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Post-glacial colonization of northwestern North America by the forest-associated American marten (*Martes americana*, Mammalia: Carnivora: Mustelidae)

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Abstract

Phylogeographic patterns were used to assess intraspecific diversification of American martens (*Martes americana*). Within martens, two morphological groups (*americana* and *caurina*) have been recognized, though the level of distinction between them has been debated. We examined mitochondrial cytochrome *b* gene haplotypes from 680 martens to explore the colonization history of the Pacific Northwest and found two clades that correspond to the morphological groups. The widespread *americana* clade extends from interior Alaska south to Montana and eastward to Newfoundland and New England (i.e. northwestern, north-central and northeastern North America). The *caurina* clade occurs in western North America, minimally extending from Admiralty Island (southeastern Alaska) south to Oregon and Wyoming. Our data indicated two colonization events for the Pacific Northwest (one by members of each clade) and were consistent with the persistence of populations throughout past glacial periods in eastern and western refugia. Due to vegetational and geological history following the past deglaciation, we hypothesize that martens of the *caurina* clade spread along the North Pacific Coast, and into southeastern Alaska, earlier than martens of the *americana* clade. Mismatch distributions for the *americana* clade were indicative of populations that recently experienced demographic expansion, while mismatch distributions for the *caurina* clade suggested that populations were at equilibrium. These clades are reciprocally monophyletic and distinctive (interclade divergence ranged from 2.5 to 3.0% (uncorrected *p*), whereas, intraclade divergence was < 0.7%), and two regions of sympatry have been identified. Genetic signatures of past admixture in hybrid zones may have been extinguished during subsequent glacial periods when ranges contracted. This recurrent pattern of relatively restricted western, or Pacific coastal, lineages and more widespread eastern, or interior continental, lineages exists across broad taxonomic groups and suggests a shared biogeographical history.

Keywords: American martens, colonization, cytochrome *b*, *Martes*, Pacific Northwest, phylogeography

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Introduction

Our understanding of the dynamics of past movements and colonization of organisms traditionally has relied on the fossil record. Information on distributions gleaned from mammalian fossils is generally limited to taxonomic units at or above the level of species because sample sizes are seldom large enough to characterize geographical variation within species. However, molecular analyses applied within the framework of phylogeography (Avise 1994; Avise & Hamrick 1996) are providing further insight into the history of range expansions and contractions of many species (e.g. Wooding & Ward 1997; Bernatchez & Wilson 1998; Conroy & Cook 2000). DNA sequences have

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been crucial in identifying lines of descent both at the intra- and interpopulation levels (e.g. Gilbert *et al*. 1990; Craighead *et al*. 1995), reconstructing colonization histories (Wooding & Ward 1997), and even exploring temporal variation in effective population size (Rogers & Harpending 1992; Rogers 1995; Schneider & Excoffier 1999). Molecular (and morphological) studies of extant species, in combination with palaeoecology, may provide opportunities to test hypotheses related to the effects of dramatic fluctuations during Pleistocene ice ages on genetic diversity in extant populations (Hewitt 1996).

Morphological analyses of recent specimens (Wright 1953; Anderson 1970; Giannico & Nagorsen 1989) and fossils (Graham & Graham 1994) have investigated the history and taxonomy of American martens, *Martes americana*. Although 14 subspecies of *M. americana* have been described (Hall 1981), these are traditionally placed in two morphologically distinct groups (*americana* and *caurina*). The *americana* group is distributed from Montana and Idaho northward to Alaska and eastward to the Atlantic Coast, while the *caurina* group is described from parts of the West Coast (California to British Columbia), Wyoming, Montana and Idaho (Fig. 1, inset map; Wright 1953; Hall 1981; Carr & Hicks 1997).

Although several studies (e.g. Merriam 1890; Anderson 1970; Hall 1981; Clark *et al*. 1987; Carr & Hicks 1997) have corroborated the separation of *M. americana* into these two groups, the level of distinctiveness between them has been debated. Originally described as distinctive species based on morphology (Merriam 1890), Wright (1953) reports intergradation between the groups and suggests they were conspecific. Molecular data from Carr & Hicks (1997) compares the divergence of these two groups to that of three Palearctic species of *Martes*. Because levels of divergence are as great between the *americana* and *caurina* clades as levels are among these Palearctic species, Carr & Hicks (1997) conclude that *americana* and *caurina* should be recognized as distinct species, *Martes americana* and *M. caurina*. We expand on this work by documenting the extent of geographical variation in the mitochondrial cytochrome *b* (cyt *b*) gene across populations of this species from the Pacific Northwest. We place a particular focus on southeastern Alaska, where secondary contact between these groups has been hypothesized (Giannico & Nagorsen 1989).

Southeastern Alaska is a heterogeneous landscape that encompasses the vast Alexander Archipelago (2000+ islands) and adjacent mainland with deep fjords, glaciers, temperate rainforest and alpine habitats. These features and a dynamic glacial history during the Pleistocene have contributed to a highly fragmented flora and fauna. Numerous nominal species and subspecies are endemic to the region (MacDonald & Cook 1996; Cook & MacDonald 2001). Phylogeographic investigations have revealed distinct evolutionary lineages of ermine (*Mustela erminea*; Fleming & Cook 2002), dusky shrew (*Sorex monticolus*; Demboski *et al*. 1999), brown bear (*Ursus arctos*; Talbot & Shields 1996), black bear (*U. americanus*; Stone & Cook 2000) and long-tailed voles (*Microtus longicaudus*; Conroy & Cook 2000). This high degree of endemism and diversity of lineages suggests a complex colonization history for deglaciated areas within the Pacific Northwest (Cook *et al*. 2001).

The existence of ice-free refugia during full glacial advances in the Pacific Northwest has been debated (e.g. Demboski *et al*. 1999). During the past glaciation, the Cordilleran Ice Sheet, in combination with portions of the Laurentide Ice Sheet, covered most of southeastern Alaska, Yukon Territory and British Columbia (Cowan 1989). The large number of coastal endemic taxa combined with molecular and palaeontological investigations of plants, insects, fish and mammals suggest, however, that portions of the Alexander Archipelago of southeastern Alaska and Haida Gwaii (Queen Charlotte islands) of British Columbia may have remained devoid of ice (Kavanaugh 1980; Warner *et al*. 1982; Heusser 1989; O'Reilly *et al*. 1993; Heaton *et al*. 1996; Byun *et al*. 1997). The high degree of endemism and repeated pattern of intraspecific lineage diversity across taxa of the North Pacific Coast may be the result of the persistence of refugial populations in these ice-free areas (palaeoendemic), or secondary contact of populations that have recently expanded into the region (neoendemic). Thus far, fossils of purported palaeoendemics that span the periods of glacial maxima have not been identified (Heusser 1989).

Some investigators suggest that other species have tracked the northern expansion of forests into previously glaciated regions of North America following the Pleistocene. We examined genetic differentiation of North American martens to elucidate the colonization history of this medium-sized carnivore and to compare this data set with a growing body of evidence for common phylogeographic history across forest-associated species in the Pacific Northwest.

