anderson 1998).

Program *Capwire*

Program *Capwire* (Miller et al. 2005) was used as an additional method to estimate population size of both martens and fishers. *Capwire* accounts for multiple observations of an individual within a session and was developed to accommodate non-invasive genetic sampling (Miller et al. 2005). Population estimation in traditional mark-recapture studies is based on the probability of capture of each individual in each trapping period. An animal is either observed or not observed. Therefore, data is potentially wasted if an individual is observed more than once within a trapping session. Although

MARK allows for differences in capture responses by occasion, it does not account for multiple captures within an occasion. By combining all observations of all individuals across sampling events into a single session, *Capwire* maximizes the information that can be used to generate a population estimate. *Capwire* has been reported to be effective at estimating size of small ($N < 100$ individuals) populations (Miller et al. 2005), as was expected in this study. Assumptions of this method include individuals are correctly identified by their genotypes, individual genotypes are unique, and no trap response (Miller et al. 2005).

Capwire was run for both fishers and martens using the Likelihood Ratio Test to choose between two models: the even capturability model (ECM) and the two innate rates model (TIRM). The ECM is based on equal capture probability for all individuals. The TIRM allows for heterogeneity (Miller et al. 2005). The ECM was rejected when the likelihood value was less than 0.10 as suggested by Miller et al. (2005).

Chapman estimator

Due to a very small sample size ($N < 25$) of marten observations anticipated on the study area, a Chapman estimator (Chapman 1951) was used to generate a baseline population estimate to which $\hat{N}$ from CAPTURE, MARK, and *Capwire* could be compared. The Chapman estimator is represented as:

$$\hat{N}_c = \frac{(M+1)(n+1)}{(m+1)} - 1 \qquad (4)$$

Where N is the population size, M is the number of animals captured and marked during

the first trapping session, n is the number of animals captured in a subsequent trapping session, of which m already have marks. The Chapman estimator has a variance of:

$$\mathrm{Var}(\hat{N}_c) = \frac{(M+1)(n+1)(M-m)(n-m)}{(m+1)^2(m+2)} \qquad (5)$$

Martens were grouped into two sessions for the population estimation. Session one included trapping periods one and two. Session two included trapping periods three and four. When harvest of martens on the study area was included, the captures were added to session two. The assumptions of the estimator include population closure, equal catchability, and permanence of marks.

Use of harvest samples and assumption of population closure

The assumption of population closure in mark-recapture analyses is of fundamental importance, and can be separated into demographic and geographic closure components (White et al. 1982). Demographic closure assumes no births, deaths or permanent immigration or emigration during the course of the study. Errors based on lack of demographic closure in fishers and martens are expected to be small for the four weeks over which hair samples were collected. However, it is not known if dispersal or mortality occurred between the hair snaring period and harvest, effecting this assumption.

Geographic closure assumes no individuals move on or off the study area between trapping periods. Presence of animals with home ranges overlapping study boundaries will tend to positively bias the population estimate (White et al. 1982, Boutin 1984). Bias can be minimized by physical boundaries that prevent movement onto or off the study

area. Bias is also minimized by sampling a large study area compared to average home range size of target species and sampling over a short duration to reduce probability of long-range movements (e.g., dispersal; White et al. 1982). Although relatively high-use roads were used as boundaries of our study area, geographic closure was likely to not have been absolute.

Harvest samples were used as a final recapture period in the mark-recapture population estimation methodology. These samples were also used to test the assumptions of geographic closure of each species on our study area. In December of 2004, tissue samples were collected from harvested martens and fishers by the MDNR. All fishers and martens harvested on the study area were used as a recapture period. In addition, a series of sequential buffers of the study area were created to examine the validity of the assumption of closure in the mark-recapture model (Figure 5.3). The width of each buffer was calculated as the diameter of maximum male home range for each species, a distance used to detect movements of both sexes from the study area. Three sequential buffer strips were delineated for fishers (6.96 km, 13.92 km, and 20.88 km) and martens (3.21 km, 6.42 km, and 9.63 km). Individuals harvested from these areas were added to the final recaptures to detect matches with animals on the study area.

Results

Probability of identity

Mean H_E in the baseline data was 0.708 for the six markers used for fishers. Mean H_E was 0.715 for the six marten microsatellite markers. No deviations from Hardy-Weinberg or linkage equilibrium were detected in the baseline fisher samples. No

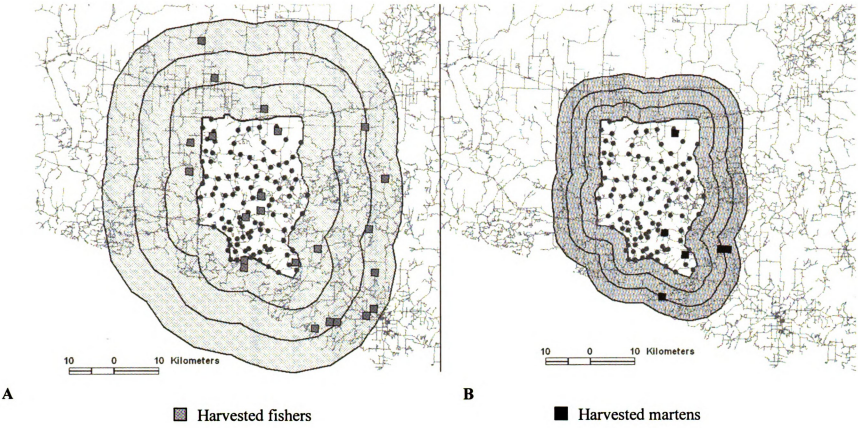

Figure 5.3. Buffers extended beyond the boundary of the study site to detect harvest reporting error and violations of the assumption of closure using harvest samples. Buffers were calculated as the diameter of a mean male home range. Fisher buffers are shown in A and are each 6.96 km wide. Marten buffers shown in B are each 3.21 km wide. Locations of individuals harvested to 1.6 km^2 during the 2004 season are displayed as shaded squares. Dots represent trap locations.

deviations from Hardy-Weinberg were detected in the baseline marten samples, although linkage disequilibrium was detected in the locus-locus combination of Ma-2 and Ma-8.

Probability of identity was another indicator of appropriateness of the markers selected. $P_{(ID)}$ calculated across all six loci for the fishers in this study was 3.39×10^{-6}. $P_{(ID)sib}$ was estimated to be 5.49×10^{-3} ($N = 453$). Although the true probability of identity value most likely lay between the calculations, the number of fishers in the immediate area of the study site allowed for a very low probability of two individuals sharing the same genotype based on the conservative estimate ($P_{(ID)sib}$).

$P_{(ID)}$ calculated across the six loci used to distinguish martens on the field site was 3.49×10^{-6}. The estimate of $P_{(ID)sib}$ was 5.56×10^{-3} ($N = 112$). Similar to the fishers, the estimated number of martens around the study area was lower than the conservative probability that two individuals share the same genotype.

The microsatellite markers used in this study for both martens and fishers appeared sufficient to distinguish individuals. The expected population genotype frequencies based on estimates of $P_{(ID)sib}$ were 179 and 147 for fishers and martens, respectively, and an order of magnitude larger than the estimated population size based on density.

Trap success and quality control

During the four trapping sessions, hair was collected from 87.2% of the traps. Between 41.4% and 48.1% baits were removed each trapping period (Table 5.1). Bait removed from a trap did not necessarily result in hair deposited on the glue pads. In

Table 5.1. Summary of animal visitations at fisher and marten traps during September and October 2004 and harvests on the study site and within each buffer strip.

	Trap period 1	*Trap period 2*	*Trap period 3*	*Trap period 4*	Harvest			
					Study area	*Buffer 1*	*Buffer 2*	*Buffer 3*
# of traps with baits taken	55 (41.4%)	61 (45.9%)	64 (48.1%)	64 (48.1%)	-	-	-	-
# traps with hair	58 (43.6%)	61 (45.9%)	74 (55.6%)	66 (49.6%)	-	-	-	-
# traps with hair and no bait	9 (6.8%)	7 (5.3%)	16 (12.0%)	10 (7.5%)	-	-	-	-
Number of fishers captured	17	22	35	13	6	6	5	6
Number of fishers recaptured	-	21	26	10	1	0	1	0
Cumulative unique fishers	11	25	36	37	42	48	53	59
Number of martens captured	3	6	3	10	3	0	2	1
Number of martens recaptured	-	6	3	8	1	0	0	0
Cumulative unique martens	3	8	8	12	14	14	16	17
Number of non-target mustelids	9	7	7	8	-	-	-	-

contrast, hair was sometimes found on glue pads in traps where the bait was not taken (N = 42). The number of hair samples collected increased from 58 in trapping session one to 74 in session 3 followed by a decrease to 66 hair samples in session 4. The number of hair samples deposited when the bait remained in the trap followed a similar trend (9 samples collected in trapping period 1 increasing to 16 in period 3 and decreasing to 10 in period 4). The number of traps per session resulting in hair samples ranged from 44% during trapping period one to 56% during the third trapping period. The trapping success in period 4 was 49.6%.

Two hundred fifty-nine hair samples were collected during the four trapping occasions for genetic analysis. Following inspection of the quantity and quality of each sample, 19 were culled prior to extraction. One hundred nine samples were identified as fisher. Forty-three samples were identified as marten. There were 9 unknown species and 79 non-amplifications. The mammal sexing primer amplified for 34 of the 79 samples where no Gg-3 genotype was resolved, suggesting collection of 34 non-mustelid mammal samples, and 45 samples of poor quality or with negligible amounts of DNA. Three samples were removed due to presence of three or more alleles, indicating visitation by more than one animal. Forty-five samples did not amplify at both the Gg-3 locus and the sexing locus and were culled from further analysis.

Eighty-eight fisher samples and 22 marten samples were tested for mismatches (1-MM and 2-MM) including all harvested samples (N = 23 fishers, N = 6 martens; see below). Mismatches between harvested individuals were ignored due to the certainty of presence of two different individuals. One-hundred nineteen matched, eight 1-MM, and six 2-MM fisher genotypes were identified. Twenty-one matched, zero 1-MM, and one

2-MM marten genotypes were identified.

After the final reanalysis, no further culls were made. One hundred-twenty-seven matched, three 1-MM, and twelve 2-MM fisher genotypes were recorded. Twenty-one matched, zero 1-MM, and three 2-MM marten genotypes were identified. All matches were assigned a $P_{(ID)sib}$ value < 0.05.

Thirty-one samples were identified as putative *Mustela* spp. These samples were collected from traps located across the entire study area. No further analyses were performed using these individuals.

Eighty-seven fisher samples were detected by the hair snares across all trapping periods (Figure 5.4, Table 5.1). Detections increased from 17 fisher samples in trapping period 1 to 35 fisher samples in trapping period 3, followed by a decrease to 13 fisher samples in period 4. The percentage of traps that yielded fisher observations over the course of the four trapping sessions was 36.8%. Thirty-seven unique fisher genotypes were identified for use in the population estimation models. Twenty-one individuals resulted in 33 recaptures. Average recapture rate was 64%. Six fishers were legally harvested on the study area during the December 2004 trapping season. One harvested fisher was a recapture. The spatial dispersion of the captures and recaptures are summarized in Figure 5.4. Six trapped individuals were added through extension of the first buffer of the field site (Figure 5.3). Five additional harvested fishers, including one recapture, were included with the second buffer. The third and final buffer added six fishers. The mean maximum distance between fisher recaptures was 2.71 km (range 0 – 11.42 km). This distance includes harvest recaptures. If harvested individuals were removed, mean distance between recaptures was 2.30 km (range 0 – 8.05 km).

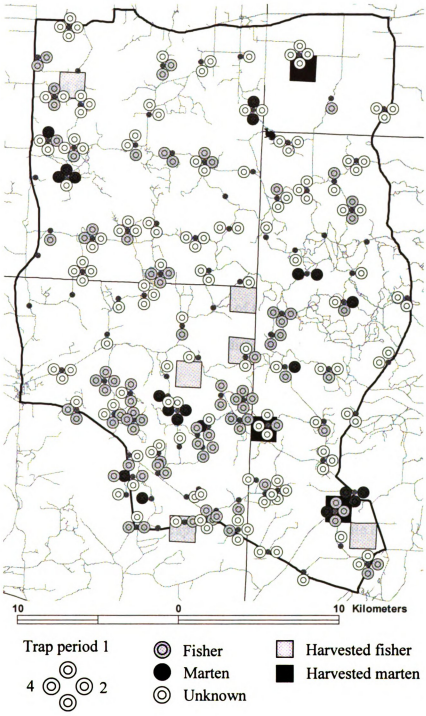

Figure 5.4. Map of the study area in the Upper Peninsula showing results from four trapping sessions and harvest. Shaded circles shown in chronological order of trapping period represent the locations of genetically determined marten and fisher trap visitations. Squares represent locations of individuals legally harvested in December 2004 and used as a final recapture for mark-recapture population estimation.

Twenty-two marten samples were detected over the course of the four trapping sessions (Figure 5.4, Table 5.1). The greatest number of marten samples was collected during the fourth and final trapping session. Twelve unique marten genotypes were identified to estimate population size. The percentage of traps in which marten hairs were collected over the duration of the trapping periods was 9.8%. Ten recaptures were made by five individuals, resulting in an average recapture rate of 32%. Three martens were legally harvested within the boundaries of the study area. One trapped marten was a recapture. The spatial dispersion of these martens is shown in Figure 5.4. No harvested martens were added in the first buffer. A total of two and one martens were added in the second and third buffers, respectively. Mean distance between marten recaptures was 0.91 km (range 0 – 3.15 km).

Many more fishers than martens were identified, indicating a difference in the abundance of each species on the study area. Figure 5.4 shows a greater dispersion of fishers across the study area than martens, which appeared to be located in discrete areas. The mean distance between fisher recaptures was more than double the average distance between marten recaptures.

Population estimation

Program CAPTURE

The model selection process in CAPTURE indicated M_t as the most appropriate model (1.00) followed by M_{th} (0.76) in the fisher dataset including harvest on the study area. Heterogeneity was not significantly supported against the null model ($P = 0.06$), nor was a behavioral response ($P = 0.77$). However, M_t was significantly supported ($P =$

0.00003). The population estimate using the time response model was 50 (95% CI = 46 – 61).

When harvest was removed as a final capture-recapture period, M_t was again the most appropriate model (1.00) followed by M_{th} (0.73). Neither heterogeneity nor a behavioral response was significantly supported against the null ($P = 0.20$, $P = 0.46$, respectively), whereas M_t was significantly supported ($P = 0.0001$). The population estimate using M_t without harvested fishers on the study site was 40 (95% CI = 38 – 48).

The analysis of marten observations including harvests on the study area using CAPTURE yielded the null (M_o) as the most appropriate model (1.00) followed by M_h (0.92). No models were significant against the null ($P > 0.05$). This is likely a result of the very small number of captures and recaptures. Due to the unrealistic nature of M_o, the population estimate was calculated using the jackknife model M_h. The resulting population estimate was 29 (95% CI = 21 – 48).

The removal of marten harvest as a final observation resulted in a sample size too small to test all models. However, similar to above, the null was suggested as most appropriate (1.00) followed by M_h (0.83). No models that were run were significant against the null ($P > 0.05$). The jackknife model M_h presented a population estimate of 20 (95% CI = 16 – 34).

Program MARK

Due to the small number of fisher and marten captures and recaptures used for the population estimate, significant differences were not expected in the results generated

from each model within MARK. In general, the models incorporating genotyping error resulted in lower estimates of population size than the models run in CAPTURE.

The model best supported by the data for fishers including harvests on the study area was M_t as indicated by the lowest AIC_c value (Table 5.2). The population estimate derived from this model was 39 fishers (95% CI = 27 – 51). This estimate was less than the number of unique fisher genotypes used in the calculation ($N = 43$).

With the removal of the harvested fishers, M_t was again the model best supported by the data (Table 5.3). However, model M_b could not be run, presumably due to overparameterization. M_t resulted in a population estimate of 34 fishers (95% CI = 24 – 44), a value less than the number of unique genotypes entered into the calculation ($N = 37$).

Model M_b, incorporating a behavioral response, was best supported by the marten data including harvests on the study area (Table 5.4). Model $M_{b+harvest}$ resulted in a confidence interval that included a negative number and was removed from the model selection process. The population estimate derived from M_b was 9 martens (95% CI = 3 – 15). Similar to the fishers, the estimate is less than the number of unique genotypes used to calculate $\hat{N}$ ($N = 14$).

With removal of martens harvested on the study area, the model best supported by the data was M_b (Table 5.5). The resulting population estimate was 8 (95% CI = 3 – 13). This value was again less than the number of unique genotypes entered into the calculation ($N = 12$).

Table 5.2. Closed capture estimation models using program MARK for observed and harvested fishers on the study area including model name, parameters, AIC_c, Δ_i, w_i, population estimate, and the 95% confidence interval about the estimate.

