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DEMOGRAPHIC AND GENETIC EVALUATION OF AN AMERICAN MARTEN REINTRODUCTION

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Reintroduced populations are generally smaller and more isolated than native populations; thus even when reintroduced populations are demographically stable, a lack of genetic variation may present a threat to long-term persistence. We examined the demographic structure and genetic variation of the marten reintroduction into the Upper Peninsula of Michigan. Male : female and juvenile : adult female ratios indicate that the Michigan population is demographically stable. Michigan martens had higher allelic diversity ($A = 7.4$) compared to the average diversity found among Canadian populations ($A = 5.8$) and similar levels of observed heterozygosity ($H_{\text{Canadian}} = 0.64$, $H_{\text{Michigan}} = 0.63$), excluding Newfoundland martens. We found no significant differences in the allelic diversity or heterozygosity between the reintroduced Michigan population and the source population for the reintroduction, that of Chapleau, Ontario. Surprisingly, we found no evidence of a genetic bottleneck in the Michigan population. We suggest that the genetic success of this reintroduction is a result of the multiple reintroductions and subsequent intrastate translocations that mimicked gene flow. The success was further aided by the presence of small remnant populations that remained in Michigan, as evidenced by the presence of private alleles in Michigan.

Key words: marten, *Martes americana*, microsatellites, reintroduction, translocation

Reintroduced populations face a host of interrelated demographic and genetic problems as a result of their small effective population sizes (O'Brien and Evermann 1988), isolation due to fragmented habitat (Slough 1994), and reduced genetic variation compared to their source population (Stockwell et al. 1996). The increased isolation and reduced population size results in a rapid loss of genetic variation (Maruyama and Fuerst 1984; Nei et al. 1975; Wright 1969) from an already genetically depauperate population. Reduced genetic variation decreases evolutionary potential (Day et al. 2003; Soulé 1980) and has been implicated in the inability to fight off disease (Frankham 1995a; O'Brien and Evermann 1988), and increases the likelihood of deleterious inbreeding (Day et al. 2003; Reed et al. 2003; Wright 1969), mutational meltdown (Lynch et al. 1995), and genetic load (Rowe and Beebee 2004). All of these factors increase the probability of a population entering an extinction vortex (Lacy 1993; Lande and Barrowclough 1987; Nieminen et al. 2001). Given the negative effects associated with low levels of genetic variation, a major concern following a reintroduction should be to evaluate the genetic variation and insularity of a reintroduced population.

In this paper we examine the genetic variation and insularity of an American marten (*Martes americana*) population following its reintroduction to Michigan. By the mid-1900s the American marten had been extirpated from much of its original range, including Michigan (Earle et al. 2001). Two major factors led to the regional extirpation of martens, overharvesting and the loss of suitable habitat (Soutiere 1979). The marten is considered a habitat specialist requiring some type of conifer component (Buskirk and Powell 1994), canopy closure of 50–70% (Thompson and Harestad 1994), and a large amount of coarse woody debris (Harden 1998), although the habitat specificity and requirements have been questioned (Bowman and Robitaille 1997; Kyle and Strobeck 2003; Potvin et al. 2000). Marten habitat requirements result in both large-scale (Soutiere 1979; Thompson and Colgan 1987) and small-scale (Hargis et al. 1999; Potvin et al. 2000; Robitaille and Aubry 2000) fragmentation negatively impacting martens. Large- and small-scale fragmentation occurred in Michigan between the late 1800s and early 1900s due to land settlement and logging practices (Earle et al. 2001). Isolation of Michigan's Upper Peninsula also influenced the extirpation of martens by reducing the likelihood of a rescue effect from nearby populations. The only source for a rescue effect during

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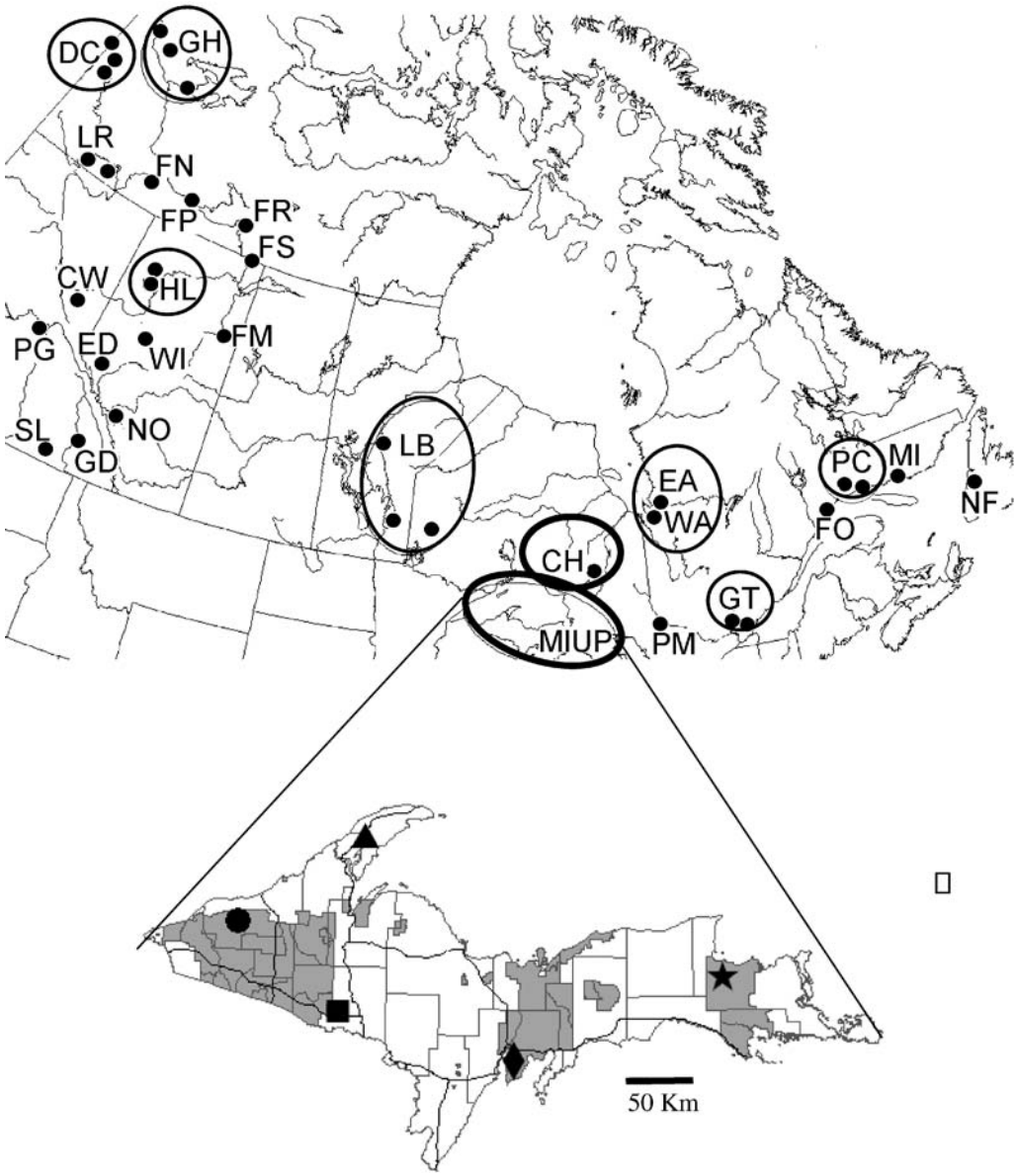