Materials and methods

DNA extractions, polymerase chain reaction and sequencing of the cytochrome b *gene*

DNA was extracted from marten tissues (heart, kidney, liver, spleen, skeletal muscle, skin, or blood) archived in the Alaska Frozen Tissue Collection of the University of Alaska Museum (AFTC). Methods for extracting, amplifying and sequencing DNA, and aligning sequences were carried out according to Lessa & Cook (1998) unless otherwise noted. Amplifications were in 50 L volumes containing 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 1.0 M of each primer, 1.25 units of Perkin-Elmer Ampli*Taq* DNA

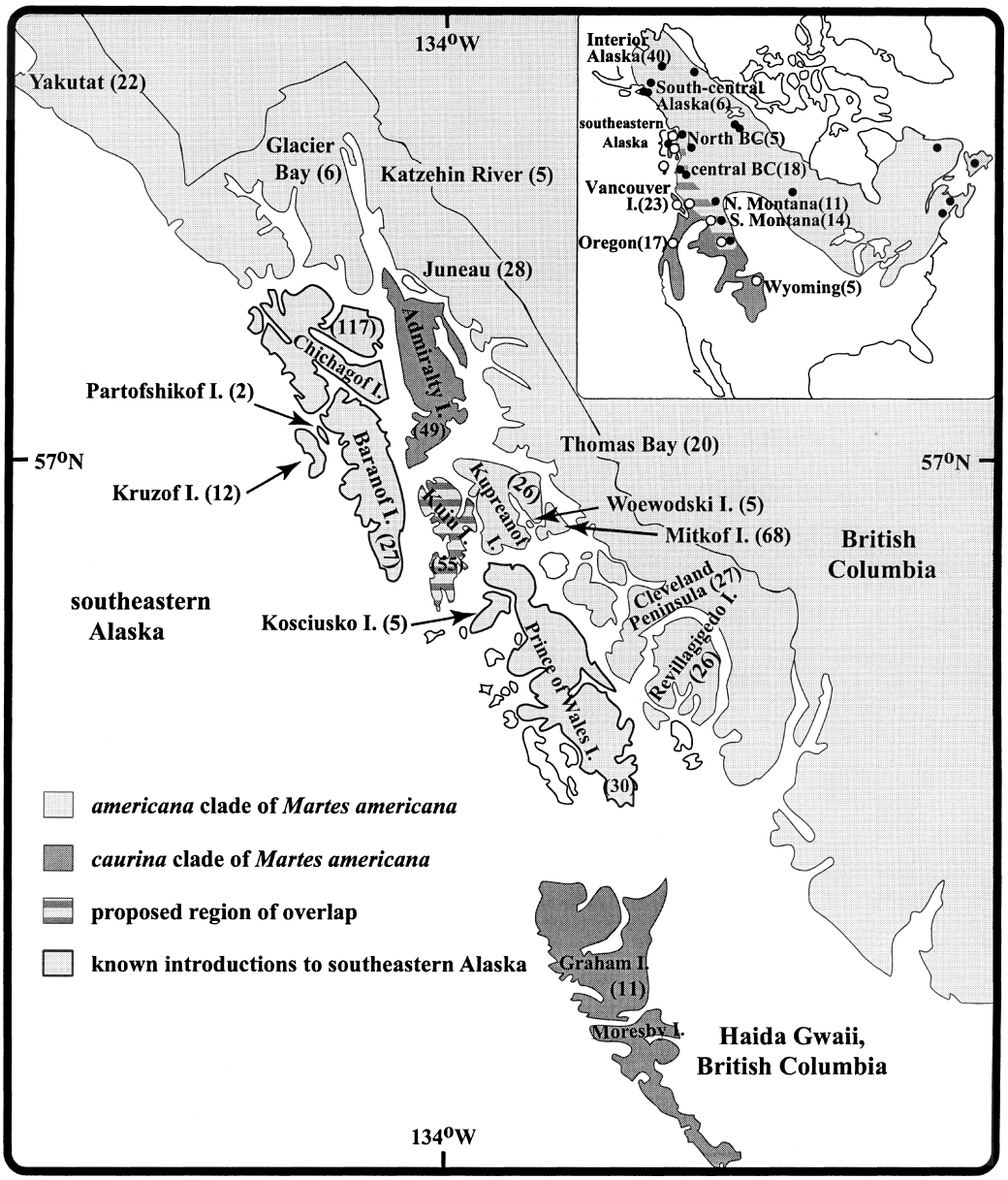

Fig. 1 Distribution of mitochondrial clades of American martens (*Martes americana*) in southeastern Alaska. Numbers in parentheses indicate sample sizes for locations analysed. Inset map shows the North American distribution of martens modified from Hall (1981) and plots sample localities from this study, Carr & Hicks (1997) and Hosoda *et al*. (1997). ● and ○ represent marten samples belonging to the *americana* and *caurina* clades, respectively.

polymerase, Perkin-Elmer 10× polymerase chain reaction (PCR) buffer and 1–100 ng whole genomic DNA. The mitochondrial (mt) marker, cyt *b*, was amplified using a Perkin-Elmer GeneAmp PCR System 2400 with the following PCR conditions: one cycle of 94 C for 45 s, followed by 35 cycles of denaturation at 94 C for 10 s, annealing at 45 C for 15 s, and an extension at 72 C for 45 s, followed by one cycle of 72 C for 3 min. Negative controls were included in each PCR experiment. The following primer pairs amplified cyt *b*: MVZ4 and 5, 14 and 23, 16 and Marten37 (Table 1). Both forward and reverse strands were sequenced for each individual.

A total of 680 American martens were examined. Partial cyt *b* sequences [441 base pairs (bp) using primers MVZ16/Marten37; corresponding to sites 14498–14938 of *Mus musculus*; Bibb *et al*. 1981] were generated from 151 *Martes americana*, complete cyt *b* sequences (1140 bp; corresponding to sites 14139–15282 of *Mus musculus*; Bibb *et al*. 1981) were generated from 30 *M. americana*, and restriction fragment length polymorphism (RFLP) profiles were

Table 1 Sequences and associated references for primers used to amplify the mitochondrial cytochrome *b* gene

Primer	Sequence (5′ to 3′)	Reference
MVZ4	GCAGCCCCTCAGAATGATATTTGTCCTC	Smith & Patton (1993)
MVZ5	CGAAGCTTGATATGAAAAACCATCGTTG	Smith & Patton (1993)
MVZ14	GGTCTTCATCTYHGGYTTACAAGAC	Smith & Patton (1993)
MVZ23	TACTCTTCCTCCACGAAACJGGNTC	Smith & Patton (1993)
MVZ16	AAATAGGAARTATCAYTCTGGTTTRAT	Smith & Patton (1993)
Marten37	TATATATACCCCGAAACATGGA	Demboski *et al*. (1999)

determined for the remaining 499 individuals (Appendix I). All DNA sequences were deposited in GenBank under accession numbers: AF154964–74, AF268272–4, AF448237–8, AY121187–93, and AY121195–AY121352. Complete cyt *b* sequences were generated from one European pine marten (*M. martes*; GenBank AF448239) and one sable (*M. zibellina*; GenBank AF448244) and used as outgroups.