Model Name	Parameters	N[a]	AIC_c[b]	Δ_i[c]	w_i[d]	$\hat{N}$[e]	95% CI
M_t with genotyping error	$p_1, p_2, p_3, p_4, p_5, c_2, c_3, c_4, c_5, N$	43	-2.0401	0.000	0.984	39	27-51
M_b with genotyping error	$p_{1\text{-}4,5}, c_{2\text{-}4,5}, N$	43	6.1817	8.222	0.016	37	23-52
$M_{b+harvest}$ with genotyping error	$p_{1\text{-}5}, c_{2\text{-}5}, N$	43	33.0556	35.100	0.000	47	22-73

[a] Number of unique fisher genotypes used in estimation

[b] Akaike's information criterion including a bias correcting term.

[c] Difference in AIC_c

[d] AICc weighting factor representing the weight in evidence between models. This value is scaled to 1.

[e] Population estimate

Table 5.3. Closed capture estimation models using program MARK for observed fishers (excluding harvest) on the study area including model name, parameters, AIC_c, Δ_i, w_i, population estimate, and the 95% confidence interval about the estimate.

Model Name	Parameters	N[a]	AIC_c[b]	Δ_i[c]	w_i[d]	$\hat{N}$[e]	95% CI
M_t with genotyping error	$p_1, p_2, p_3, p_4, p_5, c_2, c_3, c_4, c_5, N$	37	-5.4669	0.000	0.999	34	24-44
$M_{b+harvest}$ with genotyping error	p_{1-5}, c_{2-5}, N	37	8.2707	13.74	0.001	41	20-61

[a] Number of unique fisher genotypes used in estimation

[b] Akaike's information criterion including a bias correcting term.

[c] Difference in AIC_c

[d] AICc weighting factor representing the weight in evidence between models. This value is scaled to 1.

[e] Population estimate

Table 5.4. Closed capture estimation models using program MARK observed and harvested martens on the study area including model name, parameters, AIC_c, Δ_i, w_i, population estimate, and the 95% confidence interval about the estimate.

Model Name	Parameters	N[a]	AIC_c[b]	Δ_i[c]	w_i[d]	$\hat{N}$[e]	95% CI
M_b with genotyping error	$p_{1\text{-}4,5}$, $c_{2\text{-}4,5}$, N	14	38.0475	0.000	0.812	9	3-15
M_t with genotyping error	$p_1, p_2, p_3, p_4, p_5, c_2, c_3, c_4, c_5, N$	14	40.9721	2.925	0.188	9	3-14

[a] Number of unique marten genotypes used in estimation

[b] Akaike's information criterion including a bias correcting term.

[c] Difference in AIC_c

[d] AIC_c weighting factor representing the weight in evidence between models. This value is scaled to 1.

[e] Population estimate

Table 5.5. Closed capture estimation models using program MARK for observed martens (excluding harvest) on the study area including model name, parameters, AIC_c, Δ_i, w_i, population estimate, and the 95% confidence interval about the estimate.

Model Name	Parameters	N[a]	AIC_c[b]	Δ_i[c]	w_i[d]	$\hat{N}$[e]	95% CI
M_b with genotyping error	$p_{1\text{-}4,5}$, $c_{2\text{-}4,5}$, N	12	27.0225	0.000	0.799	8	3-13
M_t with genotyping error	$p_1, p_2, p_3, p_4, p_5, c_2, c_3, c_4, c_5, N$	12	29.0225	2.755	0.201	8	3-13

[a] Number of unique marten genotypes used in estimation

[b] Akaike's information criterion including a bias correcting term.

[c] Difference in AIC_c

[d] AICc weighting factor representing the weight in evidence between models. This value is scaled to 1.

[e] Population estimate

***Program* Capwire**

The population estimate calculated using *Capwire* was 60 fishers (95% CI = 47 – 76) when harvest was included. With the removal of harvest samples, the population estimate was 49 (95% CI = 38 – 61). For both estimates ECM was not rejected, indicating heterogeneity did not have a significant effect on individual capture probabilities.

Capwire provided a population estimate of 28 martens (95% CI = 14 – 47) with the inclusion of individuals harvested on the study area. ECM was rejected, indicating heterogeneity was a factor in this dataset. When harvested individuals were removed, the population estimate was 17 (95% CI = 12 – 25). ECM was not rejected, suggesting an equal probability of capture across the four sampling sessions.

Chapman estimator

Because so few martens were observed on the study site, the Chapman estimator was used to approximate population size. Based on the estimator with the inclusion of individuals harvested on the study area, 17 ± 6 martens resided on the study area. When the harvested martens were removed, the estimate was 14 ± 4.

Assumption of population closure

Seventeen fishers were harvested in the three buffer strips (Figure 5.3, Table 5.2). One fisher in the second buffer strip (between 6.96 and 13.93 km from the study area boundary) matched hair samples collected on the study site, suggesting violation of the assumption of geographic closure.

Three martens were harvested in the buffer strips (Table 5.2). None of those martens matched hair samples collected on the field site, suggesting geographic closure. However, the limited number and dispersion of harvest samples in the buffer strips does not eliminate the possibility of movement onto or off of the study area.

Discussion

Success of trapping method

Peak overall trapping success was achieved during the third trapping session. Due to the use of prebait, no pattern in hair collection was expected. An increase in the number of captures through trapping periods has been documented in other non-invasive hair snaring studies (Mowat and Paetkau 2002, Dreher 2004). The decrease in overall success from the third to fourth trapping periods was likely a result of environmental factors, specifically a sudden decrease in temperature and snow that fell at the end of trapping occasion three. Fisher captures followed this overall trend. However, marten captures increased during the fourth capture period. This pattern could be due to sampling artifact. Heterogeneity in capture probabilities could also be a result of differing movement patterns and home range size between martens and fishers. The probability that a trap was placed near the center fisher's home range was likely greater than for martens based on territory size, thus differentially affecting detectability.

The overall trap success rate of 87.2% suggested that methods employed were very successful at collecting hair for DNA-based studies. The trapping method could be modified to target a variety of tree climbing species, such as long-tailed weasels, short-tailed weasels, red squirrels, and flying squirrels. The lower trap success rate for each

target species supported harvest distribution data that suggested relatively low densities of both species in the study area. However, the percent of traps visited by fishers was 36.8%, which was similar to reported values from additional population estimate studies (48.3% for grizzly bears, Mowat and Strobeck 2000; 31.4% for black bears, Eason et al. 2002).

The fisher recapture rate was high (64%), which suggested a positive behavioral response (due to baiting) or narrow trap spacing relative to home range size. However, a behavioral response was not found using CAPTURE or MARK. To accommodate the demographic characteristics of both martens and fishers, traps were spaced at an intermediate distance that would be dense relative to fishers and sparse relative to martens. The average distance between fisher recaptures using the hair snares was 2.30 km, suggesting that if this technique were to be modified to estimate the population abundance of fishers only, a systematic grid would be best employed with trap spacing between 2 and 2.5 km.

Fisher observations were not evenly distributed across the study site. Fishers were most often detected using traps in hardwoods stands. However, fisher samples were also collected from traps in conifers or mixed conifer-hardwood stands. These forest types provided necessary overhead cover and further describe the habitat specialization of the species.

In contrast to the fishers, the low rate of trap success for martens (9.77%) indicated a low density of the species on the study area. Similarly, the patchy dispersion of observations suggested non-random distribution of martens across the landscape. All marten samples were collected from trap sites located in conifer or mixed conifer-

hardwood stands whereas the majority of fishers were identified from snares placed in areas predominated by hardwoods. This finding was consistent with the reported habitat specialization of martens (Mech and Rogers 1977, Wright 1999). Conifer and mixed conifer-hardwoods were not the dominant cover types on the study site, thus low marten abundance was expected. If a systematic grid design were to be employed to specifically target martens, trap spacing should be reduced to between 0.5 and 1 km.

The systematic grid design for small and mid-sized carnivores is useful for surveys conducted within small areas. However, it is not feasible due to time or cost to apply over a large area, such as a management unit or on a state-wide basis. The habitat specialization of fishers and martens in Michigan would allow for use of a stratified random sampling design to extend the methodology over larger areas. Sampling areas would first be identified for each species based on habitat. Areas of preferred habitat would be selected. Relative density could be assessed using historical harvest records to adjust trap density across areas of different habitat quality. Few historical harvests indicating potential low density would result in traps spaced further apart than in areas where many harvests were recorded.

Species identification

Thirty-four non-target species including twenty congeners (*Mustela* spp.) were detected over the course of the four trapping periods. The trap design does not exclude animals with the ability to climb trees. However, we have developed a lab protocol using microsatellite markers to identify and eliminate non-target species from the analysis with no effect on the population estimator. An alternative method of species identification

would be amplification of mitochondrial DNA (mtDNA; Foran et al. 1997a, Riddle et al. 2003). Multiple copies of mtDNA are found in each animal cell (up to 2500 copies; Kohn and Wayne 1997) in contrast to the single copy found in nuclear DNA (targeted by microsatellites). As a result, mtDNA sequences are often more easily amplified from small amounts of source sample, such as hair. The amount of variability in the mtDNA sequence is limited, and although useful to identify species in this genus, could not offer additional information. In contrast, we determined a protocol using polymorphic microsatellite markers to identify our target species and assign individual genotypes that can be used to not only estimate population, but estimate coefficients of relatedness and other important parameters.

Population estimation

The population estimates for both fishers and martens indicated low densities of both species on the study site. Low estimates were expected given the dispersion of habitat across the study area. There were differences in $\hat{N}$ provided by each method. The number of harvested martens and fishers on the study area was very low, and distribution scattered. However, the addition of harvest as a final capture period did increase the population estimate in all models and using all methods. In areas where harvest was high and more evenly dispersed across the landscape, inclusion of these individuals would greatly increase confidence in resulting population estimates.

Fishers display intrasexual territoriality, whereby male home ranges overlap those of females, but female home ranges do not overlap with neighboring females (Powell 1993). We calculated a conservative estimate of 44 fishers as the maximum number of

non-overlapping home ranges in our study area based on an average female home range size of 15 km^2 (Powell 1977). The main assumption of this calculation was that the study site was composed solely of preferred habitat. However, variability in habitat, resources, home range size and overlap would affect the population estimate.

Capwire provided the largest estimate of $\hat{N}$ for fishers (60 individuals including harvest). The program was designed to account for all observations of an individual, thereby including the most capture-recapture information of all methods used in this study. Therefore, it is not surprising that the population estimate is the largest. However, both *Capwire* and CAPTURE assume that individuals are identified correctly. The misidentification models of Luckacs and Burnham (2005) employed in MARK do not use the same assumption.

The misidentification models in MARK provided the lowest estimate of fisher abundance (39 individuals including harvest). Interestingly, this estimate was less than the number of unique genotypes identified ($N = 42$). This would be expected if genotyping error resulted in overestimation of the number of individuals. However, although it was possible for genotyping error to be > 0, it is unlikely that the error rate was high enough to result in a population estimate less than the number of observed individuals due to stringent error-proofing protocols followed in this study.

The misidentification method of Lukacs and Burnham (2005) estimates genotyping error through comparison of the number of genotypes observed once relative to the number of recaptures, or genotypes observed more than once. Lukacs and Burnham (2005) evaluated misidentification models using several forms of variation in detection. When capture probability was high (0.5), the number of genotypes observed

only once was very low. This resulted in difficulty in effectively estimating genotyping error. The capture probability in this study appeared to be high for fishers, and would have created bias in the estimate of α. Lukacs and Burnham (2005) suggested this bias would be small (1-4%). An inflated estimate of α would result in a decrease in the population estimate, explaining why the number of unique captures was greater than estimated abundance.

It is unlikely that the fisher population on the study area was as estimated using the misidentification models in MARK. To provide a population estimate in CAPTURE, multiple observations of individuals at different traps within single trapping sessions were combined. This information is retained in *Capwire*. Therefore, abundance estimated using *Capwire* is considered the most realistic in this study. However, the program has as of yet been relatively untested (Miller et al. 2005), and caution must be taken in its application.

The lowest population estimates for martens were provided by the misidentification models within MARK. Similar to the fishers, these estimates were less than the number of unique individuals used to calculate $\hat{N}$. Given the improbability of high genotyping error, we expect the small sample size and distribution of captures – recaptures artificially inflated the error rate.

The Chapman estimator was used to provide a baseline estimate to which population abundance calculated by alternative methods were compared. Similar to CAPTURE and *Capwire*, the Chapman estimator assumes all individuals are correctly identified. Observations of martens were combined into two trapping sessions, thereby

ignoring multiple observations of individuals within a single field trapping period as well as multiple observations across combined field trapping periods.

The inclusion of multiple observations within trapping sessions using *Capwire* provided a larger population estimate than the Chapman estimator. The results of *Capwire* and CAPTURE were very similar. The convergence between the two methods suggested marten abundance was approximately 30 individuals on the study site. Heterogeneity was indicated as a factor in the marten observations by both *Capwire* and CAPTURE, and may have affected capture or recapture. However, this may be an artifact of small sample size.

Harvest samples and population closure

Because the number of harvests in the study area and in each buffer was low, our ability to detect violations of geographic closure was limited. However, one fisher was recaptured during harvest in the second buffer strip approximately 12 km from the trap location of observation within the study area, indicating violation of the assumption of geographic closure. However, the low density of harvests in and around the study area did not allow for an estimate of the magnitude of the violation. Only three martens were harvested in the 3 buffer strips. Although none of these martens were recaptures, suggesting closure, the number and distribution of harvests around the study site was not sufficient to rule out movements off of the study site. In areas of dense harvest, the use of buffer strips would provide a valuable estimate of closure violation due to dispersal or migration out of an area and its effect on the population estimate.

Species presence and landscape variables

The low density of both species on the study area decreased the probability of competitive exclusion. On four occasions, fishers and martens visited the same trap during different trapping sessions. This suggested interference competition between fishers and martens did not affect trap visitations on the study area. In areas where density of martens and fishers is high, competitive exclusion would be expected to create differential patterns of observations at trap sites. We can quantitatively assess the degree of species interactions using occupancy models that account for conditional probabilities of presence or absence of a species when a second species was detected (MacKenzie et al. 2004). An understanding of negative interactions between martens and fishers would assist in developing management strategies to promote population growth and stability of both species in areas of sympatry.

We can also use occupancy models combined with habitat characteristics of the areas in which fishers or martens were observed to develop an index of abundance of both species in the western Upper Peninsula. The correlation of specific landscape components to presence of an individual allows for inference of occupation in habitats outside of the study area. Inclusion of home range data could provide theoretical densities and distributions of fishers and martens across the western Upper Peninsula that can be field tested.

Conclusion

We provided evidence that simultaneous estimates of population abundance can be determined for two secretive species of management concern, even in areas of

relatively low density. Different mark-recapture methodologies are available to estimate population size. However, care must be taken when choosing the appropriate method for a data set. We described the value of using harvest samples both as a final capture period for population estimation as well as a means to detect individuals moving off the study area, thereby violating the assumption of closure in closed-capture models. The occupancy of fishers and martens in various habitat types will allow for further investigation into species interactions as well as assist in developing stratified sampling designs to apply over a large scale.

References

Aasen E., and J.F. Medrano. 1990. Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep, and goats. Biotechnology. 8: 1279-1281.

Arthur, S.M., T.F. Paragi, and W.B. Krohn. 1993. Dispersal of juvenile fishers in Maine. Journal of Wildlife Management. 57: 868-874.

Belant, J.L. 2003. A hairsnare for forest carnivores. Wildlife Society Bulletin. 31: 482-485.

Berg, W. E. 1982. Reintroduction of fisher, pine marten, and river otter. Midwest Furbearer Management. G. C. Sanderson, Wildlife Society. North Central Section., Wildlife Society. Central Mountains and Plains Section. and Wildlife Society. Kansas Chapter., The Wildlife Society, Kansas Chapter: 158-173.

Boerson, M.R., J.D. Clark, and T.L. King. 2003. Estimating black bear population density and genetic diversity at Tensas River, Louisiana using microsatellite DNA markers. Wildlife Society Bulletin. 31: 197-207.

Boutin, S. 1984. Home range size and methods of estimating snowshoe hare densities. Acta Zoologica Fennica. 171: 275-278.

Bradle, B.J. 1957. The fisher returns to Wisconsin. Wisconsin Conservation Bulletin. 22: 9-11.

Buskirk, S.W. and R.A. Powell. 1994. Habitat ecology of fishers and American martens. Pp. 283-296 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Chapman, D.G. 1951. Some properties of the hypergeometric distribution with applications to zoological sample censuses. Univ. Calif. Berkeley, Publ. Stat. 1:131-160

Churchill, S.J., L.A. Herman, M.F. Herman, and J.P. Ludwig. 1981. Final report on the completion of the Michigan marten reintroduction program. Ecological Research Services, Inc., Environmental Report. Iron River, MI.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 2001. Fisher survey 1999. Michigan Department of Natural Resources Wildlife Division Report No. 3329. 12 pp.

