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Status of reintroduced American marten in the Manistee National Forest within Michigan's Northern Lower Peninsula

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Introduction

The American marten (*Martes americana*) was extirpated from Michigan's Lower Peninsula in the early 1900's due to habitat loss as a result of logging of old growth forests and overharvest by trappers (Buchanan 2008; McCann et al. 2010; Williams et al. 2007). The Michigan Department of Natural Resources and the United States Forest Service worked jointly to reintroduce martens in the Northern Lower Peninsula (NLP) of Michigan. They conducted a two part reintroduction with the release of 49 martens (24 female, 25 male) in the Pigeon River State Forest (PRSF) in December 1985 and the release of 36 martens (19 male, 17 female) in the Manistee National Forest (MNF) in March of 1986 (Buchanan 2008; Nelson 2006; Williams et al. 2007; [Figure 1]).

Since reintroduction in the NLP the two reintroduced populations of marten in the MNF and PRSF have genetically diverged from the source population in Ontario, Canada, into two genetically distinct populations (Nelson 2006). As a result of the genetic bottleneck associated with the reintroduction of a small number of martens, both populations have lost genetic diversity (Bicker 2007).

Martens prefer continuous old-growth mixed deciduous and coniferous forests with a canopy cover of 30 to 50% and ground cover that consists of coarse woody debris between 15 to 28 cm thick, which contains hollow trees and logs, ground burrows, and crevices to provide den sites (Buchanan 2008; Clark et al. 1987; Payer and Harrison 2002). This habitat complexity supports habitat for their prey, which consists of small mammals
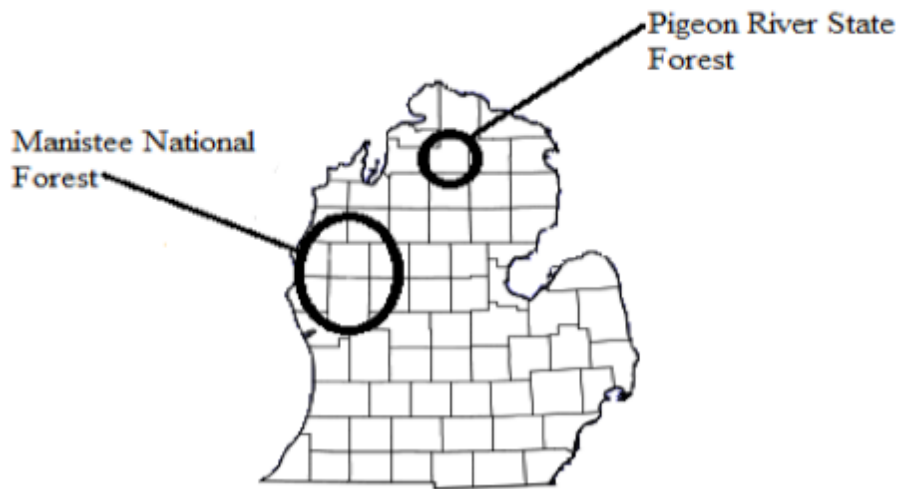
Figure 1. American marten release sites in the Manistee National Forest and Pigeon River State Forest.

such as voles, mice, chipmunks, and flying squirrels (Clark et al. 1987). Martens tend to avoid areas with large habitat openings due to their need of protection from aerial and land predators such as horned owls (Bubo virginianus), eagles (Haliaeetus leucocephalus), and coyotes (Canis latrans) (Clark et al. 1987).

Marten home ranges as calculated by McFadden (2007) using Kernel home-range estimators at 95 and 50% area contours were larger for males (avg. = 11.23 km2) than females (avg. = 7.70 km2) at the 95% area contour but were not different at the 50% area contour. The home ranges of the adult males overlap home ranges of two to six adult females, with intolerance of intrasexual competition from other adult males as evidenced by scarring in male pelts found in previous studies and the fur trade (Clark et al. 1987; McCann et al. 2010; McFadden 2007).

Many of the previously fragmented forests in the NLP of Michigan have reconnected, forming more viable habitat for martens; however, these forests are not old growth (Nelson 2006). Marten are considered to be an indicator of good forest health therefore their presence in MNF could indicate that the forests there are in good health.