Population level analyses — 441-bp cyt b (n = *181)*

All population level analyses were carried out using the 441-bp sequence fragments of cyt *b* for 181 martens and the software package ARLEQUIN (version 2.0; Schneider *et al*. 2000). A minimum spanning tree (Kruskal 1956; Rohlf 1973) was constructed to display relationships among unique haplotypes. Analysis of molecular variance (AMOVA) estimated levels of population structure at different geographical scales (Excoffier *et al*. 1992). Nucleotide diversity ($\pi \pm SD$) and haplotype diversity (h) were calculated for each population and clade, according to the formulae of Nei (1987). Mismatch distribution analyses (Schneider & Excoffier 1999) were performed for each clade to test for signatures of past population expansions. A sequence divergence estimate was calculated between individuals of the *americana* and *caurina* clades, according to Wilson *et al*. (1985). This method calculated a corrected average pairwise difference between individuals of the *americana* and *caurina* clades, $p_{AB(net)}$, by subtracting the average pairwise differences within populations (p_A and p_B) from the average pairwise difference between individuals of the two clades (p_{AB}); therefore, $p_{AB(net)} = p_{AB} - 0.5(p_A + p_B)$.

Phylogenetic analyses — 1140-bp cyt b (n = *30)*

Complete cyt *b* sequences were compared among 14 martens from southeastern Alaska (two mainland and 12 island samples), one from interior Alaska, seven from British Columbia (three mainland and four island samples), four from Montana, two from Oregon and two from Wyoming (Appendix I). Identical sequences for individuals from the same locality were removed resulting in a reduced data set of 22 sequences.

Relationships among sequences were examined using Phylogenetic Analysis Using Parsimony (PAUP*, version 4.0b3a; Swofford 1999). Phylogenetic trees were constructed using maximum parsimony (unweighted and transition/transversion weighting of 1/2, 1/5 and 1/10), maximum-likelihood, and neighbour-joining (Kimura two-parameter model of evolution; unweighted and transition/transversion weighting of 1/2, 1/5 and 1/10) methods. All searches produced trees with similar topologies therefore only the unweighted maximum parsimony analysis is shown. A strict consensus tree was generated from the four equally parsimonious trees that were constructed with a branch-and-bound search. Decay indices (Bremer 1988), reported as absolute number of steps, were computed using TREEROT (Sorenson 1996) for 100 bootstrap replicates, with maximum parsimony heuristic searches. Statistical support for the nodes of the strict consensus tree was assessed using the bootstrap (1000 replicates; Felsenstein 1985).

*RFLP profiles (*n = *499)*

We used RFLP analysis to document the geographical extent of the *americana* and *caurina* groups in the Pacific Northwest. A restriction enzyme (*Nla*III) that differentially digested PCR products from individuals of the americana and caurina clades was determined using DNA STRIDER 1.2 (written by C. Marck). A portion (approximately 830 bp) of the 3′ end of the cyt *b* gene and flanking region was amplified, using primers Marten37 and MVZ14, from 16 martens of known mt clades. A mixture of 9.0 L PCR product, 1.0 L New England Biolabs 10× buffer4, 0.10 L bovine serum albumin (10 mg/mL) and 0.2 L *Nla*III restriction enzyme (2 units) was placed in a 37 C incubator for 2–3 h. DNA fragments were visualized on a 1.5% agarose gel stained with ethidium bromide. After RFLP banding patterns were established for the divergent clades, we screened an additional 499 martens to determine clade profiles across 25 localities (Appendix I). Positive controls were included in each RFLP digestion.

Results

Population level analyses — 441-bp cyt b (n = *181)*

A minimum spanning tree (Fig. 2a) displays relationships among the 16 haplotypes (Table 2). Of the 27 populations,

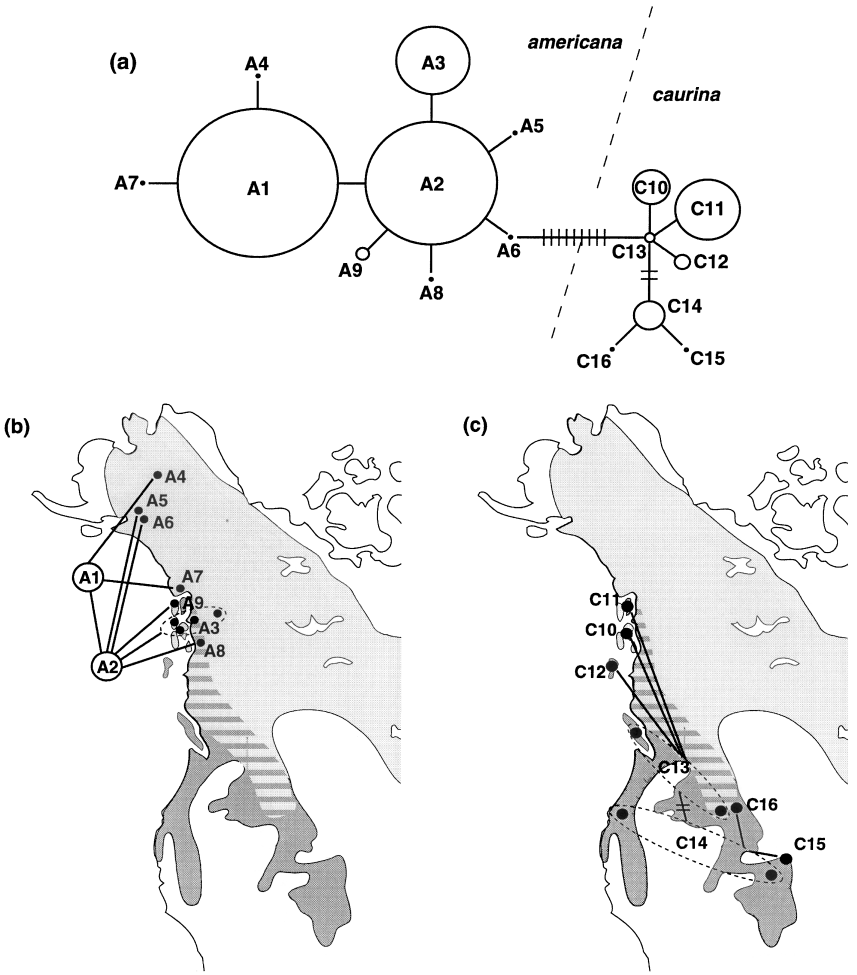

Fig. 2 (a) Minimum spanning tree showing relationships among American marten haplotypes. The circle size is proportional to the frequency of the haplotype (see Table 3 for specific values). Slash marks indicate the number of nucleotide substitutions found between haplotypes. No slash marks present indicates that a single substitution separates haplotypes. Haplotypes A1–A9 and C10–C16 are designated as belonging to the *americana* and *caurina* clades, respectively. These designations are based on the substantial division between the two clades of nine steps and analyses summarized in Fig. 3. (b) Map of western North America with the minimum spanning tree including haplotypes from the *americana* clade (see Table 3 for specific locations). Minimum spanning trees are overlaid upon the current distribution of marten (Hall 1981) with light and dark grey representing the *americana* and *caurina* clades, respectively. Haplotypes A1 and A2 were widespread throughout the mainland and many of the islands of the Alexander Archipelago. Circle size is not proportional to the frequency of the haplotypes. (c) Map of western North America with the minimum spanning tree including haplotypes from the *caurina* clade (see Table 3 for specific locations). Circle size is not proportional to the frequency of the haplotypes.

