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Quantifying dispersal rates and distances in North American martens: a test of enriched isotope labeling

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Advances in the application of stable isotopes have allowed the quantitative evaluation of previously cryptic ecological processes. In particular, researchers have utilized the predictable spatial patterning in natural abundance of isotopes to better understand animal dispersal and migration. However, quantifying dispersal via natural abundance alone has proven to be of limited utility for species exhibiting short- or mid-distance dispersal events, including most mammalian species. In previous experimental work, we demonstrated that consumption of 1 dose of isotopically enriched baits elicited a distinct “mark” in hair of captive martens (Martes spp.). Herein, we report findings from a field test of our isotopic enrichment approach to mark free-ranging animals and quantify dispersal of martens across forest stands at sites in southeastern Alaska and northern British Columbia. In the field, we supplemented bait used in single-capture hair traps with the amino acid glycine artificially enriched in 2H, 13C, or 15N. By applying unique combinations of artificially enriched isotopic markers within discrete forest stands, the isotopic signature of collected hair reflected the forest patch where the individual originated. From our isotopic marks, we were able to infer dispersal events between forested stands and, thus, estimate rates and approximate dispersal distances. Our findings demonstrate that isotopic enrichment can be a cost-effective method to mark the hair of midsized mammals for the quantification of dispersal.

Key words: carbon, deuterium, isotopic enrichment, Martes americana, Martes caurina, mesocarnivore, nitrogen

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Quantifying dispersal with deuterium is rare in mammalogical studies, particularly those featuring nonvolant and terrestrial species, which generally do not disperse sufficient distances to acquire detectable changes in natural abundance of δD (e.g., <100 km—Wunder and Norris 2008). Physiologists (Boutton 1991; Hirons et al. 2001) and ecosystem ecologists (Hall and Tank 2003) have, however, developed procedures to artificially enrich organic and inorganic compounds with stable isotopes (such as 13C and 15N) and track the flux of resources and nutrients. More recently, isotopic enrichment has been applied to mark and subsequently recapture individuals to quantify dispersal of aquatic invertebrates (Macneale et al. 2004; Wanner et al. 2006) and seeds of plants (Carlo et al. 2009). The application of enriched isotopes to mark individuals and infer dispersal distances has not been extended to the study of wild vertebrates.

Through captive feeding trials, we previously demonstrated that the hair of midsized mammals can be cost-effectively marked via artificially enriched diets (Pauli et al. 2009). For wild-caught Pacific martens (Martes caurina—MacDonald and Cook 2009) we found that during active hair growth, consumption of 1 bait containing the amino acid glycine artificially enriched with 2H, 13C, and 15N elicited a distinct “mark” in their hair. However, this approach had not been tested in the field or applied to track individual movement patterns and document long-distance dispersal events. Herein, we report findings from a test of this novel, noninvasive method to quantify dispersal rates of American (Martes americana) and Pacific (M. caurina) martens for the quantification of dispersal, 2006–2008. Symbols denote the type of enrichment applied within each area: ▲ = 15N, ■ = 2H, ● = 13C, ♦ = a combination of 15N and 2H.

Sampling areas were selected from geographic information system layers based on habitat characteristics, size, and distance from one another. Within each sampling area, 3 or 4 traplines were set. To test the effectiveness of isotope labeling for quantifying dispersal rates and distances of free-ranging individuals and explored differences in dispersal rates and distances between the 2 species.

MATERIALS AND METHODS

Our study sites were located within temperate rain forests of southeastern Alaska, and northern British Columbia (Fig. 1). Specifically, fieldwork was conducted on Prince of Wales Island (POW [55°38’N, 132°48’W]; 2006), Admiralty Island (ADM [57°43’N, 134°22’W]; 2007), and on Misty Fjords National Monument (MFNM [55°38’N, 130°37’W]) in southeastern Alaska, and Haida Gwaii (Queen Charlotte Islands; QCI [53°38’N, 132°23’W]; 2008) in British Columbia. From August to September, we deployed single-capture hair traps across 4 sampling areas within each island. Sampling areas were selected from geographic information system layers based on habitat characteristics, size, and distance from one another. Within each sampling area, 3 or 4 traplines were set. To test the effectiveness of isotope labeling for quantifying dispersal rates and distances of wild vertebrates.

