MOLECULAR EVOLUTION OF MARTENS (GENUS *MARTES*)

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MOLECULAR EVOLUTION OF MARTENS (GENUS *MARTES*)

A

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I. ABSTRACT

Molecular studies provide the opportunity to re-evaluate and further investigate hypotheses such as those related to phylogenetic relationships, inter- and intra-continental colonizations, population differentiation, and the dynamics of hybrid zones. Three sets of molecular markers, nuclear and mitochondrial, were used to examine phylogenetic relationships among species within a holarctically distributed genus (*Martes*), and intraspecific diversification and population differentiation within American marten (*Martes americana*). In American marten, two morphological groups ("americana" and "caurina") have been recognized, though the level of distinctiveness between them has been debated.

My data supported the fossil record's indication that early radiations gave rise to two subgenera of the genus *Martes* (*Pekania* and *Charronia*) and that a more recent, possibly rapid, radiation gave rise to species of the third subgenus (*Martes*). Two colonizations of North America are evident, one by members of the subgenus *Pekania*, and another by the subgenus *Martes*. However, contrary to hypotheses based on morphological evidence, the "americana" and "caurina" subspecies groups of *Martes americana* represent only one colonization. Cytochrome *b* data were consistent with the recognition of these as monophyletic clades; however, aldolase C sequences and microsatellite data indicated that these generally parapatric groups interbreed in at least one region of limited geographic overlap. These clades probably were isolated during the

late Pleistocene in eastern and western glacial refugia, but geographic separation apparently has not led to reproductive isolation.

My data also indicated two colonization events for the Pacific Northwest by American martens (one by each clade). Due to patterns of genetic variation, I hypothesize that the "caurina" clade spread along the North Pacific Coast, including southeastern Alaska, earlier than the "americana" clade, and that these clades have now formed a zone of secondary contact on Kuiu Island in southeastern Alaska. Microsatellite data revealed population differentiation among many island populations in the Pacific Northwest, but possible gene flow among several near-shore island and mainland populations was suggested. Analyses of genetic and geographic distances suggested that colonization history had a strong effect on present day population structure and that oceanic straits and possibly other physiographic features posed significant barriers to gene flow.

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VI. ACKNOWLEDGMENTS

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VII. INTRODUCTON

Evolution is represented by a continuum involving individuals, populations, species, and higher taxonomic groups. Microevolutionary processes govern evolution below the species level and have traditionally been studied through the field of population genetics. Conversely, macroevolutionary processes control evolution at and above the species level and have been predominantly studied using methods of systematics. Phylogeography, a fairly new discipline, bridges these two areas of study by examining the processes that determine the geographical distribution of genealogical lineages (Avise *et al.* 1987) and therefore involves research at several levels of the evolutionary continuum. My dissertation focuses on three areas of the continuum: (1) speciation (systematics), (2) intraspecific diversification (phylogeography), and (3) population differentiation (population genetics).

Molecular methods (*e.g.*, DNA sequencing and microsatellite analyses) and improved methods of analyses (*e.g.*, maximum-likelihood and coalescence) have recently advanced the field of evolutionary biology. For example, DNA sequences have 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 (*e.g.*, Wooding and Ward 1997), and even exploring temporal variation in effective population size (Rogers and Harpending 1992; Rogers 1995; Schneider and Excoffier 1999). In addition, microsatellites are valued as tools for genome mapping and for their ability to

characterize populations and identify individuals (Weissenbach *et al.* 1992; Bruford and Wayne 1993; Freimer and Slatkin 1996).

In mammals, two genomes exist: mitochondrial and nuclear. The mitochondrial genome is maternally inherited; whereas, the nuclear genome is both maternally and paternally inherited. The mitochondrial cytochrome *b* gene has been useful for phylogenetic reconstruction in many organisms and mutates at an accelerated rate compared to many nuclear genes. However, mitochondrial genes only give a maternal perspective; therefore, hypotheses developed from gene trees should be tested using DNA sequences from independent nuclear loci (Pamilo and Nei 1988). Another class of nuclear markers includes microsatellites. These loci consist of short tandem repeats, are found throughout the nuclear genome, and are often extremely variable because of relatively high mutation rates (Tautz 1989; Schlötterer and Tautz 1992; Ellegren 1995). I used a combination of sequence data from mitochondrial (cytochrome *b*) and nuclear (aldolase C) genes and data from nuclear microsatellites to test evolutionary hypotheses and elucidate the evolution of martens (genus *Martes*).

