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Genetic Study of Recent Samples of American Marten (Martes americana) from Vermont

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Abstract

American marten *(Martes americana)* was listed as endangered in Vermont in 1987 due to an absence of detection since 1954. Between 1989 and 1991, marten from Maine were reintroduced into southwestern Vermont but studies deemed the reintroduction unsuccessful. However, since 1998 marten have been detected in northeastern Vermont and are thought to represent colonization from a northern New Hampshire population. As of 2010 marten have also been detected in southwestern Vermont. The objective of this study was to provide insight into the source of the recently discovered marten population in southwestern Vermont by testing three hypotheses: (1) the northern New Hampshire population as the source of the northeastern Vermont population; (2) the southwestern Vermont population being derived by long distance dispersal from northeastern Vermont and/or northern New Hampshire populations; and (3) the southwestern Vermont population being remnants of the reintroduction. Three microsatellite loci were compared among 12 marten samples from northeastern Vermont, 3 samples from southwestern Vermont, and 12 samples from northern New Hampshire. No significant genetic differentiation existed between the populations and no samples from northern Vermont could be excluded as members of the northern New Hampshire source population, therefore the first hypothesis could not be rejected. There was evidence of a founder effect (lower genetic variation and loss of rare alleles) shown by the lower effective number of alleles for both the southern Vermont population (3.024) and northern Vermont population (3.512) as compared to the New Hampshire population (4.169), along with the presence of heterozygosity excess for both Vermont populations. There was also evidence of migration and assignment of samples from southern Vermont to the northern Vermont and New Hampshire source populations, thus the second hypothesis could not be rejected. Similar findings (lower genetic variation and loss of rare alleles) could also result from a recent bottleneck due to the reintroduction program. The third hypothesis, therefore, could not be rejected and additional supporting evidence was exhibited by a presence of different alleles in the southwestern Vermont population. Comparing the second and third hypotheses, a found effect is more likely than a bottleneck due to the inability to exclude northern Vermont and/or northern New Hampshire as a source of the southern Vermont population.

Introduction

Rare species exist in small and isolated populations (Lammi et al. 1999), which are more prone to extinction due to the loss of genetic variation (Frankham 1996). Peripheral populations share several characteristics, they are small, isolated, and occur in ecologically marginal habitats (Lawton 1993, Hoffman and Blows 1994, Lesica and Allendorf 1995). Thus peripheral populations of rare species are in a vulnerable position and an important focus for conservation. The value of conservation for peripheral populations has been questioned, however a study on plants by Lammi et al. (1999) found no significant differences in the measured fitness components between peripheral and central populations, which emphasizes their potential value for conservation. Genetic diversity, however, is expected to

decrease in small and isolated populations (peripheral populations and/or rare species) due to the effects of bottlenecks, founder events, inbreeding, and genetic drift (Lammi et al. 1999). American marten (Martes americana) are endangered in Vermont (Fuller 1987), consisting of small populations and the recently discovered marten in southwestern Vermont are likely a small peripheral population. The factors leading to the likely genetic diversity decrease and potential value for conservation of this peripheral population of an already rare species in Vermont makes it an important area of study.

American marten are carnivorous mammals in the family Mustelidae. They are primarily solitary and nocturnal, and are found in mature, northern forests. They have been known to swim across a lake or stream (Mech and Rogers 1977), making dispersal across water possible. Populations are regulated largely by food availability throughout the year and the home range of a male (2 to 3 km^2 using the minimum-area method or 10 to 20 km^2 using radiotelemetry) is two to three times that of a female (1 km^2 using the minimum-area method or 3 to 6 km^2 using radiotelemetry) (Clark et al. 1987). Marten populations are also shown to be dynamic, displayed from studies in Montana and Wyoming where about half of the population were residents, while the other half was made up of temporary residents and transient individuals (Clark et al. 1987).

American marten range stretches from Newfoundland and Nova Scotia west to Alaska and south into the Rocky Mountains and California (Clark et al. 1987). They are found sporadically throughout northern New England and other northern states (Clark et al. 1987). American marten have an inconsistent relationship with settlement in Vermont. At one time marten were thought to have been abundant throughout much of Vermont, but by 1850 they had decreased in numbers and were restricted to the mountainous areas of the state (Thompson 1853). Marten numbers declined between 1850 and the early 1900's due to habitat destruction and unregulated harvesting. Only four records in Vermont were known prior to American marten being listed as endangered in Vermont in 1987 (Fuller 1987). The four records include specimens from Chittenden in Rutland Co. (Kirk 1916), Glastonbury in Bennington Co. (Kirk 1916), and Stratton Mountain (Osgood 1938) and Hogback Mountain (Fuller 1987) both in Windham Co.