13 were characterized by a single haplotype, while 14 were represented by multiple haplotypes (Table 3). Haplotypes differed from common haplotypes by one or two nucleotide changes with the exception of a division of nine steps between the *americana* and *caurina* clades (Fig. 2a). The *americana* and *caurina* clades had nine and seven haplotypes, respectively (Fig. 2 and Table 3). AMOVA results confirmed the distinctive *americana–caurina* split with 88.9% of the variation accounted for between populations of the two clades. Only 7.9% of the variation was partitioned among populations within clades, and 3.2% of the variation was found within populations.

Two haplotypes from the *americana* clade (A1 and A2) were widespread throughout the mainland and many of the islands of the Alexander Archipelago (Table 3). A third haplotype from the *americana* clade (A3) was found in northern British Columbia, and the mainland and four islands of southeastern Alaska. All other haplotypes from the *americana* clade were unique to a single population and differed from either A1 or A2 by one nucleotide (Fig. 2). Haplotypes from the *caurina* clade were less widespread and more restricted to individual populations (Table 3). Of the seven populations possessing *caurina* haplotypes, five had unique haplotypes (Table 3).

Of the 27 populations, 20 are represented by only *americana* haplotypes, five populations are characterized by only *caurina* haplotypes, and two populations are represented by both *americana* and *caurina* haplotypes. Not surprisingly, nucleotide diversity (π) is highest for these two latter populations (Montana and Kuiu Island, southeastern Alaska; Table 3). Several populations had low or no nucleotide diversity (Table 3). Overall haplotype diversity (h) was 0.83 but ranged from 0.00 to 1.00 for individual populations (Table 3).

The mismatch distribution of observed number of differences between pairs of haplotypes was unimodal for the *americana* clade, possibly suggesting that members of this clade have recently passed through a demographic expansion (Rogers & Harpending 1992). However, the mismatch distribution for the *caurina* clade was bimodal — an indication of demographic equilibrium. At the 95% confidence level, ranges for ancestral (θ_0) and current (θ_1) theta values did not overlap for the *americana* clade (θ_0 = 0.000–1.019; θ_1 = 6.357–4830.938). Similarly, but less

Table 2 Condensed dot matrix displaying variable sites of the mtDNA cytochrome *b* gene in 181 American martens (*Martes americana*)

	Nucleotide position																				
Haplotype	438	445	447	468	477	480	528	565	569	576	577	585	603	618	713	722	732	770	777	783	792
A1	T	T	G	C	T	T	G	A	C	A	G	T	C	T	T	T	A	C	C	A	G
A2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·
A3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	T	·	·
A4	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·
A5	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·
A6	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	A
A7	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·
A8	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	T	·	·	·
A9	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·
C10	·	C	A	·	C	C	·	·	T	G	·	A	T	·	C	·	·	T	·	G	A
C11	·	C	A	T	·	C	·	·	T	G	·	A	T	·	C	·	·	T	·	G	A
C12	·	C	A	·	·	C	·	·	T	G	·	A	T	·	C	·	·	·	·	G	A
C13	·	C	A	·	·	C	·	·	T	G	·	A	T	·	C	·	·	T	·	G	A
C14	·	C	A	·	·	C	A	G	T	G	·	A	T	·	C	·	·	T	·	G	A
C15	·	C	A	·	·	C	A	G	T	G	·	A	T	C	C	·	·	T	·	G	A
C16	·	C	A	·	·	C	A	G	T	G	·	A	T	·	C	·	G	T	·	G	A

Haplotype names are shown on the left, and nucleotide positions are displayed at the top with position 1 representing the first nucleotide of the gene. Dots within the matrix represent identical nucleotides to the reference sequence. Haplotypes A1–A9 and C10–C16 are designated as belong to the *americana* and *caurina* clades, respectively. These designations are based on analyses summarized in Figs 2 and 3.

disparately, θ_0 and θ_1 estimates for the *caurina* clade did not overlap (θ_0 = 0.000–1.679; θ_1 = 2.755–4669.431). These results indicated that populations within the clades have expanded in the recent past, particularly within the *americana* clade. However, these analyses may be confounded by the limited levels of intraclade variation (maximum of four nucleotide differences between the most distant haplotypes within each clade).

The corrected average pairwise difference between individuals of the *americana* and *caurina* clades was 10.4 nucleotides (or 2.4%). If we assume a divergence rate of 2.5% per million years per pair of clades, as in Carr & Hicks (1997) and Carr & Hughes (1993), then individuals of the *americana* and *caurina* clades diverged approximately 1 million years ago. However, this estimate is imprecise due to the method of calibration and other sources of error (see Stone & Cook, 2002).

Phylogenetic analyses — 1140-bp cyt b (n = *30)*

Base composition (A = 28.0%, C = 31.0%, *G* = 14.5%, T = 26.5%) for cyt *b* was consistent with that of other mammals (e.g. Irwin *et al*. 1991; Talbot & Shields 1996; Stone & Cook 2000). A linear relationship (R^2 = 0.945) between third-position transitions and uncorrected *p* distances calculated for the genus *Martes* (data not shown) indicated that saturation has not been attained.

Four equally parsimonious trees (112 steps with 65 informative characters) displayed two reciprocally monophyletic clades corresponding to the *americana* and *caurina* morphological groups. Two subclades within the *caurina* clade (Fig. 3) were also apparent. Divergence between clades ranged from 2.5 to 3.0% (uncorrected *p*), whereas, intraclade divergence was < 0.5% and < 0.7% for the *americana* and *caurina* clades, respectively.

For the complete cyt *b* gene, 27 nucleotide sites (26 transitions and one transversion) differed between the *americana* and *caurina* clades (five first-position, two second-position and 20 third-position transitions). The single transversion (third-position) did not result in an amino acid change; however, three of the transitions (one first-position and both second-position) coded for different amino acids. All three amino acid differences corresponded to hypervariable residues previously identified in a cyt *b* model (Irwin *et al*. 1991).

Four nucleotide sites differed between the two subclades within *caurina* (one first- and three third-position transitions). Three third-position transitions were synonymous, whereas, the first-position transition resulted in an amino acid change. This nonsynonymous change occurred in the trans-membranous region of the protein. These results were expected for PCR amplifications of genuine mt cyt *b* (as opposed to a nuclear pseudogene).