Extant martens are primarily distributed in the holarctic region and include three subgenera: *Pekania*, *Charronia*, and *Martes*. Within one species of North American martens, American martens (*Martes americana*), two distinct evolutionary lineages exist ("americana" and "caurina"). Chapter 1 of this dissertation focuses on the phylogenetic relationships among and within the subgenera of *Martes* and the colonization of North America. Chapter 2 examines the phylogeography or intraspecific diversification of the American martens, how this diversification may relate to Pleistocene glaciations and the

colonization of the species into the Pacific Northwest. Chapter 3 concludes by focusing on population differentiation of American martens and the dynamics of a zone of hybridization between the "americana" and "caurina" lineages.

I genotyped all individuals with minimal assistance of an undergraduate student researcher, Jason Schneider, and a high school student, Kyndall Hildebrandt. In addition, I conducted all analyses for sequence and microsatellite data. Dr. Joseph Cook, my advisor and co-author on all three chapters, provided assistance in project design, laboratory facilities, financial support, and editorial comments. Rodney Flynn, a co-author on Chapter 2, provided limited editorial comments and logistical support for collecting marten specimens.

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VIII. Chapter 1

Molecular evolution of the holarctic genus *Martes*[1]

Abstract

The Bering Land Bridge has served as a major corridor of interchange between the Old and New worlds for many taxa. Molecular studies provide the opportunity to re-evaluate and further explore hypotheses related to intercontinental movements of northern mammals. I investigated the phylogeny of all extant species of *Martes* (except for *M. gwatkinsi* from India) to infer evolutionary relationships and trans-Beringian movements. Species within this genus of carnivore are distributed in the Holarctic and Oriental regions. Complete sequences of the mitochondrial cytochrome *b* gene and partial sequences of the nuclear aldolase C gene suggested that the genus *Martes* may be paraphyletic with respect to *Gulo gulo* and supported the fossil record's indication that early radiations gave rise to two subgenera (*Pekania* and *Charronia*) and that a more recent, possibly rapid, radiation gave rise to species of the third subgenus (*Martes*). Two colonizations of North America are evident, one by members of the subgenus *Pekania* and another by subgenus *Martes*. However, contrary to hypotheses based on morphological evidence, the "americana" and "caurina" subspecies groups of *Martes americana* represent only a single colonization. Cytochrome *b* data were consistent with

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the recognition of these subspecies groups as monophyletic clades; however, the aldolase C sequences indicated that these generally parapatric groups may interbreed in a region of limited geographic overlap. These clades probably were isolated during the late Pleistocene, but geographic separation apparently has not led to reproductive isolation.

Introduction

Spatial and temporal distributions of extant and extinct mammals have long been used to interpret the timing and dynamics of interchange between the northern continents (Repenning, 1967; Hoffmann, 1981). The Bering Land Bridge was an approximately 1800 km wide landmass that connected Siberia and Alaska when it was repeatedly exposed prior to and during the Pleistocene (Hopkins, 1959, 1967; Elias, 1995). Although the habitats of the region apparently were variable during its existence (Colinvaux, 1964; Hoffmann, 1985; Elias *et al.*, 1996), the land bridge played a major role in the exchange of boreal mammals between the Old and New Worlds (Hoffmann, 1985). Molecular techniques (see Avise, 1994) have provided the opportunity to re-evaluate and further investigate hypotheses related to movements of northern mammals, in particular rodents (*e.g.*, Lance and Cook, 1998; Fedorov and Goropashnaya, 1999; Steppan *et al.*, 1999; Conroy and Cook, 2000). I focus on the biogeographic history of a clade of medium-sized carnivores, the Holarctic genus of martens (*Martes*).

Several hypotheses have been formulated concerning trans-Beringian movements of the genus *Martes*. Extant martens are primarily distributed in the holarctic region and include three subgenera: *Pekania*, *Charronia*, and *Martes* (Table 1; Anderson, 1970).

The fossil record indicates that a pre-Pliocene radiation in Asia gave rise to ancestors of subgenera *Pekania* and *Charronia*; whereas, marten fossils of the subgenus *Martes* do not appear until the middle Pliocene from deposits in Poland (Anderson, 1970). The fossil record also indicates a mid-Pleistocene colonization by ancestors of the subgenus *Pekania* into North America (via the Bering Land Bridge), while members of the subgenus *Martes* did not arrive until the late Pleistocene (Anderson, 1970). The fossil history of the subgenus *Martes* may be amended, however, by the recent discovery of a North American specimen dating to $\geq$ 780,000 years ago from Porcupine Cave, Colorado (MYA; Anderson, 1997). This may indicate a North American origin for the subgenus, but more likely suggests an earlier colonization of North America across the Bering Land Bridge than previously recognized. The wolverine (*Gulo gulo*), also Holarctic in distribution, is thought to be the sister taxon to *Martes* (Bininda-Emonds *et al.*, 1999) and appears in North American fossils in the early Pleistocene (Martin, 1989).