A reintroduction program for American marten into Vermont occurred between 1989 and 1991 when 104 martens from Maine and 11 martens from New York (Moruzzi et al. 2003) were released into the Green Mountain National Forest (GMNF). Areas of release in the GMNF included East Wallingford and Mount Tabor in Rutland Co. and Stratton and Sunderland in Bennington Co. (DiStefano et al. 1990, Royar 1990, 1992). Moruzzi et al. (2003) provides details of the monitoring of

the introduced martens for a ten year time span from the time of release, 1989 until 1998. By 1991 marten had begin to disperse and several were trapped or killed on roads, including a tagged marten from New York released into the GMNF and trapped in Rangely, Maine in 1997 (Moruzzi et al. 2003). Although martens were photographed in 1996 in the areas where they were released, by 1998 no additional martens were photographed in the southern GMNF and 85% of the sets produced pictures of fishers (M. pennanti) (Moruzzi et al. 2003). However, an unmarked marten was trapped in Barton, Vermont, in the Northern Highlands in 1997 (Bernier pers. comm.). Assessments by Trombulak and Royar (2001) and Moruzzi et al. (2003) of the marten reintroduction in the southern GMNF have concluded that a viable population of martens was not reestablished in southern Vermont.

Since 1997 there has been evidence of the presence of marten in the Northern Highlands of Vermont, including a number of tracks and sightings reported from Orleans, Essex, and Caledonia Counties as well as marten incidentally taken during the fisher trapping season. In 2003 and 2004 sightings of marten in northeastern Vermont were made within 12 km of New Hampshire and therefore, are hypothesized to represent recent colonization from marten populations in northern New Hampshire (Kelly 2005). These marten populations in northern New Hampshire were documented and studied by Kelly (2005), Kelly et al. (2009) and Siren (2013).

Evidence for a recently discovered population in southwestern Vermont appeared in 2010 when two martens were incidentally taken during fisher trapping season in Bennington County (Bernier pers. comm.). In 2011, camera traps detected two additional marten and in 2012 an additional specimen was incidentally trapped (Bernier pers. comm.). Cameras failed to verify the presence of martens in the winter of 2013 though tracks were observed and fisher trappers volunteered to avoid trapping areas where they saw marten tracks. The source of this recently discovered southern Vermont population is unknown. This population in southern Vermont might be the results of the reintroduction that occurred between 1989 and 1991 that has been considered unsuccessful in establishing a viable marten population or the results of long distance dispersal from a neighboring population.

The objective of this study was to use microsatellite genetic markers to compare samples from southern Vermont with samples from potential source populations (northern New Hampshire and northern Vermont) in order to determine the source of the marten population in southern Vermont. At the start of this study the methods included other potential source populations of Maine and the Adirondacks of New York, which I was unable to accomplish due to the delay in obtaining samples. The findings were used to test three different hypotheses. The first hypothesis considered was that the

northern New Hampshire population was the source of the northern Vermont population as hypothesized by Kelly (2005) and Kelly et al. (2009). If this hypothesis was true, then little genetic differentiation was expected between these two populations and population assignment tests would not exclude samples from northern Vermont as members of the northern New Hampshire source population. If rejected, alternative hypotheses for the source of the northern Vermont population could be a source population in the Adirondacks of New York or dispersing individuals from the southern GMNF reintroduction.

The second hypothesis was that the population in southern Vermont was derived by long distance dispersal from either the population in northern Vermont and/or the population in northern New Hampshire. If this hypothesis was true, then evidence of a founder effect (lower genetic variation and loss of rare alleles) was expected as well as assignment of samples from southwestern Vermont to these source populations. The third hypothesis was that the population in southern Vermont was a remnant of the reintroduction. If this hypothesis was true, then evidence of a bottleneck (reduced genetic variation and loss of rare alleles), the presence of different alleles (alternative source population), and the exclusion of northern Vermont and/or northern New Hampshire as a source of the southern Vermont population were expected. For the third hypothesis, samples from the Maine and New York source populations were not available, therefore a direct comparison between the sources of the reintroduction (Maine and New York) and the southern Vermont population was not possible at this time.

Methods

DNA collection

Northeastern Vermont, southwestern Vermont and northern New Hampshire are defined as the three American marten geographical populations examined in this study (Fig. 1). Fifteen marten samples from Vermont (12 from northeastern and three from southwestern) incidentally captured during the fisher trapping seasons were obtained from the Vermont Department of Fish and Wildlife, and 12 samples from northern New Hampshire were obtained from a study by Siren (2013). Samples (liver or muscle tissue) were preserved in 100% ethanol or frozen.

