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Genetic diversity of reintroduced American martens in Michigan's Lower Peninsula

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Reintroductions are an important conservation and management technique used to restore extirpated populations. Negative genetic consequences (e.g., diversity loss, bottlenecks, inbreeding) are often an unintentional result of reintroductions, due to a small number of founders or suboptimal habitat at release sites. American martens (*Martes americana*) were extirpated from Michigan's Lower Peninsula in 1911 due to habitat loss and unregulated trapping. Martens were reintroduced into 2 areas of the Lower Peninsula in 1985–1986. The Lower Peninsula reintroduction was characterized by a relatively small number of founders (85 individuals) released into 2 geographically disparate, fragmented sites. We genotyped martens sampled at the 2 release sites approximately 20–25 years since reintroduction, using 11 microsatellite loci. We detected low average allelic richness (3.92 alleles per locus), moderate levels of inbreeding (mean $F_{IS} = 0.106$), and multiple loci with significant heterozygote deficiencies. Effective population size estimates were small, ranging between 6 and 27 individuals depending on the estimator and the sample group. We also detected significant population structuring between the release sites ($F_{ST} = 0.093$ using the most recent sample). With small population size and limited to no gene flow, we predict the 2 Lower Peninsula marten populations will continue to diverge and potentially further lose genetic diversity. This study highlights the importance of long-term genetic monitoring of reintroduced populations.

Key words: founders, genetic diversity, martens, *Martes americana*, microsatellites, population genetics, reintroduction

Reintroductions are used to restore extirpated populations (Griffith et al. 1989; Sarrazin and Barbault 1996; Wolf et al. 1996; Banks et al. 2002). Retaining genetic diversity in new populations is a common goal for reintroductions, yet this goal is not always achieved (Sarrazin and Barbault 1996; Wolf et al. 1996). Understanding how reintroduction characteristics (e.g., number of founders, rate of population expansion, and habitat quality of release sites) affect retention of genetic diversity is important both for species recovery and for contributing knowledge to the broader field of reintroduction biology (Armstrong and Seddon 2008).

Several factors contribute to retention of genetic diversity in reintroduced populations. First, founding population sizes of ≥ 100 individuals (Griffith et al. 1989; Fischer and Lindenmayer 2000) or an effective population size of ≥ 50 individuals (Slough 1994; Powell et al. 2012) will maximize probability of population establishment and reduce loss of genetic diversity from the source. Conversely, small founding population size is likely to impose a genetic bottleneck and heighten loss of genetic diversity (Allendorf et al. 2013). Rapid population expansion post-release will aid in maintenance of genetic diversity by reducing the number of generations of consanguineous mating, thereby decreasing inbreeding as the rate of expansion increases (Nei et al. 1975; Allendorf et al. 2013). Lastly, founding groups with equal or female-biased sex ratios will equalize genetic contributions of founders, whereas male-biased founding groups will reduce effective population size, especially in species with skewed operational sex ratios or reproductive success (e.g., a single male mates with > 1 female, or a few males dominate reproduction—Miller et al. 2009).

Reintroductions using small founding groups, skewed or male-biased sex ratios, or releases into suboptimal habitat can

have negative genetic consequences on the population at the very onset of reestablishment. These populations may suffer from low survival, reduced genetic diversity, population isolation, limited gene flow, and high inbreeding, which over time can manifest as lower reproductive success, decreased offspring survival, and reduced resistance to disease (Nei et al. 1975; Stockwell et al. 1996; Lacy 1997). Post-reintroduction genetic monitoring is therefore necessary to examine genetic diversity, inbreeding risk, long-term survival probabilities, and to measure the success of management actions (Schwartz et al. 2007).

American martens (*Martes americana*) are mid-sized mustelids native to the northern boreal forests of North America ranging throughout Canada and Alaska, portions of the northern United States including the Great Lakes Region, New England, and south along the Rocky Mountain, Sierra Nevada, and Cascade ranges (Clark et al. 1987). Martens are commonly considered a habitat specialist due to their association with late successional mixed deciduous-coniferous forests, high canopy closure (> 50%), and high levels of coarse downed wood (20–50% of ground surface cover—Buskirk and Powell 1994; Thompson and Colgan 1994; Poole et al. 2004; Slauson et al. 2007). Historically, martens were a prized fur-bearing species subject to intensive and often unregulated trapping, which in concert with habitat loss resulting from European settlement, led to extirpations of the species in many areas across its native range (Hagmeier 1956; Berg 1982). Michigan's Lower Peninsula (hereafter called "Lower Peninsula"; Fig. 1) is a region where martens were extirpated and is the focal area for this study.