Davis, C.S. and C. Strobeck. 1998. Isolation, variability and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7: 1771-1788.

Davis, M.H. 1978. Reintroduction of the pine marten into the Nicolet National Forest, Forest County, Wisconsin. M.S. Thesis. University of Wisconsin, Stevens Point: 64.

de Vos, A. 1951. Recent findings in fisher and marten ecology and management. Transactions of the Sixteenth North American Wildlife Conference. 16: 498-507.

Dieringer, D. and C. Schlötterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes. 3: 167-169.

Dreher, B.P. 2004. Estimation of black bear (*Ursus americanus*) abundance in the Northern Lower Peninsula of Michigan using microsatellite DNA markers. Dept. of Fisheries and Wildlife. East Lansing, MI, Michigan State University: 180.

Earle, R.D., L.H. Mastenbrook, and T.F. Reis. 2001. Distribution and abundance of the American marten in northern Michigan. Michigan Department of Natural Resources Wildlife Report No. 3321: 1-26.

Earle, R.D. 2002. Furbearer winter track count survey of 2001. Michigan Department of Natural Resources Wildlife Report No. 3366. 10 pp.

Eason, T.H., S.L. Simek, J.W. McCowan, and D. Ziegler. 2002. Statewide assessment of road impacts on bears in Florida. Research Work Plan. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA.

Ernest, H.B., M.C.T. Penedo, B.P. May, M. Syvanen, and W.M. Boyce. 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. Molecular Ecology. 9: 433-442.

Evett, I.W. and B.S. Weir. 1998. Interpreting DNA evidence: statistical genetics for forensic science. Sinauer: Sunderland, MA

Flagstad, Ø., E. Hedmark, A. Landa, H. Brøseth, J. Persson, R. Andersen, P. Segerström, and H. Ellegren. 2004. Colonization history and noninvasive monitoring of a reestablished wolverine population. Conservation Biology. 18: 676-688.

Fleming, M.A., E.A. Ostrander, and J.A. Cook. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology. 8: 1351-1362.

Foran, D.R., K.R. Crooks, and S.C. Minta. 1997a. Species identification from scat: an unambiguous genetic method. Wildlife Society Bulletin. 25: 835-839.

Foran, D.R., S.C. Minta, and K.S. Heinemeyer. 1997b. DNA-based analysis of hair to identify species and individuals for population research and monitoring. Wildlife Society Bulletin. 25: 840-847.

Frawley, B.J. 2002. 2001 marten harvest survey. Michigan Department of Natural Resources Wildlife Division Report No. 3369: 1-4.

Gerloff, U., C. Shlötterer, K. Rassmann, and D. Tautz. 1995. Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). Molecular Ecology. 4: 515-518.

Goossens, B., L.P. Waits, and P. Taberlet. 1998. Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. Molecular Ecology. 7: 1237-1241.

Guo S.W. and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics. 48: 361-372

Irvine, G.W., L.T. Magnus, and B.J. Bradle. 1964. The restocking of fisher in Lake States forests. Transactions of the North American Wildlife and Resource Conference. 29: 307-315.

Johnson, S.A. 1984. Home range, movements, and habitat use of fishers in Wisconsin. M.S. Thesis. University of Wisconsin, Stevens Point. 78 pp.

Jones, J.L. and E.O. Garton. 1994. Selection of successional stages by fishers. Pp. 377-387 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Kohn, M.H. and R.K. Wayne. 1997. Facts from feces revisited. Trends in Ecology and Evolution 12: 223-227.

Lofroth, E. C. 1993. Scale dependent analyses of habitat selection by marten in the sub-boreal spruce biogeoclimatic zone, British Columbia. Thesis. Simon Fraser University, Burnaby, British Columbia, Canada.

Ludwig, J.P. 1986. A short report on the Michigan marten reintroduction program Lower Peninsula in 1985-86: Comparison to trapping success with the Algonquin Park efforts of 1980-81. Ecological Research Services, Inc., Environmental Report. Boyne City, MI.

Lukacs, P.M. and K.P. Burnham. 2005. Estimating population size from DNA-based closed capture-recapture data incorporating genotyping error. Journal of Wildlife Management. 69: 396-403.

MacKenzie, D.I., L.L. Bailey, and J.D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. Journal of Animal Ecology. 73: 546-555.

McDaniel, G.W., K.S. McKelvey, J.R. Squires, and L.F. Ruggiero. 2000. Efficacy of lures and hair snares to detect lynx. Wildlife Society Bulletin. 28: 119-123.

Mech, L.D. and L.L. Rogers. 1977. Status, distribution, and movements of martens in northeastern Minnesota. U.S.D.A. For. Serv. Res. Pap. NC-14-3. 7 pp.

Miller, C.R., P. Joyce, and L.P. Waits. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. Molecular Ecology. 14: 1991-2005.

Morin, P.A., J.J. Moore, R. Chakraborty, L. Jin, J. Goodall, and D.S. Woodruff. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. Science. 265: 1193-1201.

Mowat, G. and D. Paetkau. 2002. Estimating marten *Martes americana* population size using hair capture and genetic tagging. Wildlife Biology. 8: 201-209.

Mowat, G. and C. Strobeck. 2000. Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. Journal of Wildlife Management. 64: 183-193.

Nietfeld, M.T., M.W. Barrett, and N. Silvy. 1994. Wildlife marking techniques. Pp. 140-168 in Research and management techniques for wildlife and habitats. T.A. Bookhout, ed. The Wildlife Society, Bethesda, MD.

Otis, D.L., K.P. Burnham, G.C. White, and D.R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs. 62.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology. 12: 1375-1387.

Paetkau, D. 2004. The optimal number of markers in genetic capture-mark-recapture studies. Journal of Wildlife Management. 68: 449-452.

Paetkau, D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology. 12: 1375-1387.

Palomares, F., J.A. Godoy, A. Piriz, S.J. O'Brien, and W.E. Johnson. 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Molecular Ecology. 11: 2171-2182.

Pearce, J.M., R.L. Field, and K.T. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology. 68: 471-481.

Petersen, L.R., M.A. Martin, and C.M. Pils. 1977. Status of fishers in Wisconsin, 1975. Department of Natural Resources, Research report 92.

Poole, K.G., G. Mowat, and D.A. Fear. 2001. DNA-based population estimate for grizzly bears *Ursus arctos* in northeastern British Columbia, Canada. Wildlife Biology. 7: 105-115.

Powell, R.A. 1977. Hunting behavior, ecological energetics and predator-prey community stability of the fisher (*Martes pennanti*). PhD thesis, University of Chicago.

Powell, R.A. 1993. The Fisher: Life History, Ecology, and Behavior. Second edition. University of Minnesota Press, MN.

Powell, R.A. 1994. Structure and spacing of Martes populations. Pp. 101-121 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Raine, R.M. 1983. Winter habitat use and responses to snow of fisher (*Martes pennanti*) and marten (*Martes americana*) in southeastern Manitoba. Canadian Journal of Zoology. 61: 25-34.

Raphael, M.G. 1994. Monitoring populations of fishers and American martens. Pp. 224-240 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86: 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225.

Riddle, A.E., K.L. Pilgrim, L.S. Mills, K. McKelvey, and L.F. Ruggiero. 2003. Identification of mustelids using mitochondrial DNA and non-invasive sampling. Conservation Genetics. 4: 241-243.

Schupbach, T.A. 1977. History, status, and management of the pine marten in the Upper Peninsula of Michigan. Dept. of Forestry. Houghton, MI, Michigan Technological University: 79.

Segelbacher, G. 2002. Noninvasive genetic analysis in birds: testing reliability of feather samples. Molecular Ecology Notes. 2: 367-369.

Sloane, M.A., P. Sunnucks, D. Alpers, L.B. Beheregaray, and A.C. Taylor. 2000. Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. Molecular Ecology. 9: 1233-1240.

Slough, B.G. 1994. Translocations of American martens. Pp. 165-178 *in* Martens, sables, and fishers: biology and conservation. S. W. Buskirk, A. S. Harestad, M. G. Raphael and R. A. Powell, eds. Ithaca, Comstock Publishing Associates.

Steck, M. 1988. A translocation of fisher in the Upper Peninsula of Michigan. Michigan Department of Natural Resources, Final Report. 21 pp.

Strickland, M.A., C.W. Douglas, M. Novak, and N.P. Hunziger. 1982. Marten. Pages 599-612 *in* J.A. Chapman and G.A. Feldhamer, eds. Wild mammals of North America: biology, management, economics. Johns Hopkins University Press, Baltimore.

Taberlet, P., H. Mattock, C. Dubois-Paganon, and J. Bouvet. 1993. Sexing free-ranging brown bears *Ursus arctos* using hairs found in the field. Molecular Ecology. 2: 399-403

Taberlet, P., Camarra, J.-J., Griffin, S., Uhres, E., Hanotte, O., Waits, L.P., Dubois-Paganon, C., Burke, T., and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology. 6: 869-876.

Thomasma, L.E., T.D. Drummer, and R.O. Peterson. 1991. Testing the habitat suitability index model for the fisher. Wildlife Society Bulletin. 19: 291-297.

Waits, L.P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology. 10: 249-256.

Waits, L.P. and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management. 69: 1419-1433.

Wilberg, M.J. and B.P. Dreher. 2004. GENECAP: a program for analysis of nuclear data for capture-recapture population estimation. Molecular Ecology Notes. 4: 783-785.

White, G.C., D.R. Anderson, K.P. Burnham, and D.L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, LA 8787-NERP, Los Alamos, New Mexico, USA.

White, G.C. and K.P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study. 46: S120-S139.

Woods, J.G., D. Paetkau, D. Lewis, B.N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging free ranging black and brown bears. Wildlife Society Bulletin. 27: 616-627.

Wright, J.L. 1999. Winter home range and habitat use by sympatric fishers (*Martes pennanti*) and American martens (*Martes americana*) in northern Wisconsin. Dept. of Natural Resources. Stevens Point, WI, University of Wisconsin, Stevens Point: 73.

CHAPTER 6: BIAS INTRODUCED INTO BOBCAT HARVEST SURVEYS THOUGH MISREPORTED HARVEST LOCATION AND INACCURATE SEX DETERMINATION

Introduction

Bobcats (*Lynx rufus*) are a relatively common furbearer species. However, little research has been conducted to characterize spatial genetic structure of this species. Bobcats in Michigan form two geographically distinct populations. The population segment in the Lower Peninsula is bordered on the south by large expanses of sub-optimal habitat (due to agricultural practices; Woolf and Hubert, Jr. 1998), east and west by Lake Huron and Lake Michigan, and the north by the Mackinac Strait. The bobcat population in the Upper Peninsula is contiguous with populations in northern Wisconsin to the west and south and with Canada to the east. Differences in levels of contiguity with bobcat populations in neighboring regions and differences in habitat and historical land-use patterns likely dictate that levels of genetic diversity and degree of spatial variance in allele frequency will vary within bobcats from the two regions.

Prior to 2005, different harvest regulations existed regarding bobcats in the state of Michigan. Trapping and hunting was allowed in the Upper Peninsula, whereas trapping was illegal in the Lower Peninsula. Bag limits allowed three bobcats per person in the Upper Peninsula and one per person in the Lower Peninsula (Michigan Department of Natural Resources, MDNR, 2003). Due to these differences in harvest regulations, some fur harvesters and biologists suspect cases of bobcats taken in the Lower Peninsula illegally and registered as harvests originating from the Upper Peninsula. If a fundamental discordance in genetic characteristics can be defined between bobcats from the Upper and Lower Peninsulas, it may be possible to confidently assign individual

bobcats to a population of origin.

In the mid-1970s, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was enacted. Species listed in Appendix I, including many felids, became illegal to trade commercially. As a result, there was an increase in world demand for pelts of felid species, including bobcat, not restricted by CITES regulations. From 1967-1973 the average annual bobcat harvest in the United States was approximately 10,000 (Woolf and Hubert, Jr. 1998). In 1975-1976 the harvest increased to 35,937. In 1979-1980, 86,168 bobcats were taken nationally (Woolf and Hubert, Jr. 1998). The harvest increase reflected a dramatic increase in the value of bobcat pelts. Bobcats were added to CITES Appendix II in 1975 and currently remain listed. Appendix II requires CITES member countries to demonstrate that international trade will not threaten the survival of the species before allowing export of the species or its products (Woolf and Hubert, Jr. 1998). As a result, state management agencies are required to prove that harvest is not detrimental to maintenance of stable bobcat populations. To comply, the MDNR requires registration of each bobcat harvested in the State and records all information into annual harvest surveys. Data from these surveys are compiled by the U. S. Fish and Wildlife Service (USFWS; Cooley et al. 2000). Accurate information on the number of individuals harvested and the sex and age composition of the harvest is critical for population assessments.

Beginning in 1980, entire carcasses were collected from hunters and trappers for annual surveys necessitated by CITES regulations. To reduce costs and improve efficiency, lower canine teeth have been used to sex and age bobcats by the MDNR. However, a measurable amount of error is involved with the technique used to sex

bobcats (Friedrich et al. 1983). The technique, termed MRA analysis, is a composite measure of the product of maximum canine root width and thickness. Early assessments of sex classification error based on comparisons between anatomical and MRA sex (Friedrich et al. 1983) suggested that the technique was sufficiently accurate for the purpose of harvest surveys (total error = 5.8%; Friedrich et al 1983). However, such error rates may be problematic if inferences at an individual level (e.g., defining social structure, estimating sex-biased dispersal) are required.

Population demographic parameters estimated from harvest, including sex and age, are used by management agencies to help draw conclusions regarding the sustainability of populations. Population models may be useful for testing effects of various harvest scenarios on future abundance (Bessinger and Westphal 1998). However, models are sensitive to parameter estimates (or degree of uncertainty in parameter estimates) used to develop population trends. To have confidence in the results of a population model, levels of uncertainty in estimates of demographic parameters determined using different methodologies must be identified and accounted for.

The goals of this study were to (1) describe levels of genetic diversity of bobcats in the Upper and Lower Peninsulas of Michigan; (2) quantify the magnitude of bobcat harvest location reporting error in Michigan; (3) assess sexing error rate and differences in the distribution of bias in current practices of field and canine tooth-based sex identification techniques in bobcats using genetic sexing methods, and; (4) determine effects of sexing error on the magnitude and direction of trends in population abundance using a deterministic population model.

Methods

Study area and sample collection

To minimize uncertainty in the estimation of population demographic parameters (i.e., sex and age), large sample sizes are required and sampling should be conducted systematically throughout the study area. Annual harvest returns offered the opportunity to sample a large number of bobcats across the state of Michigan. During the winters of 2001-2002 and 2002-2003 approximately 2,400 bobcats were legally harvested, and submitted to the MDNR for sex and age determination (M. Cosgrove, MDNR, personal communication). Age was estimated through presence/absence of open root apical foramina and the number of cementum annuli (Crowe 1972, Crowe 1975, Friedrich et al. 1981). Sex was estimated based on use of a composite variable created by the product of maximum canine root width and maximum root thickness for each animal (Friedrich et al. 1983). This method has been termed MRA analysis. In addition, sex was provided by the furtaker upon mandatory registration.

Bobcat skulls or undamaged canine teeth were collected from hunters and trappers by the MDNR as a mandatory harvest registration requirement (MDNR 2005). Muscle tissue was removed from the skull by MDNR employees and placed in 1.5 mL tubes. Tubes were labeled with either sequential laboratory numbers or seal numbers. Samples were stored frozen at -70°C. Lower canine teeth were removed and used to sex and age each animal. Bobcat harvest registration requires reporting of kill location to a section (1 section = 1.6 km^2). Accordingly, each animal was assigned a set of spatial coordinates representing the geographic center of the reported section.

Individuals were selected for genetic analysis from five discrete sampling

locations chosen to examine differences in genetic diversity based on contiguity of bobcat dispersal patterns (Figure 6.1). Movement of bobcats into the sample groups in the western Upper Peninsula of Michigan (WUP) and central Upper Peninsula (CUP) was expected from contiguous populations to the east and west. In contrast, dispersal into and away from the sample groups in the eastern Upper Peninsula (EUP), northern Lower Peninsula (NLP) and central Lower Peninsula (CLP) is believed to be limited by boundaries of the St. Mary's River (EUP), the Mackinac Strait (EUP, NLP, CLP), and agricultural lands (NLP and CLP). Each sample group was separated by a distance of approximately 100 km to minimize probability of bobcat dispersal from one group to another. This distance was based on average natal dispersal movements of approximately 50 km (e.g., Nielsen and Woolf 2003). Sampling areas were roughly 75 km in diameter to maximize geographic area sampled from the bobcat's range in Michigan and maximize the number of individuals within each group while maintaining discrete groups.