*RFLP profiles (*n = *499)*

Restriction enzyme digestion of the amplified fragment of cyt *b* from martens of the *caurina* clade resulted in three smaller fragments; whereas, fragments from martens of the *americana* clade remained uncut and, therefore, maintained

Table 3 Population locations, number of haplotypes (H#), nucleotide diversity ($\pi \pm SD$), haplotype diversity (h), frequency of haplotypes, and sample sizes (n) for mtDNA cytochrome *b* variation in American martens (*Martes americana*)

				Frequency of haplotype																
				americana haplotypes									*caurina* haplotypes							
Population	H#	$\pi \pm SD$	h	A1	A2	A3	A4	A5	A6	A7	A8	A9	C10	C11	C12	C13	C14	C15	C16	n
Interior Alaska	3	0.0013 ± 0.0013	0.51	7	2	·	1	·	·	·	·	·	·	·	·	·	·	·	·	10
South-central Alaska	3	0.0018 ± 0.0018	0.70	·	3	·	·	1	1	·	·	·	·	·	·	·	·	·	·	5
Northern British Columbia	3	0.0023 ± 0.0021	0.70	3	1	1	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Central British Columbia	2	0.0014 ± 0.0014	0.60	3	3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	6
Montana	4	0.0197 ± 0.0138	1.00	1	1	·	·	·	·	·	·	·	·	·	·	1	·	·	1	4
Yakutat, SE AK	1	0.0000 ± 0.0000	0.00	·	5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Glacier Bay, SE AK	2	0.0011 ± 0.0014	0.50	3	·	·	·	·	·	1	·	·	·	·	·	·	·	·	·	4
KatzehinRiver, SE AK	1	0.0000 ± 0.0000	0.00	·	5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Juneau, SE AK	2	0.0014 ± 0.0015	0.60	3	2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Thomas Bay, SE AK	3	0.0023 ± 0.0021	0.70	1	1	3	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Cleveland Peninsula, SE AK	3	0.0023 ± 0.0021	0.70	3	1	·	·	·	·	·	1	·	·	·	·	·	·	·	·	5
Chichagof I., SE AK	3	0.0013 ± 0.0012	0.51	1	10	·	·	·	·	·	·	4	·	·	·	·	·	·	·	15
Baranof I., SE AK	1	0.0000 ± 0.0000	0.00	·	·	10	·	·	·	·	·	·	·	·	·	·	·	·	·	10
Kruzof I., SE AK	1	0.0000 ± 0.0000	0.00	·	·	5	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Partofshikof I., SE AK	1	0.0000 ± 0.0000	0.00	·	·	2	·	·	·	·	·	·	·	·	·	·	·	·	·	2
Kupreanof I., SE AK	1	0.0000 ± 0.0000	0.00	·	2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	2
Mitkof I., SE AK	2	0.0014 ± 0.0015	0.60	3	2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Woewodski I., SE AK	1	0.0000 ± 0.0000	1.00	1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1
Kuiu I., SE AK	4	0.0144 ± 0.0079	0.67	5	3	3	·	·	·	·	·	·	12	·	·	·	·	·	·	23
Revillagigedo I., SE AK	1	0.0000 ± 0.0000	0.00	5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Prince of Wales I., SE AK	2	0.0008 ± 0.0010	0.35	8	2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	10
Kosciusko I., SE AK	1	0.0000 ± 0.0000	0.00	5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5
Admiralty I., SE AK	1	0.0000 ± 0.0000	0.00	·	·	·	·	·	·	·	·	·	·	21	·	·	·	·	·	21
Graham I., B.C.	1	0.0000 ± 0.0000	0.00	·	·	·	·	·	·	·	·	·	·	·	5	·	·	·	·	5
Vancouver I., B.C.	1	0.0000 ± 0.0000	0.00	·	·	·	·	·	·	·	·	·	·	·	·	2	·	·	·	2
Oregon	1	0.0000 ± 0.0000	0.00	·	·	·	·	·	·	·	·	·	·	·	·	·	6	·	·	6
Wyoming	2	0.0009 ± 0.0012	0.40	·	·	·	·	·	·	·	·	·	·	·	·	·	4	1	·	5
americana clade	10	0.0021 ± 0.0016	0.69	52	43	24	1	1	1	1	1	4	0	0	0	0	0	0	0	128
caurina clade	6	0.0041 ± 0.0027	0.76	0	0	0	0	0	0	0	0	0	12	21	5	3	10	1	1	53
Total	16	0.0125 ± 0.0067	0.83	52	43	24	1	1	1	1	1	4	12	21	5	3	10	1	1	181

Haplotypes A1–A9 and C10–C16 are designated as belong to the *americana* and *caurina* clades, respectively. These designations are based on analyses summarized in Figs 2 and 3.

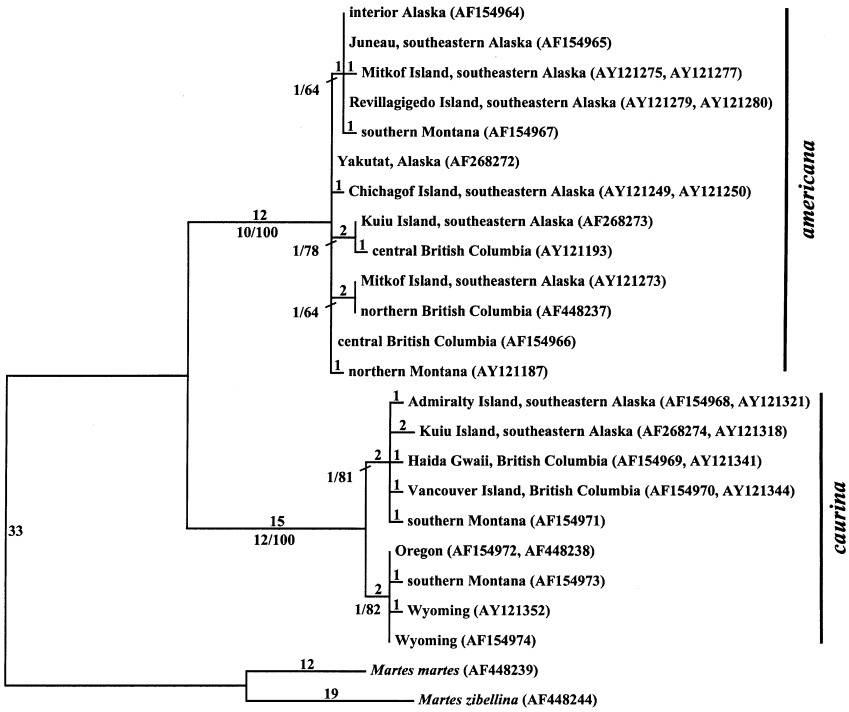

Fig. 3 Strict consensus tree of four equally parsimonious trees (length = 112 steps; CI = 0.9107; RI = 0.9669) generated from complete cytochrome *b* gene sequences of American martens (*Martes americana*) with a branch-and-bound search. Branch lengths are shown above branches, and Bremer decay indices/bootstrap values are below branches. GenBank accession numbers are in parentheses after taxon names.

only a single fragment. RFLP analysis identified 413 *americana* and 86 *caurina* haplotypes (Appendix I). These larger sample sizes allowed us to map more confidently the spatial extent of these two clades (Fig. 1). The widespread *americana* extends from interior Alaska south to Montana and eastward to Newfoundland and New England. The eastern distribution of *americana* is based on the work of Carr & Hicks (1997) and Hosoda *et al*. (1997). The *caurina* clade minimally extends from Admiralty Island, southeastern Alaska south to Oregon and Wyoming (Fig. 1, inset map). Two regions of sympatry were identified, Montana and Kuiu Island, southeastern Alaska. Northern Montana samples (n = 11) consisted of strictly *americana* haplotypes; whereas, southern Montana samples were comprised of two *americana* haplotypes and 12 *caurina* haplotypes. Samples from Kuiu Island consisted of 33 *americana* and 22 *caurina* haplotypes.