Fig. 1.—Map of the reintroduction and sampling areas of American martens (*Martes americana*) in Michigan's Lower Peninsula. Inset shows the location of Michigan within the United States. Large map shows the location of the Manistee National Forest (MNF; 2,187 km^2) and Pigeon River Country State Forest (PGR; 457 km^2) within the Lower Peninsula of Michigan. Hatched areas denote state and national forest boundaries. White circles show approximate location of original release sites of reintroduced martens.

Martens were extirpated from the Lower Peninsula by 1911 by habitat loss and over-trapping (Williams et al. 2007). Over decades, more conservative logging practices facilitated forest succession to secondary deciduous-coniferous mixed forest deemed continuous and suitable for reintroduction of martens (Shands 1991; Williams et al. 2007). The Michigan Department of Natural Resources arranged to translocate 220–240 martens from the Crown Chapleau Game Preserve, Ontario, Canada beginning in 1985, following methods similar to those implemented in previous years for marten reestablishment in the Upper Peninsula of Michigan (Williams et al. 2007). Planned releases were spaced temporally and spatially (5–6 releases over 2–3 years, spaced 32–64 km apart) in hopes of maintaining connectivity while minimizing competition at release sites (Williams et al. 2007). In 1985, 49 martens (25 males, 24 females) were reintroduced onto the Pigeon River Country State Forest (hereafter "Pigeon River") and, in 1985–1986, 36 martens (20 males, 16 females) onto the Manistee National and Pere-Marquette State Forests (hereafter "Manistee"; Fig. 1). After this initial reintroduction (and after years of previous reintroductions to the Upper Peninsula), the Ontario Ministry of Natural Resources expressed concern over the sustainability of sourcing another large group of martens from any one area of the province (Williams et al. 2007). As a result, no subsequent translocations of martens occurred from Ontario or elsewhere to the Lower Peninsula, and marten populations in the Lower Peninsula became reestablished with a small number of founders (Slough 1994) in 2 isolated patches of habitat approximately 150 km apart.

The marten reintroductions in Michigan's Upper Peninsula have been deemed both demographically and genetically successful as the Upper Peninsula martens are genetically diverse and have expanded to inhabit most of the peninsula (Swanson et al. 2006; Williams et al. 2007; Williams and Scribner 2010). Although the Lower Peninsula reintroductions did result in successful reestablishment of martens in their reintroduction areas, a genetic assessment of the Lower Peninsula martens since reintroduction was lacking.

We studied martens in the areas surrounding the 2 original reintroduction sites (Fig. 1). Secondary forests in these areas have coniferous and northern hardwood trees up to 140 years old (stand dominated by 64–83-year-old age classes—Haugen et al. 1997). The Pigeon River and Manistee are approximately 150 km apart and separated by fragmented habitat, anthropogenic development, and a 4-lane divided expressway (Interstate 75), which limits connectivity. Moderate levels of private inholdings and a network of service roads and 2-lane highways fragment habitat within each site. Our objectives were to estimate the genetic variation, spatial and temporal population differentiation, levels of inbreeding, and effective population sizes of martens in Michigan's Lower Peninsula, to measure impacts of small founding size, population isolation, and release habitat quality on genetic diversity and reintroduction success.

Materials and Methods

Sample collection.—We collected genetic samples from martens in the Manistee from 2005 to 2006 (n = 17; 10 males, 6 females, 1 unknown) and 2011 to 2013 (n = 35; 20 males, 14 females, 1 unknown), and in the Pigeon River from 2004 to 2006 (n = 24; 15 males, 8 females, 1 unknown). Samples from 2004 to 2006 were ear punches from live-captured martens (Nelson 2006; Bicker 2007). Manistee samples from 2011 to 2013 were blood from live-captured martens. Two additional tissue samples were obtained for the Manistee in 2011–2013: 1 from a road-killed marten, and a second from a dead kit. Live-captured martens from all trapping locations and periods were individually marked with a unique ear tag (2004–2006), or with an implanted passive integrated transponder tag (2011–2013; AVID, Norco, California). Martens were weighed, assigned to an age cohort (juvenile or adult based on tooth wear), and sex was determined at time of capture. All samples were stored at −80°C until DNA extraction.