Fig. 1.—Map of sample locations from Canada (from Kyle and Strobeck 2003) and Michigan. The heavily circled populations indicate the source population, Chapleau, Ontario (CH) and the Michigan population (MIUP). The enlarged map shows locations where Chapleau, Ontario martens were released in Michigan: Porcupine Mountains Wilderness State Park (black circle), Delta County (black square), McCormick Wilderness Area (black diamond) and intrastate translocations (Chippewa County (black star) and Keweenaw County (black triangle). Shaded areas are national forests. Abbreviations: MIUP = Michigan Upper Peninsula; NF = Newfoundland; EA = Eastmain, Quebec; MI = Mingan, Quebec; PC = Port Cartier, Quebec; FO = Forestville, Quebec; GT = Gatineau, Quebec; PM = Pembroke, Ontario; CH=Chapleau, Ontario; LB = Lac du Bonnet, Ontario; FM = Fort McMurray, Alberta; WI = Whitecourt, Alberta; ED = Edson, Alberta; NO = Nordegg, Alberta; HL = High Level, Alberta; PG = Prince George, British Columbia; CW = Chetwynd, British Columbia; GD = Golden, British Columbia; SL = Slocan, British Columbia; DC = Dawson region, Yukon Territory; LR = Watson Lake region, Yukon Territory.

the period of decline for the martens in Michigan would have been from Wisconsin immediately to the west, but concurrent habitat destruction and fragmentation were occurring in Wisconsin as well.

More conservative logging and land-use practices since the mid-1900s increased suitable marten habitat and allowed the Michigan Department of Natural Resources to reintroduce martens to the Upper Peninsula (Fig. 1). Between 1955 and 1957, 27 martens captured in Chapleau, Ontario, and 2 martens from British Columbia were released by the Michigan Department of Natural Resources in the Porcupine Mountains Wilderness State Park in Michigan's western Upper Peninsula (Earle et al. 2001). This was followed by a 2nd reintroduction of 99 martens from Chapleau, Ontario into the central Upper Peninsula (Delta and Alger counties) between 1968 and 1970 (Earle et al. 2001). In 1979, a 3rd release of 148 martens from Chapleau, Ontario occurred in the Huron Mountains, McCormick Wilderness Area (Baraga and Marquette counties) and

in west Iron County. Finally, a series of intrastate translocations occurred between 1989 and 1992 as the Michigan Department of Natural Resources moved 20 martens from Alger County to Chippewa County, 27 martens from Iron County to Chippewa County, and 19 martens from the western Upper Peninsula to southern Keweenaw County (Fig. 1; Earle et al. 2001).

Although there are no direct estimates of marten population size in the Upper Peninsula, the Michigan Department of Natural Resources considered the reintroduction successful enough that a limited trapping season was opened in the Upper Peninsula in 2000 (Cooley et al. 2002). During 2000–2003, an average of 130 martens were harvested in Michigan, a number that may be unsustainable depending on the current population size. Mark–recapture methods, either traditional or molecular, produce the most accurate estimates of population size but have prohibitive costs and personnel requirements for most state agencies. Recognizing this difficulty, Strickland and Douglas (1987) developed a technique to determine if a harvest is sustainable based on age and sex ratios in captured animals. Mustelids show significant trapping bias with more males than females being caught and more juveniles being caught than adults. The sex ratio of the harvested animals is proportional to harvest effort with the ratio declining from a 3:1 to a 1:1 male : female ratio as trapping pressure increases (Archibald and Jessup 1984; Quick 1953; Soukkala 1983; Stickland and Douglass 1987; Yeager 1950). Over a 10-year period Strickland and Douglas (1987) found an average of 2.81 corpora lutea per female marten; however, the juvenile : adult female ratio in the harvest was 6.1:1. Based on the greater than 2-fold difference in these ratios, Strickland and Douglas (1987) concluded that more than half of the females in the population were not harvested and were therefore available for future reproduction. With greater than 50% of the adult females producing offspring for the subsequent year, and each of them producing 2.81 offspring, they concluded that their study population was being harvested in a sustainable fashion. Strickland and Douglas (1987) suggested that marten populations are experiencing sustainable harvest if the male : female ratio is not less than 2:1 and the juvenile : adult ratio does not fall below 3:1.