Discussion

Glacial refugia and intraspecific differentiation

Mengel (1964) proposed that vicariant events, due to repeated glaciations during the Pleistocene, initiated speciation in North American warblers (Parulidae). This idea has been extended to several forest-associated species, including northern flying squirrels (*Glaucomys sabrinus*), tree squirrels (*Tamiasciurus douglasii*, *T. hudsonicus*), southern red-backed voles (*Clethrionomys gapperi*), black bears (*Ursus americanus*), and chickadees [*Poecile* (*Parus*) *hudsonica*, *P. rufescens*] (Wooding & Ward 1997; Arbogast & Kenagy 2001; Cook *et al*. 2001). A recurrent pattern of relatively restricted western, or Pacific coastal, lineages and more widespread eastern, or interior continental lineages, exists across broad taxonomic groups and suggests a shared biogeographical history.

American martens also show two distinct clades in western North America. Bootstrap support and decay indices highly supported the recognition of distinct clades (Fig. 3), and a total of 88.9% of the molecular variation is partitioned between populations of the populations of the americana and caurina clades, *americana* and *caurina*. These results corroborate and expand the findings of Carr & Hicks (1997). The *americana* and *caurina* clades differed by 2.5–3.0% (uncorrected *p*) overall sequence variation with intraclade variation < 0.7%. This level of variation reflects approximately 1 million years since divergence. We hypothesize that this divergence was accumulated through allopatry spanning several glacial cycles, as has been proposed for several taxa (Klicka & Zink 1997; Avise & Walker 1998; Avise *et al*. 1998).

Wooding & Ward (1997) propose that the existence of eastern and western forest refugia in North America during past glacial advances would account for two highly divergent clades of black bears. During much of the last 120 000 years, they contend these segregated forests formed a barrier to dispersal for other forest-associated species. While ice sheets were receding, eastern forests apparently expanded more rapidly than western forests (Williams *et al*. 1993), and therefore, populations representing the eastern clade of black bears expanded more extensively

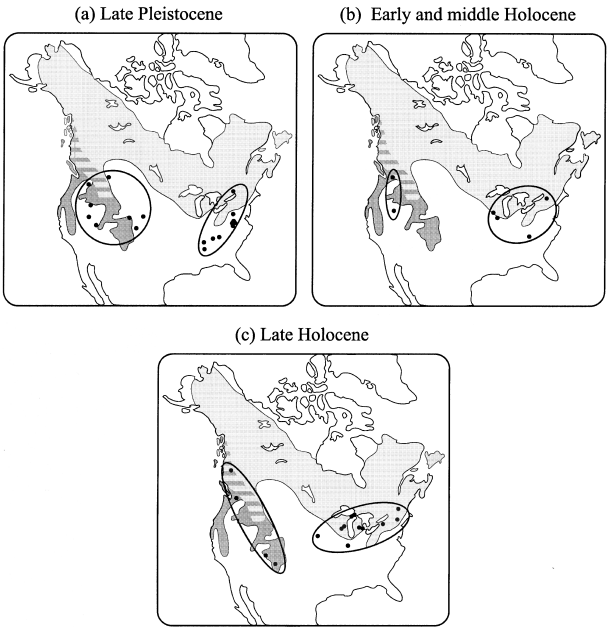

Fig. 4 Figure modified from Graham & Graham (1994) of fossil records of American martens, *Martes americana*, from (a) late Pleistocene, (b) early/middle Holocene, and (c) late Holocene. Fossil records are overlaid upon the current distribution of marten (Hall 1981) with light and dark grey representing the *americana* and *caurina* clades, respectively. Lines encircle fossil records hypothesized to belong to the *americana* clade in eastern and the *caurina* clade in western North America.

across northern North America than populations representing the western clade.

American martens show a pattern of sequence divergence and geographical diversification similar to that of black bears (Wooding & Ward 1997; Stone & Cook 2000), with relatively large interclade and small intraclade differences. Two morphologically defined groups of martens correspond to the reciprocally monophyletic clades identified with genetic analyses. Similarly, the distribution of late Pleistocene–late Holocene fossil records of martens also supports the hypothesis of separate forest refugia since the last (Wisconsin) glaciation (Fig. 4). Furthermore, the phylogeographic pattern in martens is consistent with Hoffmann's idea that taxa from large refugia in southeastern North America expanded into a larger area after the past glaciation than did taxa from smaller western refugia (Hoffmann 1985).

Broad correspondence in sequence divergence and geographical distribution of eastern and western clades in martens, black bears and other taxa (e.g. *Glaucomys sabrinus*, Arbogast 1998; *Parus hudsonicus* and *P. rufescens*, Gill *et al*. 1993; *Dendroica coronata* and *D. auduboni*, Bermingham *et al*. 1992) implicates similar vicariant events. Although we cannot effectively address the rate or timing of movement of these taxa at this time, these data suggest comparable colonization routes across diverse taxonomic units. Several taxa tend to overlap spatially (Clementsian manner) as opposed to exhibiting independent colonizations (Gleasonian), a pattern also found for some North American rodents (Riddle 1996). These concordant phylogeographic patterns seem to falsify the suggestion of Graham *et al*. (1996; p. 1605) that 'species that make up mammal communities in the contiguous United States have responded to late Quaternary environmental fluctuations in a Gleasonian manner.'

Relatively high sequence divergence indicated that vicariance between the two clades extended deeper than the last glaciation as suggested for other carnivores (e.g. 5.0% control region variation = 3.3% cyt *b* variation for black bears = 1.8 ± 0.8 million years since divergence, Wooding & Ward 1997; Stone & Cook 2000) and many other taxa (Klicka & Zink 1997; Avise *et al*. 1998). Estimates of divergence may be heavily influenced, however, by a variety of factors such as levels of ancestral polymorphisms (Edwards & Beerli 2000). Populations of the divergent clades may have come into secondary contact multiple times over the past million years during interglacial periods (e.g. Leonard *et al*. 2000), but we detected no genetic signature of past contact (e.g. individuals with divergent *americana* mitochondrial DNA located in western United States). Limited genetic admixture, occurring during the repeated northward expansions of these populations during interglacial periods, may have been eliminated by subsequent population retractions during glacial advances (Hewitt 1996).

Within the *americana* clade, little to no geographical structure was present among populations (Table 3, Figs 2 and 3). Three haplotypes (A1–3) were relatively widespread and occurred at high frequencies; whereas, the remaining six *americana* haplotypes (A4–9) were each found only in one population and occurred in low frequencies (Table 3, Fig. 2b). The unimodal mismatch distribution for this clade was indicative of a population that experienced demographic expansion (Rogers & Harpending 1992). Ancestral (θ_0) and current (θ_1) theta values were also consistent with expansion in the recent past.

Within *caurina*, several haplotypes were confined to single populations (Table 3, Figs 2c and 3). Within this clade (Fig. 3), subclade divergence apparently was initiated by the mid-Pleistocene during separation into distinctive refugial populations in western North America. The slight variation within each subclade (Fig. 3) may reflect divergence since the last glaciation. Our data were consistent with a glacial refugium along the North Pacific Coast; however, if hypotheses regarding the locations of refugia are to be critically tested with genetic data, more extensive sampling from throughout the range of the *caurina* clade should be investigated with multiple independent loci.

The mismatch distribution for the *caurina* clade was bimodal — more indicative of a population at demographic equilibrium. However, ancestral (θ_0) and current (θ_1) theta values indicated that this population may have expanded in the recent past. Again, we caution that the mismatch analysis is highly confounded by the limited levels of intraclade variation found in the cyt *b* gene. Additional molecular data from more rapidly evolving portions of DNA (e.g. mitochondrial DNA control region) are needed to test these findings.