Thirty-five bobcats were chosen randomly from each of three sampling areas in the Upper Peninsula and two sampling areas in the Lower Peninsula. An additional 16 samples were chosen to represent bobcats from unsampled areas at the fringe of the species' distribution in Michigan based on harvest distribution (total $N = 191$). These 16 bobcats were used only to examine bias in location reporting and sexing techniques. Each animal was harvested during the 2001-2002 or 2002-2003 bobcat seasons.

Microsatellite analysis

DNA was extracted from each sample using Quiagen DNeasy® Tissue Kits. DNA was quantified by spectrophotometry and diluted to a concentration of 20 ng/μL.

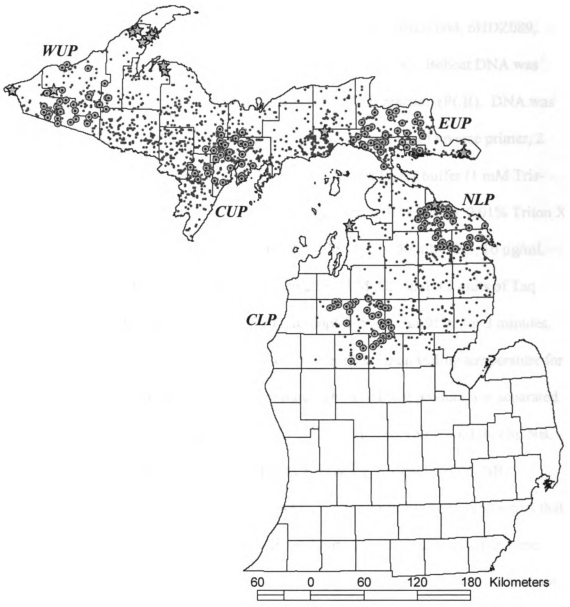

Figure 6.1. Map of Michigan showing the location of all bobcats legally harvested during the 2001-2002 and 2002-2003 seasons (small grey points). The larger points represent the locations of the 191 bobcats used in this study, including three population segments in the Upper Peninsula (WUP, CUP, and EUP), two population segments in the Lower Peninsula (NLP and CLP), and 16 additional individuals. These 16 individuals are denoted by stars.

Nine microsatellite primers selected from Williamson et al. (2002) were screened. Eight microsatellite primers were identified for use in this study based on level of polymorphism and quality of product: 6HDZ056, 6HDZ057, 6HDZ064, 6HDZ089, 6HDZ463, 6HDZ610, 6HDZ635, and 6HDZ700 (Appendix 6.A). Bobcat DNA was amplified with each primer pair using the polymerase chain reaction (PCR). DNA was amplified in a 10μL reaction with the following conditions: 2 pmol reverse primer, 2 pmol forward primer, dNTPs at 200 μM each, 200 μM 10x PCR2 buffer (1 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) or LGL buffer (1 mM Tris-HCl, pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl, 10 μg/mL BSA, 0.0025% Tween 20), variable amounts of 25 mM $MgCl_2$, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 95°C for 3 minutes, followed by 35 cycles of 95°C for 45 seconds, primer specific annealing temperature for 1 minute (Appendix 6.A), and 72°C for 1 minute. The amplified product was separated on a 6% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™), Lincoln, NB. Fragments were viewed using Saga Generation 2 software, LiCor, Lincoln, NB.

A series of quality control protocols was used to minimize genotyping errors that could result in biased estimates of genetic diversity or structure. All genotypes were scored independently by two experienced laboratory personnel. Samples that could not be genotyped at a locus were re-amplified. Following re-amplification, samples lacking genotypes at more than three of eight loci were removed from analysis.

Bobcat sex assignment

The information provided by the furtaker for bobcat registration included location

and date of capture, method of take, and sex. During the process of skinning a bobcat, a furtaker may not have inspected the carcass for testes or ovaries to definitively identify sex. It has been suspected that field sex results in most small animals called females and most large animals labeled as males (P. Friedrich, MDNR, personal communication).

The MDNR currently uses MRA analysis to determine bobcat sex from lower canine teeth based on techniques described by Johnson et al. (1981) and Friedrich et al. (1983). Maximum root width and root thickness of permanent lower canine teeth were measured to the nearest 0.01 mm. The measurements were multiplied for each individual, creating a value comparative to the maximum cross-sectional area of the root. This composite variable provides a multi-dimensional representation of tooth shape (Friedrich et al. 1983). Juveniles were identified as females based on MRA scores $\leq$ 39.14 mm^2 and males if MRA scores were greater than this value. The adult MRA criterion was 41.61 mm^2 (P. Friedrich, MDNR, personal communication).

Although bobcats are sexually dimorphic, size overlap between sexes is common (Litvaitis et al. 1984, Lovallo and Anderson 1996). Large females might display an MRA value greater than established criteria. Bias was expected to be particularly pronounced in adults. Likewise, small males might have MRA values less than the criterion. This bias was expected in juveniles when the effects of developmental differences as well as environmental factors would be most probable.

Molecular-based sexing protocols involving use of PCR were chosen to target the highly conserved mammalian zinc finger-Y-X (ZFX/Y) locus and the testes determining factor (TDF). ZFX/Y is found on both the X and Y chromosome, and the TDF locus is Y chromosome specific (S. Fain and J. Lemay, USFWS Forensic Laboratory, personal

communication, Appendix 6.B). DNA for both regions was amplified using PCR in a 25μL reaction using a Strategene® RoboCyler®. PCR amplification conditions consisted of 10 pmol reverse P2-3EZ primer, 10 pmol forward P1-5EZ primer, 10 pmol reverse Y53-3D primer, 10 pmol forward Y53-3C primer, dNTPs at 200 μM each, 200 μM 10x PCR2 buffer, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 95°C for 2 minutes, followed by 30 cycles of 94°C for 45 seconds, 54°C for 45 seconds, and 73°C for 1 minute. The PCR product was run on a 1.5% agarose gel stained with ethidium bromide with a 100bp ladder to provide known size standards. The DNA fragments were visualized using an ultraviolet light box. Two bands, 224 and 442 base pairs in size provided unambiguous identification of males (TDF and ZFX/Y). One band, 442 base pairs in size, identified females (ZFY/X).

A second set of mammal sexing primers was used to confirm all discrepancies between sex determination using the ZFX/Y - TDF primers and MRA. DNA was amplified using TET SRY/ZFX primers in a 10μL reaction with the following conditions: 2 pmol reverse TET SRY primer (Taberlet et al. 1993, Appendix 6.B), 2 pmol forward TET SRY primer (Taberlet et al. 1993, Appendix A), 2 pmol ZFX reverse primer (Aasen and Medrano 1990, Appendix 6.B), 2 pmol ZFX forward primer (Woods et al. 1999, Appendix 6.B), dNTPs at 200 μM each, 200 μM 10x PCR2 buffer, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 94°C for 2 minutes, followed by 35 cycles of 92°C for 30 seconds, 51°C for 30 seconds, 72°C for 40 seconds, and ending with a single extension of 72°C for 3 minutes. The amplified product was separated on a 6% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™), Lincoln, NB. Fragments were viewed using Saga Generation 2 software, LiCor, Lincoln,

NB. Two bands, 119 base pairs and 130 base pairs in size, signified a male (TET and ZFX). One band, 129 base pairs in size, signified a female (ZFX).

Analysis of genetic diversity

Tests of linkage disequilibrium for each pair of microsatellite loci in each population and tests for departure from Hardy-Weinberg equilibrium (HWE) using the exact test of Guo and Thompson (1992) were performed using the Markov chain Monte Carlo (MCMC) approach of GENEPOP version 3.4 (Raymond and Rousset 1995). Deviations from HWE or linkage equilibrium could bias estimates of genetic diversity or even population of origin. Bonferroni tests (Rice 1989) were used to correct for multiple tests. Lower levels of genetic diversity were expected in the bobcats in the Lower Peninsula due to the insular nature of the population, in addition to levels and duration of land use change. Genetic variation in the EUP sampling area was expected to be lower in comparison to CUP and WUP due to directionally limited dispersal. To examine differences between Peninsulas, samples were pooled from representative groups (WUP, CUP and EUP; NLP and CLP). A pairwise F_{ST} value was calculated using FSTAT 2.9.3 (Goudet 2000) to quantify the genetic differentiation between Peninsulas.

Measures of genetic diversity were also calculated for each sample group separately to assess intra-group differences. Means across sample groups were weighted for differences in sample size. Estimates of genetic diversity, including expected and observed heterozygosities calculated using MICROSATELLITE ANALYSER version 3.12 (Dieringer and Schlötterer 2003). Measures of allelic richness and F_{IS} were calculated using FSTAT 2.9.3. Current genetic structure in Michigan as a result of historic gene flow

was evaluated using the data set corrected for location bias to ensure analysis of individuals representing "true" genetic diversity in each region.

Error in harvest location reporting

Harvest regulations differed in the Upper and Lower Peninsulas of Michigan during the study period, creating the potential for intentional misreporting of bobcat harvest location by furtakers. Bag limits were higher and the bobcat season longer in the Upper Peninsula. Genetic analyses of individual bobcats can be used to identify misreporting of harvest, harvest registration recording errors, or sample number transcription errors.

Program STRUCTURE (Pritchard et al. 2000) was used to assign individuals from the Upper and Lower Peninsulas to genetically distinct subpopulations. To estimate the number of subpopulations (K), 10 independent iterations of $K = 1 - 8$ were completed at 100,000 Markov Chain Monte Carlo (MCMC) replicates and a 100,000 burnin period assuming admixture and correlated allele frequencies. This Bayesian assignment method does not require prior spatial information as is needed for many frequency-based assignment methods. STRUCTURE calculates posterior probabilities of individual assignment to each subpopulation for each K. The optimal number of subpopulations was chosen as the value of K with the maximal log-likelihood value. The number of subpopulations was confirmed using a graphical method developed by Evanno et al. (2005). An ad hoc statistic (ΔK) was calculated from the second order rate of change in the log-likelihood function between successive K values. The modal value of the distribution of ΔK was shown to accurately estimate the "true" number of subpopulations

across multiple simulations (Evanno et al. 2005). Results were visualized using ArcView3.2. Each bobcat was first assigned to a population based on the individual's largest posterior probability value (> 0.500) generated by STRUCTURE for the selected *K*. Second, thresholds of 0.950 and 0.990 were used as a measure of confidence in assignment to a genetic population (Manel et al. 2002, Cegelski et al. 2003). The threshold of 0.950 was used as the standard above which individuals assigned to an alternate population were removed for analysis of genetic diversity based on a "corrected" data set.

Program MLE 1.0 (Topchy et al. 2004), a maximum-likelihood based assignment test, was used to confirm individual membership to the number of subpopulations generated by STRUCTURE based modal ΔK criteria (Evanno et al. 2005). Bobcats from each sample group were assigned probabilities of assignment to a subpopulation of most likely origin using the leave-one-out approach (Shao 1993).

The Mackinac Strait has likely been a barrier to historical dispersal between bobcat populations from the Upper and Lower Peninsulas. At a smaller scale genetic drift or undetected barriers to dispersal and gene flow might create microgeographic structuring within each Peninsula. Program FSTAT 2.9.3 was used to estimate variance in allele frequency among population segments and between the Upper and Lower Peninsulas. Exact tests were performed for pairwise population differentiation (pairwise F_{ST}) based on the multilocus genotypes of each bobcat within each population. Cavalli-Sforza and Edwards (1967) chord distance (D_C), an additional method for describing population subdivision, was calculated using MICROSATELLITE ANALYSER version 3.12. A neighbor-joining tree (NJ), representing the population differentiation, was constructed

from a D_C distance matrix and bootstrapped over all loci using PHYLIP 3.5c (Felsenstein 1993). The resulting NJ tree with bootstrap values was visualized using TreeView 1.6.6. (Page 1998).

Multiple logistic regression was used to evaluate the potential factors that could result in the mis-assignment of location. Each bobcat was classified as correctly or incorrectly assigned to location (Upper vs. Lower Peninsula). Geographic classification was based on the results of both Bayesian (STRUCTURE) and likelihood-based (MLE 1.0) analyses. An individual with posterior probabilities of population assignment of 0.950 or greater to the alternate Peninsula was considered incorrect based on thresholds tested by Manel et al. (2002) and Cegelski et al. (2003). Independent variables in the logistic regression model included age, sex, year of harvest, Peninsula, and method of harvest (hunt or trap). Age, sex, Peninsula, and harvest method were chosen as potential descriptors of directional bias in location reporting due to the species' ecology or illegal harvest. Harvest year was included to determine if there was any bias that would affect combining the samples for all other analyses.

Error in methods of sex identification

Molecular sexing techniques were used to evaluate the magnitude and directional bias of error in field- and MRA-sexing analysis. A true measure of sex is anatomy. Fur harvesters were not required to present entire bobcat carcasses during registration. However, we assumed that a genetically determined sex based on 2 independent markers provided unambiguous classification that would have been attained with the manual inspection of each carcass. Error rates were determined for juveniles (0-1 year old) and

adults (> 1 year old) of both sexes based on comparison of genetic sex classification to field and MRA sex classification. Error rates were also calculated for juveniles (0-1 year old), yearlings (> 1 year old, but < 2 years old), and adults (> 2 years old) for input into a harvest-based population model.

Multiple logistic regression was used to evaluate the potential factors that could result in the mis-assignment of sex. Each bobcat was classified as correctly or incorrectly sexed based on a comparison of molecular to field and MRA methods. Independent variables in the logistic regression model included age (adults vs. juveniles), "true" sex as determined by molecular methods, year of harvest, location of harvest (Upper or Lower Peninsula), and method of harvest (hunt or trap). Age and sex were considered important due to variable rates of growth, and thus overlap in body size between sexes. Location and method of take were used as variables in field sexing to assess potential affects of differential harvest regulations. Degree of sexual dimorphism might be greater at higher latitudes even over limited north-south distances in the range of the bobcat in Michigan, and may affect the accuracy of the MRA sexing method. Although included in the model, harvest method was not expected to affect sex mis-classification. Harvest year was included to determine if combining samples across years was valid for determination of error rates.

Effect of error in sex identification on harvest-based population modeling

A deterministic furbearer population model developed by the Minnesota Department of Natural Resources (Berg and Snow 1989, Berg and Kuehn 1989) was used to project theoretical bobcat populations for both Upper and Lower Peninsulas. The

model requires data from annual harvest and ecological studies of the target species. The Wisconsin Department of Natural Resources implements the same model to project annual fisher and bobcat population abundance (Rolley and Roth 2004, Rolley and Roth 2005). The MDNR has experimented with the model for estimating bobcat abundance in the Upper Peninsula. However, it was determined that this model was too sensitive to variability in model inputs, particularly starting population size, to be of value for estimating bobcat abundance in Michigan (D. Etter, MDNR, personal communication).

The furbearer model does not provide direct estimation of population size. Instead, population abundance trends are estimated which can be used to estimate population abundance if corroborating population trends are projected from additional data (e.g., indices).

Assumptions of the model include: (1) a base non-harvest mortality rate upon which harvest mortality is additive; (2) rates of non-harvest mortality are not density dependent and are constant; (3) non-harvest mortality rates differ by age, but not by sex within each age category; (4) illegal harvest rates that do not vary by sex or age; (5) constant adult reproductive rates, and; (6) reproductive rates per age category can be altered over time. The output, trends in population abundance, should be supported by independent measures such as a population index (Berg and Snow 1989).

Using the Minnesota furbearer model, a theoretical pre-birth population was and the initial population was assigned an adult sex ratio and age composition. Parameters relating to production of young (e.g., mean litter size, age-specific fecundity rates) were determined and used to project future recruitment based on the initial pre-birth population. Seasonal non-harvest mortality and harvest mortality was subtracted,

resulting in a pre-birth population for year two. Calculations were continued for 19 years. Sex ratios and age composition were independently adjusted each year based on harvest data.

The model was run for Michigan harvest data from 1985 to 2003. All reported bobcat deaths, including non-harvest mortalities (e.g., road kill), were recorded in the annual bobcat harvest surveys. Individuals not legally harvested were removed from the analysis. Bobcats were sorted by year, Peninsula, MRA- or field-assigned sex, and age category. For every year and Peninsula, each age and sex category was multiplied by a constant net percentage of error based on the results of molecular sexing.

Three data sets were introduced into the model for populations from each Peninsula. The first data set included harvest records with sex estimated using MRA analysis. The sex ratios from MRA analysis were adjusted by the amount of error estimated in the sexing method to represent molecularly sexed individuals for the second data set. The third included sex ratios adjusted to account for error in field sexing.