Laboratory methods.—We extracted DNA from blood and tissue samples using Qiagen Dneasy Blood and Tissue Kit standard protocols and reagents (Qiagen Sciences, Germantown, Maryland). We amplified DNA at 11 microsatellite loci: *Ma-1*, *Ma-2*, *Ma-3*, *Ma-5*, *Ma-7*, *Ma-10*, *Ma-11*, *Ma-15*, *Gg-3*, *Gg-7* (Davis and Strobeck 1998), and *Mvis-072* (Fleming et al. 1999). PCRs were completed in 20-µl volumes using a touchdown thermal protocol to increase sensitivity and specificity of primer amplification (Korbie and Mattick 2008). Our PCR thermal protocol consisted of 95°C for 4 min, followed by 20 cycles of 95°C for 30 s, 60°C for 30 s (decreasing 0.5°C each cycle), and 72°C for 30 s, then followed by 36 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s, ending with an extension of 72°C for 7 min. Amplified PCR products were visualized on an Applied Biosystems 3130 xl Genetic Analyzer (Applied Biosystems, Carlsbad, California). Genotypes were scored using Applied Biosystems Peak Scanner Software v1.0 and 10% of all samples were randomly selected for re-scoring by TH to calculate scoring error. We corrected any errors discovered during scoring error calculation before completing downstream analyses.

Genetic analyses.—We analyzed sampling locations and time periods (Pigeon River in 2004–2006, Manistee in 2005–2006, Manistee in 2011–2013) separately to detect levels of population genetic structure and to measure temporal changes in genetic variation. We examined our loci for deviations from Hardy–Weinberg proportions using GenAlEx 6.5 (Peakall and Smouse 2006) to assess the likelihood of founder effects, genetic drift, and inbreeding due to small population sizes. We estimated the frequency of null alleles for each locus and population using the Expectation Maximization algorithm of Dempster et al. (1977) as implemented in the program FreeNA (Chapuis and Estoup 2007). We assessed genetic variation using observed heterozygosity (H_O) and expected heterozygosity (H_E), calculated in GenAlEx, as well as Wright's inbreeding coefficient (F_{IS}) and allelic richness (A_r, adjusted to the lowest sample size, n = 17), calculated in FSTAT 2.9.3 (Goudet 1995). To test for differences in genetic diversity between sampling areas and time periods, we used Friedman tests conducted in Program R (v.3.0.0—R Development Core Team 2005). We used Wilcoxon signed-rank tests for post hoc pairwise comparisons when Friedman tests were significant. Wilcoxon signed-rank tests were also used for comparisons of observed and expected heterozygosity to detect any significant reductions in heterozygosity. We evaluated genetic structure of populations using F_{ST} calculated with an Analysis of Molecular Variance in GenAlEx. All test statistics were assessed at an alpha level of 0.05. We opted to not use alpha correction for multiple comparisons in order to reduce our likelihood of making a Type II error (i.e., not detecting an effect that was present).

We estimated effective population size (N_e) to provide an additional assessment of the effects of small number of founders and subsequent genetic drift in the Lower Peninsula populations. Effective population size is an estimate of the size of an "idealized" population (i.e., with constant population size, equal family sizes, equal sex ratio, and discrete generations) that loses heterozygosity at the same rate as the focal population. For species with overlapping generations, such as martens, estimates of effective population size more closely represent the effective number of breeders per generation (N_b) (Allendorf et al. 2013). Because we lack information on census population sizes (N_c; the number of adults in the population), we used N_e to provide a rough estimate of the census population sizes based on the N_e/N_c ratios of Frankham (1995), who found effective population size estimates are typically 10–50% of the adult census population size. We used our largest estimates of N_e to estimate N_c based on the Frankham (1995) N_e/N_c ratios (0.1–0.5) as well as the 95% confidence intervals (*CI*s) around our point estimates. We estimated 95% *CI*s for N_c by dividing the lower N_e *CI* by 0.5 and the upper N_e *CI* by 0.1.