DNA extraction and sizing

DNA was extracted from samples using the Gentra Mouse Tail kit (Qiagen), first by grinding in liquid nitrogen and following the protocol provided by the manufacturer with the exception that the DNA was rehydrated in sterile water. The DNA concentration was determined by spectrophotometry using the NanoDrop ND-1000 Spectrophotometer (NanoDrop®). Three microsatellite loci (MA-1, MA-8, and MA19) detected in American marten by Davis and Strobeck (1998) were examined using fluorescent tagged reverse primers (Table 1) following the protocols provided by Kyle et al. (2000), Kyle and Strobeck (2003) and Swanson et al. (2006). Each polymerase chain reaction (PCR) contained 0.4 µl of the forward and reverse primer for a locus, 1.2 µl of 2.5 mM nucleotide triphosphates (dNTPs), 2.5 µl of 10X ThermoPol Reaction Buffer, 0.2 µl of 5,000 U/ml *Taq* DNA polymerase (New England BioLabs, #M0267S), 100 ng/µl of DNA, and sterile H_2O to bring the reaction to 25 µl. Amplifications were conducted in a GeneAmp PCR system 9600 (Applied Biosystems) with a hold at 94°C for 1 minute, three cycles at 94°C for 30 seconds, 54°C for 20 seconds, and 72°C for 5 seconds, 33 cycles at 94°C for 15 seconds, 54°C for 20 seconds, and 72°C for 1 second, and a final hold at 72°C for 30 seconds. Products of the MA-1 and MA-19 loci were diluted 1:10 and 1 µl of the PCR product (Ma-8) or diluted PCR product was combined with 10 µl of formamide and 0.3 µl of size marker LIZ 500 in a 96 well plate, before being sent to the Vermont Cancer Center DNA Analysis Core Facility for sizing by capillary electrophoresis. Data were received back electronically and the microsatellite fragments were visualized and sized with GeneMapper Software 5 (Applied Biosystems) and scored as multilocus genotypes in a spread sheet.

Data analysis

The analysis involved several computer programs to test and compare genetic data. The allele frequencies in each population were determined and alleles shared between populations and those unique to a population were graphed with GenAlEx (Peakall and Smouse 2006). The genetic variation in each population was assessed by indices including observed number of alleles, effective number of alleles (corrects bias due to sample size), observed heterozygosity, and unbiased expected heterozygosity (corrects bias due to sample size) (Nei and Roychoudhury 1974) using GenAlEx. Departure from the expectations of Castle-Hardy-Weinberg equilibrium for all three populations at the three loci and across loci and populations was tested using exact probability method of Guo and Thompson (1992), and the test for linkage disequilibrium were implemented in the program

GENEPOP (Raymond and Rousset 1995, Rousset 2008). Genetic differentiation among the three pre-defined subpopulations was tested with pair-wise F_{ST} using the "drift model," whereby drift is assumed to be the only force operating and all other forces affecting gene frequencies are excluded (Reynolds et al. 1983) and Slatkin's distance (Slatkin 1995) in the program Arlequin (Schneider et al. 2000, Excoffier and Lischer 2010). A significant reduction in the M ratio, the number of alleles compared to the size range between the largest and smallest microsatellite allele at a locus for each population, was examined using M_P_Val.exe (Garza and Williamson 2001). Population assignment tests were conducted using GeneClass 2 (Piry et al. 2004). Assignment and exclusion of individuals to populations and the detection of first generation migrants provide the probability of an individual being a member of a geographic population. Individuals collected from a geographic population that were statistically excluded as members of that population were identified as likely migrants. Detection of first generation migrants provides an estimate of the likelihood that each individual identified as a likely migrant was derived from each of the geographic populations sampled. A separate analysis relating the New Hampshire and northern Vermont source populations to the southern Vermont population was conducted in GeneClass 2 to determine the most likely source of each of the three individuals sampled from southern Vermont. All population assignment used the Bayesian assignment method of Rannala and Mountain (1997) combined with a simulation algorithm of Paetkau et al. (2004) for resampling with an allowed level of type I errors of 0.05.

Results

Of the 15 samples collected from Vermont geographical populations, 3 were female and 12 were male, resulting in large male bias. Test for departure from Castle-Hardy-Weinberg equilibrium found no significant deviations. Test for linkage disequilibrium found no significant deviation from linkage equilibrium. Estimates of genetic differentiation among populations found that pair-wise F_{ST} values were not significantly different from zero, therefore no significant genetic differentiation between populations was detected. The M ratio found no significant reductions indicative of a recent bottleneck.