Even when a marten population is considered stable enough to allow harvesting, the population may still face serious genetic challenges due to its size or isolation. The multiple reintroductions and intrastate translocations of martens in the Upper Peninsula may have partially counteracted the effects of small population size, habitat fragmentation, and geographic isolation by approximating gene flow. These movements are comparable in magnitude to the dispersal distances of martens estimated from genetic data by Kyle et al. (2000) and Kyle and Strobeck (2003). Our study examines how this reintroduction method impacted the genetic status of the marten in Michigan.

Materials and Methods

We analyzed tissue samples from 94 martens that were collected by commercial trappers from Michigan's Upper Peninsula during the 2 trapping seasons 2001–2002 and 2002–2003 and submitted to the Michigan Department of Natural Resources as required by law. All legally harvested martens are sent to the Michigan Department of Natural Resources where their sex, age, and location of capture (down to section within township and range) are recorded. Michigan Department of Natural Resources personnel took tissue samples from various muscle tissues and stored them at either −20°C or −80°C and aged each animal based on cementum analysis of the tooth. We extracted DNA from each tissue sample using Qiagen DNeasy tissue kits (Qiagen, Valencia, CA) and following Qiagen's published protocol (Qiagen 2001). We analyzed all Michigan martens at 10 microsatellite loci (Gg-3, Gg-7, Gg-14, Ma-1, Ma-7, Ma-9, Ma-10, Ma-14, Ma-19, and Tt-4) where the reverse primers were labeled on the 5′ end with one of the fluorescent dyes FAM, TET, or HEX (Davis and Strobeck 1998). Six of these loci (MA-1, MA-7, MA-9, MA-10, MA-14, and MA-19) were used by Kyle and Strobeck (2003) in their analyses of 25 Canadian populations (Fig. 1). Only subsets of the same loci were used because this project was started independently of, and before the publication of, the Kyle and Strobeck (2003) paper. We ensured that the allele sizes we scored were the same as those scored by Kyle and Strobeck (2003) by analyzing 30 Chapleau animals on the same ABI Prism™ 310 automated DNA sequencer (Applied Biosystems, Foster City, CA) used for the Michigan animals. Our comparisons were also facilitated by access to the genotypes for all of the 1,262 animals from the 25 populations of Kyle and Strobeck (2003). The number of animals analyzed by Kyle and Strobeck (2003) ranged from 17 to 149, with $n = 50$ for Chapleau, Ontario, the source population for Michigan's martens.

Each polymerase chain reaction (PCR) contained 0.16 μM of each primer, 120 μM dNTPs, 75 ng of template DNA, 0.5 units of HotMaster™ *Taq* DNA polymerase (Eppendorf, Westbury, New York), and 10× HotMaster™ PCR buffer (total volume of PCR: 20 μl). PCR temperature cycling was performed on an Eppendorf Mastercycler Gradient (Eppendorf, Westbury, New York) under the following conditions: an initial 2 min at 94°C for Hotmaster™ *Taq* activation, 4 cycles of 20 s at 94°C, 20 s at 54°C, 5 s at 65°C, followed by 33 cycles of 15 s at 94°C, 20 s at 54°C, 5 s at 65°C, and 1 min at 72°C. All DNA fragments were measured using an ABI Prism™ 310 DNA sequencer and the programs Genescan Analysis 3.1.2 (Applied Biosystems, Foster City, CA) and Genotyper version 2.0 (Applied Biosystems, Foster City, CA). All homozygous individuals were run multiple times to investigate the possibility of allelic dropout (Jeffery et al. 2001). Samples were scored as homozygotes only if 2 independent PCR analyses both showed the individual to be homozygous. Individuals who exhibited homozygous and heterozygous states in different PCR analyses were analyzed again in a 3rd independent PCR. All individuals requiring a 3rd analysis produced the same heterozygous genotype on the 3rd analysis as on the 2nd, and were scored as heterozygotes.

All our data were then examined for Hardy–Weinberg equilibrium and linkage using GENEPOP (Raymond and Rousset 1995). We compared the allelic diversity and average heterozygosity of the Michigan population to those of the Canadian populations analyzed by Kyle and Strobeck (2003), excluding the Newfoundland population, by resampling 1,000 times, with replacement, the allelic diversity (the average number of alleles per locus) and heterozygosity values for the 24 Canadian populations. Similarly, we compared the allelic diversity and average heterozygosity between the Michigan and Chapleau populations by resampling 1,000 times, with replacement, 50 animals from the Michigan population, the same number of individuals sampled from Chapleau.

We tested for bottlenecks using the m-ratio and (Garza and Williams 2001) bottleneck tests (Luikart and Cornuet 1998). Both of

TABLE 1.—Age distribution by sex for martens harvested in the Upper Peninsula of Michigan during the 2000–2001 trapping season. (Data from Cooley et al. 2002.)

Age (year)	Males (%)	Females (%)
0.5	34	40
1.5	18	31
2.5	16	14
3.5	14	9
4.5	2	6
5.5	7	0
6.5	5	0
7.5	4	0

these methods are based on rare alleles being lost more quickly than other alleles in small populations. Genetic drift removes alleles from a population; the strength of drift is inversely proportional to population size (Wright 1969), and thus small populations (e.g., reintroduced populations) are especially susceptible to drift and rare alleles are quickly removed. The rapid loss of alleles produces a powerful method for detecting if a population has been bottlenecked because the majority of alleles at a locus must be at a low frequency.