In this pilot study we evaluated the success of marten in two areas within the MNF since reintroduction. Live trapping, health assessments, success of non-invasive hair snares to estimate population, DNA analysis for genetic variance and relatedness among marten were evaluated to determine if further genetic differentiation has occurred, as well as the need for corridors, translocations, and/or reintroduction of additional martens in order to increase the gene pool to prevent further genetic bottlenecking of the two smaller populations within MNF.

Materials and Methods

Study site. – The pilot study was conducted in two areas within the 530 km2 MNF release site (Buchanan 2008; Williams et al. 2007) (Figure 2). The first area, Ward Hills, was located in Lake County near Branch, MI. The second area, Caberfae, was located in Wexford County near Cadillac, MI (Figure 3).


Figure 2. Location of Lake and Wexford Counties in the State of Michigan where the two study areas were located. The marten reintroduction area encompassed both counties in an area 530 km2 (Buchanan 2008, Williams et al. 2007).

The forests in Ward Hills and Caberfae are fragmented by such activities as timber harvest, tree thinning, agriculture, resource acquisition, and residential and urban development. They consist of a mixture of deciduous and coniferous trees such as red maple (Acer rubrum), sugar maple (Acer saccharum), quaking aspen (Populus tremuloides), red oak (Quercus rubra), white oak (Quercus alba), and black cherry (Prunus serotina). The majority of the pine trees are older, interspersed red pine (Pinus resinosa) and Jack pine (Pinus banksiana) plantations (Buchanan 2008). These forests contain the habitat requirements necessary for marten such as an understory of fallen trees and logs, high canopy cover, and locations for resting sites.

Live trapping. – Live trapping was conducted in May and August 2011 using Tomahawk live traps (Models 103 – 105;

National Live Trap Company, Tomahawk, WI, U.S.A.) that were positioned near or in coarse woody debris piles that marten would naturally use for cover while foraging. Traps were covered with tree bark, leaves, pine needles, fallen branches and other natural debris found in the nearby area to keep captured marten dry (Figure 4). Traps were baited with beaver meat or pork fat that was attached by a wire to the back of the trap to prevent it from being easily stolen by marten or other scavengers. A small amount of long distance call lure made from scent glands from skunk, beaver and fox (Caven's Gusto, F & T Harvest Trading Post), was placed on a nearby branch to attract marten to the area.
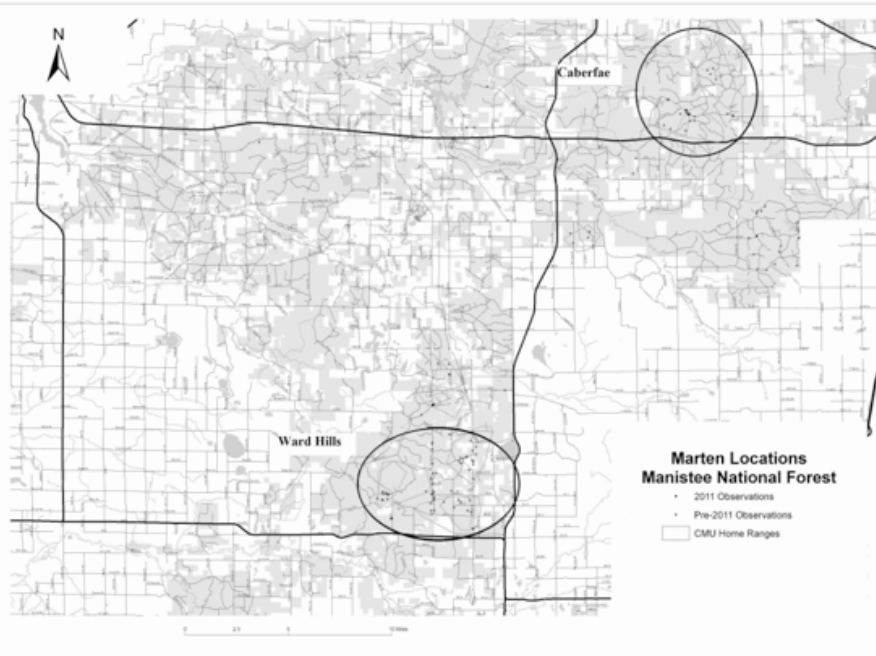
Figure 3. The two areas, Ward Hills and Caberfae, in which traps were placed in suitable marten habitat within Manistee National Forest. The black dots represent marten observations made in 2011 and the gray dots represent marten observations made prior to 2011.