Allelic Frequency

At locus MA-1, alleles were shared among all three populations and frequencies were similar (Fig. 2). The frequency of allele 214 was higher in Vermont populations, while the frequency of allele 216 was lower in Vermont populations compared to the northern New Hampshire population (Fig. 2). The MA-8 locus exhibited more diversity in the frequency of alleles (Fig. 3), which is found in other studies on marten (Broquet et al. 2006) as well. Alleles 101 and 103 were found only in northern Vermont. Allele 109 was not detected in northern Vermont, found in New Hampshire at a very low frequency but occurred in southern Vermont at a relatively high frequency (Fig. 3). At the MA-19 locus, the 206 allele was detected only in the southern Vermont population (Fig. 4).

Population pair-wise comparisons of the number of alleles shared and different across all loci showed a considerably lower number of differences between northern Vermont and New Hampshire populations and a large number of shared alleles (Table 2). The southern Vermont population shared about the same number of alleles with either the northern Vermont or the northern New Hampshire populations (Table 2).

Genetic Variation

The southern Vermont population displayed a reduction in the number of alleles compared to northern Vermont and New Hampshire populations at two of the three loci examined. Only three alleles were observed in the southern Vermont population at the MA-8 and MA-19 loci as compared with four to seven alleles in the northern Vermont and northern New Hampshire populations (Fig. 3 and Fig. 4). There was a lower effective number of alleles for northern Vermont (3.512) and for southern Vermont (3.024) as compared to the New Hampshire population (4.169) (Table 3).

Similar levels of variation, determined by unbiased expected heterozygosity, was found in the northern Vermont population (0.744), the southern Vermont population (0.756), and the New Hampshire population (0.790) (Table 3). However, the observed heterozygosity (Table 3) was higher than the expected heterozygosity resulting in heterozygosity excess (characteristic of a recent bottleneck or founding event) for both northern Vermont (0.851) and southern Vermont (0.778) populations but not in the New Hampshire population (0.778).

Population Assignment Tests

Individual 34109 captured in northern Vermont could be excluded from being derived from either the northern Vermont population (P = 0.018) or the northern New Hampshire population (P = 0.039) (Table 4). Although the probability of being derived from the southern Vermont geographical population was rather low (0.146), this population could not be rejected as a potential source. Individual MINC 2013 J captured in northern New Hampshire was excluded as being a member of the northern New Hampshire population (P = 0.01) and was likely a migrant. Although this individual had a considerably higher probability of being derived from the southern Vermont population (P = 0.680), it could not be excluded from being derived from northern Vermont (Table 4). Individual 34012 captured in southern Vermont was identified as a likely migrant from the northern New Hampshire population (P = 0.607). Similar results were obtained in GeneClass 2 for these three individuals when they were tested as likely being first generation migrants (Table 5).

None of the individuals sampled from southern Vermont could be excluded from being derived from either northern Vermont or northern New Hampshire populations (Table 6). Individual 34102 had the greatest probability (P = 0.618) of being derived from the northern New Hampshire geographical population and individual 34111 the greatest probability (P = 0.986) of being derived from the northern Vermont geographical population (Table 6). Although individual 34100 could not be excluded as being derived from the northern New Hampshire geographical population (P = 0.191), the probability of being from either of the source populations was considerable lower than the other two individuals sampled.

Discussion

Kelly (2005), Kelly et al. (2009), and Swanson et al. 2006) present a long history of extirpation due to habitat destruction and fragmentation and then recolonization or reintroduction from source populations from areas throughout the range of American marten. In 1985, the marten population in western Maine was large (6.1 ind/100 km^2) (W. Jakubas, pers. commun.) and likely served as the primary source for recolonization of the northern New Hampshire population (Kelly et al. 2009). Kelly et al. (2009) suggested that populations in New Hampshire have immigration and dispersal from adjacent populations occurring. This dispersal within New Hampshire populations appeared to be spreading into Vermont as well. Kelly (2005) presented documentation of marten occurrence from 2003 and 2004 in northeastern Vermont (K. Royar, VT Fish and Wildlife Department, pers. commun.)

from sightings made within 12 km of New Hampshire. This northeastern Vermont marten population is likely be the result of individuals dispersing from northern New Hampshire (Kelly 2005).

The hypothesis suggested by Kelly (2005) that the northern New Hampshire population of marten was the source of martens that established the northern Vermont population was examined by comparing samples from those two populations. If this hypothesis was correct, little genetic differentiation would be expected between samples from these two populations and population assignment tests would not exclude samples from northern Vermont as members of the northern New Hampshire source population. The F_{ST} test showed no significant genetic differentiation between populations in northern Vermont and northern New Hampshire. However, American marten could have low genetic differentiation among populations throughout New England, and possibly other areas as well, due to constant movement resulting from extirpations, relocations, and recolonizations.