The m-ratio evaluates whether a bottleneck occurred by determining what percentage of allelic states are filled at a microsatellite locus. The premise of the m-ratio is that a population that has not experienced a bottleneck will have a high percentage of its allelic states filled, whereas a bottlenecked population will have empty states due to drift removing rare alleles. The m-ratio is calculated as $M = k/r$ where k is the number of alleles and r is the range in allele size measured in repeat units. During a bottleneck, as the rare alleles are lost, k will be reduced more quickly than r. By comparing the observed m-ratio to a 95% confidence interval generated by simulating the population of interest, the null hypothesis that there is no difference in the observed m-ratio to the m-ratio expected under an equilibrium population can be tested. We used Critical M and M P Val (available at http://santacruz.nmfs.noaa.gov/staff/carlos_garza/software.php) to estimate the m-ratio and its significance. The m-ratio requires that all alleles of a locus are multiples of the repeat unit, only permitting us to use loci Ma-1, Ma-7, Ma-14, Ma-19, Gg-14, and Tt-4 for the Michigan martens and Ma-1, Ma-2, Ma-3, Ma-7, Ma-8, Ma-11, Ma-14, and Ma-19 for the Chapleau, Ontario population. We estimated the pre-bottleneck effective population size (N_e) of the Michigan population using the average heterozygosity of the Chapleau population ($H_{observed} = 0.55$) and followed suggestions of Garza and Williams (2001) for the other parameters; mutation rate (μ) $= 5 \times 10^{-4}$, average size of nonsingle step mutations = 3.5, and the frequency of nonsingle step mutations = 0.20.

The program Bottleneck is based on the equilibrium reached between the number of alleles in a population and that population's heterozygosity (Luikart and Cornuet 1998). When a population is at drift, mutation equilibrium heterozygosity is correlated with the number of alleles at the locus. Following a bottleneck the low-frequency alleles will be lost, but because low-frequency alleles have little influence on heterozygosity, the heterozygosity at the locus will be greater than expected for the number of alleles present (Garza and Williams 2001). We used the program Bottleneck (available at http://www.montpellier.inra.fr/URLB/) to compare percentage of low-frequency alleles in a population to the frequency expected under the 2-phase model of mutation for an equilibrium population. Because low-frequency alleles are lost more quickly than other alleles, a population with a low percentage of low frequency alleles is assumed to have passed through a bottleneck. Specifically, Bottleneck tests the null hypothesis that there is no difference in the percentage of low-frequency alleles in the observed population compared to the expected percentage of low-frequency alleles in a population at drift-mutation equilibrium.

We produced an unrooted neighbor-joining tree based on Nei's genetic distance (D_S) values (Nei et al. 1975) using the PHYLIP 3.6 (Felsenstein 2005) tree, using the 6 loci common to this and Kyle and Strobeck's (2003) study.

It is possible that the spatial and temporal variation associated with the Michigan marten reintroductions produced some degree of population genetic structure. We used the Bayesian clustering program STRUCTURE (Pritchard et al. 2000) to determine if population structure existed. STRUCTURE clusters individuals into a user-chosen number of populations (K) and determines the most likely number of populations as the number of clusters, which minimizes deviations from Hardy–Weinberg equilibrium and minimizes linkage disequilibrium. We examined the Michigan population for K = 1–4. A burn-in of 100,000 iterations of the Markov chain was followed by a run of 100,000 iterations and was repeated 10 times at each k. These parameters produced consistent estimates of the posterior probability of having k populations in replicate runs. Values are presented as mean ± *SE*.

RESULTS

The harvested martens exhibited a male-skewed sex ratio across all ages (1.5:1), which increased to 1.86:1 when only adults were considered and was not significantly different (chi-square test; $n = 94$, $\chi^2 = 0.19$, $P > 0.2$) from the 2:1 ratio, indicating a sustainable harvest level (Strickland and Douglas 1987). Similarly, the ratio of juvenile martens ($\leq$1.5 years old) to reproductive adult females ($\geq$2.5 years old) was 3.3:1 (Cooley et al. 2002), not significantly different (chi-square test; $n = 94$, $\chi^2 = 0.35$, $P > 0.5$) than the recommended ratio of 3:1 (Strickland and Douglas 1987). The age structure of the harvested population showed that in both sexes subsequent age classes typically contributed a smaller proportion to the total population than did the prior age class (Table 1).

All of the loci in the Michigan population were in Hardy–Weinberg equilibrium, as was also found in the Chapleau and other Canadian populations (Kyle and Strobeck 2003). The allelic diversity of the Michigan marten population (7.4 ± 0.7) was significantly greater ($P = 0.002$) than the allelic diversity across 25 Canadian populations ($A = 5.8 \pm 0.09$; Table 2) as reported by Kyle and Strobeck (2003). In contrast, we found no significant difference ($P = 0.96$) in the average heterozygosity between Kyle and Strobeck's (2003) Canadian marten populations ($H_{observed} = 0.64 \pm 0.006$) and the Michigan ($H_{observed} = 0.63 \pm 0.07$) marten population. The analyses of allelic diversity and heterozygosity did not include the island of Newfoundland because of its significantly reduced genetic variation relative to the rest of mainland Canada (Kyle and Strobeck 2003).

Allelic diversity was not significantly different ($P = 0.37$) between the Michigan and Chapleau populations for the matched loci (Fig. 2). We found 42 alleles across the 6 loci we analyzed in common with Kyle and Strobeck (2003). Of these alleles, 4 were only found in western Canadian populations (Alberta [$n = 1$], Dawson [$n = 2$], Deline [$n = 2$],

TABLE 2.—Significantly higher allelic diversity, but no significant difference in heterozygosity was found for the reintroduced Michigan population (MIUP) and 25 Canadian populations, including the Chapleau, Ontario (CH) source population for Michigan's reintroduction. All abbreviations are as in Fig. 1.