Colonization history of a forest-associated mammal and contact zones

The close relationship of martens with late-successional forests has been compared to that of spotted owls (*Strix occidentalis*) and red-cockaded woodpeckers (*Picoides borealis*) (Thomas *et al*. 1988; Thompson 1991). Dependence of martens on overhead tree cover and an associated complex ground structure (Buskirk & Powell 1994) is consistent with an expected correspondence between its range expansion and that of forests. Lodgepole pine, *Pinus contorta*, was established as early as 10 500 years before present (BP) along the southeastern Alaskan coast, but establishment on the inland (eastern) side of the Coast Mountain Range did not occur until about 2300 BP (Peteet 1991). Mathewes (1989) and Fedje & Josenhans (2000) suggest the arrival of coniferous trees as early as 12 200 BP to the coastal region just south of southeastern Alaska (Haida Gwaii of British Columbia). The coastal corridor may have been broader due to a lower sea level at that time (Barrie *et al*. 1993; Fedje and Josenhans 2000). Because we expected a general correlation between range expansion of vegetation and associated animals (Hewitt 1996), the ice-free, coastal corridor may have served as a route for forest-associated species, such as martens, to colonize the coast from a southerly refugial population (MacDonald & Cook 1996).

Due to this forest association and the geographical proximity of purported western forest refugia to the North Pacific Coast, we hypothesize that the individuals belonging to the *caurina* (coastal) clade colonized along the North Pacific Coast (including southeastern Alaska) earlier than individuals from the more widespread *americana* clade (see Fig. 4C). We suspect that individuals representing the *americana* clade colonized the coastal region during the Holocene when recession of the Cordilleran Ice Sheet would have allowed western movement through low elevation passes and rivers that transect the Coast Range.

Individuals of the *caurina* clade extend northward to Admiralty Island, and are not found in interior Alaska. This pattern is repeated among several forest-associated taxa (MacDonald & Cook 1996). The Pacific coastal, or western, clade is often restricted to the coast and does not extend farther north than southeastern Alaska but the interior continental, or eastern, clade projects across northern North America into interior Alaska (see Cook *et al*. 2001 and Arbogast & Kenagy 2001 for examples). This common pattern among several taxa suggests that similar forces have shaped current distributions, such as barriers to dispersal north of southeastern Alaska. Alternatively, the pattern may be due to competition among individuals of the two clades, whereby, individuals from one clade are able to outcompete individuals from another clade under certain ecological circumstances. These are suppositions and should be investigated with future work.

Admiralty, Kuiu and Graham (Haida Gwaii) islands supported only *caurina* haplotypes (Table 3 and Fig. 3) suggesting that these populations have been isolated. Although haplotypes were unique, differentiation was minimal (one or two mutations) suggesting differentiation within the Holocene and supporting Giannico & Nagorsen's (1989) idea that the distinct phenotype of martens from Haida Gwaii (Queen Charlotte islands) evolved very recently. Martens carrying *americana* haplotypes apparently followed the westward progression of the eastern refugial forest. This colonization could have resulted in the shared occurrence of *americana* haplotypes on the mainland and near-shore islands (Table 3 and Figs 2b and 3).

The limited distribution of the *caurina* clade to several islands may be partially the result of genetic swamping of this clade elsewhere by the *americana* clade following its later arrival to the coast. When gene flow is relatively high between two taxa, interbreeding may cause outbreeding depression or even extinction via hybridization or genetic assimilation (Ellstrand 1992). Extensive sampling in southeastern Alaska revealed only one region of contact, Kuiu Island, and we suspect this area was colonized recently by individuals of the *americana* clade as a result of island hopping across Mitkof and Kupreanof islands (peninsular effect) due to shallow water channels (Fig. 1). Other typically mainland species show a similar distribution (MacDonald & Cook 1996). Possible introgression and/or genetic swamping of *caurina* by *americana* individuals should be investigated using a combination of mitochondrial and nuclear markers, and possibly an experimental breeding programme. Individuals of the *caurina* clade may persist on Admiralty, Graham (Haida Gwaii) and Vancouver because these islands are sufficiently isolated from individuals of the *americana* clade. Only one other region of contact, southern Montana, was revealed in this study, though that contact zone presumably extends from Montana northwest through British Columbia (also see Wright 1953).

Human introductions of martens

In the 1930s, introductions of martens were made by the Alaska Game Commission to Baranof and Prince of Wales

islands followed by the introduction of martens to Chichagof Island during 1949–52. These transplantations were made without knowledge of the underlying morphological and genetic variation that exists across the region (Elkins & Nelson 1954; Burris & McKnight 1973; MacDonald & Cook 1996). Marten populations were thought to not exist on these islands before introductions, but this presumption was questioned due to the rapid increase in numbers on Prince of Wales Island following transplantation (Elkins & Nelson 1954). Extensive sampling of these introduced populations (e.g. Chichagof Island, $n = 117$) suggested that the individuals representing the *americana* clade had been the sole source of these introductions. Giannico & Nagorsen's (1989) morphological assessment of samples from Baranof and Chichagof islands indicated that these populations belong to the *americana* clade. Our data corroborated their findings. However, contrary to their conclusion that *americana* was found exclusively throughout the region, our extensive sampling indicated that some individuals (martens from Admiralty Island and some martens from Kuiu Island) also belong to the *caurina* clade.

Our analyses provided no indication that martens existed on Chichagof, Baranof, or Prince of Wales islands prior to introductions, but this conclusion may be premature because it was derived from a mitochondrial gene that may not effectively detect genetic swamping. Additional nuclear markers should be used to test this hypothesis (see for example, Paetkau *et al*. 1998). If the islands were naturally colonized first by individuals of the *caurina* clade, perhaps when sea levels were lower at the end of the Pleistocene, then the persistent populations of *caurina* on Admiralty and Kuiu islands may be remnants of a previously widespread clade across the archipelago. Although the disjunct distribution of the *caurina* clade (i.e. *caurina* on Admiralty and Kuiu islands) may conversely appear to be the result of introductions, we doubt this is the case because both populations have fixed, unique haplotypes unlike those from documented population introductions on Chichagof and Baranof islands.

The genetic substructure, displayed by marten populations along the North Pacific Coast, is consistent with independent colonizations of the region by representatives of the two divergent clades. These data further exemplify the need to develop a historical framework for the biota of a region through extensive sampling, if we are to hope to effectively understand and manage the complexities associated with environmental change (Wilson 2000).

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This work comprises a portion of the PhD dissertation of Karen Stone on molecular evolution of martens (genus *Martes*). The study was conducted at the University of Alaska Fairbanks in the laboratory of Joseph Cook, whose interests lie in evolution and conservation of mammals. Karen Stone is currently an assistant professor of biology at Southern Oregon University and is conducting mammalian research. Joseph Cook is now a professor of biology at Idaho State University. Rodney W. Flynn is a research wildlife biologist with the Alaska Department of Fish and Game and project leader for marten ecological studies in southeastern Alaska.

