Estimating marten Martes americana population size using hair capture and genetic tagging

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Estimating marten *Martes americana* population size using hair capture and genetic tagging

Garth Mowat & David Paetkau


We tested non-invasive genetic methods for estimating the abundance of marten *Martes americana* using baited glue-patch traps to pull hair samples from individual animals. We divided our 800-km² study area into 3 × 3 km cells and put one hair trap in each cell. We trapped 309 sites for an average of 15 days each between 15 January and 14 March 1997. Based on tracks in snow and hair morphology, we captured hair from marten, red squirrels *Tamiasciurus hudsonicus*, flying squirrels *Glaucomys sabrinus*, short or long-tailed weasels *Mustela erminea* and *M. frenata*, and several unidentified mouse and vole species. Of 309 sites, 58% collected a marten hair sample while 8% of sites removed weasel hair. When roots were embedded in adhesive, a xylene wash was used to remove them before extracting DNA. All marten samples were genotyped at six microsatellite loci to identify individuals. Xylene-washed samples yielded similar genotyping success to samples that had never been exposed to xylene, and genotyping success increased with the number of hairs in the sample. Genetic data allowed 139 samples to be assigned to 88 individual martens, constituting 124 capture events during the four trapping sessions. The population estimate for our study area was 213 (95% CI: 148-348) and the average capture probability was 0.15. The density of marten in our study area was 0.33/km² when inhospitable habitat was removed from the calculation. We believe hair sampling and genetic analysis could be used to measure population distribution, trend and size for marten, and perhaps also for other carnivores.

**Key words:** Canada, density, hair removal, mark-recapture, marten, *Martes americana*, microsatellite genotyping

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Monitoring the abundance of carnivores is problematic; track plate, remote camera, and track count methods have been developed for marten and many small carnivores; however, these methods detect the target species at a site but do not allow the identification of individuals. Hence, detection methods can be used to measure relative abundance and population trend but not to estimate population size (Raphael 1994, Zielinski & Kucera 1995). Detection methods are well suited for comparing changes in distribution, but they may lack power to detect trends in abundance (Strayer 1999). Intensive sampling may be required to detect even gross changes in abundance (Zielinski & Stauffer 1996). Also, using detection data to examine population trend assumes
that detection probabilities are similar among surveys (Harris 1986). Variation in sampling techniques, weather and food abundance among surveys may make this assumption questionable. Using estimates of abundance may allow the detection of more subtle trends in abundance, and there is no assumption that capture probabilities are equal among surveys.

The development of microsatellite markers for analyzing patterns of genetic variation is recent (Snow & Parker 1998). These markers are highly variable and therefore well suited to comparing genetic variation among individuals. Microsatellite analysis, using poly­erase chain reaction (PCR), can be performed using relatively small DNA samples, allowing the use of non-invasive collection techniques which typically yield small amounts of low quality DNA. Modern equipment allows the repeatable analysis of large numbers of samples, making microsatellite markers particularly useful for consistently assigning identity to samples from unknown individuals. Match statistics can be used to ensure that the available genetic data are able to resolve individuals even when the study population contains many close relatives (Woods, Paetkau, Lewis, McLeod, Proctor & Strobeck 1999). It is also possible to use genetic markers to determine gender and species (Taberlet, Mattock, Dubois-Paganon & Bouvet 1993, Foran, Minta & Heineneyer 1997b).

The goal of our study was to test the efficacy of non-invasive DNA sampling for estimating population size of marten Martes americana. We were also interested in finding out whether the field methods presented by (Foran, Crooks & Minta 1997a) worked for weasels Mustela erminea and M. frenata and other similar-sized mammals. Our long-term goal was to use marten, and perhaps weasels, as focal species in monitoring forest biodiversity (sic McLaren, Thompson & Baker 1998).