Figure 4. Placement of a live trap covered with natural debris for marten.

A total of 43 traps were deployed May 11 – 14, 2011 in what appeared to be suitable marten habitat in the Ward Hills study area. The traps were checked in the morning and evening each day. On the evening of May 14, twelve traps were relocated to the Caberfae study area (Figure 3). Trap checks continued in both Ward Hills and Caberfae using the same intervals, May 15- 17, for a total trapping period from May 11, 2011 to May 17, 2011. A second trapping session following the same schedule was conducted from July 30, 2011 to August 2, 2011. The total number of trap nights for the pilot study equaled 473.

Captured marten were first transported to a mobile field laboratory where they were safely allowed to exit the trap into a denim cone (Figures 5a and 5b) that fit over the opening of the trap. Once a marten reached the end of the cone, they were sedated using Isoflurane inhalation anesthesia (Desmarchelier et al. 2007) overseen by Dr. Maria Spriggs, DVM, from Mesker Park Zoo, Evansville, Indiana. Marten were given an overall health assessment in which hair samples, blood samples and data such as body mass, gender, age, ectoparasites, fecal, urine, ear, pictures of dentition and throat patterns were recorded. Captured males were fitted with a radio-collar (Holohil Systems Ltd., Ontario, Canada) and micro-chipped (AVID Personal Identification Tag [PIT], Norco, California). Captured females were micro-chipped with AVID PIT tags only. All research on live marten captured in this study followed ASM guidelines (Gannon et al. 2007) and was approved by institutional animal care and use committee (IUCAC).

Hair snares. – Hair snares (Figures 6a and 6b) were based on the design used by Mowat and Paetkau (2002) with a few modifications. They were made of 1.27 cm thick plywood and secured with screws so they were more structurally stable. Also, two holes were drilled in the roof where a U-shaped wire was used to attach the bait instead of the side location recommended by Mowat and Paetkau (2002). Four equal-sized glue patches were cut from a Victorinox mouse trap, a cardboard backed non-toxic adhesive laced with peanut butter, and attached with a staple gun. The glue patches were placed in the

Figure 5. (a) The opening at the end of the denim cone where the marten is sedated using the Isoflurane unit.

Figure 5. (b) The denim cone shown wrapped around the cage front and tightened with heavy duty Velcro.
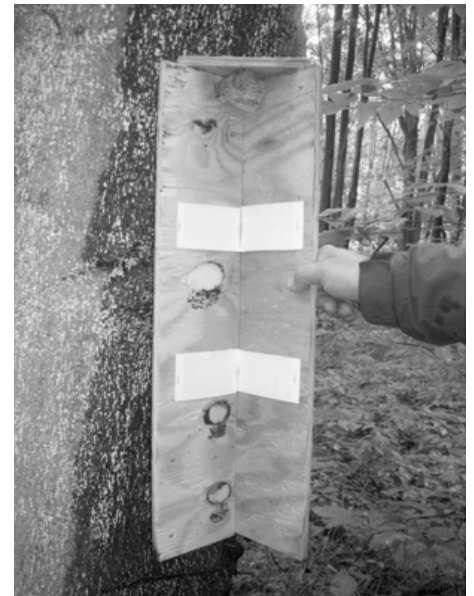
Figure 6. (a) Inside of hair snare showing positioning of glue patches.