FIG. 1.—Location of study sites within southeastern Alaska (Admiralty Island [ADM], Misty Fjords National Monument [MFNM], and Prince of Wales Island [POW]) and northern British Columbia, Canada (Queen Charlotte Islands [QCI]) where we used artificially enriched isotopic baits to mark American (Martes americana) and Pacific (M. caurina) martens for the quantification of dispersal, 2006–2008. Symbols denote the type of enrichment applied within each area: ▲ = 15N, ■ = 2H, ● = 13C, ♦ = a combination of 15N and 2H.
Martens between forest stands, we supplemented bait used in hair traps with the amino acid glycine enriched with 99% $^2$H, $^{13}$C, or $^{15}$N (ISOTEC, Miamisburg, Ohio). We applied a unique label to each forest stand so the isotopic signature of the hair reflected the location where the individual originated (Fig. 1). We estimated the quantity of glycine needed to enrich the guard hair of martens for each isotope following the equations provided in Pauli et al. (2009).

We simultaneously collected marten fur and isotopically labeled martens with a single-capture hair trap that allowed visiting animals to escape, but prevented subsequent access by additional animals (Pauli et al. 2008). Hairs of the departing animal were collected by wire brushes. We baited each trap with deer scraps and jam (the latter containing the unique isotopic label), and applied fish oil and essence of skunk as olfactory lures. Traps were checked every 5th day for 5 weeks. Hair samples were collected from the wire brushes using forceps, placed in 1.5-ml microcentrifuge vials containing silica bead desiccant, identified to species based on dorsal guard-hair characteristics, and stored frozen. We also opportunistically collected fresh marten scat samples found near hair traps on ADM for analyses of $^{13}$C and $^{15}$N. Finally, we purchased carcasses from commercial trappers that caught martens within or in close proximity to our study sites during the winter trapping season (December–January) that followed our autumn labeling effort. Sampling procedures adhered to the guidelines for use of mammals in research set forth by the American Society of Mammalogists (Sikes et al. 2011) and methods were approved by the Institutional Animal Care and Use Committee at the University of Wyoming. Sampling permits were obtained through the Alaska Department of Fish and Game.

Hair samples from both carcasses and traps were rinsed 3 times with 2:1 chloroform : methanol solution to remove surface oils (Cryan et al. 2004), dried for 72 h at 60°C, and homogenized with surgical scissors. Fecal samples were dried and ground to a fine powder. All samples were weighed, placed in tin ($^{13}$C and $^{15}$N) or silver ($^2$H) capsules, and submitted to the Stable Isotope Facility at the University of Wyoming. Analysis of $^{13}$C and $^{15}$N was conducted with a Costech 4010 elemental analyzer (Costech Analytical, Valencia, California) and $^2$D with a Finnigan TC/EA (High Temperature Conversion Elemental Analyzer; Thermo Finnigan, Bremen, Germany) attached to a Thermo Finnigan Delta$^{^18}$LUS XP Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Finnigan). Results are provided as per mil (parts per thousand [%]) ratios relative to the international standards of PeeDee Belemnite (PDB; $^{13}$C), atmospheric nitrogen (AIR; $^{15}$N), and Standard Mean Ocean Water (SMOW; $^2$D) with calibrated internal laboratory standards. Because all samples were processed and analyzed in the same geographic location we did not correct the raw $^2$D data for atmospheric exchange (Wassenaar and Hobson 2000).