**Study area**

Our study area covered 797 km² of the central Selkirk Mountains in southeastern British Columbia (Fig. 1). In this area, cedar-hemlock forests are found below approximately 1,400 m a.s.l., where western hemlock Tsuga heterophylla, western red cedar Thuja plicata, Douglas fir Pseudotsuga menziesii and spruce hybrids Picea spp. are the dominant canopy species. Engelmann spruce Picea engelmannii - subalpine fir Abies lasiocarpa forests are found between roughly 1,400 and 2,300 m a.s.l., where these two species dominate the canopy, although many early seral stands are dominated by lodgepole pine Pinus contorta. Extensive areas of alpine tundra are found above ca 2,300 m. The area is mountainous with little flat ground, scattered rock outcrops and cliffs, steep-sided watercourses and lakes, and few well-developed riparian areas. The study area is in the interior wetbelt and receives approximately 80 cm of precipitation per year (Environment Canada, New Denver weather station, climate normals). Marten and short and long-tailed weasels occur throughout both forest zones, but fisher Martes pennanti, which have morphologically similar hair to marten, are absent from this region (Cowan & Guiguet 1965, Gribilisco 1994).
Table 1. Hair capture results using glue-patch traps to sample individual marten in southeast British Columbia during January-March 1997.

<table>
<thead>
<tr>
<th>Session</th>
<th>Start</th>
<th>End</th>
<th>Number of sites</th>
<th>Mean duration</th>
<th>Marten detections N (%)</th>
<th>Weasel detections N (%)</th>
<th>Marten captures N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 January</td>
<td>27 January</td>
<td>71</td>
<td>13</td>
<td>33 (46)</td>
<td>7 (10)</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>27 January</td>
<td>10 January</td>
<td>77</td>
<td>13</td>
<td>45 (58)</td>
<td>3 (4)</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>10 February</td>
<td>23 February</td>
<td>77</td>
<td>15</td>
<td>48 (62)</td>
<td>9 (12)</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>23 February</td>
<td>14 March</td>
<td>84</td>
<td>18</td>
<td>54 (64)</td>
<td>5 (6)</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>309</td>
<td>15</td>
<td>180 (58)</td>
<td>24 (8)</td>
<td>124</td>
</tr>
</tbody>
</table>

**Methods**

We divided the study area into 3 × 3 km cells (see Fig. 1). Study area boundaries were largely along heights of land between major watersheds. Most boundaries were at high altitudes and above the treeline. These boundaries probably provided geographic closure to the resident marten population, especially in winter. There were 86 cells in the study area, and we tried to set one marten capture site in each cell during each trapping session. However, some cells with very poor access or no marten habitat (i.e. no forest) were not trapped. In the first session, 15 cells were not trapped and during the second and third sessions nine cells were missed; during the final session, all but two cells were trapped because we used a helicopter for access (Table 1). After approximately 14 days, each capture site was changed to another location in the same cell. We trapped four 14-day sessions during January-March 1997.

The field personnel were instructed to install sites in places they felt would be most likely to catch marten while attempting to space sites at least 1 km apart. Both principal technicians had previous experience trapping marten. We used baited glue traps to remove hair from animals. Traps were fashioned after the design of Foran et al. (1997a). They consisted of two pieces of wood (2 × 14 × 60 cm), screwed together at the edge of each board to form a triangle, and were attached to trees with wood screws. Inside the triangle formed by the trap, four patches (1.5 × 5 cm) of Stick-em™ (Woodstream Trap Corporation, Ithaca, NY, USA) were attached with thumb tacks about one third of the way in from the trap opening. Stick-em™ is a mouse trap based on a cardboard-backed, peanut butter-laced industrial adhesive. In the middle of the wooden triangle, a piece of rotten chicken was attached to a fence staple with wire. We also applied commercial marten lure inside the trap. Above the trap we attached a film canister with a cotton ball soaked in fish oil (rendered fish) inside. Traps were attached to trees vertically and the top part of the trap was protected by a small wooden porch.