Studies have found that geographic distance between marten populations does not effect genetic differentiation. Kyle et al. (2000) found that marten populations sampled from the Yukon and Northwest Territories had very little genetic differentiation between them suggesting extensive gene flow across the entire region. They found that even large mountain ranges have little effect on gene flow between marten populations (Kyle et al. 2000). In addition Koen et al. (2012) found no independent support for isolation by increased landscape resistance in Ontario and Broquet et al. (2006) found only a weak correlation between genetic distance and geographic distance for marten in Ontario. Wasserman et al. (2010) found relatively strong patterns of genetic differentiation as a function of elevation in the Rocky Mountains, but those were independent of geographical distance.

Three individuals, one collected from each of the three geographic populations sampled, were excluded as being members of the population from which they were sampled (Table 4) and were considered to be migrants. One individual, 34102 was likely an emigrant from the northern New Hampshire population into the southern Vermont population (Tables 4-6) lending support to long distance dispersal. The other two individuals identified as likely being migrants (Table 5), 34109 and MINC 2013J from northern Vermont and northern New Hampshire, respectively, both showed the greatest likelihood as being derived from the southern Vermont sample. The likelihood values showing these other two migrants as being most likely derived from southern Vermont may result from the lack of genetic differentiation found among the three populations and the small number of loci examined.

The hypothesis of the northern New Hampshire population as the source of the northern Vermont population could not be rejected by the the F_{ST} test, the supporting previous work, and other

supportive results. Results such as the inability of population assignment tests to exclude samples from northern Vermont as being members of the northern New Hampshire source population, along with the presence of characteristics that support a recent founding (high amount of shared alleles (Table 2), reduced effective number of alleles, and observed heterozygosity excess (Table 3)). Heterozygosity excess results from greater than expected observed heterozygosity, which occurs when genetic drift eliminates low frequency alleles. The elimination of low frequency alleles has little influence on heterozygosity when compared to allelic diversity, producing the heterozygote excess that is expected from a founder event (Garza and Williamson 2001).

The second hypothesis, that the southern Vermont population was derived by long- distance dispersal of the northern Vermont and/or northern New Hampshire populations, if true, would expect to show evidence of a founder effect with low genetic variation and the absence of rare alleles. There was a reduction in the number of alleles and the effective number of alleles (Table 3) for the southern Vermont population compared to the New Hampshire population. Observed heterozygosity was greater than unbiased expected heterozygosity (Table 3) as expected from a founder effect. Allelic diversification, increase in the frequency of an allele compared to the source populations, was also observed in the southern Vermont population (Fig. 2 and 3) and is characteristic of a founder event.

The sources of the three individuals sampled from southwestern Vermont was examined by population assignment test. Individual 34102 was likely an emigrant from the northern New Hampshire population (Tables 4 & 6) and the other two individuals, 34100 and 3411, could not be excluded as being derived from populations in northern Vermont or New Hampshire (Table 6).

The majority of the samples from Vermont geographical populations were males. Male martens have a home range about twice the size of females (Clark et al. 1987) and they are more easily trapped than are females. Thus, a male bias may be common in the sampling of martens because males are more easily lured and have a larger home range, making it easier for them to be captured, incidentally trapped, or killed on the road just due to the larger space they occupy. However, this bias is very high (12/15), which would be consistent with male biased dispersal and reports that males have larger home range and therefore larger dispersal distance (Clark et al. 1987), supporting a recently founded population by dispersing individuals. Mustelids, most notably wolverines (*G. gulo*) have large home ranges and large dispersal distances (Banci 1987). Similar to marten, wolverines have low genetic distance no matter the amount of geographic distance (Kyle and Strobeck 2003). All of these findings are consistent with a founder effect resulting from long distance dispersal, and the hypothesis that

southern Vermont population being derived from the northern Vermont and northern New Hampshire population could not be rejected.

The third hypothesis proposed that the individuals in the population in southern Vermont were remnants of the reintroduction that occurred between 1889 and 1991 but then went through a major bottleneck where the population was undetected from 1997 to 2010. If this hypothesis was true, evidence of a recent bottleneck including reduced genetic variation and a presence of different alleles (from the sources used in the reintroduction but not sampled in this study) were expected. The results showed evidence of a bottleneck such as reduced allelic variation and heterozygosity excess, however the M ratio test (Garza and Williamson 2001) failed to find a significant signal of a recent bottleneck. The presence of a unique allele in the southern Vermont population (Fig. 4), however is evidence of colonization from an alternative source. Further research including samples from other source populations (Maine and New York) would provide insight into the source of this unique allele.