We estimated N_e using Bayesian and linkage disequilibrium methods, as well as the temporal method for the Manistee sampling periods, for which 2 sets of samples separated by > 1 generation were available. The approximate Bayesian computation estimator ONeSAMP (Tallmon et al. 2008) was selected based on its ability to calculate robust estimates with small sample sizes and low levels of polymorphic data (Beebee 2009), which we expected based on the elusive behavior of martens and small founding sizes of our study populations, respectively. We calculated linkage disequilibrium (bias-corrected version—Hill 1981; Waples 2006; Waples and Do 2010) and temporal method (Pollak 1983; Waples 1989) estimates for N_e in NeEstimator V2.01 (Do et al. 2014). For temporal estimates, we set the Manistee samples collected in 2005–2006 as generation zero, and the Manistee samples collected in 2011–2013 as generation 1.3 based on the length of time between these sampling periods (5–8 years), and marten generation time (4–6 years—Clark et al. 1987). We calculated multiple estimates for N_e to account for estimator tendencies to produce large or infinite *CI*s when tested using small sample sizes (Beebee 2009). We rounded all N_e estimates up to the nearest whole number to represent complete individuals. This study conformed to the American Society of Mammalogists guidelines for the use of wild mammals in research (Sikes et al. 2016) and was approved by Grand

Valley State University's Institutional Animal Care and Use Committee (protocol number 12-05-A).

Results

Estimates of genetic diversity.—We genotyped a total of 76 American martens from Manistee (n = 52) and Pigeon River (n = 24). Scoring error rate of samples over 11 loci was 2.4%. The average estimated frequency of null alleles across all loci and populations was 5.1%, and no locus showed consistently high frequencies of null alleles across all populations and time periods. Because there were no locus-specific patterns indicative of true null alleles, we retained all loci for downstream analyses. We detected departures from Hardy–Weinberg proportions due to heterozygote deficits at 1 locus in the Manistee 2005–2006 sample (*Ma-10*), 7 loci in the Manistee 2011–2013 sample (*Ma-1*, *Ma-2*, *Ma-5*, *Ma-7*, *Ma-11*, *Ma-15*, *Mvis-072*), and at 2 loci in the Pigeon River sample (*Ma-1*, *Ma-10*). Excess heterozygosity caused departures from Hardy–Weinberg proportions at 2 loci in the Manistee 2005–2006 sample (*Ma-11*, *Gg-3*). F_{IS} was highest for the Manistee 2011–2013 sample (F_{IS} = 0.238, range = 0.016–0.238; Table 1), indicating this population has experienced moderate inbreeding. Because departure from Hardy–Weinberg equilibrium was not detected across all populations for any 1 locus, we retained all loci in subsequent analyses with the exception of *Ma-3* in the Manistee 2005–2006 because it was monomorphic.

Observed heterozygosity was 0.544 across all loci and allelic richness averaged 3.924 (Table 1). There were no significant differences in genetic diversity measures (A, H_O, F_{IS}) between sampling areas or time periods (Table 1; Friedman, $\chi^2_3 = 7.514$, $P = 0.057$ for A; $\chi^2_3 = 3.165$, $P = 0.367$ for H_O; and $\chi^2_3 = 7.200$, $P = 0.066$ for F_{IS}). Overall, H_O was significantly lower than H_E in the Manistee sample from 2011 to 2013 (Wilcoxon signed-rank, P = 0.014), but overall heterozygote deficiencies were not detected in either the earlier Manistee or Pigeon River samples ($P > 0.05$). We observed significant population structure between all sampling areas and time periods (pairwise F_{ST} range = 0.043–0.093, $P < 0.001$; Table 2).

Effective population size.—We observed low estimates of N_e for martens in the Lower Peninsula, with both sampling areas and periods falling below 30 individuals. N_e estimates for the Pigeon River were 17 martens (95% *CI* = 10–34) and 23 martens (95% *CI* = 19–32), for linkage disequilibrium and Bayesian methods (Fig. 2). Linkage disequilibrium and Bayesian N_e estimates for the Manistee in 2005–2006 were 10 martens (95% *CI* = 4–24, linkage disequilibrium) and 17 martens (95% *CI* = 14–22, Bayesian), and for 2011–2013 were 13 martens (95% *CI* = 10–18, linkage disequilibrium) and 27 martens (95% *CI* = 22–42, Bayesian), respectively (Fig. 2). Our N_e estimate for the Manistee using the temporal method provided the lowest N_e estimate of all methods at only 6 martens (95% *CI* = 4–11; Table 1).