Population	Average heterozygosity	Allelic diversity
MIUP	0.63	7.4
CH	0.59	5.8
NF	0.40	2.6
EA	0.62	6.2
MI	0.62	6.3
PC	0.62	6.6
FO	0.59	5.6
GT	0.62	6.4
PM	0.57	5.1
LB	0.64	6.0
FM	0.61	4.8
WI	0.66	5.8
ED	0.68	5.5
NO	0.68	5.6
HL	0.65	5.6
PG	0.64	5.8
CM	0.63	5.5
GD	0.65	5.6
SL	0.60	5.6
DC	0.67	6.2
LR	0.67	6.4
FP	0.64	5.6
FS	0.66	6.0
FN	0.67	5.9
FR	0.64	5.3
FG	0.64	5.5

Fort Smith [$n = 1$], Fort Good Hope [$n = 1$], and Prince George [$n = 2$]). There were also 3 alleles that were not found in any of the Canadian populations. We found no significant difference in heterozygosity between the Michigan and Chapleau populations (ranked-sign test, $P = 0.16$; Fig. 3).

STRUCTURE found that the most likely number of populations of martens in Michigan was one ($P = 0.999999$). The neighbor-joining tree for marten populations east of Saskatchewan showed that the Michigan population was almost as unique as the Newfoundland population (Fig. 4). All Canadian populations, other than Newfoundland, clustered tightly together compared to the Michigan population. Genetically, the closest population to the Michigan population was the source population of Chapleau, Ontario (Fig. 4).

Using the ranked-sign test, the program Bottleneck failed to reject the null hypothesis that all loci were in drift-mutation equilibrium under the 2-phase mutation model, for both Chapleau ($P = 0.38$) and the Michigan population ($P = 0.50$). The Chapleau population showed 6 loci deficient in heterozygotes and 5 loci with an excess of heterozygotes compared to 4 loci with heterozygote deficiency and 7 loci with heterozygote excess in the Michigan population. Both populations showed the characteristic L-shaped distribution (Fig. 5) as opposed to the mode shift expected following a bottleneck. The m-ratio test also failed to find evidence of a bottleneck in either the Michigan ($M = 0.66$, $SE = 0.07$, $P = 0.63$) or the Chapleau ($M = 0.74$, $SE = 0.08$, $P = 71$)

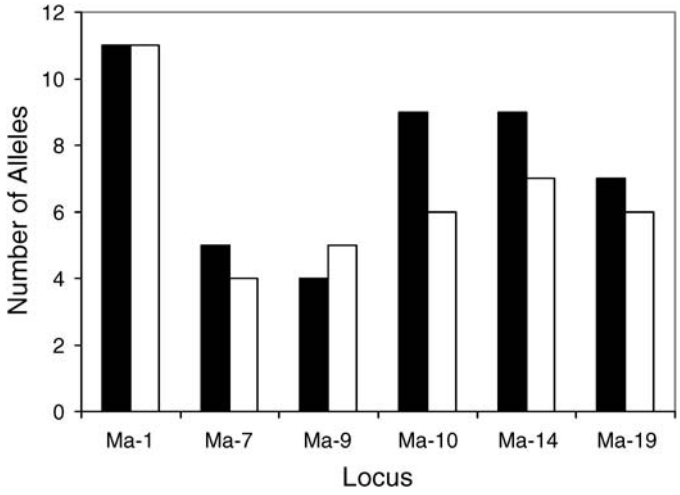

FIG. 2.—Comparison of number of alleles found in the Chapleau, Ontario, source population ($n = 50$; white bars) and the reintroduced Michigan population ($n = 93$; black bars) for matched loci.

population. Based on overlapping 95% confidence intervals, there was no significant difference in the m-ratio of the Michigan population and the Chapleau population.

DISCUSSION

The reintroduction of martens in Michigan could serve as a model for future mesocarnivore reintroductions because it appears to be a demographic and genetic success. Demographically, the population should be considered stable if the current harvest level is sustainable. The age structure of the harvested population (Table 1) is not a reliable indicator of the true age structure because juvenile *Martes* are trapped disproportionately often (Powell 1994; Strickland and Douglas 1987). Nevertheless, it is still possible to evaluate the sustainability of the harvest following established guidelines (Strickland and Douglas 1987). Given that males and juveniles are more likely

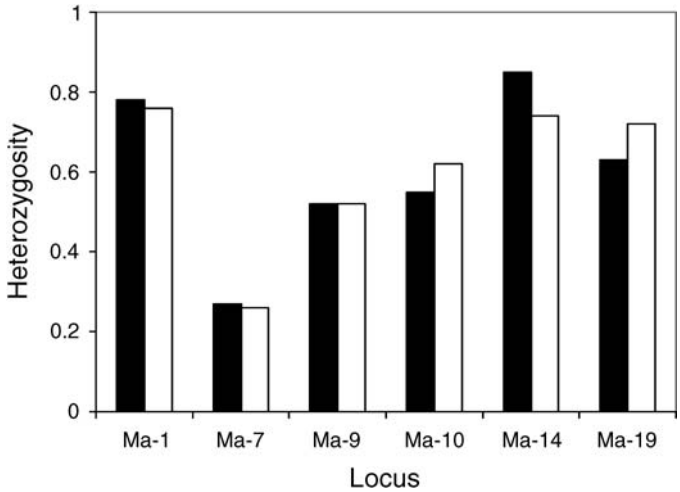

FIG. 3.—Comparison of heterozygosity found in the Chapleau, Ontario, source population ($n = 50$; white bars) and the reintroduced Michigan population ($n = 93$; black bars) for matched loci.

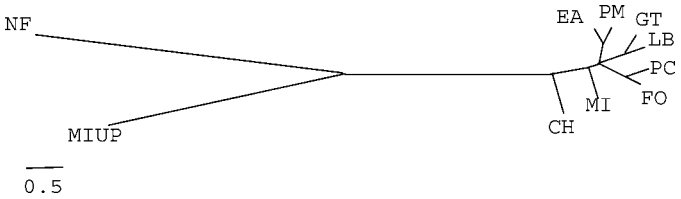

FIG. 4.—Neighbor-joining tree of pairwise genetic distances (D_S) between marten populations in Manitoba, Ontario, Quebec, Newfoundland, and Michigan. Abbreviations as in Fig. 1.

to be trapped (Powell and Zielinski 1994; Smith and Brisbin 1984), sufficiently high ratios of adult males : females and juveniles : adult females provide evidence of a sustainable harvest (Strickland and Douglas 1987); however, this method has not been empirically tested. The male : female, and the adult female : juvenile, ratios did not differ from those indicating a sustainable harvest level (Strickland and Douglas 1987), which, by extension, indicates that the population itself must be demographically stable.