When a glue patch had hair on it, we covered the patch with a piece of clean flagging tape and stored it in a sealed plastic bag. All samples were stored in a freezer. We recorded observations of tracks in snow at all sites to aid in species identification and to record the presence of a target species that was not detected in the hair trap.

Hair samples were sorted to species based on morphology and colour. Weasels had short white hair, although a few samples contained longer black hairs, presumably from the tail. Red squirrels *Tamiasciurus hudsonicus* and flying squirrels *Glaucomys sabrinus* had short red-brown to gray hair with little difference between the length of the guard hair and under fur. Mink *Mustela vison* hair is usually darker than marten hair, but the two species could be confused based on hair morphology alone. Mink were not abundant in our study area due to the scarcity of riparian habitat.

We removed hair from glue patches by washing the entire patch in xylene until the glue was soft enough to easily remove hairs from the patch (C. Strobeck, pers. comm.). In many cases we were able to cut enough roots from hair that was suspended beyond the glue patch to avoid the xylene wash. We normally extracted hair from only one of the glue patches at a site, although on rare occasions we took hair from several patches to increase the number of hairs put into the extraction. Entire hairs were put in 1.5 ml microfuge tubes and DNA was extracted using QIAamp™ DNA Mini Kits (QIAGEN Inc., Santa Cruz, California), eluting in a final volume of 200 µl. We used 15 hairs per sample when possible, but fewer hairs were used when necessary. We screened 12 marten microsatellite markers: Ma1, Ma2, Ma3, Ma4, Ma7, Ma8, Ma10, Ma11, Ma14, Ma15, Ma18, Ma19 (Davis & Strobeck 1998) and chose a suite of six markers (Ma1, Ma2, Ma8, Ma10, Ma18, Ma19) based on demonstrated variability and amplification strength with small quantities of DNA. We designed new primers for some loci to improve amplification characteristics and to enable all loci to be analyzed in a single lane (Table 2). PCR conditions were as described by Davis & Strobeck (1998) using a volume of 15 µl containing 10 µl of template DNA. PCR products were analyzed under standard conditions using an Applied Bio-
systems 310 automated sequencer, and genotypes were scored with the aid of Genotyper software (Applied Biosystems).

Davis & Strobeck (1998) provided information on amplification patterns (presence of product, size, variability) in mink for the markers we used. To provide similar information on short-tailed weasels, we ran two known short-tailed weasel samples as well as four samples identified as weasel using tracks and hair morphology. We assumed that squirrel samples would not produce scorable PCR products in the size range observed for marten. A likelihood of occurrence ($P_{\text{random}}$; Woods et al. 1999) was calculated for each genotype to identify extremely rare genotypes. These sources of information allowed us to confirm species for the samples we genotyped.

Genotyping was carried out in several distinct phases. An initial attempt was made to obtain genotypes for every extracted sample. Samples producing scorable products for fewer than three loci in this initial screen were excluded from subsequent analyses. Samples that produced data for 3-5 loci were then subjected to a second round of PCR and scoring for those loci that could not be scored on the first screen. After the second attempt, samples with genotypes at less than four loci were excluded. We wrote a computer program to assign samples with identical genotypes to individuals, and to identify pairs of genotypes that differed at only one locus. These highly similar pairs of genotypes were checked for scoring or typing errors. Similar genotypes that could not be explained by these errors were then subjected to another round of PCR and scoring for the loci where the differences were observed (see also Poole, Mowat & Fear 2001).

In addition to restricting the analysis to samples with 4-locus data or better, we calculated the probability that a full sibling would have the same genotype ($P_{\text{sib}}$; Woods et al. 1999) for each pair of matching samples, limiting the calculation to only those loci that were complete for both samples under consideration. We required that this probability be less than 0.05 to assign a sample to an individual.