Using the range of N_e/N_c ratios (0.1–0.5) of Frankham (1995), and our largest N_e estimates for each population and time period, we estimated adult census sizes of 46–230 martens (95% *CI* = 38–320) in the Pigeon River, 34–170 martens (95% *CI* = 28–220) in the early Manistee (2005–2006) samples, and 54–270 martens (95% *CI* = 44–420) in the recent Manistee (2011–2013) samples.

Discussion

We found low levels of allelic diversity, relatively low heterozygosity, and moderately high levels of inbreeding in both Manistee and Pigeon River populations of American martens reintroduced to Michigan's Lower Peninsula in 1985–1986. Manistee in particular showed significant heterozygote deficiencies across numerous loci, and a positive F_{IS} value, which were both consistent with a small population experiencing considerable genetic drift and inbreeding (Waples 2015). Significant population genetic structure existed between the

Table 1.—Summary of genetic diversity measures for reintroduced American marten (*Martes americana*) populations in Michigan's Lower Peninsula based on 11 microsatellite loci. Samples were obtained from reintroduction release sites in Manistee National Forest (MNF) and Pigeon River Country State Forest (PGR), with years of sampling indicated in parentheses. Number of martens sampled (n), mean number of alleles per locus (A), allelic richness (A_r) with 95% confidence intervals (*CI*s), number of loci in Hardy–Weinberg Equilibrium (HWE), observed heterozygosity (H_O) with *SE*, expected heterozygosity (H_E) with *SE*, Wright's inbreeding coefficient (F_{IS}), and effective population size (N_e) estimates with 95% *CI*s. Bolded values indicate significant heterozygote deficiency at P = 0.014.

Sampling area (years sampled)								N_e		
	n	A	A_r (95% *CI*)[a]	HWE	H_O (*SE*)	H_E (*SE*)	F_{IS}	Bayesian[b]	LD[c]	Temporal[d]
MNF (2005–2006)	17	3.727	3.585 (2.723–4.447)	7	0.576 (0.074)	0.565 (0.066)	0.016	17 (14–22)	10 (4–24)	6 (4–11)
MNF (2011–2013)	35	5.364	4.344 (3.415–5.273)	4	**0.494 (0.068)**	**0.636 (0.066)**	0.238	27 (22–42)	13 (10–18)	6 (4–11)
PGR (2004–2006)	24	4.091	3.842 (3.106–4.578)	9	0.561 (0.070)	0.585 (0.058)	0.063	23 (19–32)	17 (10–34)	
Average		4.394	3.924		0.544	0.595	0.106			
Source population[e]	61	6.0		11	0.626 (0.051)	0.656	0.027			

[a] Allelic richness estimates for the MNF and PGR adjusted to a sample size of n = 17.
[b] Bayesian estimator ONeSAMP (Tallmon et al. 2008).
[c] Linkage disequilibrium (LD) method, bias-corrected version (Hill 1981; Waples 2006; Waples and Do 2010), estimated using program NeEstimator V2.01 (Do et al. 2014).
[d] Temporal method (Pollak 1983; Waples 1989), generations set to 0 and 1.3, estimated using program NeEstimator V2.01.
[e] Source population located in Crown Chapleau Game Preserve, Ontario, Canada. Values originally reported in Williams and Scribner (2010).

Manistee and Pigeon River populations. N_e estimates were low (< 30 individuals) for both populations. Our findings reflect some negative genetic effects of small founder groups and warrant additional genetic monitoring and management to maintain long-term viability of Lower Peninsula martens.

Levels of allelic diversity for reintroduced martens in Michigan's Lower Peninsula fall between estimates for large marten populations in mainland Canada (Kyle et al. 2003; Kyle and Strobeck 2003; Williams and Scribner 2010), and isolated peninsular and island populations of martens from the Pacific Northwest and Europe (*M. caurina*—Small et al. 2003; *M. martes*—Pertoldi et al. 2008; *M. americana*—Williams and Scribner 2010). Allele counts for Lower Peninsula martens were below 75% of mainland Canadian populations studied with similar sampling effort ($n \leq 35$—see table 1 of Kyle and Strobeck 2003); nevertheless, there was considerable overlap across all studies. Allele counts in this study ranged from 3.73 to 5.36 alleles/locus (mean 4.39 alleles/locus) compared to 4.82–6.64 alleles/locus (mean 5.89 alleles/locus) across mainland Canada (Kyle and Strobeck 2003; Williams and Scribner 2010), and 1.29–5.36 alleles/locus (mean 3.57 alleles/locus) in isolated peninsular and island populations of the Pacific Northwest and Europe (Kyle et al. 2003; Small et al. 2003; Pertoldi et al. 2008). Our observed estimates of heterozygosity were also comparable to findings from reintroduced and natural populations elsewhere, with values from mainland Canada and the Pacific Northwest (0.390–0.680—Kyle et al. 2000; Kyle and Strobeck 2003; Small et al. 2003; Williams and Scribner 2010) fully encompassing the range of values estimated for Lower Peninsula martens (0.494–0.576). Based on these 2 parameters, current estimates of genetic diversity for reintroduced martens in Michigan's Lower Peninsula are within values observed elsewhere for isolated, yet presumably viable marten populations.