All members of the subgenus *Martes*, with the exception of *M. foina*, are morphologically similar, maintain allopatric or parapatric distributions, and possibly form a superspecies (Anderson, 1970). Although 8 subspecies of *M. americana* are recognized (Clark *et al.*, 1987), two distinct subspecies groups ("americana" and "caurina") have been identified based on morphology (Merriam, 1890) and mitochondrial DNA sequence (Carr and Hicks, 1997; Stone *et al.*, submitted). Anderson (1970, 1994) suggests the "americana" group colonized North America in the early Wisconsinan, moved eastward, and was subsequently isolated in eastern North America south of the ice sheets. The "caurina" group is thought to have crossed the Bering Land Bridge later than the

"americana" group, because the "caurina" group is postulated to be more closely related to its Siberian counterpart, *M. zibellina*, due to cranial and dental similarities (Anderson, 1970; Kurtén and Anderson, 1980).

If these hypotheses are correct, the "caurina" group of *M. americana* and *zibellina* should form a clade that is sister to the "americana" group of *M. americana*. Carr and Hicks (1997) and Stone *et al.* (submitted) did not find support for these hypotheses based on sequence of the mitochondrial cytochrome *b* gene (cyt *b*). Both studies report the "americana" and "caurina" groups form a sister clade apart from other extant species of the same subgenus. Furthermore, Carr and Hicks (1997) suggest the "caurina" group should be recognized as a distinct species (originally described by Merriam, 1890) based on sequence divergence (1.5-2.0%).

I investigated the phylogeny of *Martes* using sequences from mitochondrial and nuclear genes. In particular, I used phylogenetic reconstruction to test: (1) monophyly of the genus *Martes*, (2) validity of subgenera (*i.e.*, *Pekania*, *Charronia*, and *Martes*), (3) relationships among species of the subgenus *Martes*, and (4) whether North American *M. americana* are the result of multiple colonizations from Eurasia.

Materials and Methods

DNA extractions, PCR, and sequencing of the cytochrome b gene

DNA was extracted from marten tissues (heart, spleen, or skeletal muscle) 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 and Cook (1998) unless otherwise noted. Amplifications were in 50-µl volumes containing 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 1.0 µM of each primer, 1.25 units of Perkin-Elmer AmpliTaq DNA polymerase, Perkin-Elmer 1X 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 sec, then 35 cycles of denaturation at 94°C for 10 sec, annealing at 45°C for 15 sec, and an extension at 72°C for 45 sec, 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* (corresponding to sites 14139-15282 of *Mus musculus*; Bibb *et al.*, 1981): MVZ4 and MVZ5, MVZ14 and MVZ23, MVZ16 and Marten37 (Table 2). Both forward and reverse strands were sequenced for each individual.

Phylogenetic analyses of cytochrome b

The complete cyt *b* gene (1140 base pairs) was sequenced for two martens of the "americana" group of *M. americana*, two of the "caurina" group of *M. americana*, three *M. zibellina*, four *M. martes*, two *M. foina*, and two *M. pennanti* (Table 3). DNA sequences will be deposited in GenBank. *Martes americana* samples were drawn from a larger data set including 680 individuals, which represented two clades (Stone *et al.*, submitted). Only two individuals from each clade were chosen for this paper because little intra-clade variation existed. Sequences were compared among these individuals,

two *M. melampus* (GenBank AB012347 and AB012355; Kurose *et al.*, 1999), two *M. flavigula* (GenBank AB012362-3; Kurose *et al.*, 1999), and *Gulo gulo* (GenBank X94921; Ledje and Arnason, 1996). *Mustela erminea* and *M. vison* were used as outgroups for all analyses (GenBank AF057127 and AF057129, respectively; Koepfli and Wayne, 1998).