Bottlenecked populations typically exhibit reduced allelic diversity or heterozygosity due to the increased genetic drift experienced by small populations (e.g., otters [*Lutra lutra*], Arrendal et al. 2004; fishers [*Martes pennanti*], Kyle et al. 2001; Larson et al. 2002; red foxes [*Vulpes vulpes*], Swanson et al. 2005; fisher, Wisely et al. 2003; and black-footed ferrets [*Mustela nigripes*], Wisely et al. 2004). When compared to all of the Canadian populations, the Michigan marten population showed little evidence of a bottleneck; the Michigan population had a higher allelic diversity than did the Canadian populations and heterozygosity that was equal to these populations (Table 2). We expected to find a reduced amount of genetic variation in the Michigan population given the bottleneck caused by the reintroduction compared to the more natural and stable status of the Canadian populations (Kyle et al. 2000; Kyle and Strobeck 2003).

The high allelic diversity likely arose from alleles contributed from the 2 martens released in Michigan from British Columbia and from remnant Michigan populations. We found 9.5% (4 of 42) of the alleles in Michigan only occurred in western provinces and an additional 7.1% of the Michigan alleles were not found in any of the 1,252 Canadian martens. This highlights the importance of incorporating multiple populations to maximize allelic diversity during reintroductions.

Alternative explanations for the high allelic diversity of Michigan include differences in sample size and sampling over multiple time periods. We discount the possibility that the high allelic diversity in Michigan is the result of differences in sample sizes because the allelic diversity of the Michigan population exceeded every Canadian population, including the 3 Canadian populations with larger sample sizes (Dawson region, Yukon Territory, $n = 108$, Lac du Bonnet, Ontario, $n = 106$, Gatineau, Quebec, $n = 149$; Table 2). It also seems unlikely that the high allelic diversity arose from changes in allele frequency occurring in the source population over the 37 years of the reintroduction process. The time span over which the multiple reintroductions in Michigan occurred would allow time for the Chapleau population to drift. The frequency of alleles that were rare during one translocation would then drift to the point where they were sampled in subsequent translocations and established in the Michigan population. An additional 20 years passed before the subsequent sampling by Kyle and Strobeck (2003), allowing time for further changes in allele frequencies. However, because continental Canada seems to function as a single large population (Kyle and Strobeck 2003), genetic drift should have been negligible.

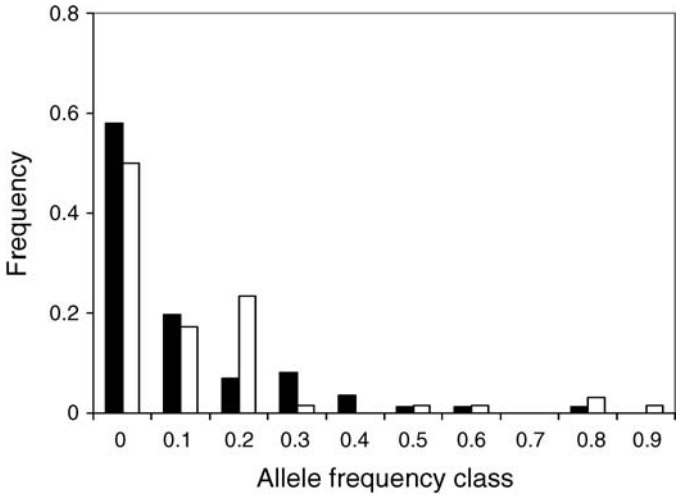

FIG. 5.—Frequency of alleles in each allele class across all loci for the Chapleau, Ontario, source population ($n = 50$; white bars) and the reintroduced Michigan population ($n = 93$: black bars).

The neighbor-joining tree supports the idea that Michigan marten followed an independent evolutionary trajectory. The Michigan population is most similar to the Chapleau, Ontario population from which the majority of the reintroduced animals originated (Fig. 4). However, there is still considerable genetic differentiation between the Michigan population and its source population; the Michigan population is nearly as unique to the rest of the Canadian populations as is the Newfoundland population, which has been isolated from mainland Canada since the Wisconsin glaciation.

No evidence of a bottleneck was found in the Michigan or the Chapleau populations using Bottleneck, the m-ratio test, or the distribution of allele frequencies (Fig. 5). The similarity in m-ratios between the Michigan and Chapleau populations is further evidence that the methods used in the reintroduction of the Michigan population facilitated the genetic recovery. Considering that our study had sufficient power to detect a bottleneck (Cornuet and Luikart 1996; Luikart and Cornuet 1998), the absence of any genetic signature of a bottleneck is likely due to several factors. First, the high degree of polymorphism of our loci would allow the loci to return quickly to mutation-drift equilibrium (Cornuet and Luikart 1996), relative to the time period since the reintroduction. The reintroductions occurred from 1955 to 1979, a period of 10–22 generations; Cornuet and Luikart (1996) indicate that a bottleneck should be detectable for $0.5N_e - 5N_e$ generations following the bottleneck. Wright (1969) defined N_e as the size of an ideal

