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SHORT COMMUNICATION

Characterizing the molecular variation among American marten (*Martes americana*) subspecies from Oregon and California

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Abstract We investigated the subspecific identity of a rediscovered population of American martens within the range of a presumed extinct subspecies (*Martes americana humboldtensis*) by comparing mitochondrial DNA sequence diversity from contemporary individuals within the described ranges of *M. a. humboldtensis*, nearby ranges of *M. a. caurina* and *M. a. sierrae*, and a museum specimen of *M. a. humboldtensis*. Martens from the rediscovered population shared a haplotype (#2) with the museum specimen. This haplotype was found only in the coastal regions of Oregon and California, suggesting that the rediscovered population represents descendants of a relictual population that previously existed in coastal California. The subspecific boundary between *M. a. humboldtensis* and *M. a. caurina* may not be valid, because haplotype #2 was shared between coastal Oregon and coastal California populations and no known contemporary or historical biogeographic barriers prevent north–south movement. Thus, marten populations currently located in coastal forests of California and Oregon should be managed collectively to preserve the connectivity that our data suggest occurred historically. *M. a. sierrae* differed substantially from both *M. a. humboldtensis* and *M. a. caurina*, suggesting marten populations were not a historically genetically homogeneous population and divergence may have occurred in separate glacial refugia.

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Introduction

The American marten (*Martes americana*) is a carnivorous, forest-dwelling mustelid associated with late-successional coniferous forests (Powell et al. 2003). During the last glaciation, martens were restricted to Pleistocene refugia in the southeastern and southwestern portions of continental North America (Graham and Graham 1994; Stone et al. 2002). Since then, martens have recolonized northern regions and are currently recognized as 14 subspecies (Hall 1981). This species has long been of conservation concern due to its sensitivity to the loss and fragmentation of mature forests (Bissonette et al. 1997; Potvin et al. 1999; Heinemeyer 2002) and its value as a furbearer, which caused dramatic declines from trapping in the late 1800s and early 1900s (Dixon 1925; Zielinski et al. 2001).

In California and Oregon, martens were historically found throughout most mesic coniferous forests (Grinnell et al. 1937; Bailey 1936) and are represented by four subspecies (Fig. 1). Of these subspecies, *M. a. humboldtensis* has received the greatest conservation attention (Zielinski and Golightly 1996) and was considered extirpated until 1996 when a single population was rediscovered in its historical range (Zielinski et al. 2001; Slauson 2003). *M. a. caurina* in coastal Oregon also appears to have declined, known only from two disjunct populations (Slauson and Zielinski 2004). The contemporary distributions of *M. a. caurina* in the Oregon Cascades, and *M. a. sierrae* in the California Cascade and Sierra Nevada mountains have remained relatively similar to their historical ranges except in the northern Sierra Nevada (Zielinski et al. 2005; Fig. 1).

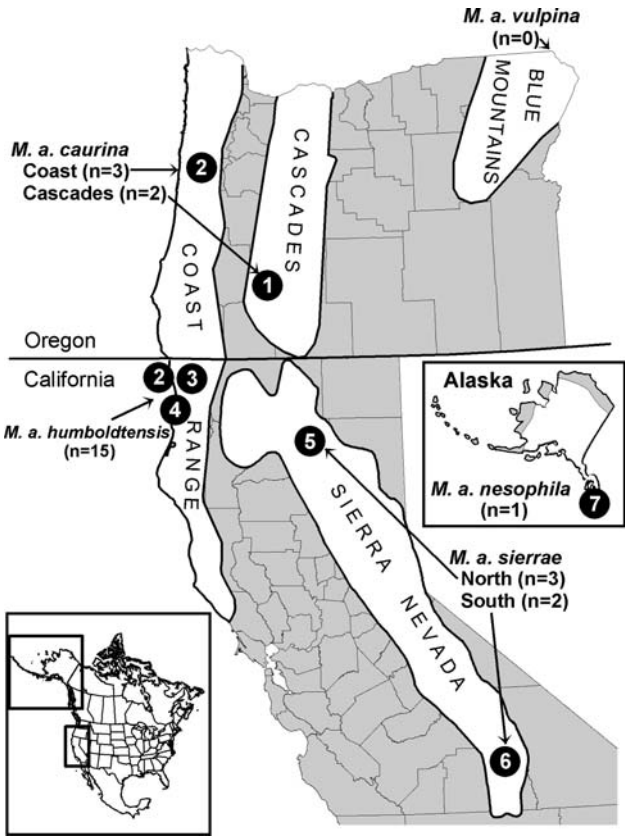

Fig. 1 Historical distribution of the American marten in Oregon and California (modified from Zielinski et al. 2001) and Alaska (Giblisco 1994). Solid black lines represent ranges of recognized subspecies. Thin black lines represent county lines. Numbers in solid black circles represent haplotypes from Table 1. The rediscovered population of M. a. h. is in the region under haplotype 3, all other subspecies sampling locations are under their single hapltoype numbers

The contemporary distribution of *M. a. vulpina* in the Blue mountains of northeastern Oregon is unknown.