Relationships among *Martes* and *Gulo* cyt *b* sequences were examined using PAUP* (Version 4.0b3a; Swofford, 1999). A neighbor-joining (NJ) tree, employing the HKY85 model (Hasegawa *et al.*, 1985), was generated to approximate tree topology. This tree was used to evaluate different likelihood models (in order of decreasing complexity, GTR+I+Γ > GTR+I = GTR+Γ > GTR > HKY85+I+Γ > HKY85+I = HKY85+Γ > HKY85 > K2P+I+Γ > K2P+I = K2P+Γ = JC+I+Γ > K2P = JC+I = JC+Γ > JC) following Sullivan *et al.* (1997). Likelihood scores generated for each model were compared using a χ^2 test. The least complex model that was significantly better than other simpler models was chosen for further analyses.

A successive approximation approach was used for the maximum likelihood (ML) search, whereby the general time-reversible rate matrix (GTR rmat), the proportion of invariable sites (I), and the gamma-distribution shape parameter (α) were estimated from the NJ tree. These values, along with empirical base frequencies, were used in the original ML search (GTR+I+Γ model; heuristic search with TBR branch swapping). That run was stopped after 10 min and values were re-estimated. The subsequent ML

search used the new estimates. The optimal tree was subjected to 200 bootstrap replicates using ML heuristic searches.

Phylogenetic trees were also constructed using maximum parsimony (unweighted and transition/transversion weighting of 1/2, 1/5, 1/10, and 1/20), and NJ (Tamura-Nei distance) methods. All searches produced trees with similar topologies; therefore, the unweighted maximum parsimony analysis is shown. A strict consensus tree was generated from the two 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) with 100 replicates and maximum parsimony heuristic searches. The strict consensus tree was then subjected to 1000 bootstrap replicates.

Molecular clock and other constraints

Using the same parameters as the final ML search and midpoint rooting, a maximum likelihood score was calculated when enforcing a molecular clock. Likelihood scores of trees generated when enforcing and not enforcing a molecular clock were compared using a χ^2 test to determine whether taxa evolved at equal rates. The two-cluster test (Takezaki *et al.*, 1995) was used to identify non-clock-like nodes (discussed in Voelker, 1999) for both ML and NJ topologies. For the ML topology, the two cluster test used Tamura-Nei+Γ distances (where $\Gamma = 2.0783$) to determine whether two daughter lineages at a node have evolved at significantly different rates. Tamura-Nei distances were used to assess the NJ topology.

In addition, constrained trees were generated to test alternative hypotheses and identify well-resolved nodes as opposed to unresolved relationships. ML scores for these trees were compared to the optimal ML score using the Kishino-Hasegawa test (Kishino and Hasegawa, 1989). Due to multiple comparisons (N = 5), I initially adjusted α to 0.01 using a sequential Bonferroni correction (Rice, 1989). Constraints included monophyly of: (a) the genus *Martes*, (b) "caurina" and *M. zibellina*, (c) "caurina", *M. zibellina*, and *martes*, (d) *M. melampus*, *zibellina*, and *martes*, and (e) *M. melampus* and *americana*.

PCR, sequencing, and single-strand conformation polymorphism of the aldolase C gene

A portion (241 base pairs) of nuclear aldolase C (ald C) exon 5 and following intron (corresponding to sites 2756-2996 of *Rattus norvegicus*; Mukai *et al.*, 1991) was amplified using primers Ald-1 and Ald-2 (Table 2). A third primer, Ald-1B (Table 2), was designed to amplify an additional 25 base pairs at the 5' end. An annealing temperature of 60°C was used for these amplifications. The portion of ald C was sequenced for 17 martens of the "americana" group of *M. americana*, 18 martens of the "caurina" group of *M. americana*, two *M. zibellina*, two *M. martes*, two *M. foina*, and one *M. pennanti* (Table 3). Individuals detected as possibly heterozygous (through sequence analysis) were subjected to single-strand conformation polymorphism (SSCP) to test sequencing results (Pfau *et al.*, 1999). Variable sites were mapped onto the phylogeny constructed under maximum parsimony.

Results

Mitochondrial cytochrome b

Base composition (A = 28.2%, C = 30.6%, G = 14.4%, T = 26.8%) for cyt *b* was consistent with other mammals (*e.g.*, Irwin *et al.*, 1991; Talbot and Shields, 1996a). A linear relationship ($R^2 = 0.945$) between third position transitions and uncorrected *p* distances calculated for species of *Martes* suggested saturation had not been attained (Lara *et al.*, 1996). After evaluating different likelihood models, the general time-reversible with 6 rates of substitution (Yang, 1994) + proportion of invariable sites + gamma-distribution shape parameter (GTR+I+Γ) was determined to be the significantly best model tested with the least complexity. The ML search, employing GTR+I+Γ and run to termination with preset values, resulted in one tree (Fig. 1).