Natural abundance values of $^{13}$C and $^{15}$N are highly correlated for martens of southeastern Alaska because these terrestrial carnivores also consume salmon (Oncorhynchus spp.) and other marine organisms that are naturally enriched in both $^{13}$C and $^{15}$N (Ben-David et al. 1997). Therefore, we constructed a baseline regression model from 69 marten hair samples collected from 3 locations in southeastern Alaska (POW, ADM, and MFNM) that were either outside of our sampling areas or sampling period, and therefore could not have come from animals that ingested an isotopically labeled bait. Hair samples collected within our study area with isotopic values exceeding the 99% prediction interval of the regression equation were classified as originating from individuals that ingested a $^{15}$N-enriched bait; those with values less than the lower 99% prediction interval were classified as having been marked with $^{13}$C. We explored whether natural abundance of $^{13}$C, $^{15}$N, and deuterium differed between locations with 1-way analyses of variance. Because natural abundance values of $^2$D are influenced by latitude and distance from the coastline (Bowen et al. 2005; Wunder 2012), we pooled samples collected along the coast of MFNM with those from coastal POW (because they were collected from the same latitude). We did not attempt to identify animals marked with deuterium from QCI because we lacked isotopically unmarked (i.e., reference) individuals. We identified marked individuals as those for which the signature of hair samples exceeded the upper 99% prediction limit of means for each location.

We identified fecal samples as isotopically marked using dual criteria of elemental abundance of nitrogen, and isotopic enrichment of $^{15}$N and $^{13}$C. First, samples were considered marked when they possessed $<5.5%$ nitrogen, which is indicative of feces free of animal tissue (Ben-David et al. 1998), but were enriched in $^{15}$N and $^{13}$C above available vegetation in the region (i.e., $>5\%$ for $^{15}$N or $>25\%$ for $^{13}$C—Ben-David et al. 1997). Second, scat samples with $>5.5\%$ nitrogen, indicating that animal tissues were present in the excreta, were deemed isotopically marked when $^{15}$N and $^{13}$C exceeded the signature of the most enriched animal diet items (i.e., $^{15}$N $>15\%$ or $^{13}$C $>17\%$).

In southeastern Alaska, the average home-range size of male martens is 7.8 km$^2$ and females have a mean home-range size of 5.3 km$^2$ (Flynn and Schumacher 1999). Thus, based on isotopic labeling and location of sample collection we identified potential dispersers as those animals that moved a distance of $\geq2$ home-range diameters, assuming a circular home range, away from their original location of capture (i.e., males moving $\geq6.3$ km; females moving $\geq5.2$ km). We estimated minimum dispersal distances by measuring the distance between nearest trap location supplemented with the enriched isotope to the final trapped location in ArcMap (ESRI, Redlands, California). We compared dispersal rates (the frequency of observed dispersal events relative to the number of marked animals) between American (for combined samples from POW and MFNM) and Pacific (ADM and QCI) martens with a $G$-test. Similarly, we compared dispersal distances for the 2 species with a 2-sample $t$-test (Zar 1999).

**Results**

In total, we collected 372 hair samples with our hair traps and obtained 366 hair samples from trapper-killed marten...
Table 1.—Number of samples, average capture success (number of hair captures/trap night), and number of carcasses collected from commercial trappers for martens (Martes americana and M. caurina) inhabiting sampling sites during autumn for Admiralty Island (ADM), Misty Fjords National Monument (MFNM), and Prince of Wales Island (POW), Alaska, and Queen Charlotte Islands (QCI), British Columbia, Canada.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>No. samples</th>
<th>No. trap occasions</th>
<th>No. samples/no. trap occasions</th>
<th>No. carcasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADM</td>
<td>M. caurina</td>
<td>231</td>
<td>760</td>
<td>0.304</td>
<td>49</td>
</tr>
<tr>
<td>QCI</td>
<td>M. caurina</td>
<td>2</td>
<td>246</td>
<td>0.008</td>
<td>71</td>
</tr>
<tr>
<td>MFNM</td>
<td>M. americana</td>
<td>45</td>
<td>425</td>
<td>0.106</td>
<td>78</td>
</tr>
<tr>
<td>POW</td>
<td>M. americana</td>
<td>94</td>
<td>1,650</td>
<td>0.057</td>
<td>168</td>
</tr>
</tbody>
</table>