(b) Image of the outside of a snare when mounted vertically on a tree.

approximate locations where the shoulders and mid- back of the marten would be while eating the bait. A roof was nailed on the top end to help prevent debris such as pieces of bark and rain from falling in and sticking to or wetting the glue patches, rendering them useless. The hair snares were baited with a piece of salted pork by inserting the U-shaped wire into the salted pork, through the two holes drilled into the roof, and twisted on the outside to secure. They were then mounted vertically to the tree with wood screws and spaced 2 km apart when deployed in the field. Gusto was placed approximately 2 meters from the snare location to attract the marten.

Thirty-three hair snares were deployed in known marten habitat within the Caberfae study area to collect marten hair samples. Hair snares were checked every seven days for a total of 10 checks during the 2011 summer field season. Collected hair samples were covered with wax paper and placed in a clean, dry envelope that was labeled with the snare location and date of sample collection. Samples were stored in the freezer at -80° C until examination.

Using a stereo microscope, we visually compared collected hair samples to known marten hair samples and to other possible non-target mammals in the study area. These included southern flying squirrels, red squirrels, eastern gray squirrels and mice. We then sorted the samples based on color and morphology (i.e., texture, thickness, etc.) (Mowat and Paetkau 2002).

DNA extraction and amplification. – We extracted DNA from blood samples using the QIAGEN DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) following the extraction protocol for blood with non-nucleated erythrocytes (Spin-Column). Twenty percent diluted DNA was used for PCR in a 1.5 ml microcentrifuge tube for each sample. The diluted samples were stored at 20 C and the remainder of the undiluted DNA was stored at -80 C.

Blood samples were genotyped using forward and reverse primers for 8 microsatellite loci: Ma-1, Ma-2, Ma-5, Ma-8, Ma-9, Ma-10, Ma-18, and Ma-19. We selected these primers due to their success in previous studies on marten (Bicker 2007; Davis and Strobeck 1998; Mowat and Paetkau 2002; Smith 2007; Williams et al. 2007).

We amplified PCR products using a tailed protocol. PCR was performed using 20 µl reactions which included 9.2 µl deionized (ddH2O), 2.0 µl 10x buffer (1.5 mM MgCl2), 1.6 µl 2.5 mM dNTPs, 2.0 µl 1 µM forward and reverse primer mix, 2.0 µl M13F (-29) labeled primer (FAM, NED, VIC or PET), 0.2 µl Syzygy DNA Polymerase (Syzygy Biotech, Grand Rapids, MI), and 3.0 µl genomic DNA. Two microsatellite loci (Ma-1 all samples and Ma-2 sample 8) were amplified once more using the original master mix reagent except for 7.0 µl DNA, 5.1 µl ddH2O, and 0.3 µl Syzygy DNA Polymerase in the 20 µl PCR sample. Amplification of the microsatellite loci was done using a BIO-RAD MyCycler ThermoCycler with the following cycling protocol: Initial cycle of 95 C for 5 min, 46 cycles of 95 C for 20 sec, 50 C for 20 sec, and 72 C for 30 sec, a termination step of 72 C for 3 min, and a final holding temperature of 4 C. For microsatellite locus Ma-18, an additional protocol was attempted with the same procedures except for the annealing temperature of 52 C. PCR products were visualized using an Applied Biosystems 3130xl Genetic Analyzer, GS-500 LIZ 3130 size standard, and PeakScanner software (Applied Biosystems, Foster City, CA).

We extracted DNA from four marten hair samples obtained during live trapping using the QIAGEN DNeasy Blood & Tissue Kit following a user-developed protocol for purification of total DNA from nails, hair, or feathers. Hair samples were tested in increasing increments to determine if there was a minimum (smallest tested = 5 - 10 hairs) or maximum (largest tested = 20 - 30 hairs) of hair that could be used for extraction. After DNA extraction, samples were amplified using the PCR master reagent mix above.

Genetic analysis. -- The program KINGROUP (Konovalov et al. 2004) was used to calculate relatedness among individual marten within Ward Hills and Caberfae populations and to determine whether any were more likely to be full-siblings or unrelated, as well as whether they were more likely parent-offspring or unrelated at a significance level of $p < 0.05$. The program Arlequin (version 3.5) was used to calculate a pairwise FST value between the Ward Hills and Caberfae populations to measure the genetic

difference and dispersal between them (Konovalov et al. 2004). The number of alleles and expected heterozygosity (HE) at each microsatellite locus was calculated to determine if there had been any loss in heterozygosity of the alleles or allelic dropout at these loci since the last similar genetic study of MNF martens (Bicker 2007; Davis and Strobeck 1998; Mowat and Paetkau 2002; Nelson 2006; Smith 2007).