Original subspecific designations were based on the comparisons of morphological and pelage characteristics and have been challenged (Hagmeier 1958, 1961) and defended (Dillon 1961). Additionally, several of the subspecies in California and Oregon have nearby boundaries (e.g., *M. a. sierrae*, *M. a. caurina*, and *M. a. humboldtensis*). Most interesting is the subspecific boundary between *M. a. humboldtensis* and *M. a. caurina*, drawn largely coincident with the California/Oregon state boundary (e.g., Grinnell et al. 1937; Bailey 1936; Fig. 1). We are aware of no contemporary or historical biogeographic barrier that would represent a boundary to north–south movement of martens and support the location of a subspecific boundary in this region.

Although the recently rediscovered population is within the boundaries of *M. a. humboldtensis* (Grinnell et al. 1937), it is on the eastern edge and its genetic affinity with martens that were originally described as *M. a. humboldtensis* by Grinnell and Dixon (1926) is unknown. One hypothesis is that the existing population has descended from the population originally described in 1926. Alternatively, the extant population may have been founded from dispersers from adjacent subspecies. Therefore, we compared mitochondrial DNA sequence diversity of contemporary martens from within the described ranges of *M. a. humboldtensis*, *M. a. caurina* and *M. a. sierrae*, with a 1927 museum specimen of *M. a. humboldtensis* representing the historic subspecies in order to clarify their genetic relationships.

Materials and methods

Sampling

Most source material from contemporary populations came from small (2 × 2 mm) pieces of tissue collected from ear margins of live-trapped or road-killed martens. Live-trapping occurred in three locations in California: (1) extreme northwestern California (Del Norte County, *M. a. humboltensis*) (2) northern Sierra Nevada (Lassen National Park, northern *M. a. sierrae*) (3) southern Sierra Nevada (Sequoia National Forest, southern *M. a. sierrae*; Table 1, Fig. 1). Live capture and chemical immobilization were carried out using standard protocols (Slauson 2003). Once chemically immobilized, a tissue sample was collected from pinna margin, which was then cauterized. Coastal Oregon samples were obtained from road killed individuals provided by the Oregon Department of Fish and Wildlife (ODFW) and Portland State University. All tissue samples were placed in a plastic microcentrifuge tube with silica desiccant and frozen prior to extraction. Contemporary samples from the southern Cascades of Oregon (*M. a. caurina*) and southeastern Alaska (*M. a. nesophila*; included as an out-group) were provided by trappers through ODFW and the Alaska Department of Fish and Game, respectively (Table 1, Fig. 1). Clippings of museum skins of *M. a. humboldtensis* were obtained from eight specimens at the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley.

DNA sequencing and data analysis

Whole genomic DNA was extracted from skin clippings using DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). DNA was extracted under a laminar flow hood, where UV lights were used to sterilize surfaces before and after extractions. Additionally, special care was used while extracting DNA from museum specimens by using new reagents. DNA amplification, PCR product purification,

Table 1 Population locations, subspecific designations according to Hall (1981), Genbank accession numbers, number of haplotypes (H#), frequency of haplotypes, and sample sizes (*n*) for mtDNA cytochrome *b*, tRNA-Pro and partial control region variation in American martens (*Martes americana*)

Population	County	Subspecies	Genbank accession #	H#	Frequency of haplotype							
					1	2	3	4	5	6	7	*n*
Coastal California	Del Norte	*humboldtensis* (presumed)	EU73287-EU873300	3	–	3	10	1	–	–	–	14
Coastal California (1927 museum skin)	Humboldt	*humboldtensis*	MVZ38869	1	–	1	–	–	–	–	–	1
Coastal Oregon	Lane	*caurina*	EU873302-EU873304	1	–	3	–	–	–	–	–	3
Southern Cascade Mountains of Oregon	Klamath	*caurina*	AF154972, AF448238, EU873305, EU873306	1	2	–	–	–	–	–	–	2
Southern Cascade Mountains of California	Shasta	*sierrae*	EU873307-EU873309	1	–	–	–	–	–	–	–	3
Southern Sierra Nevada of California	Tulare	*sierrae*	EU873310-EU873311	1	–	–	–	–	–	2	–	2
Southeastern Alaska	Kuiu Is.	*nesophila*	AY121318, AFTC17552	1	–	–	–	–	–	–	1	1
Total				7	2	7	10	1	3	2	1	26