carcasses (Table 1). As predicted, δ13C and δ15N were strongly correlated ($r = 0.930$) for unmarked martens in our external samples collected across southeastern Alaska (Fig. 2A). We detected significant differences among sites in the δ13C signature of unmarked martens ($F_{2,68} = 4.26, P = 0.018$; Fig. 3A), and marginally significant differences in δ15N ($F_{2,68} = 2.76, P = 0.071$; Fig. 3B). Site-based differences were driven by samples from ADM being slightly enriched in both 15N and 13C. Nonetheless, omitting ADM samples from the regression model resulted in overlapping parameter estimates ($\beta_0 = 34.5 \pm 1.4 \text{ SE}$ and $\beta_1 = 1.2 \pm 0.06$) compared to the model that included ADM samples (Fig. 2A). Using the 99% prediction interval from the regression model that included ADM unmarked samples, we identified 21 carcasses marked by 13C and 9 marked by 15N (Fig. 2B). For hair obtained from the 372 samples collected in our noninvasive traps, we identified 37 instances in which the captured animal was marked by 15N and 26 instances in which the marten was marked by 13C (Fig. 2C). Of the 112 marten scat samples we collected along traplines on ADM, we deemed 35% (n = 41) as enriched in 15N (Fig. 4A) and 5% (n = 6) enriched in 13C (Fig. 4B).

Values of δD were correlated with both δ13C ($r^2 = 0.198, F_{1,63} = 15.3, P = 0.001$) and δ15N ($r^2 = 0.203, F_{1,63} = 15.8, P < 0.001$) among the unmarked samples, indicating that martens obtained substantial quantities of structural hydrogen for hair growth from their diet. Because both δ13C and δ15N were only weakly related to δD, however, we did not include estimates of carbon or nitrogen enrichment in identifying martens that had been marked with enriched deuterium. We detected strong differences among sites in the δD signature of unmarked martens ($F_{2,63} = 43.4, P < 0.001$; Fig. 5). Samples collected from the mainland (MFNM) were most depleted, followed by samples from the northernmost (ADM) and southernmost (POW and MFNM) coastal locations. We found that deuterium for marked animals (23 noninvasive samples and 48 carcasses) ranged from −55.6% to −15.1% in the continental MFNM sample, −37.5% to +2.2% in the ADM sample, and −16.7% to +11.5% in the POW and coastal MFNM samples.

The majority of male and female carcass samples (19 Pacific martens and 59 American martens) were obtained from the forest stand in which they were originally labeled and were within our threshold value for dispersal distance. However, we identified 22 individuals (3 Pacific martens and 19 American martens) as potential dispersers. Of these, 10 were males and 12 were females, but all were either young of the year (0 years old) or yearlings. Three dispersing individuals (2 Pacific martens and 1 American marten), all 0 years old, moved >25 km, presumably dispersing from their natal territories. Although the exact distances moved by martens are incalculable without individual identity for the noninvasively collected samples, we were able to detect dispersal events and estimate the minimum Euclidean distance moved. Of the 86 marked hair samples (68 Pacific martens and 18 American martens) we identified, 62 were collected within the forest stand where the isotopic label was applied and were presumed to be resident animals. The remaining 24 samples (19 Pacific martens and 5 American martens) appeared to have been collected from long-distance dispersers, moving 15–40 km. The combination of carcasses and noninvasive samples suggested that Pacific and American martens exhibited similar rates of dispersal (28% of marked individuals dispersing; $G = 0.70, P = 0.40$), and similar dispersal distances ($\bar{X} = 15.5 \text{ km} \pm 1.01 \text{ SE}; t_{45} = 1.01, P = 0.16$).