Results

Six martens (4 female, 2 male) were captured in Ward Hills at 5 different trap locations during the May 2011 trapping period. Two of these martens (1 male, 1 female) were caught in the same trap on different days. Four martens (3 male, 1 female) were captured in two traps (3 males in same trap, 1 female in nearby trap) in Caberfae. One additional male was caught in the Ward Hills area during the second trapping period in August 2011. The sample size for this study was 11 martens.

DNA extraction was successful for all blood samples. Three microsatellite loci (Ma-1, Ma-5, Ma-19) amplified successfully for all eleven samples. A fourth microsatellite locus (Ma-2) amplified 10 of 11 samples successfully. Locus Ma-10 was considered unreliable for analysis due to inconsistent binning of alleles. Locus Ma-9 was also unreliable in this study because it was monomorphic. We used six loci (Ma-1, Ma-2, Ma-5, Ma-9, Ma-10, and Ma-19) for final calculations of gene diversity and FST. The number of alleles and HE for each loci were A = 5; HE = 0.72, A = 4; HE = 0.66, A = 4; HE = 0.62, A = 1; HE = 0, A = 2; HE = 0.49, A = 6; HE = 0.81, respectively. The number of alleles was lower for each locus in this study except in Ma-19, in which the number of alleles remained the same. Similarly, the HE was lower at each locus except for locus Ma-19, in which it was slightly higher than in previous studies (Bicker 2007, Nelson 2006). A comparison of the number of alleles and the expected heterozygosity for each microsatellite loci used in this study and in previous studies is shown in Table 1.

DNA extraction from known marten hair samples was also successful and was weakly amplified in PCR for three of the

four samples tested. The amount of DNA extracted seemed to be independent of the amount of hair used for extraction until a minimum was reached. The largest sample tested took longer for the non-DNA components to be broken down during incubation without significantly increasing the amount of DNA extracted. However, the smallest sample tested was too small and barely produced products during PCR amplification.

Five marten from Ward Hills (IDs: 2, 3, 5, 8, and 9) were more likely to be full-siblings with each other than unrelated with a type II error of 36%. Two martens (IDs 9 and 5) from Ward Hills and three martens (with IDs 7, 6, and 10) from Caberfae were more likely to be parent-offspring than unrelated with a type II error of 32% (Tables 2a and 2b). Type II error for comparison of parent-offspring versus full-siblings in related martens was 73%.

A calculated FST value of 0.141 represents a small genetic difference among the two populations with little differences among the alleles that exist in each. Statistical analysis shows no significance (p = 0.054) in the genetic difference between Ward Hills and Caberfae populations. Despite not showing significance, the FST value does indicate a trend of genetic differentiation between the two study sites.

The hair snares produced 37 total samples with 10 classified as rodent (flying squirrel or unidentified mice), 17 red squirrels, and 8 possible martens.

Discussion

The loss of alleles and decreased heterozygosity at sampled loci (Table 1) suggested the genetic diversity in the two populations has decreased since previous genetic evaluations (Bicker 2007). Interestingly, the MNF population founded with a smaller number of individuals had not experienced a bottleneck effect as severe as the larger PRSF population. This could be due to a greater amount of habitat fragmentation in the PRSF area and a smaller reintroduction area (Nelson 2006). The fact that we caught mostly relatives within each site, but not between sites suggests that dispersal may be limited between the Ward Hills and Caberfae populations. Relatedness could explain why

martens with IDs 6 and 10, two males, were captured in the same trap and potentially share all or a portion of their home ranges, which is uncharacteristic of usual marten behavior (Clark et al. 1987). All of these observances in behavior could also be representative of phylopatric behavior, meaning that the two populations have become more or less two family groups that experience little dispersion between them.