The deterministic nature of the model allows for stochasticity only in situations where temporal differences in input parameters are known. Due to the lack of input data specific to Michigan's bobcat population as it might have varied from 1985 to 2003, all parameters were assumed to have been constant except sex and age composition. This is not realistic, as demographic parameters change as a result of environmental variation or even stochastically. However, the objective of using the population model was not to describe a real population, or provide a realistic population estimate. The purpose of the modeling exercise was to show how error in sex identification could bias population assessment, both in direction and magnitude.

Due to the sensitivity of the Minnesota furbearer model to input values, specific parameters were selected that would likely vary over time and to which the model was sensitive. Although remaining constant over time, these parameters were increased and decreased individually per run of the model to assess their effects on the overall magnitude of difference between sexing methods. The initial inputs included an adult litter size of kittens/female of 3.1 (Hoppe 1979), and an adult female pregnancy rate of 80%. Some female bobcats may experience estrus in their first year, but most breed during the second year (Rolley 1985). As a result, yearling pregnancy rate was assumed to be 20%. Yearlings have also been reported to have smaller litter sizes than adults (Knick et al. 1985). Yearling litter size was set at 2.8 kittens/female. Different values of initial population size for both Peninsulas were also used in the model to assess effects on magnitude of error of field and MRA sex identification.

Results

Genetic diversity

Following Bonferroni correction, two population-locus comparisons significantly deviated from Hardy-Weinberg equilibrium (EUP and locus 6HDZ463; NLP and locus 6HDZ635). These deviations were both due to homozyogote excess. Linkage disequilibrium was not detected. The deviations were determined to be random occurrences and no loci were removed from analysis. No change occurred following removal of the individuals ($N = 10$) flagged as having misreported locations.

The data corrected for misreported harvest location were used to describe genetic variation between Peninsulas as well as between sample groups within Peninsulas. Mean

number of alleles per locus, and consequently allelic richness, were higher in the Upper Peninsula than the Lower Peninsula (Table 6.1). Mean heterozygosity was also greater in the Upper Peninsula than in the Lower Peninsula. F_{IS}, the inbreeding coefficient was greater in the Lower Peninsula population. Genetic diversity measures of sample groups were similar except for a lower F_{IS} value in CUP relative to neighboring groups of EUP and WUP.

Error in harvest location reporting

Location and genetic differentiation

Considerable genetic divergence was apparent between the Upper Peninsula and Lower Peninsula. Pairwise F_{ST} values between Peninsulas ranged from 0.049 between NLP and EUP to 0.104 between CLP and CUP. There was no significant difference between populations within the Lower Peninsula ($\alpha = 0.05$; NLP and CLP). A significant difference was evident between CUP and EUP ($\alpha = 0.05$; pairwise F_{ST} value = 0.011). However no other pairwise F_{ST} comparisons were found among the Upper Peninsula subpopulations. Cavalli-Sforza and Edwards chord distance also revealed statistically supported variance between the Upper and Lower Peninsula populations (Figure 6.2). The percent bootstrap value assigned to the node separating the Upper from the Lower Peninsula was 100, indicating a clear support for the separation of the two Peninsulas. In addition, the longest branch of the neighbor-joining tree was between EUP and NLP, suggesting the greatest genetic divergence occurred between Peninsulas. The large degree of genetic differentiation between the Upper and Lower Peninsulas allowed for a statistically powerful means by which assign individuals to location.

Table 6.1. Summary of estimates of genetic diversity for each subpopulation and averaged across the Upper and Lower Peninsula in Michigan. Estimates were made after the data was corrected for suspected mis-reported harvest location.

		Summary means of genetic diversity					
		N	# alleles	A[A]	H_0[B]	H_e[C]	F_{IS}
Upper Peninsula		90	5.9	5.80	0.694	0.711	0.025
WUP	West	32	6.0	5.87	0.704	0.733	0.040
CUP	Central	33	5.9	5.66	0.698	0.694	-0.007
EUP	East	25	5.9	5.88	0.675	0.707	0.047
Lower Peninsula		69	4.9	4.73	0.596	0.620	0.038
NLP	North	*34*	5.1	4.88	0.603	0.632	0.046
CLP	Central	*35*	4.8	4.58	0.589	0.608	0.031

[A] Allelic richness
[B] Observed heterozygosity
[C] Expected heterozygosity

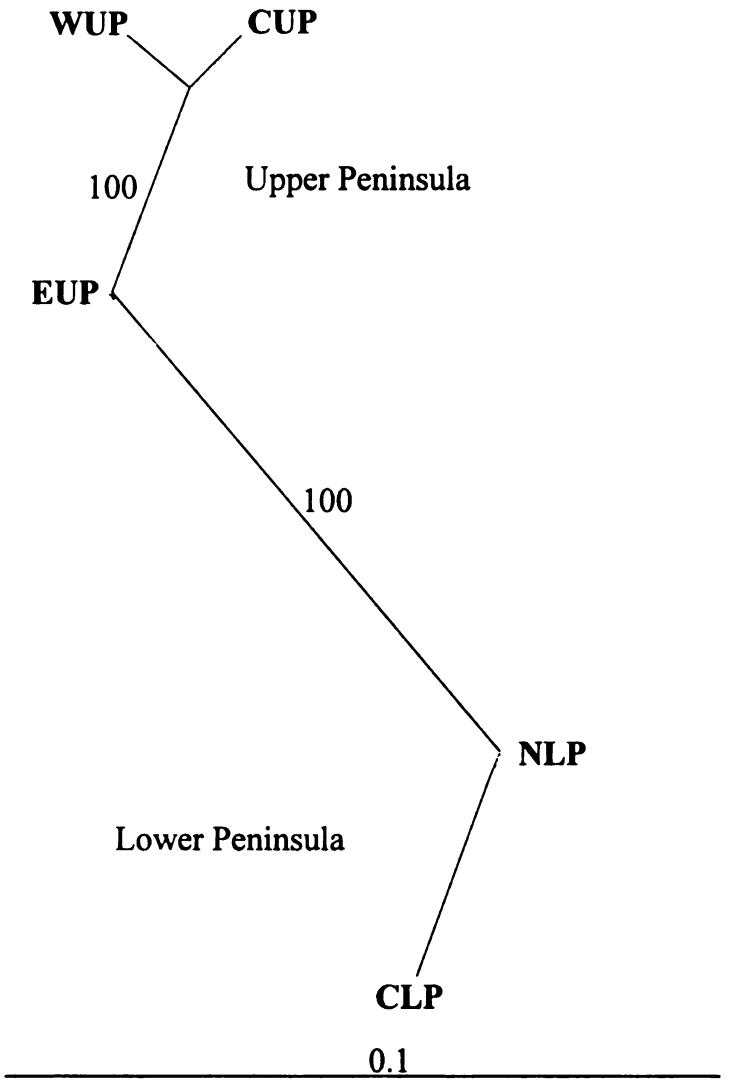

Figure 6.2. Cavalli-Sforza and Edwards (1967) chord distance based unrooted neighbor-joining cluster diagrams showing relationships among Michigan bobcat subpopulations. Values are provided showing the percentage of all bootstrap replicates supporting the branches.

Methods of assignment

Differential harvest regulations could result in directional bias in location reporting of bobcats. Assignment methods were used to identify individuals with probable misreported harvest locations. One-hundred ninety individuals were used in Bayesian-based (STRUCTURE analysis) assignment methods. Estimates of the most statistically supported number of genetic clusters based on likelihood ratio tests was two subpopulations of bobcats ($K = 2$) in Michigan. Following the methods of Evanno et al. (2005) the modal value of ΔK was greatest at $K = 2$ supporting the results generated by program STRUCTURE. One-hundred and four of 118 (88.1%) bobcats registered as Upper Peninsula harvests showed maximum posterior probabilities of assignment to an Upper Peninsula population. Fourteen bobcats (11.9%) reported to have been harvested in the Upper Peninsula exhibited posterior probabilities of assignment to a Lower Peninsula population. Seventy of 72 (97.2%) individuals registered as harvested in the Lower Peninsula exhibited maximum posterior probabilities of assignment to a Lower Peninsula population. Two bobcats (2.8%) showed maximum posterior probabilities of assignment to an Upper Peninsula population (Figure 6.3). When a threshold of 0.95 was imposed, 94 of 118 bobcats registered as Upper Peninsula harvests were assigned to an Upper Peninsula population; 12 bobcats registered as Upper Peninsula harvests were assigned to a Lower Peninsula population. Sixty-seven bobcats of 72 bobcats registered as Lower Peninsula harvests were assigned to a Lower Peninsula population; one bobcat registered as a Lower Peninsula harvest was assigned to an Upper Peninsula population.

A maximum-likelihood based assignment test implemented in MLE 1.0 was used to corroborate results from program STRUCTURE. Two bobcats reported harvested in the

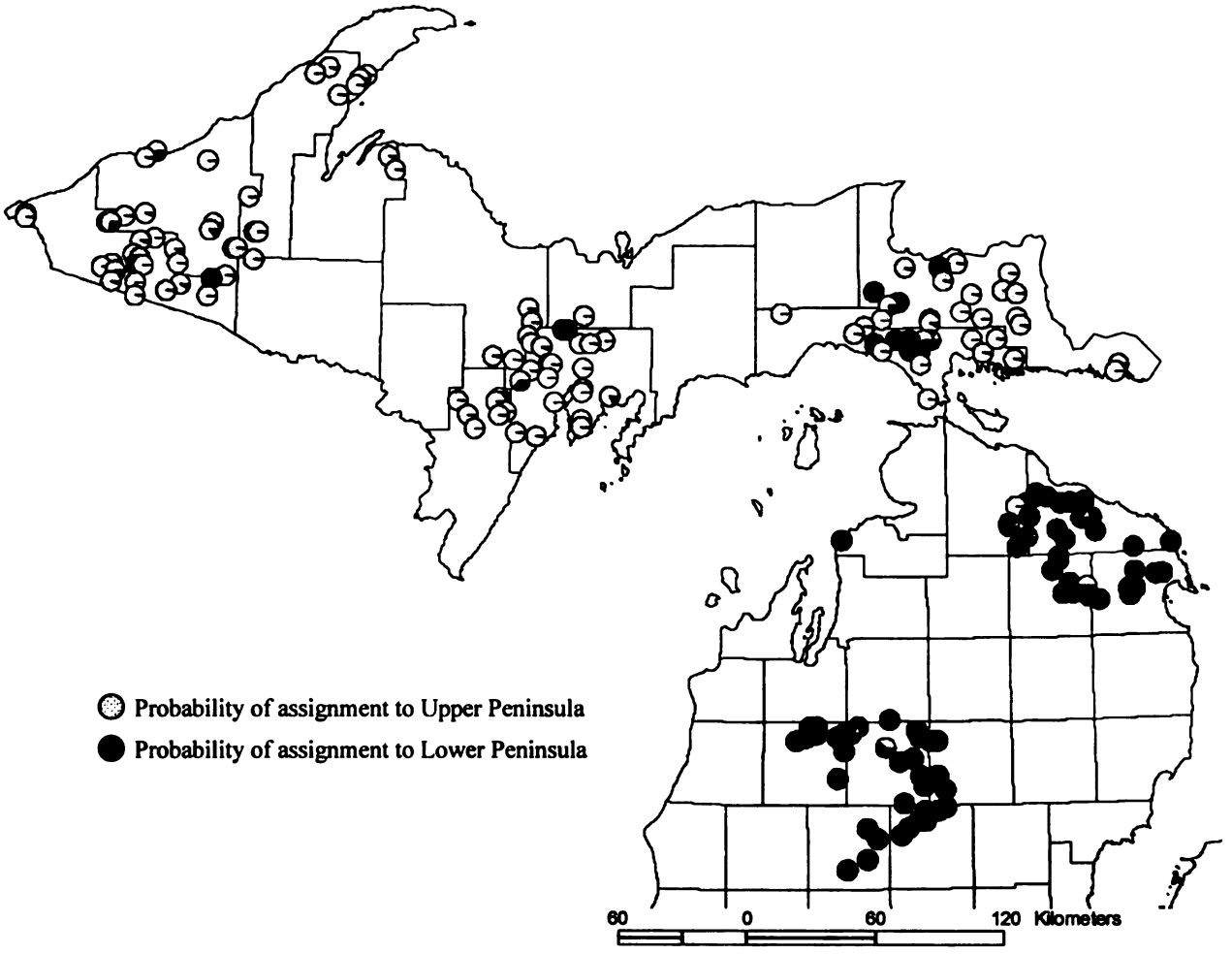

Figure 6.3. Results of STRUCTURE analysis for all assayed bobcats showing posterior probabilities of individual membership in two identified genetic clusters that corresponded to Upper and Lower Peninsula regions. One bobcat registered in the Lower Peninsula was assigned to the Upper Peninsula population with a posterior probability of >0.900 and 0.950. Twelve bobcats registered in the Upper Peninsula clustered with the Lower Peninsula group with posterior probabilities of >0.900 and 0.950. These individuals were assumed to have been mis-reported.

Lower Peninsula were assigned to the Upper Peninsula population. The two individuals corresponded to those identified using program STRUCTURE. Fifteen bobcats registered in the Upper Peninsula were assigned to the Lower Peninsula. Fourteen of the animals corresponded to the fourteen bobcats identified by STRUCTURE. Based on STRUCTURE results, one bobcat displayed a very low posterior probability of assignment to the Upper Peninsula population (0.503). However, the confidence level of assignment of the individual to the Lower Peninsula using program MLE 1.0 was 84.6%.

The original data set was corrected for location by removing individuals with posterior probabilities of assignment to the alternate population above thresholds of 0.950 and 0.990. Using the threshold of 0.950, 12 bobcats reported harvested in the Upper Peninsula, including 9 animals from EUP, and 1 bobcat reported harvested in the Lower Peninsula were removed. When the threshold was raised to 0.990, 6 bobcats reported harvested in the Upper Peninsula were assigned to the Lower Peninsula and no bobcats harvested in the Lower Peninsula were assigned to the Upper Peninsula.

Logistic regression

Multiple logistic regression analysis was used to determine potential factors associated with bias of harvest location reporting. A significant association of harvest location reporting error was detected for location of harvest ($\chi^2 = 9.884$, $P = 0.002$; Table 6.2). No significant association ($P > 0.05$) was found for age, sex, year of harvest, or harvest method. The overall regression model for harvest location was also significant ($\chi^2 = 16.537$, $P = 0.005$).

Table 6.2. Summary of results from logistic regression analysis of bobcat harvest location reporting error as a function of year, location of harvest, harvest method, and age. Also provided are the test statistics for the final model of the regression.

Independent variable	Chi-square	Degrees of freedom	*P*-value
Year	3.546	1	0.600
Location	9.884	1	0.002
Harvest method	2.515	1	0.113
Sex	0.877	1	0.349
Age	1.904	1	0.168
Final model	16.537	5	0.005

Comparison of sexing methods

Nine bobcats were removed from the analysis of bias in field sex identification based on lack of field sex or age information. Likewise, seven bobcats were removed from analysis of bias in the MRA method. One-hundred eighty two and 184 individuals remained for the analysis of field and MRA sexing methods, respectively. The ZFX/Y-TDF and TET SRY/ZFX markers were concordant assignment of sex for all individuals. The bobcats consisted of 26 juvenile males ($N = 25$ for field sexing analysis), 18 juvenile females, 74 adult males (>1 year old), and 66 adult females (>1 year old; $N = 65$ for field sexing analysis) based on the aging method used by the MDNR. The inclusion of yearling as an additional age category resulted in the same number of juveniles, but 22 yearling males, 28 yearling females, 52 adult males (>2 years old), and 38 adult females (> 2 years old; $N = 37$ for field sexing analysis). These data were compared with the sex determinations for each individual provided by the MDNR using MRA analysis and field sexing.

Sex and age were significantly associated with correct determination of field sex in the logistic regression ($\chi^2 = 6.193$, $P = 0.013$ and $\chi^2 = 6.831$, $P = 0.009$, respectively; Table 6.3). The overall regression model was also significant ($\chi^2 = 20.231$, $P = 0.001$). However, there was no significant association of year of harvest, location of harvest, or harvest method to correct identification of sex using field methods ($P > 0.05$). Accordingly, error rates by age and sex were totaled across bobcats from both Peninsulas and both years sampled.

Genetic sexing methods resulted in determination of 64.0% (16/25) juvenile males incorrectly classified as females based on field sex assignment and 16.7% (3/18)

Table 6.3. Summary of results from logistic regression analysis of bobcat field sex identification error as a function of year, location of harvest, harvest method, and age. Also provided are the test statistics for the final model of the regression.