Appendix I

Collection locations, clade profiles, molecular methods used and voucher number for *Martes americana* specimens

Locality*	Clade	Method(s)†	Alaska Frozen Tissue Collection number
Chichagof Island, SE AK	*americana*	full cyt *b*	10755–6
Chichagof Island, SE AK	*americana*	441-bp cyt *b*	10758–60, 14524–6, 14540, 14550, 14553, 19996–7, 30673–4
Chichagof Island, SE AK	*americana*	R	10761–2, 14495–512, 14514–42, 14544–75, 15999, 16000, 16067–70, 19889–97, 19964–74, 19996–8
Baranof Island, SE AK	*americana*	441-bp cyt *b*	19902–3, 19907, 19916, 19918, 19934, 19975–8
Baranof Island, SE AK	*americana*	R	19908–11, 19917, 19919–22, 19926, 19929–33, 19935–6
Kruzof Island, SE AK	*americana*	441-bp cyt *b*	19904–6, 19913, 19923
Kruzof Island, SE AK	*americana*	R	19914–5, 19924–5, 19927, 24019–20
Partofshikof Island, SE AK	*americana*	441-bp cyt *b*	19912, 19928
Kupreanof Island, SE AK	*americana*	441-bp cyt *b*	10823, 16027
Kupreanof Island, SE AK	*americana*	R	20074, 20081, 24440, 24442–4, 24448, 24498–9, 24527–30, 24533, 24539–43, 24550–4
Mitkof Island, SE AK	*americana*	full cyt *b*	10829–30, 10832
Mitkof Island, SE AK	*americana*	441-bp cyt *b*	10831, 14475
Mitkof Island, SE AK	*americana*	R	14476–90, 14492–4, 16028–32, 16034–53, 16057, 19937–40, 19947–61
Woewodski Island, SE AK	*americana*	441-bp cyt *b*	10822
Woewodski Island, SE AK	*americana*	R	20075–6, 24416–7
Kuiu Island, SE AK	*americana*	full cyt *b*	17541
Kuiu Island, SE AK	*americana*	441-bp cyt *b*	17534, 17536–9, 17543, 17546, 17549, 17551, 19888
Kuiu Island, SE AK	*americana*	R	24471, 24473–4, 25302, 25304–6, 25310–15, 25318, 25324–6, 25328–9, 25332–4
Prince of Wales Island, SE AK	*americana*	441-bp cyt *b*	10665–8, 10670, 10673, 10678–9, 10684–5
Prince of Wales Island, SE AK	*americana*	R	14629–32, 15903, 15909–12, 15917–8, 15924–6, 15940, 15942, 15948, 15977, 15980, 15997
Kosciusko Island, SE AK	*americana*	441-bp cyt *b*	15904–8
Revillagigedo Island, SE AK	*americana*	full cyt *b*	10707–8
Revillagigedo Island, SE AK	*americana*	441-bp cyt *b*	10709, 10711–2
Revillagigedo Island, SE AK	*americana*	R	10690–4, 10710, 10713–5, 10721–2, 10724, 10726–7, 14634–5, 14639–43
Yakutat, SE AK	*americana*	full cyt *b*	10769
Yakutat, SE AK	*americana*	441-b cyt *b*	10770–3
Yakutat, SE AK	*americana*	R	10774–89, 24454
Glacier Bay, SE AK	*americana*	441-bp cyt *b*	10848–51, 14628
Glacier Bay, SE AK	*americana*	R	19990
KatzehinRiver, SE AK	*americana*	441-bp cyt *b*	14591–5
KatzehinRiver, SE AK	*americana*	R	14592
Juneau, SE AK	*americana*	full cyt *b*	14952
Juneau, SE AK	*americana*	441-bp cyt *b*	14954, 14956–7
Juneau, SE AK	*americana*	R	10763–6, 10833, 10852–3, 14951, 14953, 14955, 14958–65, 19962–3, 20063, 20065, 20068, 20070
Thomas Bay, SE AK	*americana*	441-bp cyt *b*	19941–5
Thomas Bay, SE AK	*americana*	R	19946, 20071–3, 20077–80, 24500–6
Cleveland Peninsula, SE AK	*americana*	441-bp cyt *b*	10695–9
Cleveland Peninsula, SE AK	*americana*	R	10700–6, 10717, 14653–7, 14659–64, 14666–7, 14669
Interior Alaska	*americana*	full cyt *b*	53
Interior Alaska	*americana*	441-bp cyt *b*	50–2, 54, 144, 146, 148, 30671–2
Interior Alaska	*americana*	R	24601–15, 24627, 24629, 24631–2, 24636–46
South-central Alaska	*americana*	441-bp cyt *b*	14111–2, 14114–6
South-central Alaska	*americana*	R	13559
northern BC	*americana*	full cyt *b*	16004
northern BC	*americana*	441-bp cyt *b*	16005–8
northern BC	*americana*	R	16007
central BC	*americana*	full cyt *b*	16010, 16020
central BC	*americana*	441-bp cyt *b*	16009, 16014–5, 16019
central BC	*americana*	R	16011–3, 16016–8, 16021–3, 16026, 16033, 20612

Appendix I *Continued*

Locality*	Clade	Method(s)†	Alaska Frozen Tissue Collection number
northern Montana	*americana*	full cyt *b*	23185
northern Montana	*americana*	R	23180–2, 23185–92
southern Montana	*americana*	full cyt *b*	23183
southern Montana	*americana*	R	23183–4
Admiralty Island, SE AK	*caurina*	full cyt *b*	14470, 14972
Admiralty Island, SE AK	*caurina*	441-bp cyt *b*	14973, 16063, 16073–4, 16076–81, 19898–901, 19979–82, 19993
Admiralty Island, SE AK	*caurina*	R	19983–8, 19994–5, 20069, 24424–37, 24439, 24464–7
Kuiu Island, SE AK	*caurina*	full cyt *b*	17533, 17552
Kuiu Island, SE AK	*caurina*	441-bp cyt *b*	17535, 17540, 17542, 17544–5, 17547–8, 17550, 17553, 19887
Kuiu Island, SE AK	*caurina*	R	24472, 25301, 25303, 25307, 25309, 25316, 25319, 25321, 25327, 25330
Graham Island, Haida Gwaii, BC	*caurina*	full cyt *b*	20601, 20604
Graham Island, Haida Gwaii, BC	*caurina*	441-bp cyt *b*	20603, 20605–6
Graham Island, Haida Gwaii, BC	*caurina*	R	20602, 20607–11
Vancouver Island, BC	*caurina*	full cyt *b*	24477–8
Vancouver Island, BC	*caurina*	R	24475–8, 24479–97
southern Montana	*caurina*	full cyt *b*	23169, 23171
southern Montana	*caurina*	R	23168–79
Oregon	*caurina*	full cyt *b*	15936–7
Oregon	*caurina*	441-bp cyt *b*	15931, 15935, 15938–9
Oregon	*caurina*	R	15941, 19543, 15945–7, 15950–5
Wyoming	*caurina*	full cyt *b*	20613–4
Wyoming	*caurina*	441-bp cyt *b*	20615–7

*SE AK = southeastern Alaska; BC = British Columbia.

†Automated sequencing of the complete mitochondrial cytochrome *b* gene (full cyt *b*), partial cytochrome *b* gene (441-bp cyt *b*) or screening with a restriction enzyme digestion (R).