population which experiences the same amount of genetic drift as does the focal population, where an ideal population is one with equal sex ratio, Poisson distribution of reproductive success, nonoverlapping generations, and constant population size. In most cases, when a population violates one or more of the assumptions of an ideal population the N_e is less than the census size (N_c). In a review of the relation of N_e to N_c values, Frankham (1995b, 1996) found that N_e values are typically only 0.1–0.5 times as large as the census size (Frankham 1995b, 1996). The length of time over which a bottleneck can be detected depends upon N_e with the more severe bottlenecks being detectable for shorter periods of time. In Michigan, a total of 274 martens, with an approximately equal sex ratio, were released. Assuming the minimal $N_e : N_c$ of 0.1 (Frankham 1996), which produces the smallest N_e, indicates that a bottleneck would be detectable for 13.7–137 generations, well within our analysis time frame. However, because the Michigan martens were reintroduced serially, the N_e of each of the specific reintroductions was significantly lower, reducing our ability to still detect the signature of the bottleneck. Assuming the same $N_e : N_c$ ratio of 0.1, the initial 1957 reintroduction of 27 animals would only have been detectable for 1.4–13.5 generations, followed by a 2nd reintroduction in 1970 of 99 animals detectable for 5–49.5 generations, and lastly a 3rd reintroduction of 148 animals in 1979 detectable for 7.4–74 generations. The lower end of the detection times for all of these reintroductions falls outside our window of detection, which, taken in conjunction with the translocations and dispersal of individuals in the expanding population, likely erased the genetic signature of the bottleneck in Michigan's marten population.

The Michigan marten reintroduction appears to be a success despite relatively limited human manipulations compared to other reintroduction programs such as the black-footed ferret (e.g. Wisely et al. 2003). Over a period of 49 years, the only intensive management performed was a series of 3 introductions and 3 translocations. In the 25 years since the last set of martens was reintroduced and the 12 years since the last marten translocation, martens in Michigan's Upper Peninsula have returned to a demographically stable population with $N_e =$ 1,576 martens, assuming the appropriate stepwise mutation model (Ohta and Kimura 1973) for microsatellites (Sainudiin et al. 2004). This value is similar to the current N_e (1,237) of their source population in Chapleau, Ontario. Although there are concerns regarding the accuracy of estimating population size (N_c) from N_e, they can be used as rough approximations if the ratio of N_e to N_c is known (Frankham 1995b, 1996), suggesting the marten census size is approximately 3,150–15,760 animals. This value also supports the indication that the approximately 130 animals harvested per year is a sustainable level for this population as suggested by the ratios of the harvest indices.

Several factors likely played a role in the success of Michigan's marten reintroduction. System-wide, the 276 animals reintroduced are likely sufficient to meet Slough's (1994) suggestion that reintroductions consist of an effective population size of 50 animals, even if no single site exceeded an $N_e = 50$. The multiple reintroductions and translocations were equivalent to large-scale long-distance dispersal events and could have restored alleles on the verge of being lost in the population. Perhaps most importantly, the martens were reintroduced into areas with relatively low human population density (average county density of 6.6 $\pm$ 3.3 *SD* individuals per km^2, Census 2000, available at: http://www.census.gov/main/www/cen2000.html) and, when examined on a coarse scale, large amounts of contiguous suitable habitat.

Although Michigan's marten populations are recovering and appear to be on a sound genetic and demographic footing, we suggest that they continue to be monitored because (1) they are exposed to increasing anthropogenic disturbances; (2) they are on the southern periphery of the North American marten distribution where marten populations are fragmented (Gibilisco 1994); (3) they live on a peninsula and experience less natural immigration and more isolation than mainland populations; and (4) they may be experiencing a net loss of individuals to Wisconsin (Earle et al. 2001), reducing their gene pool.

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Literature Cited

Archibald, W. R., and R. H. Jessup. 1984. Population dynamics of the pine marten (*Martes americana*) in the Yukon Territory. Pp. 81–97 in Northern ecology and resource management: memorial essays honouring Don Gill (R. Olson, R. Hastings, and F. Geddes, eds.). University of Alberta Press, Edmonton, Alberta, Canada.

Arrendal, J., C. W. Walker, A. K. Sundqvist, L. Hellborg, and C. Vila. 2004. Genetic evaluation of an otter translocation program. Conservation Genetics 5:79–88.

Bowman, J. C., and J.-F. Robitaille. 1997. Winter habitat use of American marten *Martes americana* within second-growth forest in Ontario, Canada. Wildlife Biology 3:89–101.

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, and M. G. Raphael, eds.). Cornell University Press, Ithaca, New York.

Cooley, T. M., S. M. Schmitt, P. D. Friedrich, and T. F. Reis. 2002. American marten survey—2001. State of Michigan Department of Natural Resources Wildlife Report No. 3381.

Cornuet, J. M., and G. Luikart. 1996. Description and evaluation of two tests for detecting recent population bottlenecks. Genetics 144: 2001–2014.

Davis, C., and C. Strobeck. 1998 Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Molecular Ecology 7:1776–1778.

Day, S. B., E. H. Bryant, and L. M. Meffert. 2003. The influence of variable rates of inbreeding on fitness, environmental responsiveness, and evolutionary potential. Evolution 57:1314–1324.

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.

Felsenstein, J. 2005. PHYLIP [Phylogeny Inference Package] version 3.6. Available from the author: Department of Genome Sciences, University of Washington, Seattle.

Frankham, R. 1995a. Inbreeding and extinction: a threshold effect. Conservation Biology 9:792–799.

FRANKHAM, R. 1995b. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66:95–107.

FRANKHAM, R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

GARZA, J. C., AND E. G. WILLIAMS. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

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, and M. G. Raphael, eds.). Cornell University Press, Ithaca, New York.

HARDEN, J. 1998. Status of the American marten in the Manistee National Forest of lower Michigan. M.S. thesis, Central Michigan University, Mount Pleasant.

HARGIS, C. D., J. A, BISSONETTE, AND D. L. TURNER. 1999. The influence of forest fragmentation and landscape pattern on American martens. Journal of Applied Ecology 36:157–172.

JEFFERY, K. J., L. F. KELLER, P. ARCES, AND M. W. BRUFORD. 2001. The development of microsatellite loci in the song sparrow, *Melospiza melodia* (Aves) and genotyping errors associated with good qualtiy DNA. Molecular Ecology Notes 1:11–13.