Independent variable	Chi-square	Degrees of freedom	*P*-value
Year	2.087	1	0.149
Location	1.76	1	0.185
Harvest method	0.094	1	0.759
Sex	6.193	1	0.013
Age	6.831	1	0.009
Final model	20.231	5	0.001

juvenile females incorrectly classified as males (Table 6.4). Nineteen of seventy-four (25.7%) adult males were incorrectly identified as females, whereas 7.7% (5/65) of adult females were misclassified as males. The addition of the yearling age category and reassignment of the adult category resulted in 45.5% (10/22) yearling males misidentified as females, 11.0% (3/28) yearling females misclassified as males, 17.3% (9/52) adult males incorrectly classified as females, and 5.4% (2/37) adult females incorrectly identified as males. The overall error rate in the field sexing method was 23.6% (43/182).

In the logistic regression model, "true" sex was significantly associated with correct determination of sex using the MRA method ($\chi^2 = 4.259$, $P = 0.039$; Table 6.5). The overall regression model was also significant ($\chi^2 = 11.807$, $P = 0.038$). No association of incorrect determination of sex was detected as a function of year of harvest, harvest method, location or age ($P > 0.05$). The error appears to be random in respect to the variables used in this study. Therefore, MRA error rates were calculated using all bobcats sampled from both Peninsulas and both years.

Based on sex identification using genetic techniques, 34.6% (9/26) of juvenile males were incorrectly classified as females using MRA analysis, and 5.6% (1/18) of juvenile females were incorrectly classified as males (Table 6.4). Five of 74 (6.8%) adult males were classified incorrectly as females, and 24.2% (16/66) of adult females were incorrectly classified as male. Addition of the yearling age category resulted in identification of 4.5% (1/22) of yearling males misclassified as females, 25.0% (7/28) of yearling females misidentified as males, 7.7% (4/52) adult males misclassified as females, and 23.7% (9/38) adult females incorrectly identified as males. The overall

Table 6.4. Summary of error rates determined for MRA and field sexing methods based on molecular sexing techniques. The total number of misclassified individuals/the total number in a given age and sex category are provided in addition to percent error.

	Field sexing method		MRA sexing method	
Sex and age category	# errors/total	% error	# errors/total	% error
Adult male (>1 ya)	19/74	25.7%	5/74	6.8%
Adult male (>2 ya)	9/52	17.3%	4/52	7.7%
Yearling male (>1ya, <2 ya)	10/22	45.5%	1/22	4.5%
Juvenile male (<1 ya)	16/25	64.0%	9/26	34.6%
Adult female (>1 ya)	5/65	7.7%	16/66	24.2%
Adult female (>2 ya)	2/37	5.4%	9/38	23.7%
Yearling female (>1ya, <2 ya)	3/28	11.0%	7/28	25.0%
Juvenile female (<1 ya)	3/18	16.7%	1/18	5.6%
Total error rate:	43/182	23.6%	31/184	16.8%

Table 6.5. Summary of results from logistic regression analysis of bobcat MRA sex identification error as a function of year, location of harvest, harvest method, and age. Also provided are the test statistics for the final model of the regression.

Independent variable	Chi-square	Degrees of freedom	*P*-value
Year	1.763	1	0.184
Location	0.046	1	0.830
Harvest method	3.053	1	0.081
Sex	4.259	1	0.039
Age	1.143	1	0.285
Final model	11.807	5	0.038

error rate in the MRA sex identification method was 16.8% (31/184).

Population modeling incorporating sexing error

Logistic regression analysis of sexing error showed no relationship between either field or MRA-based sexing error and year or location. As a result, error rates in both sexing methods were assumed to be the same for the Upper and Lower Peninsulas and constant over the modeled time period. The magnitude and direction of sexing error based on molecular methods was considered representative of the population trend across years and areas of the state.

Population trends modeled for the Upper Peninsula were similar to those developed for the Lower Peninsula (Figure 6.4 and Figure 6.5) based on the minimum initial population size and initial input parameters as described above. In both locations, population trends projected using bobcat sex determined using MRA analysis resulted in greater overall abundance (positive bias) over estimates when molecular-based sexing methods were used. In the final year modeled (2003), the bobcat population abundance trend representing MRA sex identification overestimated the true population by 25.1% in the Upper Peninsula and 30.1% in the Lower Peninsula. Conversely, field sexing resulted in underestimation of population abundance (negative bias) compared to molecular sexing. Field sex classification resulted in underestimation of the true population abundance by 87.3% and 79.4% in the Upper and Lower Peninsulas, respectively.

Increasing initial population size resulted in a decrease in the magnitude of error due to sex misidentification. As initial population size increased, the direction of the

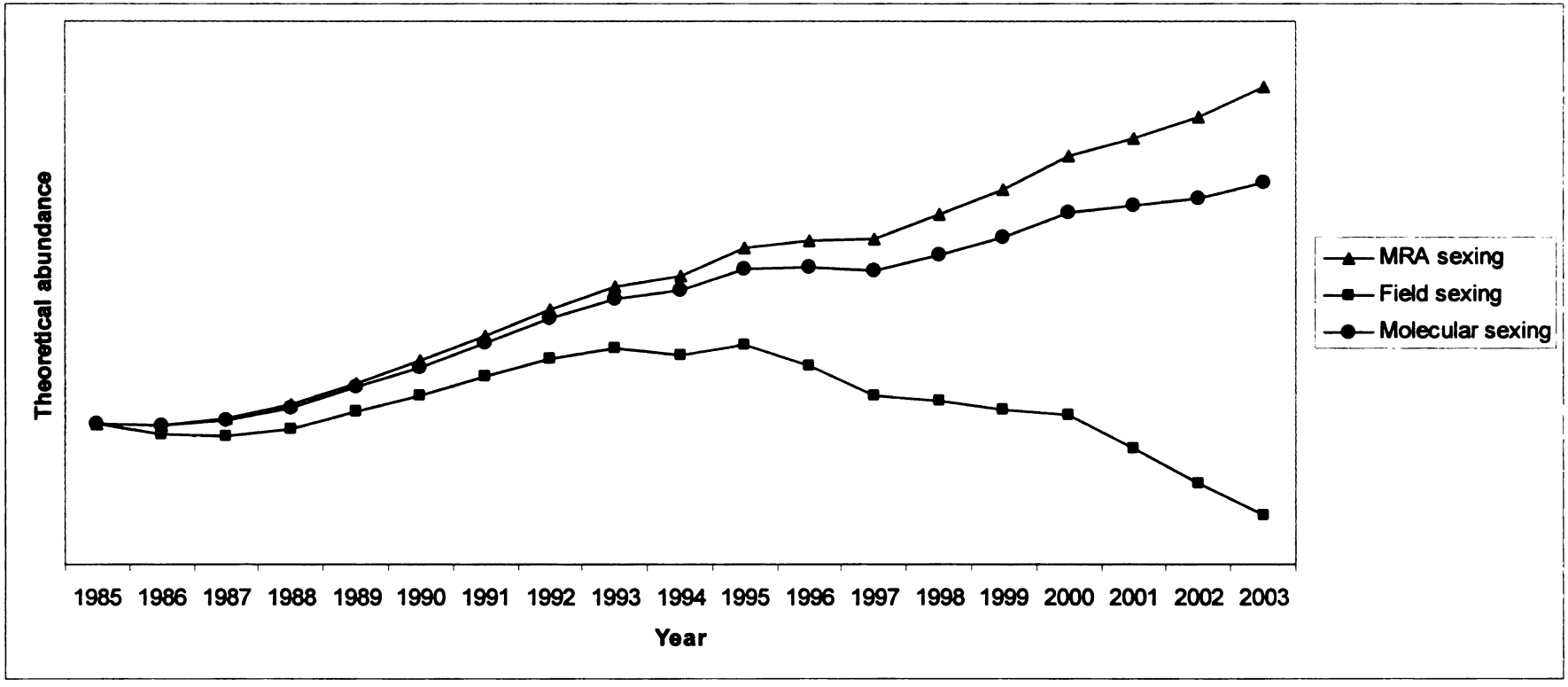

Figure 6.4. Bobcat population projections in the Upper Peninsula based on the Minnesota furbearer population model. The trends were calculated using sex ratios from harvest data representing MRA-, field-, and molecular-based sex identification.

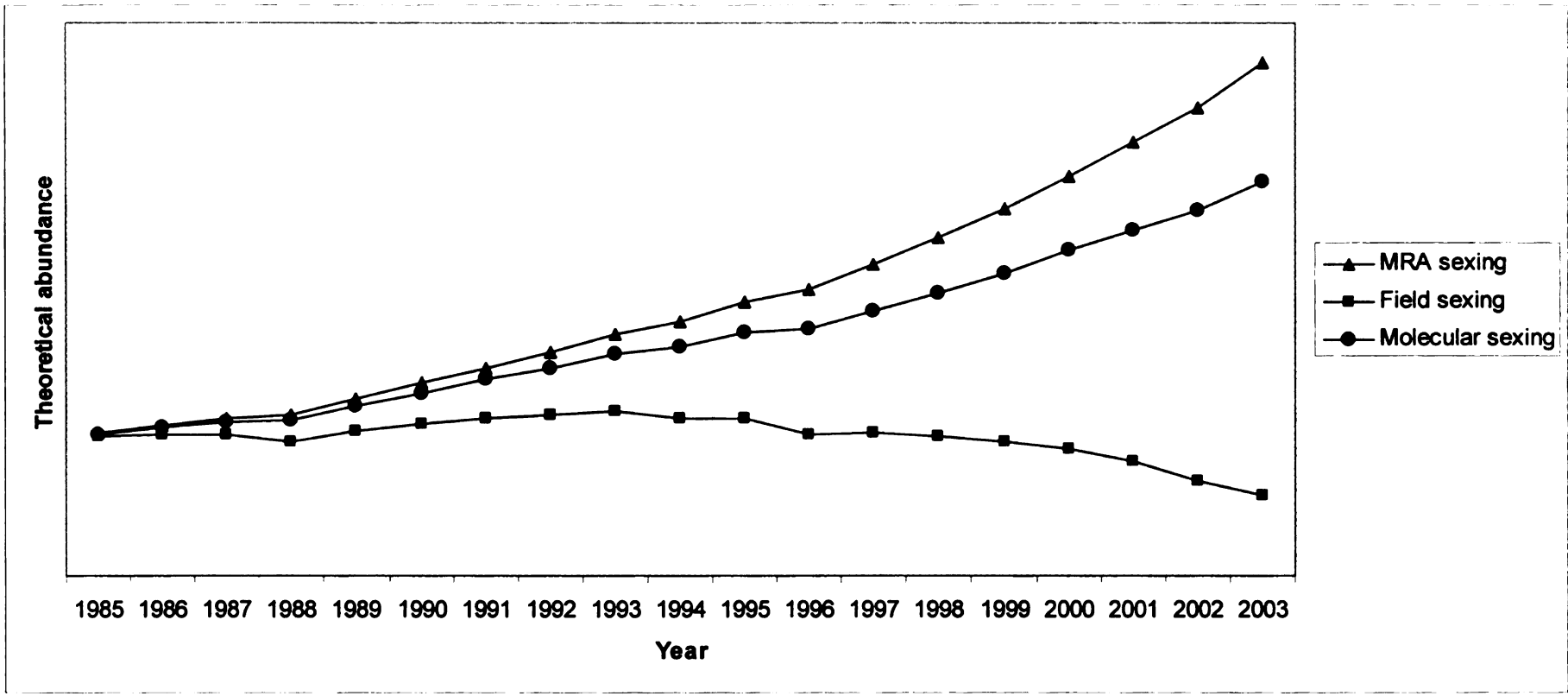

Figure 6.5. Bobcat population projections in the Lower Peninsula based on the Minnesota furbearer population model. The trends were calculated using sex ratios from harvest data representing MRA-, field-, and molecular-based sex identification.

field sexing trend leveled out and began to show an overall increase in abundance. The population trends representing MRA- and molecular-based sex ratios increased as with the minimum initial population size, but at a faster rate, resulting in a greater estimate of abundance.

Increasing adult female litter size resulted in a decrease in the magnitude of error due to sex misidentification. Conversely, the magnitude of error was greater when adult litter size was decreased. Adjusting yearling litter size had a similar directional effect on magnitude of error, but to a lesser degree than adult female litter size, likely due to the small percentage of yearling female breeders. When percentage of female yearling pregnancies was increased, the magnitude of error increased. Similarly, increasing the percentage of pregnant adults increased the overall magnitude of error. A decrease in the percentage of pregnant adult females resulted in a decrease in the magnitude of error in the MRA- and field-based sexing methods (results not shown).

Discussion

Genetic diversity and genetic population structure

Bobcats in Michigan display a high degree of spatial genetic structuring between the Upper and Lower Peninsulas. Large variance in allele frequency between bobcats from these areas was expected due to prolonged periods of separation since the last glacial epic. Likewise, the lower overall genetic diversity found in the Lower Peninsula is consistent with the population's historical insularity and greater extent of land-use change and expected concomitant decline in population abundance. Dispersal, and thus gene flow, was likely reduced from areas outside of the northern Lower Peninsula,

resulting in lower heterozygosity, lower allelic diversity, and higher inbreeding coefficients (F_{IS}) than was observed for bobcats from the Upper Peninsula. Pairwise F_{ST} values and the neighbor joining tree provided strong evidence of genetic differentiation between the EUP and the two sample groups to the west (CUP and WUP) even following removal of individuals of probable misrepresented harvest location. However, expanding distribution and increases in bobcat abundance in areas to the south may result in increased immigration to the Lower Peninsula population in the future (Woolf et al. 2000, D. Etter, MDNR, personal communication).

Error in harvest location reporting

Results from analyses using both Bayesian and maximum-likelihood assignment methods showed bobcats generally assign very highly to location of origin (Peninsula), underlying the usefulness of genetic information for detection of misreported bobcat harvest location.

A threshold posterior probability of assignment generated using STRUCTURE $\geq$ 0.950 resulted in identification of 13 bobcats that were likely misreported. Twelve of the 13 bobcats were registered as harvested in the Upper Peninsula, but genetically assigned to the Lower Peninsula population. This bias was clearly directional, shown by a significant relationship in the multiple logistic regression analysis between location misclassification and location (Table 6.2).

Although the Mackinac Straits are a likely formidable barrier to bobcat movements, as is supported by the genetic divergence between the Upper and Lower Peninsulas, infrequent dispersal may occur, and might explain the pattern of bias in

location misreporting in this study. Genetic analysis of these dispersers would result in the observation of genotypes consistent with the opposite Peninsula from where the animal was reported. However, a directional bias would not be expected, and the dispersers would likely be juvenile male biased (Kitchings and Story 1984, Kamler et al. 2000). Neither sex nor age was found to have a significant relationship with location mis-classification in the multiple logistic regression analysis (Table 6.2). In addition, the high posterior probabilities of assignment of the 13 misreported bobcats suggested animals were likely first-generation dispersers. Lower posterior probabilities of assignment would be expected from an F1 hybrid individual or individual of higher filial generation when harvested. If the number of mis-classified bobcats detected in the sample size used for this study was extrapolated to an estimate of the true bobcat population in Michigan, the number of dispersers becomes unfeasible based on the lack of observations of dispersal as well as contemporary genetic discontinuity between the Upper and Lower Peninsulas.

Unintentional database error may have contributed to the error in harvest location reporting. Long seal numbers are often used to identify individual animals. Error can occur in transcription of those numbers from the seal to the database. Errors can also occur in the laboratory (see Paetkau 2003 for a review). However, these errors would be stochastic and would not result in a strongly directional bias. To minimize future potential recording errors, the MDNR is experimenting with a data collection system using personal digital assistants (PDAs) and scanners for collecting harvest information (D. Etter, MDNR, personal communication).

The directional bias of the location reporting error might be explained by the

differential bobcat harvest regulations that existed through the sampling period. Bag limits were lower, the harvest season was shorter, and trapping was not permitted in the Lower Peninsula. If one chose to illegally harvest a bobcat in the Lower Peninsula, either by exceeded the bag limit, harvesting out of season, or even trap, there may be an inclination to register the animal as having been killed in the Upper Peninsula. Nine of the 12 bobcats removed from the Upper Peninsula as a result of strong genetic assignment to the Lower Peninsula population were registered as harvested in the EUP. Bobcats illegally harvested in the Lower Peninsula may often be registered in the eastern Upper Peninsula due to proximity to the bridge connecting the two Peninsulas. It has been suspected that poachers would drive to the closest MDNR office on the north side of the Mackinac Straits to falsely register their bobcat as a legal harvest.

There is no harvest-based rationale explaining why bobcats reported as harvested in the Lower Peninsula were genetically assigned to the Upper Peninsula. The most likely explanation for the error in location reporting detected in this study was a combination of illegal harvest, which may have resulted in the directional bias, recording error, and potential, but limited dispersal.