A consensus tree of two equally parsimonious trees (672 steps with 265 informative characters) was constructed (Fig. 2). The NJ tree differed from the parsimony consensus tree only with respect to the placement of *M. pennanti*. *Martes pennanti* was found basal to *Gulo gulo* and the remaining *Martes* species in the NJ tree while both ML and parsimony trees placed *M. pennanti* and *G. gulo* as sister taxa. Well-resolved nodes corresponded across all three methods (ML, parsimony, and NJ) of tree reconstruction.

Enforcing a molecular clock significantly increased the likelihood score (= -4561.77537; $P < 0.001$); therefore, I assumed these taxa have not evolved at equal rates. The two-cluster test identified non-clock-like nodes (Figs. 1 and 2). Constraining the monophyly of (a) a clade of "caurina" and *M. zibellina*, and (b) a clade of "caurina",

M. zibellina and *martes* also significantly increased the likelihood score (Table 4). However, all other constrained trees were not significantly different from the initial ML tree (Table 4). Other constraints tested included the monophyly of (a) the genus *Martes*, (b) *M. melampus*, *zibellina*, and *martes*, and (c) *M. melampus* and *americana*. Therefore, I concluded: (1) the genus *Martes* may be paraphyletic with respect to *Gulo*; (2) three distinct clades were apparent corresponding to the morphologically defined subgenera *Pekania*, *Charronia*, and *Martes*; (3) the inability for my data to resolve the placement of *M. foina* and *melampus* may have been due to a rapid radiation within the subgenus *Martes*, which gave rise to *M. foina*, *melampus*, *martes/zibellina*, and *americana*; (4) *M. martes* and *zibellina* formed a monophyletic group; (5) *M. martes* was paraphyletic with respect to *M. zibellina*; (6) the "americana" and "caurina" groups of *M. americana* were sister taxa; and (7) several daughter lineages have not evolved in a clock-like manner.

Nuclear aldolase C

Less variation was found in ald C sequence (Table 5), which is consistent with studies that have compared nuclear introns and mitochondrial gene sequence variation in mammals (*e.g.*, Slade *et al.*, 1994). This limited variation, however, corroborated relationships supported by cyt *b* data (see Fig. 2, ald C variable sites mapped on cyt *b* tree). One silent, third position transition (site 2763; Table 5) found in exon 5 distinguished the "americana" and "caurina" clades of *M. americana*, and heterozygous individuals were detected on Kuiu Island, southeastern Alaska (a region of sympatry; Stone *et al.*, submitted). Eight individuals from Kuiu Island, analyzed with SSCP,

revealed 4 homozygotes (2 of each genotype) and confirmed the 4 presumed heterozygotes determined via automated sequencing.

Discussion

The Bering Land Bridge has intermittently permitted the interchange of many organisms between the Old and New worlds over the past several million years. The Bering Strait first opened 4.8-7.4 MYA (Marincovich and Gladenkov, 1999) forming a connection between the Pacific and Arctic oceans. Since that time, the Bering Land Bridge has experienced a series of exposures and inundations; the latest inundation occurred approximately 11,000 years ago (Elias *et al.*, 1996; Sher, 1999). The timing of these connections and the habitat composition of the bridge have been extensively debated (*e.g.*, Colinvaux, 1964, 1980; Guthrie, 1985, 1990; Elias *et al.*, 1996). Undoubtedly, the land bridge has been a mosaic of habitats (Elias *et al.*, 1996) both temporally and spatially filtering organisms that were exchanged at this high latitude crossroads (Rausch, 1994; Sher, 1999).

The timing of interchange of carnivores among continents has been extensively investigated using fossils (*e.g.*, Anderson, 1970; Martin, 1989; Hunt, 1996) and comparative morphology (*e.g.*, Anderson, 1970; Rausch, 1994). Molecular studies provide another opportunity to interpret the sequence of colonizations by examining tree topology (*e.g.*, Lance and Cook, 1998; Steppan *et al.*, 1999) and potentially the timing of these events through estimates of genetic divergence (*e.g.*, Wayne *et al.*, 1989; Talbot and Shields, 1996b; Fedorov and Goropashnaya, 1999; Conroy and Cook, 2000).