**Discussion**

Our results demonstrate that enriched isotopic baits combined with hair collection can be an effective method to quantify dispersal for midsized mammals. Using this approach, and without additional information, we were able to quantify dispersal rates and distances, and explore the dispersal power of 2 species of martens with putatively different evolutionary histories. Both the rates and distances of dispersal we documented using enriched isotopes generally corresponded with those recently found using radiotelemetry among juvenile martens from Ontario (Johnson et al. 2009). Our ability to identify dispersers and quantify attributes of dispersal for American and Pacific martens using isotopic labeling and noninvasive sampling should be of use to future researchers, and particularly relevant for those studying Pacific marten populations on ADM. To date, identification of individuals and, therefore, estimation of dispersal were unattainable because of low diversity in microsatellite DNA loci (Small et al. 2003). Our findings that Pacific and American martens may possess similar dispersal propensity and dispersal power should be considered with caution; site-specific differences in habitat quality, marten density, and population dynamics are currently unknown for our study sites, and likely exert a strong influence on dispersal patterns.
Our field test of isotopic labeling revealed that some elements were more effective in marking the hair of martens. In particular, we found that $^{15}$N-labeled bait produced an unambiguous mark, especially among martens on ADM, whereas $^{13}$C and $^2$H marks were less obvious. This finding contrasts with our previous results from captive experiments, where consumption of a single dose yielded unequivocal marking in all 3 isotopes (Pauli et al. 2009). Our results for deuterium likely reflect both high spatial variation in values of this element isotope and high individual variation in isotopic incorporation. Wolf (2011) recently documented large individual variation in the incorporation of H into several types of tissues, including feathers, in house sparrows (Passer domesticus) and Japanese quails (Coturnix japonica). Because the birds were fed controlled diets, Wolf (2011) attributed some of the variation to individual differences in the amount of water consumed and metabolic activity. Our sampling locations on POW and MFNM, which share the same latitude ($55^\circ38^\prime$N), exhibited significant differences in
deuterium between inland and coastal sites in unmarked animals. Also, the observed individual variation in \(dD\) values among animals that we considered unmarked within each study site was large, ranging from \(299\%\) to \(218\%\). Although these data highlight some potential limitations of using natural abundance in dispersal studies, these factors also may influence our ability to use \(^2H\) in marking studies. It is possible that the high \(\delta^{15}N\) values we observed among martens on ADM resulted from a few individuals consuming multiple doses of enriched bait. However, repeated consumption of bait cannot fully explain the differences between \(\delta^{13}C\) and \(\delta^{15}N\) among individuals on this island. During our sampling on ADM, small mammal abundance was low, but capture rates of martens were high (Table 1), suggesting that these animals could have been nutritionally stressed. When undernourished, predators will allocate some consumed amino acids to respiration (Ben-David et al. 2012; Martínez del Rio and Wolf 2005; Whiteman et al. 2012). This is particularly relevant for nonessential amino acids, including glycine. Thus, martens consuming enriched glycine may have allocated a portion of it to respiration. The deamination of glycine and subsequent nitrogen recycling could have led to an elevation in \(\delta^{15}N\) (Hobson et al. 1993; but see Ben-David et al. 1999) and a pronounced signature in ADM martens. To reduce the effects of elemental routing on isotopic labeling, we recommend the use of essential amino acids in field labeling studies. Although currently cost prohibitive, cysteine, a sulfur-bearing amino acid and a main component of keratin (Richards et al. 2003; Thomas et al. 2007) would likely be a superior vehicle for isotopic enrichment. Researchers exploring isotopic labels for free-ranging mammals, especially with \(^{13}C\), also should consider increasing the amount of enriched amino acid applied to bait in the field to ensure that unambiguous marks are achieved.