In 2005, bobcat harvest regulations in Michigan were changed to allow trapping and hunting in both the Upper and Lower Peninsulas. In addition, bag limits were reduced to two bobcats per person in the Upper Peninsula and a mandatory field tag was created. These regulation changes were made in part to address concerns of mis-registration (D. Etter, MDNR, personal communication). However, the seasons remain different, with an abbreviated harvest in the Lower Peninsula (MDNR 2005). Therefore, it is likely that illegal misreporting of harvested bobcats may continue in a

directional manner.

Error in sex determination

Friedrich et al. (1983) calculated an overall error rate in MRA sex determination of 5.8%. However, comparison of bobcats sexed using the MRA method to two independent molecular sexing techniques found a much higher level of overall error (16.8%). The overall error in field sexing was even higher (23.6 %).

Molecular sexing was considered to reflect "true" sex. Mammal molecular sexing markers target the X and Y chromosomes, which are permanently present in the animal's DNA. Therefore, body size, rate of development, or age does not affect the accuracy of sex determination. Lab errors, such as allelic dropout, might create error in molecular techniques. However, the concordance in results of two independent molecular sexing markers in this study provided evidence of the accuracy of the sex determination. An additional error of concern would be contamination of the tissue sample used for molecular analysis. If DNA from a male contaminates DNA from a female, the sample will be identified as male, as both possess the X chromosome, but only males possess the Y chromosome. Likely sources of contamination would be from the separation of frozen heads following submission for registration, or from accidental submersion of tissue in blood from a different individual (P. Friedrich, MDNR, personal communication). All bobcats molecularly sexed were genotyped across eight additional microsatellite loci. Any contamination would have appeared in the scoring of these microsatellite loci. No contamination was detected, further supporting the assumption of molecular sex representative of "true" sex in this study.

Sexing error in the MRA method was directionally biased. The greatest error rates were observed in juvenile males (34.6%), yearling females (25.0%), and adult females (23.7%). This suggests the error was driven by overlap in size of the sexually dimorphic bobcat. The MRA method is based on physical characteristics that are size dependent. Juvenile animals tend to mature at different rates based both on environmental and genetic factors. Small or slowly developing males might have MRA measurements that fall below the criterion used to determine sex. The low error in misclassification of juvenile females (5.6%) suggested that higher than average rates of development and growth in female bobcats are rare relative to the MRA criterion. The attainment of potential breeding age (yearling and adult) resulted in a shift in error of the MRA sexing method. Error rates in all males were small, likely due to the accuracy of the MRA criterion to account for a majority of the physically larger males in comparison to the smaller females. However, the high rates of error in yearling and adult females indicated the MRA criterion was set at a value that resulted in directional bias. MRA measurements of large females in the harvest would be greater than the established criterion. Larger females might be older females. Due to generally smaller home ranges (Bailey 1974), a greater proportion of females relative to males may survive harvest, thereby shifting an observed sex and age distribution. Similarly, more conservative movements (daily and dispersal) of females in comparison to males (Buie et al. 1979, Knowles 1985, Knick and Bailey 1986) might make it less likely for females to be harvested. However, the high error rate in yearling females and a lack of significant association between sex classification error and age in the multiple logistic regression suggested female size is not related to age in the context of MRA sex identification.

Sexing error in the field method was also directionally biased. The highest rates of error were found in juvenile males (64%) and yearling males (45.5%). Unlike the MRA method which used scientifically tested and defined threshold values to identify males and females, field sex is determined by each individual furtaker. It has been suspected that field sexing is often based solely on body size of the harvested bobcat (P. Friedrich, MDNR, personal communication). Small animals would be classified as females and large animals classified as males. Although body size directs both MRA and field sexing, subjectivity in the field method would result in different biases than observed in the scientifically-based MRA method. The high rates of error in juvenile and yearling males suggested that younger males were considered small, and therefore female. In addition, undeveloped males (i.e., young males with undescended testes) of any size may be classified as females due to the lack of obvious male secondary characteristics. The high sex- and age-dependent error rates explained the significant association of field sex classification with both sex and age described by the multiple logistic regression analysis.

The high overall error rates calculated for MRA and field sexing methods indicated that an alternative method of sex determination should be explored for future use. Subjectivity in the field sexing method resulted in unreliability in accurate sex determination. The unidirectional bias in error in MRA analysis indicates the accuracy of the method in determining sex for certain sex and age classes. However, the bias suggests that overlap in size of females and males will result in error regardless of the MRA criterion. For example, if the current criterion for adults is increased to account for larger adult females, error will increase in the number of smaller adult males

misclassified as females. One alternative method would be to resume carcass collection at registration and manually sex each individual in the laboratory. However, resources may not be available to collect, store, and process each whole carcass. Molecular sexing requires only a small amount of tissue to be collected at registration, limited storage space, and the ability to process hundreds of samples in a short period of time. Although aging would continue to be determined using tooth analysis, molecular sex analysis might reduce overall cost in terms of money and resources.

Effects of error in sex determination on population assessment

Sex and age are key population demographic parameters estimated from annual bobcat harvests and are used by the MDNR to help determine population sustainability. Identification of error in estimation of these parameters is important. However, it is critical to evaluate the effects of the error within the framework of how those estimates are used.

Deterministic accounting-type population models are heavily influenced by female abundance. The number of breeding females in a population is directionally related to the number of offspring produced. Therefore, the magnitude of sexing error might affect the accuracy of the conclusions drawn from population assessment through either over- or under-estimation of abundance of females. Error in one sex-age category resulting in overabundance of harvested females may offset error in a different sex-age class causing underestimation of the number of females in the harvest, thereby minimizing overall bias in abundance. The degree to which females are directionally misrepresented in the harvest determines the degree of bias in the population estimate.

High rates of MRA-based sexing error in bobcat yearling and adult females resulted in an underestimate of the number of potentially breeding females in the harvest. As a result, overall population abundance was overestimated.

High rates of field-based sexing error were defined in males of all age categories, resulting in overestimation of female representation in the harvest. The observed higher percentage of females harvested resulted in an underestimation of overall abundance.

The magnitude of underestimation due to field sexing error was greater than the magnitude of overestimation due to MRA sexing. Since the highest field sexing error was found in males of all age groups, the misrepresentation of the number of females in the harvest was more grossly biased than the number of harvested females estimated using MRA analysis, whereby the underestimation of yearling and adult females was somewhat offset by an overestimation of juvenile females.

The Minnesota furbearer model was used as means to describe how error in field- and MRA-based sex identification could bias population assessment in direction and magnitude. The purpose of the modeling exercise was not to describe a real population, or to calculate a realistic estimate of population abundance. Stochasticity or environment-driven fluctuations in parameter estimates would affect the population estimate, and therefore the bias caused by sexing methodology. Although such temporal fluctuations could not be accounted for in the model, a simplistic sensitivity analysis of selected parameters provided relative effects on magnitude or direction of each adjustment. This information would allow managers to evaluate how known population parameters, in combination with applied sexing methods, might affect the accuracy of harvest-based population assessments.

The theoretical Michigan bobcat populations were modeled to describe how error in field- and MRA-based sexing methods affected overall trends in abundance over time. Since the population trends for each sexing method were started at an equal initial population size for each run of the model, any difference in the trends due to error was cumulative. Although the results presented here are theoretical error-based differences in magnitude in 2003, after 19 harvest seasons, they represent a singular moment in time. The magnitude of difference between trends calculated using sex ratios determined by the different sexing methods would continually increase over time.

Although the magnitude of error between the population trends representing each sexing method occurred with adjustments to input parameters, the overall trends remained the same. Harvest records maintained using field sexing methods to estimate sex ratios always underestimated abundance of bobcats. Conversely, harvest records with sex ratios determined using MRA analysis always overestimated population numbers. However, the magnitude of bias in the MRA-based population trend was much less than that depicted by the field-based trend. The consequences of using either method to estimate sex as a critical harvest-based demographic parameter are important to consider. Conclusions regarding probability of population sustainability using the Minnesota furbearer model would be very different if based on harvest population assessment and field-based sex determination versus MRA analysis. However, deterministic harvest-based models are only one of many methods concurrently used by agencies to make management decisions (e.g., setting future harvest regulations). An understanding and minimization of the potential biases in harvest records would increase the accuracy of harvest-based population models, and together with estimates of input

parameters, might make such methods more useful for describing and projecting bobcat population trends.

References

Aasen E. and J.F. Medrano. 1990. Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep, and goats. Biotechnology. 8: 1279-1281.

Bailey, T.N. 1974. Social organization in a bobcat population. Journal of Wildlife Management. 38: 435-446.

Berg, W. and D. Kuehn. 1989. Fisher, 1988-89. pp. 42-43 in Status of wildlife populations, fall 1989 and 1981-1988 hunting and trapping harvest statistics. Section of Wildlife, Minnesota Department of Natural Resources, St. Paul 120 pp. (unpublished report)

Berg, W. and Snow. 1989. Furbearer population model. Minnesota Department of Natural Resources.

Bessinger, S.R. and M.I. Westphal. 1998. On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management. 62: 821-841

Buie, D.E., T.T. Fendley, and H. McNab. 1979. Fall and winter home ranges of adult bobcats on the Savannah River Plant, South Carolina. Proceedings of the Bobcat Research Conference, National Wildlife Federation Scientific and Technical Series, 6: 42-46.

Cavalli-Sforza, L.L. and A.W.F. Edwards. (1967) Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics. 19: 233-257.

Cegelski, C.C., L.P. Waits, and N.J. Anderson. 2003. Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. Molecular Ecology. 12: 2907-2918.

Cooley, T.M., S.M. Schmitt, P.D. Friedrich, and T.F. Reis. 2000. Bobcat survey 1999-2000. Michigan Department of Natural Resources Wildlife Division Report No. 3325. 19 pp.

Crowe, D.M. 1972. The presence of annuli in bobcat tooth cementum layers. Journal of Wildlife Management. 36: 1330-1332.

Crowe, D.M. 1975. Aspects of aging, growth, and reproduction of bobcats from Wyoming. Journal of Mammalogy. 56: 177-198.

Dieringer, D. and C. Schlötterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes. 3: 167-169.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14: 2611-2620.

Felsenstein, J. 1993. PHYLIP (*Phylogeny inference package*), Version 2.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Friedrich, P.D., R.D. Earle, and T.M. Cooley. 1981. Comparison of bobcat age estimates from annuli in the cementum of incisor and canine teeth. Michigan Department of Natural Resources Wildlife Division Report No. 2895. 6 pp.

Friedrich, P.D., G.E. Burgoyne, Jr., T.M. Cooley, and S.M. Schmitt. 1983. Use of lower canine teeth for determining the sex of bobcats in Michigan. Michigan Department of Natural Resources Wildlife Division Report No. 2960. 5 pp.

Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. Journal of Heredity. 86: 485-486.

Guo S.W. and E.A. Thompson. 1992. Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. Biometrics. 48: 359.

Hoppe, R.T. 1979. Population dynamics of the Michigan bobcat (*Lynx rufus*) with reference to age structure and reproduction. Proceedings of the Bobcat Research Conference. National Wildlife Federation Scientific and Technical Series. 6: 111-115.

Johnson, N.F., B.A. Brown, and J.C. Bosomworth. 1981. Age and sex characteristics of bobcat canines and their use in population assessment. Wildlife Society Bulletin. 9: 203-206.

Kamler, J.F., P.S. Gipson, and T.R. Snyder. 2000. Dispersal characteristics of young bobcats from northeastern Kansas. Southwestern Naturalist. 45: 543-546.

Kitchings, J.T. and J.D. Story. 1984. Movement and dispersal of bobcats in east Tennessee. Journal of Wildlife Management. 48: 957-961.

Knick, S.T., and T.N. Bailey. 1986. Long-distance movements by two bobcats from southeastern Idaho. The American Midland Naturalist. 116: 222-223.

Knick, S.T., J.D. Brittell, and S.J. Sweeney. 1985. Population characteristics of bobcats in Washington state. Journal of Wildlife Management. 49: 721-728.

Knowles, P.R. 1985. Home range size and habitat selection of bobcats, *Lynx rufus*, in north-central Montana. The Canadian Field-Naturalist. 99: 6-12.

Litvaitis, J.A., C.L. Stevens, and W.W. Mautz. 1984. Age, sex, and weight of bobcats in relation to winter diet. Journal of Wildlife Management. 50: 110-117.

Lovallo, M.J. and E.M. Anderson. 1996. Bobcat (*Lynx rufus*) home range size and habitat use in northwest Wisconsin. American Midland Naturalist. 135: 241-252.

Manel, S., P. Berthier, and G. Luikart. 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conservation Biology. 16: 650-659.

Michigan Department of Natural Resources. 2003. 2003 Michigan Hunting and Trapping Guide. Lansing, MI. 46 pp.

Michigan Department of Natural Resources. 2005. 2005 Michigan Hunting and Trapping Guide. Lansing, MI. 46 pp.

Nielsen, C.K. and A. Woolf. 2003. Dispersal of juvenile male bobcats (*Lynx rufus*) in southern Illinois. Transactions of the Illinois State Academy of Science. 96: 313-318.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology. 12: 1375-1387

Page, R.D.M. 1998. TREEVIEW: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences. 12: 357-358. Available at: http://taxonomy.zoology.gla.ac.uk/rod/treeview.html

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86: 248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225.

Rolley, R.E. 1985. Dynamics of a harvested bobcat population in Oklahoma. Journal of Wildlife Management. 49: 283-292.

Rolley, R.E. and A.M. Roth. 2004. Fisher population analyses 2004. Wisconsin Department of Natural Resources. 4 pp.

Rolley, R.E. and A.M. Roth. 2005. Bobcat population analyses 2005. Wisconsin Department of Natural Resources. 3 pp.

Shao, J. 1993. Linear model selection by cross-validation. Journal of the American Statistical Association. 88: 486-494.

Taberlet, P., H. Mattock, C. Dubois-Paganon, and J. Bouvet. 1993. Sexing free-ranging brown bears *Ursus arctos* using hairs found in the field. Molecular Ecology. 2: 399-403.

Topchy, A., K.T. Scribner, and W. Punch. 2004. Accuracy-driven loci selection and assignment of individuals. Molecular Ecology Notes. 4: 798-800.

Williamson, J.E., R.M. Huebinger, J.A. Sommer, E.E. Louis, Jr., and R.C. Barber. 2002. Development and cross-species amplification of 18 microsatellite markers in the Sumatran tiger (*Panthera tigris sumatrae*). Molecular Ecology Notes. 2: 110-112.

Woods, J.G., D. Paetkau, D. Lewis, B.N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin. 27: 616-627.

Woolf, A. and G.F. Hubert, Jr. 1998. Status and management of bobcats in the United States over three decades: 1970s-1990s. Wildlife Society Bulletin. 26: 287-293.

Woolf, A., C.K. Nielsen, and T.J. Gibbs-Kieninger. 2000. Status and distribution of the bobcat (*Lynx rufus*) in Illinois. Transactions of the Illinois Academy of Science. 93: 165-173.

CHAPTER 7. CONCLUSION

Furbearers and management

Furbearer species played an important role in the history of the Midwestern United States as an economic resource. However, unregulated harvest and deforestation resulted in severe declines in abundance and distribution throughout the 19th and early 20th centuries (Hubert 1982). Many species were extirpated, or became locally extinct, as early as the mid-1800s. Population declines and local extinction represent a historical legacy of many species and must be considered in present furbearer management planning. Following severe declines in population abundance and/or extirpation of a species from an area, managers must determine the necessity and feasibility of reestablishment of the species, either through reintroduction, translocation, or supplementation. It is critical to monitor abundance and distribution of both remnant and reestablished populations to help make informed management decisions to minimize potential of additional population declines.

Harvest is often the ultimate goal of management agencies for many furbearer populations due to the species' value as a recreational and economic resource. Once a population has been determined to be feasibly harvestable, managers must monitor the population to ensure sustainability of the harvest. Monitoring can occur through collections of demographic parameters from annual harvests. However, uncertainty in estimation of harvest parameters might lead to erroneous conclusions regarding population trends and stability. Knowledge of uncertainty in harvest surveys and of understanding of the effects of uncertainty on harvest-based population assessment is necessary to effectively use harvest data to monitor population sustainability and prevent

future population declines and extirpation.

The preceding chapters of this thesis illustrated how three furbearer species, the American marten (*Martes americana*), fisher (*Martes pennanti*), and bobcat (*Lynx rufus*) exemplify the furbearer management cycle.

Extirpation, reintroduction and the American marten

Martens were extirpated from Michigan and Wisconsin in the early 1900s. Four genetically divergent putative source populations (and a fifth unsampled source) were used to restore the species to both states. The genetic differentiation between source populations provided the ability to evaluate differential success of each reintroduction event (i.e., genetic contribution). Three genetically distinct populations were discovered in the Upper Peninsula of Michigan, geographically corresponding to release locations from three different sources. The populations in Michigan and Wisconsin in proximity to release sites of the two most recent reintroduction events were genetically similar to their putative sources. In contrast, the populations in Michigan closest to release sites of the first two reintroductions did not show genetic affinity to their sources, likely due to a large effect of stochastic genetic processes (e.g., drift) on the reintroduced population following release. Although distribution of marten harvest locations was continuous across much of the Upper Peninsula, dispersal barriers appeared to limit interaction between genetic populations. Levels of genetic diversity in the reintroduced populations were generally not lower in comparison to genetic diversity in the source populations, suggesting demographic bottlenecks, if occurred, were of short duration. However, significant bottleneck effects were not detected in any of the reintroduced populations.