sequencing and alignment of sequences were carried out using standard protocols (Fleming and Cook 2002). The following primer pairs amplified cyt *b* for most samples: MVZ4 and 5, 14 and 23, 16 (Smith and Patton 1993) and Marten37 (Demboski et al. 1999). Primers CYTB1F-CYTB4R (1F:5′CCGAGCTTGATATGAAAAACC3′, 1R:5′AGTATAGGCCYCGTCCRACGT3′, 2F:5′TACGGCTGAATTATCCGATACA3′, 2R:5′ACTGCTGCYAATGCTRAGA3′, 3F:5′ATCTGAGGAGGATTCTCGGTA3′, 3R:5′GGATCGYARGATTGCATATGC3′, 4F:5′CCCRCTCAACACRCCACC3′, 4R:5′GACCAAGGTAATCAATATACT3′) were designed and used to amplify shorter fragments of cyt b in the museum skin. Primers CTRL-L (Bidlack and Cook 2001) and TDKD (Kocher et al. 1993) were used to amplify tRNA-Pro and 263 base pairs of the 5′ end of the control region. Both forward and reverse strands were sequenced for each individual.

1428 base pairs were sequenced (1140 bp from cyt *b*, 25 bp from tRNA-Pro and 263 bp for the control region) from 26 *M. americana*. All DNA sequences were deposited in GenBank under accession numbers: EU873287-EU873312, AF154972, AF448238, AY121318 (Table 1). A minimum spanning tree (Kruskal 1956; Rohlf 1973) was constructed to display relationships among haplotypes using Arlequin (version 2.0; Schneider et al. 2000).

Results

Genetic variation among individuals was slight and distributed across both the cyt *b* gene (10 mutations) and control region (6 mutations). Of the 10 mutations in the coding cyt *b* gene, we observed: 7 third position synonymous transitions; 1 third position non-synonymous transversion coding for an amino acid located within the mitochondrial membrane; 1 first position non-synonymous transition coding for an amino acid located within the mitochondrial membrane but this mutation was functionally conservative; and 1 first position non-synonymous transversion coding for an amino acid located on the inner surface of the protein but this mutation was also functionally conservative. These results were expected for PCR amplifications of genuine mt cyt *b* (as opposed to a nuclear pseudogene). Inter-subspecific sequence divergence ranged from 0 to 1.0%; whereas, within subspecies, sequence divergence ranged from 0 to 0.4%. The number of haplotypes per location and per subspecies ranged from 1 to 3 (Table 1). Of the eight skins sampled from MVZ, only one sample (MVZ38869) was successfully sequenced. This 1927 museum skin possessed the second most common haplotype (#2; Table 1) which was shared across martens from coastal Oregon and coastal California, a region included within range maps for both *M. a. caurina* and *M. a. humboldtensis* (Fig. 1).

Two groups were apparent in the minimum spanning network (Fig. 2), one including the closely related haplotypes from *M. a. caurina* and *M. a. humboldtensis* (coastal California and Oregon samples) and another including haplotypes from *M. a. sierrae* and *M. a. nesophila* (interior California and Alaska samples). Individuals from these two groups share 6 fixed mutations, including both first position mutations in cyt *b*.

Discussion

Two distinct clades are evident, separated by 7 base pairs (0.5%); one containing samples from southern Cascade