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. 2003. Genetic homogeneity of Canadian mainland marten populations underscores the distinctiveness of Newfoundland pine martens (*Martes americana atrata*). Canadian Journal of Zoology 81:57–66.

LACY, R. C. 1993. VORTEX: A computer simulation model for Population Viability Analysis. Wildlife Research 20:45–65.

LANDE, R., AND G. R. BARROWCLOUGH. 1987. Effective population size, genetic variation, and their use in population management. Pp. 87–124 in Viable populations for conservation (M. E. Soulé, ed). Cambridge University Press, Cambridge, United Kingdom.

LARSON, S., R. JAMESON, J. BODKIN, M. STAEDLER, AND P. BENTZEN. 2002. Microsatellite DNA and mitochondrial DNA variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy 83:893–906.

LUIKART, G., AND J.-M. CORNUET. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12:228–237.

LYNCH, M., J. CONERY, AND R. BURGER. 1995. Mutation accumulation and the extinction of small populations. American Naturalist 146: 489–518.

MARUYAMA, T., AND P. A. FUERST. 1984. Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 108:745–763.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

NIEMINEN, M., M. C. SINGER, W. FORTELIUS, K. SCHOPS, AND I. HANSKI. 2001. Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. American Naturalist 157:237–244.

O'BRIEN, S. J., AND J. F. EVERMANN. 1988. Interactive influence of infectious disease and genetic diversity in natural populations. Trends in Ecology and Evolution 3:254–259.

OHTA, T., AND M. KIMURA. 1973. The model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a genetic population. Genetic Research 22:201–204.

POTVIN, F., L. BÉLANGER, AND K. LOWELL. 2000. Marten habitat selection in a clear-cut boreal landscape. Conservation Biology 14: 844–857.

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, and M. G. Raphael, eds.). Cornell University Press, Ithaca, New York.

POWELL, R. A., AND W. J. ZIELINSKI. 1994. Fisher. Pp. 38–73 in The scientific basis for conserving forest carnivores in the western United States: American marten, fisher, lynx and wolverine (L. F. Ruggiero, K. B. Aubry, S. W. Buskirk, L. J. Lyon, and W. J. Zielinski, eds.) United States Department of Agriculture Forest Service, General Technical Report RM-254.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959

Qiagen. 2001. DNeasy Tissue Kit Handbook. Qiagen Inc., Valencia, California.

QUICK, H. F. 1953. Wolverine, fisher and marten studies in a wilderness region. Transactions of the North American Wildlife Conference 18:512–533.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP version 3.1 A population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

REED, D. H., E. H. LOWE, D. A. BRISCOE, AND R. FRANKHAM. 2003. Inbreeding and extinction: effects of rate of inbreeding. Conservation Genetics 4:405–410.

ROBITAILLE, J. F., AND K. AUBRY. 2000. Occurrence and activity of American martens *Martes americana* in relation to roads and other routes. Acta Theriologica 45:137–143.

ROWE, G., AND T. J. C. BEEBEE. 2004. Reconciling genetic and demographic estimators of effective population size in the anuran amphibian *Bufo calamita*. Conservation Genetics 5:287–298

SAINUDIIN, R., R. T. DURRETT, C. F. AQUADRO, AND R. NIELSEN. 2004. Microsatellite mutation models: insights from a comparison of humans and chimpanzees. Genetics 168:383–395.

SLOUGH, B. G. 1994. Translocations of American martens: an evaluation of factors in success. Pp. 165–178 in Martens, sables, and fishers: biology and conservation (S. W. Buskirk, A. S. Harestad, and M. G. Raphael, eds.). Cornell University Press, Ithaca, New York.

SMITH, L. M., AND I. L. BRISBIN, JR. 1984. An evaluation of total trapline captures as estimates of furbearer abundance. Journal of Wildlife Management 48:1452–1455.

SOUKKALA, A. M. 1983. The effects of trapping on marten populations in Maine. M.S. thesis, University of Maine, Orono.

SOULÉ, M. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pp. 151–170 in Conservation biology (M. E. Soulé and B. A. Wilcox, eds.). Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

SOUTIERE, E. C. 1979. Effects of timber harvesting on marten in Maine. Journal of Wildlife Management 43:850–860.

STOCKWELL, C. A., M. MULVEY, AND G. L. VINYARD. 1996. Translocations and the preservation of allelic diversity. Conservation Biology 10:1133–1141.

STRICKLAND, M. A., AND C. W. DOUGLAS. 1987. Marten. Pp. 530–546 in Wild furbearer management and conservation in North America (M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch, eds.). Ministry of Natural Resources, Ottawa, Ontario.

SWANSON, B. J., R. T. FURHMANN, AND R. L. CRABTREE. 2005. Elevational isolation of red fox populations in the Greater Yellowstone ecosystem. Conservation Genetics 6:123–131.

THOMPSON, I. D., AND P. W. COLGAN. 1987. Numerical responses of martens to a food shortage in northcentral Ontario. Journal of Wildlife Management 51:832.

THOMPSON, I. D., AND A. S. HARESTAD. 1994. Effects of logging on American martens, and models for habitat management. Pp. 355–367 in Martens, sables, and fishers: biology and conservation (S. W. Buskirk, A. S. Harestad, and M. G. Raphael, eds.). Cornell University Press, Ithaca, New York.

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.

WISELY, S. M., D. B. MCDONALD, AND S. W. BUSKIRK. 2003. Evaluation of the genetic management of the endangered black-footed ferret (*Mustela nigripes*). Zoo Biology 22:287–298.

WRIGHT, S. 1969. Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago, Illinois.

YEAGER, L. E. 1950. Implications of some harvest and habitat factors on pine marten management. Transactions of the North American Wildlife Conference 15:319–334.

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