In addition to assessment of population-level effects of reintroduction, genetic data were used to elucidate important aspects of the ecology of the marten over known time periods. Extirpation of the species and subsequent reintroduction offered opportunities to evaluate spatial genetic structure as a function of ecological patterns (i.e., dispersal) following release. Overall patterns of isolation by distance were found in two of three genetic populations in the Upper Peninsula, consistent with release from a single location and lack of distinct subpopulations. In contrast, an overall pattern of isolation by distance was not detected in the third genetic population, likely due to dispersal from four different release sites, creating a non-linear pattern of overall genetic spatial autocorrelation. Distance over which effective gene flow occurred, or genetic patch size, differed between the three populations in the Upper Peninsula, ranging from 30 km – 120 km. However, this 4-fold difference in genetic patch size indicated differences in dispersal ability of martens in the three populations, either due to genetic or environmental factors. Two-dimensional spatial autocorrelation was used to identify fine-scale patterns indicative of dispersal or presence of kin groups within each genetic population in the Upper Peninsula. Evaluation of correlations between inter-individual genetic relationships and landscape features, including habitat, might help elucidate causes for differential patterns of gene flow (i.e., dispersal), creating genetic structure at both population and individual levels.

Extirpation, reintroduction and the fisher

Similar to martens, fishers were extirpated from Michigan and Wisconsin in the early 20th century, and subsequently reestablished, providing a unique opportunity to

examine the genetic effects of reintroductions and infer ecological processes, such as dispersal. Evaluation of genetic diversity and structure in Minnesota and New York, the two source populations used to restore fishers to Michigan and Wisconsin indicated the New York population experienced a recent bottleneck and potential current gene flow from a neighboring population. However, genetic divergence between the two source populations facilitated evaluations of genetic contributions of source populations to reintroduced populations in Michigan and Wisconsin. Although there was no spatial genetic structure in the reintroduced populations suggesting panmixia, genetic contributions to the recipient populations by founders from the New York source (the numerical minority genetic contributor) appeared to be greatest around the release location for the New York founders and into western Michigan along a northeast to southwest ordination.

Reintroductions of fishers into Michigan and Wisconsin had little effect on overall genetic diversity contrary to expectations based on small numbers of founding individuals. Fishers in this area appeared to highly successful colonizers, capable of high levels of gene flow across large geographic distances. Recent bottlenecks were detected in locales at the periphery of the population in the Upper Peninsula, reflecting potential areas of recent population expansion through dispersal.

Success of the reintroduction of fishers into Michigan and Wisconsin is apparent in the wide distribution, lack of population genetic structure, and high harvests. However, evaluation of correlation of genetic affinities with landscape variables would further elucidate causes for differential patterns of gene flow based on distance and ordination.

Marten and fisher population estimation

Wildlife managers frequently struggle to make effective management decisions for furbearers due to a paucity of ecological and demographic information owing to the solitary and secretive nature of the species. Estimation of population abundance is needed for effective population management. Simultaneous estimates of population abundance were determined for two secretive species of management concern, martens and fishers. Different mark-recapture methodologies are available to estimate population size. However, care must be taken when choosing the appropriate method for a data set. Harvest samples were valuable in this study, both as a final capture period for population estimation as well as a means to detect individuals moving off the study area, thereby violating the assumption of closure in closed-capture models. In addition to use for estimation of population abundance, the genetic data collected during the course of the study can be used to investigate marten – fisher interactions. The presence of observed martens and fishers in various habitat types would also provide an opportunity to develop an index of occupancy that could be applied at a large or management-based scale.

Uncertainty in bobcat harvest parameters

Bobcats in Michigan were represented by two geographically and genetically distinct populations. Genetic differentiation between the Upper and Lower Peninsulas not only indicated lack of historic gene flow, and therefore movement between Peninsulas, but also provided an opportunity to investigate error in harvest location reporting. Genetic methods were shown to be effective for identification of bobcats that originated in the Peninsula opposite the one in which harvest location was reported, due

to harvest misreporting, recording errors, or potential dispersal. Estimates of demographic parameters in annual harvest surveys are used by management agencies to help draw conclusions regarding population sustainability. Genetic sexing methods were used to assess the magnitude and direction of error in current practices of field and canine tooth-based sex identification techniques for bobcats. An understanding and minimization of error in harvest records would increase the accuracy and utility of harvest-based population models used to help make management decisions.

APPENDICES

Appendix 3.A. List and characteristics of primers acquired for use with American martens including annealing temperature, mM of Mg used in the PCR reaction and the sizes of the PCR product.

Locus name	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	$MgCl_2$ [mM]	Genbank acc. No.	Size (bp)
(Davis and Strobeck 1998)						
Ma-2	**F:** ACC CAT GAA TAA TGT CTT AT **R:** ATC TTG CAT CAA CTA AAA AT	$(TG)_{17}$	52	1	AF075139	166-178
Ma-5	**F:** TAA AAC CAG GAA ACA GAT AC **R:** AGT ATG GAT AAA GCA CAA AC	$(TG)_{17}$	54	2	AF075141	245-273
Ma-8	**F:** GTT TTC TAA TGT TTC GTG TG **R:** CAG TGG TTG ACT ACA AGA AA	$(TG)_{21}$	50	3	AF075143	116-132
Ma-14	**F:** GAC CTG AGC CGA AGG CA **R:** AGG TGT GGA AAC AAA CGA G	$(TG)_{15}$	64	2	AF075147	184-208
Ma-19	**F:** AAG GCT TAT GGA TAC CAC AT **R:** GAT CAT TTG GTA TTT GTC TTT C	$(TG)_{16}$	48	1	AF075150	201-211
Gg-3	**F:** CCT TTC TCT GAC TGA CTT TT **R:** TGT GTC TTG CTC ACT CTC TA	$(TG)_{15}$	54	2	AF075151	152-166
Gg-7	**F:** GTT TTC AAT TTT AGC CGT TCT G **R:** GTT TAT CTC CCT CTT CCT ACC C	$(TG)_{20}(T)_3(TG)_5$	54	3	AF075153	144-162
Tt-1	**F:** AAC GGC TTC TAA CCA CTC CA **R:** CCC CGC TTT TCA TTT CTT TA	$(TG)_{20}$	50	3	AF075156	168-170
Tt-4	**F:** GGT GAG ACC CTG GAA ATA GAA A **R:** GCT AAC CAA ACC TAG CAA TGA T	$(TG)_{18}$	50	3	AF075159	161-175
(Fleming et al. 1999)						
Mvis072	**F:** CTG CAA AGC TTA GGA ATG GAG A **R:** CCA CTA CAC TGG AGT TTC AGC A	$(CA)_{15}$	54	2	AF132104	255-305
Mer022	**F:** CCA TGC TTT GGG TAG GAG AA **R:** CCT TGT TCT CAG GTG GTT GG	$(CA)_{15}$	54	2	AF132109	239-261
Mer041	**F:** TGT GTG ATC TCT GGG AAT TCT C **R:** TCT GCT CCC CAG ATA AAA GC	$(CA)_{11}$	52	1	AF132111	151-155

Appendix 4.A. List of primers acquired for use with fishers including annealing temperature, mM of Mg used in the PCR reaction and the sizes of the PCR product.

Locus name	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	Mg [mM]	Genbank acc. No.	Size (bp)
(Davis and Strobeck 1998)						
Ma-1	**F:** ATT TTA TGT GCC TGG GTC TA **R:** TTA TGC GTC TCT GTT TGT CA	$(TG)_4TA(TG)_{19}$	48	2	AF075137	199-213
Ma-2	**F:** ACC CAT GAA TAA TGT CTT AT **R:** ATC TTG CAT CAA CTA AAA AT	$(TG)_{17}$	48	2	AF075139	168-180
Ma-19	**F:** AAG GCT TAT GGA TAC CAC AT **R:** GAT CAT TTG GTA TTT GTC TTT C	$(TG)_{16}$	48	2	AF075150	203-215
Gg-7	**F:** GTT TTC AAT TTT AGC CGT TCT G **R:** GTT TAT CTC CCT CTT CCT ACC C	$(TG)_{20}(T)_3(TG)_5$	54	3	AF075153	159-173
Tt-1	**F:** AAC GGC TTC TAA CCA CTC CA **R:** CCC CGC TTT TCA TTT CTT TA	$(TG)_{20}$	50	3	AF075156	166-182
Tt-4	**F:** GGT GAG ACC CTG GAA ATA GAA A **R:** GCT AAC CAA ACC TAG CAA TGA T	$(TG)_{18}$	50	3	AF075159	165-173
(Fleming et al. 1999)						
Mvis002	**F:** TGG GAA AAA TAG TGC TCC AAA G **R:** AAA CAG CAG AGA GCA TAC AGC C	$(CA)_{11}$	56	1	AF132100	202-218
Mvis072	**F:** CTG CAA AGC TTA GGA ATG GAG A **R:** CCA CTA CAC TGG AGT TTC AGC A	$(CA)_{15}$	54	2	AF132104	272-282
Mer022	**F:** CCA TGC TTT GGG TAG GAG AA **R:** CCT TGT TCT CAG GTG GTT GG	$(CA)_{15}$	54	2	AF132109	244-266
Mer041	**F:** TGT GTG ATC TCT GGG AAT TCT C **R:** TCT GCT CCC CAG ATA AAA GC	$(CA)_{11}$	52	1	AF132111	157-169

Appendix 5.A. Characteristics of PCR primers for microsatellites (Davis and Strobeck 1998, Fleming 1999) and sexing primers (Aasen and Medrano 1990, Woods et al. 1999, Taberlet et al. 1993) used in analyses of fishers.

Locus name	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	Mg [mM]	Genbank acc. No.	Size (bp)
Ma-1	**F:** ATT TTA TGT GCC TGG GTC TA **R:** TTA TGC GTC TCT GTT TGT CA	$(TG)_4TA(TG)_{19}$	48	2	AF075137	199-213
Ma-2	**F:** ACC CAT GAA TAA TGT CTT AT **R:** ATC TTG CAT CAA CTA AAA AT	$(TG)_{17}$	48	2	AF075139	168-180
Ma-19	**F:** AAG GCT TAT GGA TAC CAC AT **R:** GAT CAT TTG GTA TTT GTC TTT C	$(TG)_{16}$	48	2	AF075150	203-215
Gg-3	**F:** CCT TTC TCT GAC TGA CTT TT **R:** TGT GTC TTG CTC ACT CTC TA	$(TG)_{15}$	54	2	AF075151	137
Gg-7	**F:** GTT TTC AAT TTT AGC CGT TCT G **R:** GTT TAT CTC CCT CTT CCT ACC C	$(TG)_{20}(T)_3(TG)_5$	54	3	AF075153	159-173
Tt-1	**F:** AAC GGC TTC TAA CCA CTC CA **R:** CCC CGC TTT TCA TTT CTT TA	$(TG)_{20}$	50	3	AF075156	166-182
Mer022	**F:** CCA TGC TTT GGG TAG GAG AA **R:** CCT TGT TCT CAG GTG GTT GG	$(CA)_{15}$	54	2	AF132109	244-266
X chromosome						
ZFX	**F:** CTC CTT TTT CTT TAT GCA CC		51	-	-	129
	R: TET ATA ATC ACA TGG AGA GCC ACA AGC T		51	-	-	
Y chromosome						
TET SRY	**F:** AAC GCA TTC ATG GTG TGG TC		51	-	-	119
	R: TET GCT TCT GTA AGC ATT TTC CA		51	-	-	

Appendix 5.B. Characteristics of PCR primers for microsatellites (Davis and Strobeck 1998) and sexing primers (Aasen and Medrano 1990, Woods et al. 1999, Taberlet et al. 1993) used in analyses of American martens.

Locus name	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	Mg [mM]	Genbank acc. No.	Size (bp)
Ma-2	**F:** ACC CAT GAA TAA TGT CTT AT	$(TG)_{17}$	52	1	AF075139	166-178
	R: ATC TTG CAT CAA CTA AAA AT					
Ma-5	**F:** TAA AAC CAG GAA ACA GAT AC	$(TG)_{17}$	54	2	AF075141	245-273
	R: AGT ATG GAT AAA GCA CAA AC					
Ma-8	**F:** GTT TTC TAA TGT TTC GTG TG	$(TG)_{21}$	50	3	AF075143	116-132
	R: CAG TGG TTG ACT ACA AGA AA					
Ma-19	**F:** AAG GCT TAT GGA TAC CAC AT	$(TG)_{16}$	48	1	AF075150	201-211
	R: GAT CAT TTG GTA TTT GTC TTT C					
Gg-3	**F:** CCT TTC TCT GAC TGA CTT TT	$(TG)_{15}$	54	2	AF075151	152-166
	R: TGT GTC TTG CTC ACT CTC TA					
Gg-7	**F:** GTT TTC AAT TTT AGC CGT TCT G	$(TG)_{20}(T)_3(TG)_5$	54	3	AF075153	144-162
	R: GTT TAT CTC CCT CTT CCT ACC C					
X chromosome						
ZFX	**F:** CTC CTT TTT CTT TAT GCA CC		51	-		129
	R: TET ATA ATC ACA TGG AGA GCC ACA AGC T		51	-		
Y chromosome				-		
TET SRY	**F:** AAC GCA TTC ATG GTG TGG TC		51	-		119
	R: TET GCT TCT GTA AGC ATT TTC CA		51	-		

Appendix 6.A. List of primers for use with bobcats (Williamson et al. 2002).

Locus name	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	Mg [mM]	Genbank accession No.	Size (bp)
6HDZ056	**F:** ACT AAG TCT GTA ACC ACG CCC **R:** CAG TCA AAC AAC TGC CCT TTC	$(CA)_{15}$	52	1	AF296743	172-176
6HDZ057	**F:** CTA CCT TTC TTT CAC CTT CTT TTT G **R:** TCG TGC GTT AGA GGA ATT GG	$(CT)_{16}$	52	1	AY045524	92-100
6HDZ064	**F:** ATG GTA TTT GCC ATT CTC TGA C **R:** CAG ATT TAA TTG TGT GTA GTA TAT GAG C	$(CA)_{17}$	48	2	AF296745	184-186
6HDZ089	**F:** GCA TAA AAC TCT AAC ACA GCA TCT **R:** TTC TGA AAT AGG ATT GGC AAA	$(GT)_{22}$	50	3	AF296749	207-221
6HDZ463	**F:** GCA TGA AGG AAC AAG CCA G **R:** CCT CAG GTA GCT TCT TTA TTC AG	$(CA)_{18}$	46	2	AY45522	116-124
6HDZ610	**F:** ATC AGG AGT TCT ATC ACC AAC CC **R:** CAC ATG ATT AGG GAG TTG AGA AGT C	$(CA)_{17}$	56	3	AF296744	168-172
6HDZ635	**F:** TGC AGT GAC AGT AGG GAG CC **R:** CCA GAA TGA AAG GTA GCC AAA C	$(CT)_{16}$	54	2	AF399645	166-174
6HDZ700	**F:** TCC TCC TTC CAG GAT GCC A **R:** AGG ATG GGG GAA AAT CTC TC	$(GT)_{19}$	52	1	AF296747	141-143

Appendix 6.B. Sexing primers used for bobcats.

Sexing primers	Primer Sequence (5' to 3')	T_a (°C)	References
X chromosome			
ZFY/X P1-5EZ	**F:** ATA ATC ACA TGG AGA GCC ACA AGC T	54	*(S.R. Fain, pers. comm.)*
ZFY/X P2-3EZ	**R:** GCA CTT CTT TGG TAT CTG AGA AAG T	54	
Y chromosome			
TDF Y53-3C	**F:** CCC ATG AAC GCA TTC ATT GTG TGG	54	
TDF Y53-3D	**R:** ATT TTA GCC TTC CGA CGA GGT CGA TA	54	
X chromosome			
ZFX	**F:** CTC CTT TTT CTT TAT GCA CC	51	*(Woods et al. 1999)*
ZFX	**R:** TET ATA ATC ACA TGG AGA GCC ACA AGC T	51	*(Aasen and Medrano 1990)*
Y chromosome			
TET SRY	**F:** AAC GCA TTC ATG GTG TGG TC	51	*(Taberlet et al. 1993)*
TET SRY	**R:** TET GCT TCT GTA AGC ATT TTC CA	51	