We used the mark-recapture models available in the program CAPTURE (Otis, Burnham, White & Andersen 1978, White, Andersen, Burnham & Otis 1982) for estimation of population size. Model selection was based on our knowledge of marten biology and movements and the goodness-of-fit tests available in CAPTURE. We used Pearson correlation to assess the relationship between the number of hairs in a sample and the number of loci that could be scored. We used logistic regression to test the relationship between the number of hairs in a sample and the probability of scoring a 6-locus genotype. We used the logit function and tested assumptions regarding residuals. All analysis was done using SAS 6.12 (SAS Institute Inc., Cary, NC, USA).

### Results

In November and December 1996, we tested hair removal methods near Nelson, British Columbia. We found that it was easier to attach traps on trees vertically and that this placement generated hair samples more consistently than horizontal placement. We experimented with a number of glue patch placements and found that we got larger hair samples if the patches were high.
up in the V of the trap and placed to capture hair midway down the back of the animal, i.e. midway between the center of the trap and the end. Upper and lower hair patches were equally successful at removing hair. For marten, 92% of upper patches removed hair vs 87% of lower patches (N = 180 sites that detected marten). For weasel, 96% of upper patches removed hair while 100% of lower patches removed hair (N = 24). Snow, and especially rain, caused the paper backing on the back of the patches to soften, occasionally rendering the patches useless. Using large live trees and wooden porches above traps minimized this problem.

Based on tracks in snow and hair morphology, we captured hair from marten, red squirrels, flying squirrels, short or long-tailed weasels, and several unidentified mouse and vole species. Several glue patches obviously had hair from more than one species. However, except for the two weasel species and between marten and mink, hair morphology was so different for the species in our study area that they could be separated visually. Of 309 sites, 58% removed a marten hair sample while 8% of sites produced weasel samples (see Table 1). Based on tracks in snow, 14 sites were approached by weasels but did not capture hair. Likewise, 15 sites approached by marten failed to collect samples. The trap failure rate, calculated as the proportion of visited sites that did not remove a hair sample, was much higher for weasels (37%) than for marten (8%).

Marten were detected throughout the study area (see Fig. 1). The linear fashion in which sites were distributed through the study area is largely a result of the very mountainous terrain and the fact that we relied heavily on the road network for access. Individual marten were detected at different sites 36 times, and the average movement distance was 2.6 km (range: 0.5-8.9 km). Movements appeared to be confined to low-elevation forested areas except for one movement in the north end of the study area, where an individual probably crossed through 1 km of alpine habitat to access the adjacent drainage (see Fig. 1).

We extracted DNA from 180 marten hair samples. Of these, 139 produced enough genetic data to be assigned to individuals. Of those samples that could not be assigned to individuals, two were excluded because $P_{sib}$ was >0.05, and the remainder because <4 loci could be scored. Two samples with 4-locus genotypes, and 21 samples with 5-locus genotypes, were assigned to individuals. Two scoring errors and two cases of allelic dropout (Taberlet, Griffin, Goossens, Questiau, Manceau, Escaravage, Waits & Bouvet 1996, Gagneux, Boesch & Woodruff 1997) were detected by checking pairs of similar genotypes. One additional sample had to be discarded because there may have been allelic dropout, but the DNA sample was used up before the suspicious genotype could be confirmed. After correcting these errors, all pairs of genotypes in the data set differed at two or more loci. Given that we checked every case where a single error could have occurred and how rare these events were (4/139), we believe that the probability of having errors at two loci in the same genotype is negligible, and that each of the 88 different genotypes in our data set corresponded to a unique individual.

The xylene treatment appeared to have little effect on genotyping success, with 83% (N = 101) of clipped samples and 75% (N = 69) of xylene extracted samples generating 4-locus genotypes or better. The number of roots in a sample was positively correlated with the number of scorable loci for both clipped ($r = 0.39, N = 99, P < 0.0001$) and xylene treated ($r = 0.32, N = 66, P = 0.01$) samples. Logistic regression showed a significant relationship between the number of roots in a sample and the event of scoring a complete 6-locus genotype ($\chi^2 = 12.9, df = 1, P = 0.0003$; Fig. 2). We also ran the logistic analysis for clipped and xylene treated samples separately. The logit probabilities and the shape of the logit curves were similar for both extraction methods, so we present only the combined analysis.