When trying to clarify if related martens are more likely to be parent-offspring or full-siblings a type II error of 73% is calculated for the results. This indicates that we had low power and would likely miss 73% of these relationships given our small sample size.

Four of the loci I screened during this study need to be further analyzed for their usefulness in additional studies. Microsatellite locus Ma-8 failed to amplify with multiple attempts so there may be an issue with using a tailed protocol which could alter the dynamics of PCR causing amplification to fail. It could be resolved by directly labeling these loci, rather than using a tailed protocol. Microsatellite locus Ma-9 should be included in further analysis on marten in the MNF despite being monomorphic in this study. A larger sample size may result in limited polymorphism at this locus or reveal whether the locus is truly monomorphic where it was previously polymorphic, which would suggest a loss in diversity. The alleles at locus Ma-10 did not consistently bin, did not amplify for two of the eleven samples, and therefore may not be a worthwhile addition to future studies. Locus Ma-18 was used successfully on marten from the same geographic area by increasing the annealing temperature (Tann) to 58 C to decrease the amount of secondary amplification at that locus (Bicker 2007). We only increased the Tann to 52 C for samples at locus Ma-18 which still produced too much secondary amplification. In addition to experimenting with a touch-down protocol for locus Ma-18, other loci included in Appendix I should be screened to increase the number of alleles genotyped for each individual and increase the ability to determine genetic relatedness among individual marten.

DNA extraction from known marten hair samples was not thoroughly explored during this study. Only four samples were

analyzed based on being the largest so they would not be exhausted during the screening process. The protocol used for the hair samples needs to be more thoroughly tested by making modifications to components within the master mix reagent and attempting a touch-down protocol to determine the most effective means for extraction and amplification of even the smallest hair sample acquired.

In our study, marten were caught in May even though trapping is not usually attempted or successful during the summer months (Mowat and Paetkau 2002; Nelson 2006). The hair snare design we used was successful in a study by Mowat and Paetkau (2002) during drier winter months but not during the summer months. The low number of marten hair samples acquired in locations where martens were previously captured may suggest that the use of snares to gather data on martens during the summer season is not effective. The increase in their prey selection during these months may prevent them from seeking food elsewhere as in winter months. Since our study was conducted in more humid summer months that experienced greater rainfall than usual, hair snares may have worked better with a few modifications.

One modification we suggest is the addition of water sealant before deployment to prevent the cardboard backing on the glue patches from becoming soaked. A better hair snare to use in the summer months may be found by experimenting with the design used by Pauli et al. (2008), which is made of plastic, substitutes the use of the glue patches for a bristled brush, and has a door that prevents multiple visits to the same snare between checks. These snares should also be checked at a higher frequency to refresh bait and re-set them if they have been visited and are found closed. Experiments with additional snare designs should be attempted during the summer months before the use of snares for non-invasive data collection should be abandoned.

The bait, salted pork, used in the hair snares was not effective for use in the wettest or hottest part of the summer field season. The roof may have prevented rain from falling into the snares. However, the rain soaked into the wood and absorbed into the cardboard backing on the glue

patches, causing mold to grow. The salted pork would mold rather quickly in the wettest weather and reached its melting point during episodes of high temperatures causing it to liquefy and run down the tree on which the snare was mounted. I would recommend bait with less fat such as canned tuna, chicken, beef or beaver to prevent loss of hair samples due to the absence of bait in the snare between visits.

Continued investigation of marten in the MNF would potentially increase the sample size in this study, which would increase our statistical power and produce more accurate calculations on population structure and health. Martens could benefit from management plans that attempts to develop and preserve existing corridors that connect Ward Hills and Caberfae, two areas of high quality habitat. The FST value of 0.141 could display a bias toward higher levels of relatedness within the two populations than between them. Translocation of some of the members in each population could help overcome phylopatric behavior exhibited by marten, lack of dispersal, and enable gene flow, increasing genetic diversity within both populations. However, further investigation of the fragmentation between the two populations in social structure, home range use, habitat selection, genetic variation, and population demographics need to be conducted to understand the best care and management guidelines for marten in the Manistee National Forest.

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