Relative timing of divergence

My analyses suggested the genus *Martes* may be paraphyletic with respect to *Gulo gulo*; however, the Kishino-Hasegawa test could not exclude the monophyly of *Martes* (Table 4). Additional nuclear genes should be explored to test this possible result. Cyt *b* sequences supported fossil data (Anderson, 1970) indicating early radiations gave rise to subgenera *Pekania* and *Charronia*, and that a more recent radiation led to species in the subgenus *Martes*. *Martes pennanti* (subgenus *Pekania*) was consistently the most basal species of the genus, followed by *M. flavigula* (subgenus *Charronia*); whereas, most species of the subgenus *Martes* formed a monophyletic polytomy (Figs. 1 and 2).

The marten fossil record suggests the genus *Martes* arose in the Palearctic and that the three subgenera diverged there (Anderson, 1970). Based on this record, the paraphyletic relationship between the endemic North American species *M. pennanti* and *americana* supports two colonizations across the Bering Land Bridge into North America, one by members of the subgenus *Pekania* and the other by subgenus *Martes*. Because of the strong association between *Martes* species and forested habitats (except for *M. foina*), as seen throughout their current distributional range and fossil history (Powell, 1981; Clark *et al.*, 1987; Graham and Graham, 1994), continuous forested habitats most likely existed in Beringia during these colonization events. Therefore, estimating when these taxa crossed Beringia may help determine the habitat composition of the region during those times.

The "americana" and "caurina" groups of *M. americana* formed a monophyletic clade to the exclusion of all closely related Eurasian species (*i.e.*, *M. martes*, *zibellina*,

melampus, and *foina*). Anderson (1970) indicates that "americana" represents an earlier colonizations of North America than "caurina" because of the similarity of "caurina" to *M. zibellina*. However, the cyt *b* sequence supported a single colonization of North America. These clades may have arisen due to separation into different southerly refugia in North America throughout past glacial and inter-glacial cycles (Carr and Hicks, 1997; Stone *et al.*, submitted) as suggested for black bears (*Ursus americanus*; Wooding and Ward, 1997; Stone and Cook, 2000), and other western North America taxa with multiple lineages, including plants (Soltis *et al.*, 1997), amphibians (Templeton *et al.*, 1995; Green *et al.*, 1996), birds (Bermingham *et al.*, 1992; Gill *et al.*, 1993), and mammals (Arbogast, 1998; Cook *et al.*, in press).

The fossil record suggests that isolation between "americana" and "caurina" may have occurred in eastern and western refugia south of the ice sheets. Many fossils have been recovered from these two regions (Graham and Graham, 1994); whereas few fossils have been found in Beringia (Youngman, 1993). In addition, these Beringian fossils have not been dated and may represent Holocene taxa (Youngman, 1993).

Fossil records exist for another North American species, *M. nobilis*, belonging to the subgenus *Martes*. This questionable species may belong to the "caurina" clade (Anderson, 1970, Kurtén and Anderson, 1980; Youngman and Schueler, 1991; Anderson, 1994; Graham and Graham, 1994). Sequences of ancient DNA from these fossils may provide a definitive answer.

Molecular clocks

The molecular clock hypothesis has been extensively debated (*e.g.*, Ayala, 1999; Strauss, 1999). Hillis *et al.* (1996) discuss the dangers of estimating absolute times of divergence under the assumption of a molecular clock. A clock can be estimated if several conditions, albeit generally unrealistic, are met (Hillis *et al.*, 1996). These include, but are not limited to: (1) molecular change is linear with time, (2) rate of change is equal across positions and lineages, (3) the phylogenetic tree can be correctly reconstructed, and (4) accurate calibration dates are available to calculate the rate of the molecular clock. Because these conditions are often not met, confidence limits should be calculated incorporating the standard error of the calibration point(s) and stochastic variation in the clock. Stochastic variation exists even in a "perfect" clock, whereby the 95% confidence limits after 15 million years of isolation, for a clock ticking at one substitution per million years, equals 15 ± 7 substitutions (Hillis *et al.*, 1996). These large standard errors reflect considerable imprecision (Hillis *et al.*, 1996; Ayala, 1999).