The source populations for the 1989-1991 reintroduction (Maine and New York) were not sampled in this study, resulting in the inability to directly compare alleles between the source populations and the southern Vermont population. The inability to exclude northern Vermont and/or northern New Hampshire as a source of the southern Vermont population does not support reintroduction, however a bias exists in the small number of loci examined. Also the lack of presence of a significant reduction in population size from the M ratio test could be possible evidence against reintroduction since strong evidence of a recent bottleneck would be expected. Williams and Scribner's (2010) study on American marten reintroductions found evidence for the effects of drift following a bottleneck of the initial founding event, however no bottleneck was detected as would be expected. Luikart and Cornuet (1998) state that it is difficult to detect a recent bottleneck in a population because historical population sizes and levels of genetic variation are rarely known. Their study (1998) examining recent bottlenecks used the sign test of heterozygosity excess (Cornuet and Luikart 1996) detected a little over half of recently bottlenecked natural populations. The populations detected were severe and/or recent and examined a large number (10-20) of polymorphic loci (Luikart and Cornuet 1998). The lack of detection of a recent bottleneck does not reject the reintroduction as a source of the southern Vermont population because a small number of microsatellites were examined, which along with other factors, is supported by previous studies as a hinderance to the ability to detect a recent bottleneck. Thus, the hypothesis of a recent bottleneck can not be rejected due to inconclusive results (from the small number of loci and absence of source population samples) and the similar effects

produced by a founder effect and a bottleneck, however the results are not in support of the reintroduction hypothesis.

Conclusions

Northern New Hampshire as the source of the northeastern Vermont population could not be rejected by either the F_{ST} values nor the inability of population assignment tests to exclude samples from northern Vermont as being members of the northern New Hampshire source population. These two populations share a large number of alleles. Evidence that the northern Vermont population may have experienced a recent founding event includes both lower genetic variation (reduced effective number of alleles) and observed heterozygosity excess. Neither the second (founder effect resulting from long distance dispersal) nor third (a bottleneck resulting from a population crash associated with what is thought to have been a failed reintroduction) hypotheses could be rejected due to the similarity of the expectations of a founder event and a bottleneck (lower genetic variation and loss of rare alleles). Evidence of the effects of a bottleneck or a founder event was shown by the lower effective number of alleles when compared to the New Hampshire population, the presence of heterozygosity excess, and allelic diversification in the southern Vermont population. Supporting evidence for the founder effect included the presence of migration and the assignment of samples from southern Vermont to the northern Vermont and New Hampshire source populations, which does not support a bottleneck from the reintroduction. Additional support for the founder effect was found in the high male bias, which was consistent with a recently founded population by dispersing individuals since males have a greater likelihood of undergoing long distance dispersal due to their greater dispersal distances. In comparing the founder effect by dispersal and a bottleneck from the reintroduction hypotheses, without the samples from the reintroduction source population, the founder effect hypothesis is better supported in this study.

The results of this study are bias due to the small number of microsatellite loci examined, small sample size (result of the small southern Vermont population) and lack of samples from Maine and New York (source of reintroduction). Further research needs to incorporate the Maine source population samples, along with the Adirondacks of New York source samples (another possible source for the population), especially since the presence of a unique allele in the southern Vermont population is indicative that the southern Vermont population was founded at least in part from a source not

currently included in the samples. Additional American marten microsatellite loci (Davis and Strobeck 1998) also needs to be incorporated into further research. This further research will hopefully provide insight into the population dynamics of American marten, and allow differentiation between the opposing hypotheses for the origin of the southwestern Vermont population of either long distance dispersal or a reintroduction where martens persist for decades without detection.

The study of this southern Vermont peripheral population of marten either established by a founder event or a bottleneck will not only provide insight into marten population dynamics, but also provide data from a peripheral population of a rare species in that area. Genetic diversity of the small, isolated peripheral population was expected to decrease (Lammi et al. 1999), which findings show did happen. However as previous studies note, there are no differences observed in the measured fitness components between peripheral and central populations (Lammi et al. 1993). Thus studying the southern Vermont peripheral population of American marten, which are endangered in Vermont (Fuller 1987), will provide invaluable insight into the stability of peripheral populations of rare species with various conservation efforts.

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Figure 1: Map of Vermont and New Hampshire with circles representing the roughly estimated areas of the geographical populations. The thin circle represents the southern Vermont marten population, the thick circle represents the northern Vermont population, and the dotted circle represents the northern New Hampshire population.