Table 2.—Pairwise F_{ST} values (below diagonal) between sampling areas and time periods of reintroduced American martens (*Martes americana*) in Manistee National Forest (MNF) and the Pigeon River Country State Forest (PGR) in Michigan's Lower Peninsula. *P*-values are presented above the diagonal.

	MNF 2005–2006	MNF 2011–2013	PGR 2004–2006
MNF 2005–2006		0.001	0.001
MNF 2011–2013	0.068		0.001
PGR 2004–2006	0.043	0.093	

The reintroduction of martens to the Lower Peninsula does not appear to have adequately captured the genetic diversity of the source population (Crown Chapleau Game Preserve, Ontario—Williams and Scribner 2010), likely due to small founding population size. Goals of genetic management for reintroduced populations are to retain 90–95% of the heterozygosity of the source population over 100–200 years (Allendorf and Ryman 2002). Compared with the source population, the Manistee population has retained 79% of heterozygosity since reintroductions in 1985–1986. Small founding population size likely imposed loss of allelic diversity and heterozygosity that will continue to worsen with time, particularly with the apparent absence of gene flow.

A confounding result was that our more recent Manistee sample showed higher allelic diversity than the earlier sample. As mutation is unlikely to generate new alleles in this short time period, and gene flow was unlikely (see below), this result could be an artifact of increased sample size in the 2011–2013 Manistee sample, which was twice as large as the 2005–2006 sample from that population. Allelic diversity is highly sensitive to sample size (Allendorf et al. 2013), and if we take the overlapping 95% *CI*s into account, our estimates of allelic richness (which account for differing sample sizes) are not different across time periods. In spite of this, the Lower Peninsula

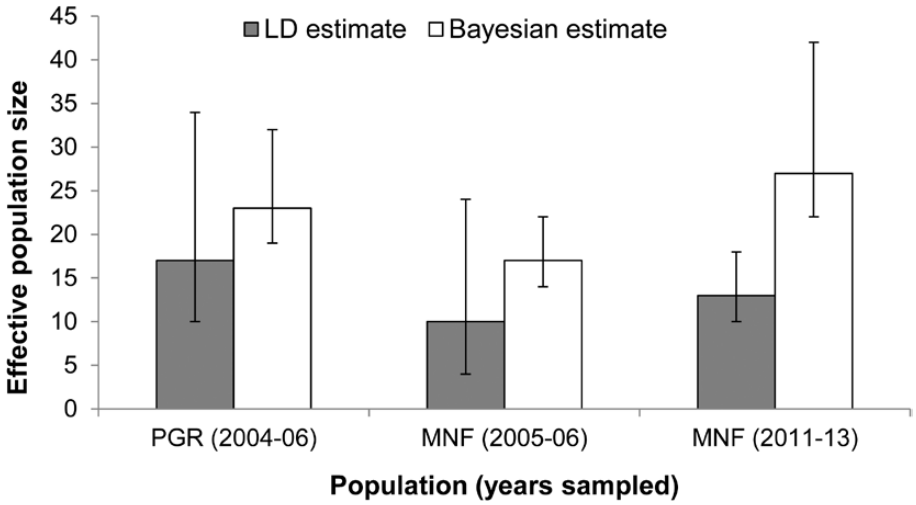

Fig. 2.—Effective population size (*Ne*) estimation of American marten (*Martes americana*) populations from the 2 reintroduction sites (Manistee National Forest [MNF], in 2005–2006, $n = 17$ and 2011–2013, $n = 35$, and Pigeon River Country State Forest [PGR] 2004–2006, $n = 24$) in Michigan's Lower Peninsula. Gray bars represent estimates using the linkage disequilibrium method (NeEstimator V2.01, bias-corrected version—Do et al. 2014; Hill 1981; Waples 2006; Waples and Do 2010). White bars represent estimates using the Bayesian method (ONeSAMP—Tallmon et al. 2008). Error bars represent 95% confidence intervals.

marten reintroduction has not met standard genetic goals for reintroductions (outlined in Weeks et al. 2011) as it was likely founded with too few individuals to adequately capture > 95% of the source population's heterozygosity.