Because these sources of error exist, attempts to determine times of divergence have yielded mixed results. Flynn (1996) concludes from his preliminary analyses of molecular data for carnivores that there is "little or no support for a strong relationship between divergence time and amount of molecular change, as would be necessary for the application of a molecular clock" (Flynn, 1996:571). Additional difficulties may occur with calibration points. A relatively good fossil record exists for carnivores (Martin, 1989); however, many calibration points are at relatively deep levels. Koepfli and Wayne (1998) used the procyonid-mustelid split, estimated at 33 MYA based on the

appearance of the first fossil mustelid, to calculate a divergence rate of 0.46% per million years for third position transversions. Although third position transversions accumulate approximately linearly with time for up to 80 MYA or more (Irwin *et al.*, 1991), too few of these mutations accumulate between sister species to allow reasonable estimates at this level (Fig. 2).

In addition, Bininda-Emonds *et al.* (1999) display the danger of casually estimating times of divergence. Using date estimates from the literature and a composite tree generated for Mustelidae, they dated an internal node 770% older than an ancestral one within the subgenus *Martes*. Because of difficulties associated with deep calibration points and the finding that several nodes on my trees (Figs. 1 and 2) have daughter lineages that are not evolving clock-like, I discussed relative times of divergences rather than absolute times (Hillis *et al.*, 1996).

Hard versus soft polytomies

Both ML and parsimony trees showed weak support for the placement of *M. foina* and *melampus* (Figs. 1 and 2). My data therefore suggested a polytomy containing four clades (*americana*, *foina*, (*martes*, *zibellina*), and *melampus*). Some unresolved polytomies have been attributed to bursts of speciation (*e.g.*, Lessa and Cook, 1998; Conroy and Cook, 1999; Steppan *et al.*, 1999; Waits *et al.*, 1999). Although the fossil record indicates an earlier divergence for *foina* (which is supported by the ald C data), the remaining four species are thought to possibly form a superspecies (suggesting a close phylogenetic relationships; Hagmeier, 1961; Anderson, 1970). Sequence from other

genes may resolve the relationships among species within the subgenus *Martes*, implying that this clade represents a "soft" polytomy (Maddison and Maddison, 1992), and that my cyt *b* data lack the resolution to provide a dichotomous phylogenetic tree (Mooers and Heard, 1997). Conversely, additional data may confirm that the species within the subgenus represent a rapid radiation, in which case this clade represents a "hard" polytomy (Maddison and Maddison, 1992) and would remain unresolved even with additional nuclear markers.

Hybridization

Within the monophyletic *M. zibellina/martes* clade, *M. martes* was paraphyletic with respect to *zibellina* (Figs. 1 and 2). Although incomplete lineage sorting can result in paraphyletic relationships whereby a gene tree does not have the same topology as a species tree (Pamilo and Nei, 1988; Davison *et al.*, 1999), this paraphyletic relationship is probably due to hybridization. Hybridization between *zibellina* and *martes* (Grakov, 1994) and between the "americana" and "caurina" groups of *M. americana* (Wright, 1953; Hagmeier, 1961) has been documented. *Martes americana* of both the "americana" and "caurina" cyt *b* lineages coexist in some regions, such as Kuiu Island, southeastern Alaska, and sequences of the nuclear ald C gene were consistent with hybridization. Samples from Kuiu Island included mtDNA "americana" individuals with nuclear DNA (nDNA) "caurina" signatures, mtDNA "caurina" individuals with nDNA "americana" signatures, mtDNA "americana" and mtDNA "caurina" individuals as heterozygotes, and all combinations thereof (Table 5), indicating that martens of both

groups have interbred. Complete sequences of cyt *b* consistently recognized two historical groups within *Martes americana*, with partial sequence from the nuclear ald C gene indicating that the two groups have interbred on Kuiu Island. These groups probably experienced isolation from one another during the Pleistocene, but this geographic separation apparently has not led to reproductive isolation.

Carr and Hicks (1997) conclude that the "americana" and "caurina" clades are distinct species. Under the Phylogenetic Species Concept (Cracraft, 1989), these reciprocally monophyletic clades could be identified as distinct species; however, the apparent zones of hybridization in southeastern Alaska and Montana (this study; Wright, 1953) may not meet the criterion set forth by the Biological Species Concept (Mayr, 1942). More importantly, the gene tree should be tested using DNA sequences from other, independent loci (Pamilo and Nei, 1988; Maddison, 1997). Independent genes, such as the nuclear ald C locus, will provide the opportunity to explore concordant phylogenetic partitions as proposed by Avis and Ball (1990). The dynamics of speciation and hybridization will require evaluation of additional nuclear loci and the examination of possible ecological, behavioral, or physiological differences between these two clades of *M. americana*. Investigations centered on contact zones may be particularly informative because hybrid zones allow us "to quantify the genetic differences responsible for speciation [and] to measure the diffusion of genes between diverging taxa" (Barton and Hewitt, 1989:497).