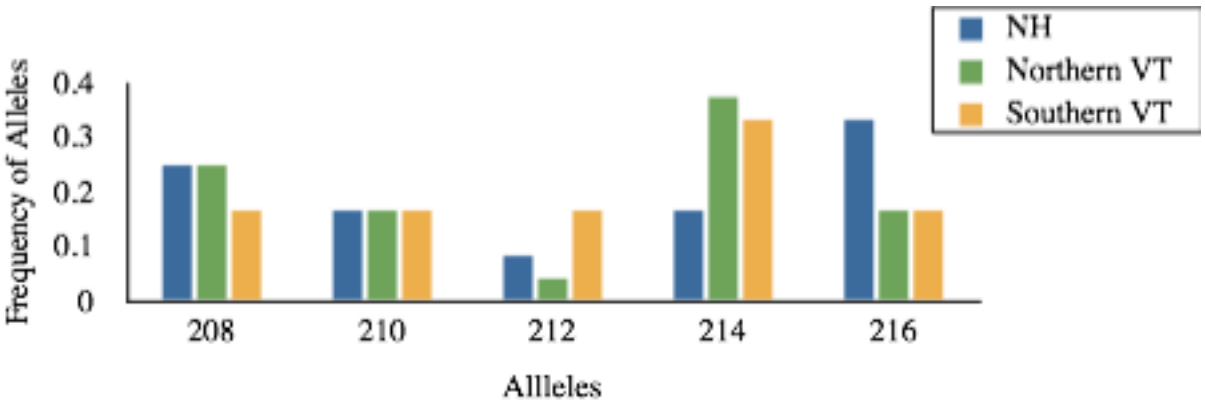

FIGURE 2. Frequency of alleles in each population at the MA-1 locus.

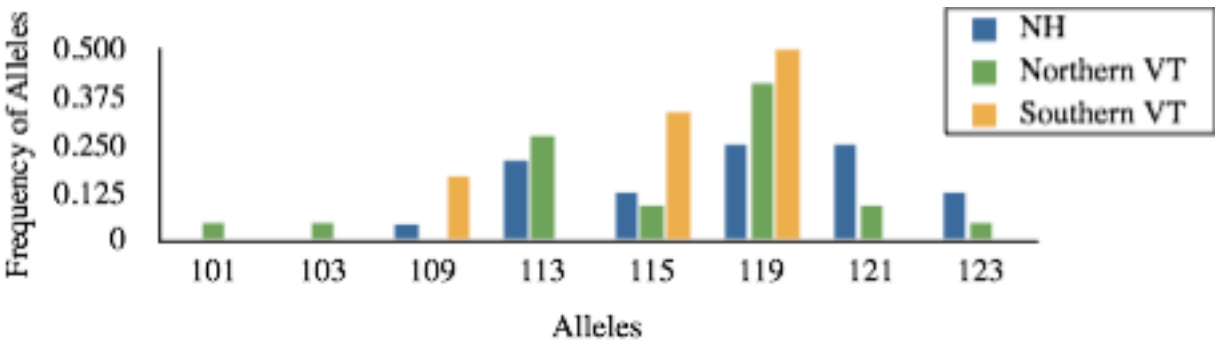

FIGURE 3. Frequency of alleles in each population at the MA-8 locus.

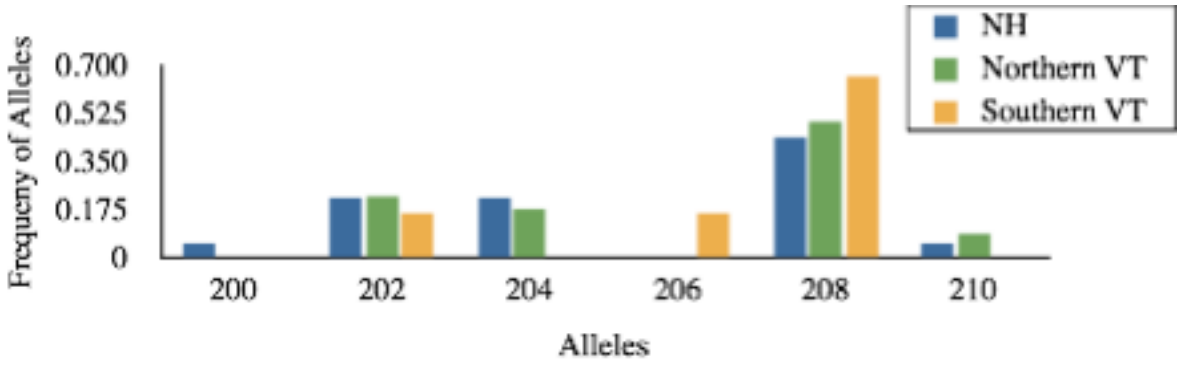

FIGURE 4. Frequency of alleles in each population at the MA-19 locus.

TABLE 1. Forward (F) and reverse (R) primer sequences with a concentration 10.0 μM and amount of 0.4 μl added to PCR for each locus.