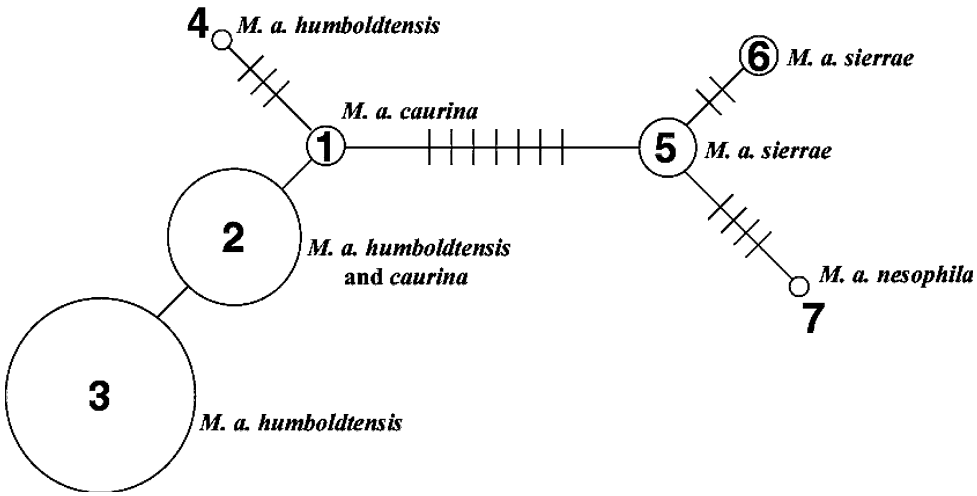

Fig. 2 Minimum spanning tree showing relationships among American marten haplotypes. Size of circle is proportional to the frequency of the haplotype (see Table 1 for specific values). Slash marks indicate the number of nucleotide substitutions found between haplotypes. No slash marks present indicates a single substitution separates haplotypes. Subspecific names listed refer to the location of the haplotype relative to the range of recognized subspecies. Numbers represent haplotypes from Table 1

Mountains of California, Sierra Nevada and southeastern Alaska and another including samples from coastal California, coastal Oregon and southern Cascades of Oregon. This substantial split supports the subspecific designation of *M. a. sierrae* versus *M. a. humboldtensis* and *M. a. caurina*, despite the relatively short geographic distances between these populations and lack of significant biogeographic barriers. At a minimum, this suggests that marten populations were not one large, genetically homogeneous population in the Pacific states. Past glaciations had profound genetic consequences on populations (Hewitt 1996). The relatively deep divergence between these clades, as indicated by the number of mutations and position (i.e., first), is presumably one of these consequences, suggesting divergence in separate glacial refugia. The close affinity of *M. a. nesophila* (southeastern Alaska) and *M. a. sierrae* (southern Cascades and Sierra Nevada mountains of California), to the exclusion of *M. a. caurina* (Oregon), indicates a possibly complex history of recolonization of northerly regions. Schwartz et al. (2007) similarly found that wolverines historically occupying the Sierra Nevada of California were more closely related to geographically distant populations than those in the western U.S.

Our results support the hypothesis that the population currently in coastal California (Del Norte County) represents descendants of a relictual population of martens that previously existed along the coast; supported by a common haplotype (#2) present in both the historical Humboldt marten museum skin of 1927 and coastal California and coastal Oregon populations but not found in the 8 individuals analyzed from outside of this region (Fig. 1). Further resolution of either the relationship of the historical museum skins or subspecific validity within the *M. a. caurina*/*M. a. humboldtensis* clade is hampered due to failure of 7 of 8 museum skins to amplify and small sample sizes from the coastal and Cascades populations of Oregon, respectively. Furthermore, because we are only using mtDNA, we are limited to examination of matrilineal patterns of genetic variation. The 2 unique haplotypes (#3, #4) present in the coastal California population is most likely due to increased sample size. Additional sampling in this region (coastal and Cascade mountains of Oregon) and the adjacent Salmon, Trinity and Marble mountains in the western extent of the range of *M. a. sierrae* may allow for a more detailed analysis of the region and mapping of haplotypes #3 and #4. Finally, museum samples should be re-analyzed to document the extent of genetic variation and distribution of haplotypes possessed by the historic population.

Despite the aforementioned sample size limitations, the occurrence of haplotype #2 in coastal Oregon and in coastal California populations, and its absence in the individuals from Oregon Cascades, suggest that coastal populations of California and Oregon are more similar to each other than to Oregon Cascades populations. While additional samples will be needed to confirm this relationship, it suggests the subspecific boundary between *M. a. humboldtensis* and *M. a. caurina* at the Oregon–California state border may not be valid and that *M. a. humboldtensis* may occur along the Oregon coast; this result would not be surprising due to the apparent absence of a biogeographical barrier in this region.

Our results support that marten populations currently located in the coastal forests of California and Oregon should be managed collectively to preserve the connectivity that our data suggest occurred historically. These populations are small and isolated due to natural distribution of suitable habitat, historical and contemporary effects of timber harvest, and historical effects of fur trapping (Zielinski et al. 2001; Slauson 2003). We have serious concerns about the viability of these coastal populations of martens. Maintaining and restoring suitable habitat to encourage population expansion as well as enhancing

functional connectivity between populations should benefit the marten populations in California and Oregon.

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