Artificially enriching keratin will be particularly useful when other emerging approaches to quantifying dispersal rates, such as satellite tracking or spatial variation in the natural abundance of deuterium- or DNA-based analyses, are intractable. Animal movement data from global positioning system or ARGOS technology are rich in detail, but can be prohibitively expensive ($2,000–8,000 per collar [Hebblewhite and Haydon 2010] versus $0.45–6.60 per isotopically enriched bait [Pauli et al. 2009]) and currently are limited to the study of large or midsized mammals. Natural deuterium levels are effective at reconstructing dispersal at continental scales (Hobson 1999; Wunder 2012), but provide insufficient...
resolution for species that disperse short to middistances (Wunder and Norris 2008). Dispersal inferred from genetic divergence among populations (e.g., \( F_{ST} \)--Wright 1951) or via the assignment of individuals to subpopulations based on genotypes (Pritchard et al. 2000) fail for systems featuring a high degree of connectivity because allele frequencies are nearly identical among subpopulations (Palsbøll et al. 2010). Genetic kinship methods, characterizing dispersal via spatial distribution of close relatives (Peery et al. 2008), are effective for functionally connected systems, but require that the majority of the population is sampled (Palsbøll et al. 2010). Thus, artificial isotopic enrichment seems particularly applicable for small-bodied species within systems that exhibit a high degree of functional connectivity and where the majority of the population cannot be sampled. Many of the collection techniques already developed for noninvasive, DNA-based approaches (e.g., hair traps [Pauli et al. 2008] or hair snares [DePue and Ben-David 2007]) can be used with the isotopic method we describe. Isotopic labeling offers the added benefit that, unlike DNA (Roon et al. 2003), the keratin is resilient to degradation (Macko et al. 1999).

For enriched isotopic baits to be effective, researchers will need to calculate dose requirements (Pauli et al. 2009), and conduct feeding trials to ensure that isotopic marks are unambiguous. In the field, enriched baits should be deployed during a period of keratin growth, ideally at the onset of a molt. Because most amino acids incorporated in hair are from recently consumed food items (Ayliffe et al. 2004), isotopic enrichment of ingested amino acids will provide a rapid mark within those tissues. Surprisingly, we found that a reasonable proportion of scat samples collected from martens on ADM were enriched with nitrogen or carbon (deuterium was not analyzed for feces). By using elemental abundance of nitrogen in fecal samples, we 1st separated animal-free scat samples from those with animal tissue. Based on the extreme isotopic signatures of plant and animal tissues, we then discriminated scat samples isotopically enriched in \( ^{15}N \) and \( ^{13}C \). Although having reference unmarked fecal samples for comparison would have been ideal, our general approach in identifying marked scat seems promising, especially for detecting scats marked with \( ^{15}N \). Examination of these preliminary data suggests that collection of fresh scat may provide complementary information on dispersal when collected concurrently with hair sampling. Finally, site- and tissue-specific baseline values for \( ^{15}N \), \( ^{13}C \), and \( ^{12}C \) are needed. Where these elements in the natural diet are not correlated, individuals that have consumed an enriched bait can be identified using the upper 99% prediction intervals constructed from baseline values. In cases where the correlation between elements in the diet is high, such as the relationship we observed for salmon-consuming martens, more involved analytical methods will be necessary to identify animals marked with enriched isotopes. Of course, without quantifying the individual identity for noninvasively sampled martens, our estimates of dispersal are prone to bias, particularly if dispersing animals possess different likelihoods of visiting baited traps or exhibit strong responses to sampling (e.g., becoming trap-happy or trap-shy—Pollock 1981).

Dispersal, the main process connecting animal subpopulations, is a critical aspect of a species’ ecology, life history, and evolution. Knowledge of dispersal characteristics is particularly relevant given the current level of global habitat fragmentation (Foley et al. 2005) and forecasted consequences of climate change (Thomas et al. 2004). Although we developed this method for 2 species of mesocarnivores, providing artificially enriched isotopic bait and subsequently collecting hair or other tissue represents a safe (Koletzko et al. 1997) and cost-effective (Pauli et al. 2009) approach to mark and track small and medium-sized animals, thereby, enabling the quantification of this previously cryptic process.

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**Literature Cited**


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