Locus	Primer Sequences
MA-1	F:ATTTTATGTGCCTGGGTCTA
	R: TTATGCGTCTCTGTTTGTCA
MA-8	F: GTTTTCTAATGTTTCGTGTG
	R: CAGTGGTTGACTACAAGAAA
MA-19	F: AAGGCTTATGGATACCACAT
	R: GATCATTTGGTATTTGTCTTTC

TABLE 2. Population pair-wise comparisons of number of alleles shared above the diagonal and number of alleles different below the diagonal using the program GenAlEx (Peakall and Smouse 2006).

Pop	Northern VT	Southern VT	NH
Northern VT	-	9	14
Southern VT	9	-	10
NH	4	7	-

TABLE 3. Genetic variation analyses with observed heterozygosity, unbiased expected heterozygosity (corrects for bias due to sample size), number of alleles, and number of effective alleles (corrects for bias due to sample size), using the program GenAlEx (Peakall and Smouse 2006).

Pop	Observed heterozygosity	Unbiased expected heterozygosity	Number of alleles	Number of effective alleles
Northern VT	0.851	0.744	5.333	3.512
Southern VT	0.778	0.756	3.667	3.024
NH	0.778	0.790	5.333	4.169

TABLE 4. GeneClass 2 (Piry et al. 2004) exclusion test where values represent the probability of an individual being derived from a geographical population based on its multilocus genotype. Only individuals statistically excluded as members of the geographic populations they were collected are included. The Bayesian methods of assignment (Rannala and Mountain 1997) combined with the resampling simulation algorithm of Paetkau et al. (2004) were used.

		Northern VT	Southern VT	NH
ID	Home Population	Probability	Probability	Probability
34109	Northern VT	0.018*	0.146	0.039*
MINC 2013 J [M]	NH	0.061	0.680	0.01**
34102	Southern VT	0.092	>0.001**	0.607

[M] = MA-19 locus not resolved in analysis

TABLE 5. GeneClass 2 (Piry et al. 2004) detection of first generation migrants with the likelihood values identifying the population from which each migrant was likely derived. Only individuals statistically excluded as members of the geographic populations they were collected are included. The Bayesian methods of assignment (Rannala and Mountain 1997) with the resampling simulation algorithm of Paetkau et al. (2004) were used.

ID	Home Population	Probability	Likelihood of Origin		
			Northern VT	Southern VT	Northern NH
34109	Northern VT	0.019*	-5.676	-4.694	-6.12
MINC 2013J	Northern NH	0.009**	-3.941	-2.094	-4.447
34102	Southern VT	0.001**	-4.962	-6.056	-3.688

TABLE 6. GeneClass 2 (Piry et al. 2004) detection of source populations and exclusions test with the three southern Vermont samples showing the geographical population from which they were most probably derived, with the highest probability bolded. Settings used were the Bayesian methods of assignment (Rannala and Mountain 1997) and the simulation algorithm of Paetkau et al. (2004).

	Northern VT	NH	Northern VT and NH combined
ID	Probability	Probability	Probability
34100	0.121	**0.191**	0.124
34102	0.089	**0.618**	0.395
34111	**0.986**	0.914	0.980

APPENDIX 1. Alleles found for each individual at the three American marten microsatellite loci in the three geographical populations.

ID	Pop	Ma 1		Ma 8		Ma 19	
25679	No VT	210	210	113	119	202	208
28783	No VT	214	216	113	119	204	208
34105	No VT	214	216	115	119	202	208
34106	No VT	208	210	119	121	0	0
34107	No VT	208	216	113	113	202	204
34108	No VT	208	214	119	119	208	210
34109	No VT	208	214	101	103	208	208
34110	No VT	214	216	113	123	204	208
34112	No VT	212	214	113	119	208	208
34148	No VT	208	214	115	119	204	208
34150	No VT	208	214	119	121	202	210
6224	No VT	210	214	0	0	202	208
34100	So VT	212	214	115	119	206	208
34102	So VT	210	216	109	115	202	208
34111	So VT	208	214	119	119	208	208
F8	No NH	210	216	113	119	200	208
F16	No NH	210	211	113	121	204	204
M13	No NH	208	216	123	123	202	202
F2	No NH	208	214	113	119	204	208
MINC 2013 A	No NH	216	216	113	121	0	0
MINC 2013 B	No NH	214	216	119	123	208	210
MINC 2013 C	No NH	214	216	119	121	202	208
MINC 2013 D	No NH	208	212	115	119	0	0
MINC 2013 E	No NH	208	208	113	121	202	208
MINC 2013 F	No NH	210	216	115	119	208	208
MINC 2013 G	No NH	208	216	121	121	204	208
MINC 2013 J	No NH	210	214	109	115	0	0