Decreases in genetic diversity as a result of small founding population size are well documented in reintroduced populations (Stockwell et al. 1996; Williams et al. 2000; Vernesi et al. 2003; Mock et al. 2004). An effective size of 50 individuals is recommended for mitigating negative genetic effects in a reintroduction (Slough 1994; Powell et al. 2012), and 100 individuals is recommended to maximize the probability of success (Griffith et al. 1989; Fischer and Lindenmayer 2000). The proportion of genetic variability from the source population represented in founding individuals will decrease with founding group size, particularly in terms of overall allelic diversity and especially rare alleles occurring at low frequencies (Nei et al. 1975; Allendorf et al. 2013). Both populations in the Lower Peninsula were founded with fewer than 50 individuals, and current effective population sizes are less than 30. Over time, loss of diversity and inbreeding can cause inbreeding depression or increase genetic load, as evidenced by reduced survival of juvenile Scandinavian wolves (*Canis lupus*—Liberg et al. 2005), and congenital bone deformities in both Florida panthers (*Puma concolor coryi*—Roelke et al. 1993) and Isle Royale wolves (Räikkönen et al. 2009). We currently lack the data to assess fitness or vital rates in Lower Peninsula martens, which could provide an indication of any existing inbreeding depression. In the absence of management interventions, diversity levels and inbreeding rates may worsen over time.

Although we did not calculate trends in population growth over the past 2 decades, our current N_e and extrapolated estimates of census population size indicate the populations are small (i.e., $N_e < 50$, N_c likely < 300 total individuals—Slough 1994; Frankham 1995). Populations that expand quickly after a reintroduction or bottleneck are able to retain more diversity than populations with slow growth rates (Nei et al. 1975; Allendorf et al. 2013). Slow population growth post-reintroduction could also explain reduced diversity and increased inbreeding levels detected in Lower Peninsula martens compared to the source population.

Population isolation has resulted in reduced genetic diversity in populations of martens elsewhere (*M. martes* in Europe—Kyle et al. 2003; *M. americana atrata* in Newfoundland—Kyle and Strobeck 2003; *M. caurina* in Alaska and British Columbia—Small et al. 2003), as well as in other carnivore species (brown bear, *Ursus arctos*, on Kodiak Island—Paetkau et al. 1998; wolves on Isle Royale—Wayne et al. 1991). Isolated populations lose genetic diversity over time because the number of new alleles being integrated into the population is scarce, and the effects of genetic drift are more pronounced (Allendorf et al. 2013). Gene flow between the 2 release sites could counteract the effects of genetic drift, but it is unlikely martens are able to disperse between the release sites (Epps et al. 2005; Howell et al. 2016). Manistee and Pigeon River are separated by approximately 150 km of fragmented habitat, including a 4-lane divided expressway, many additional roads of moderate-to-high traffic volume, and areas of agricultural, residential, and commercial development. Martens are capable of dispersing long distances (up to 40–80 km—Thompson and Colgan 1987), but avoid large tracts of open land (Thompson and Colgan 1994; Chapin et al. 1998; Hargis et al. 1999), and occupancy rates drop sharply in areas with > 30% non-forested habitat (Fuller 2006). Although our F_{ST} estimates do not reflect complete isolation, these estimates could equally be reflective of the fact these 2 populations were sourced from the same genetic stock. For context, our F_{ST} estimate between the recent Manistee sample and Pigeon River ($F_{ST} = 0.093$) is on par with previous estimates between marten populations as distant as Pembroke, Ontario and Chetwynd, British Columbia or Slocan, British Columbia and Gatineau, Quebec (Kyle and Strobeck 2003).

Our genetic assessment of martens in Michigan's Lower Peninsula demonstrated multiple negative genetic consequences that are likely resulting from populations being founded by too few individuals and lacking connectivity between populations. This study highlights the importance of long-term genetic monitoring of reintroduced populations.

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