The 139 marten samples identified were all from different sites and corresponded to 124 capture events during the four trapping sessions (see Table 1). The number of capture events per session is less than the total number of captures because several marten were detected at multiple sites within a session. Individual marten were captured at as many as eight different sites, and multiple captures of an individual within a session occurred 16 times during the study.
Discussion

Marten density in our area was low and most similar to other areas where food abundance was low (Weckworth & Hawley 1962, Thompson & Colgan 1987), or where there was significant trapping mortality (Payer 1999), or to areas which had been clear-cut in the preceding 35 years (Soutiere 1979, Thompson & Colgan 1987, Payer 1999). The only other study of marten population size done in temperate western forest did not present densities. We calculated approximate densities from the measures of population size given in Weckworth & Hawley (1962) using the study area size of ~15.5 km² cited in Hawley & Newby (1957). Winter densities varied between 1.9 and 0.8 marten/km² in a mixture of forest types typical of the Rocky Mountain west slopes. It is likely that some previous density estimates are biased high by 'edge effect' (White et al. 1982), or may not be typical because of the tendency to select good quality habitat as study sites (Smallwood & Schonewald 1996). Our density estimate is a landscape-scale estimate for an area that has many recent logging blocks, little trapping, no human residents, many logging roads and a mixture of forest types from low elevation cedar-hemlock forests to upper elevation subalpine parkland.

We demonstrated that non-invasive sampling and genetic analysis can be used to measure distribution and estimate population size for areas large enough to be relevant to marten population management or landscape-scale forest planning. This study area encompassed the greater part of three registered traplines (areas of public land for which the licensee holds the exclusive right to trap furbearing animals) and one entire forest license area. In our study area 12 marten were trapped during the winter we worked there; this is <6% of the winter population. Marten were readily detected in our traps as demonstrated by the low number of instances when animals had approached a trap but did not leave a hair sample (8%). Further, marten did not seem to be deterred from re-entering traps as demonstrated by the relatively high recapture rate. The method may work for other carnivores as demonstrated by our ability to collect weasel hair, although modifications are needed to adjust the size of the trap to the smaller species.

We screened for species based on tracks at the site,
hair morphology and microsatellite results. While we believe these efforts were sufficient in our study area, an objective species test similar to the one presented by Foran et al. (1997b) or Woods et al. (1999) would likely be necessary in areas where mink or fisher are common. The data we obtained for the two known weasel samples and the four samples identified as weasel in the field were consistent in terms of size range, which loci amplified, and which loci were variable. No loci produced products in the same size range as marten samples. Similarly, Davis & Strobeck (1998) provided data which indicated that a mink genotype could not be confused with a marten genotype. In addition, none of the 88 genotypes we recorded were unusually rare. Combined with our confidence in field species assignments, we feel certain that the 139 samples included in our analysis are from marten. In areas where hair samples can be visually sorted to exclude most non-target individuals, it may be cheaper to simply use microsatellite results to exclude the few non-target species remaining, rather than to run a species test on all samples.

The xylene wash did not appear to reduce the quality or quantity of DNA in a sample compared to clipped samples, similar to chloroform (Foran et al. 1997a). However, unlike Foran et al. (1997a) we removed entire hairs from the xylene wash solution. We demonstrated that the probability of scoring a genotype for a sample is related to the number of roots available for the extraction. Similar results have been shown for marmots *Marmota marmota* and bears *Ursus arctos* (Gossens, Waits & Taberlet 1998; G. Mowat, unpubl. data). We achieved the greatest success with 20 hairs in the extraction, but the relationship in Figure 2 did not asymptote. The probability of scoring six loci increased from 0.78 to 0.88 when the number of hairs was increased from 15 to 20. Genotyping failures were numerous (41 of 180 samples) and 38% of our samples had <10 hairs in them. Future workers may be able to increase genotyping success by combining hair from several glue patches and putting more hairs in a sample. We avoided combining samples because we felt that it would increase the risk of mixing individuals in a sample. However, on three occasions we combined hairs from more than one pad but none appeared to contain DNA from more than one individual (we expect mixed samples to show three or four alleles at one or more loci given the variability of the markers used). Nor did any of the 136 single samples analyzed appear to be from different individuals. Marten may not have entered previously visited traps because bait was no longer present. Mixed samples may be more common in areas where the target species occurs at higher density. It may be possible to separate multiple individuals on a single patch by carefully selecting clumps of hair, if adequate sample remains. As noted by Foran et al. (1997a), restricting the trap in some way such that only one individual could enter would reduce the risk of mixed samples during DNA analysis.

Shorter intervals between trap checks should also reduce the number of mixed samples. However, trap check intervals must be long enough to ensure reasonable capture success. Trap-check interval was the most important variable affecting detection success in a detailed study done by Zielinski, Truex, Ogan & Busse (1997) using track plates to detect fisher and marten. We checked hair traps weekly during our prototype testing and found that detection success increased through the four weeks we trapped (G. Mowat, unpubl. data).

Our population estimate was imprecise and perhaps biased because capture probabilities varied. Subsequent workers can probably increase precision by increasing trap density and minimizing capture variation. And, genotyping success can probably be increased by combining samples from several glue patches at a site. Capture success varied among sessions (see Fig. 1), which is common (White et al. 1982). Longer sessions, which offer similar mean weather, and shorter study duration (i.e. fewer capture sessions) may help minimize time variation in a hair sampling study where traps must remain set for multiple days to ensure reasonable capture success. Lab failures may also add to variation in captures among sessions (Mowat & Strobeck 2000) which will be reduced as field and genetic analysis methods improve.

Heterogeneity variation, defined by Otis et al. (1978) as random variation among individuals, is also common in field data (Conner & Labisky 1985, McCullough & Hirth 1988, Corn & Conroy 1998), and was detected in our data. Low trap density can cause heterogeneity; hence, Otis et al. (1978) recommended four traps per home range per session. This trap density would be difficult to achieve in large-scale studies such as ours, but moving traps each session should increase effective trap density and reduce heterogeneity (Pollock, Nichols, Brownie & Hines 1990). Targeting preferred habitat should also increase effective trap density.

Marten may also have shown a positive behaviour (‘trap-happy’) response, probably because they received a meat reward when they entered a trap. This form of capture variation can cause negative bias in models that do not accommodate this variation explicitly, and behav-
ior models often require large sample sizes and deliver poor precision (Boulanger & Krebs 1996). Given that both time and heterogeneity variation are common in field data, it is advisable for field biologists to minimize all three forms of variation explicitly. Avoiding any form of reward at a capture site should eliminate positive behaviour response. However, it is not clear that marten and weasels could be enticed into hair traps with a scent bait such as those used for bears (Mowat & Strobeck 2000). Access to individual cells was often restricted to relatively short sections along one or two roads on our study area, and so we had trouble moving traps more than 1 km between sessions. Short trap movement distances may have allowed previously caught martens to successfully seek out our traps in following sessions. Greater movement of sites between sessions leading to more even trap coverage is preferred.

Geographic closure can lead to overestimates of population size (White et al. 1982). In most cases, marten would have had to traverse alpine areas to cross the study area boundary, and the movements of animals documented in this work suggest that this was rare. Also, the effect of closure bias declines as the ratio of study area size to home range size increases (White et al. 1982). Mean home-range size is likely to be less than 10 km² in our area (Buskirk & Ruggiero 1994) suggesting that the ratio of study area to home range size is at least 80 times, which is much larger than many contemporary mark-recapture studies (Garshelis 1992). Geographic closure probably did not cause an important bias in our study.

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