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Table 1. Taxonomy and general distributions of extant *Martes* species (Anderson, 1970).

Subgenus	Species	General distribution
Pekania	*M. pennanti*	northern North America
Charronia	*M. flavigula*	Asia
	M. gwatkinsi	southern India
Martes	*M. foina*	Europe and southwestern Asia
	M. martes	Europe and northwestern Asia
	M. zibellina	Siberian taiga, Mongolia, and northern Japan
	M. melampus	Japan and Korea
	M. americana	northern North America

Table 2. Sequences and associated references for primers used to amplify the mitochondrial cytochrome *b* gene and a portion of the nuclear aldolase C exon 5 and following intron.

Primer	Sequence (5' to 3')	Reference
MVZ4	GCAGCCCCTCAGAATGATATTTGTCCTC	Smith and Patton, 1993
MVZ5	CGAAGCTTGATATGAAAAACCATCGTTG	Smith and Patton, 1993
MVZ14	GGTCTTCATCTYHGGYTTACAAGAC	Smith and Patton, 1993
MVZ23	TACTCTTCCTCCACGAAACJGGNTC	Smith and Patton, 1993
MVZ16	AAATAGGAARTATCAYTCTGGTTTRAT	Smith and Patton, 1993
Marten37	TATATATACCCCGAAACATGGA	Demboski *et al.*, 1999
Ald-1	TGTGCCCAGTATAAGAAGGATGG	Lessa and Applebaum, 1993
Ald-1B	GCTGGATGGRCTCTYRAAAC	this study
Ald-2	CCCATCAGGGAGAATTTCAGGCTCCACAA	Lessa and Applebaum, 1993

Table 3. Specimen numbers and locations of *Martes* samples sequenced for this study.

Species	Group[a]	Method[b]	Location	Alaska Frozen Tissue Collection #
M. pennanti		cyt b	Alberta, Canada	21217-8
M. pennanti		ald C	Southeast Alaska, USA	16072
M. foina		cyt b	Germany	17568-9
M. foina		ald C	Germany	17568-9
M. martes		cyt b	Germany	17559-60
M. martes		cyt b	Sweden	21213-4
M. martes		ald C	Germany	17559
M. martes		ald C	Sweden	21214
M. zibellina		cyt b	Russia	25268, 25270, 25274
M. zibellina		ald C	Russia	25270, 25274
M. americana	"americana"	cyt b	Interior Alaska, USA	53
M. americana	"americana"	cyt b	British Columbia, Canada	16004
M. americana	"americana"	ald C	Interior Alaska, USA	53, 147
M. americana	"americana"	ald C	Southeast Alaska, USA	10667, 10756, 10771, 14952, 17536-8, 17551, 19888, 19996-7
M. americana	"americana"	ald C	British Columbia, Canada	16004, 16006
M. americana	"americana"	ald C	Montana, USA	23183, 23185
M. americana	"americana"	SSCP	Interior Alaska, USA	53
M. americana	"americana"	SSCP	Southeast Alaska, USA	17536
M. americana	"caurina"	cyt b	Southeast Alaska, USA	14470
M. americana	"caurina"	cyt b	Oregon, USA	15937

Table 3 continued.

M. americana	"caurina"	ald C	Southeast Alaska, USA	16076, 17533, 17540, 17545, 17547, 17552, 19982, 19993-5
M. americana	"caurina"	ald C	Haida Gwaii, British Columbia, Canada	20601, 20603-4
M. americana	"caurina"	ald C	Oregon, USA	15931, 15936
M. americana	"caurina"	ald C	Montana, USA	23169, 23171
M. americana	"caurina"	ald C	Wyoming	20614
M. americana	"caurina"	SSCP	British Columbia, Canada	16004
M. americana	"caurina"	SSCP	Southeast Alaska, USA	17533, 17540, 17547, 19982
M. americana	"caurina"	SSCP	Oregon, USA	15931

[a]determined by cytochrome *b* gene sequence

[b]cyt *b* = sequencing of the cytochrome *b* gene, ald C = sequencing of a portion of the aldolase C gene, SSCP = single-strand conformation polymorphism to distinguish homozygous versus heterozygous individuals at position 2763 of the aldolase C gene (see Table 5).

Table 4. Comparison of optimal (unconstrained) maximum likelihood tree score (= -4523.20501) with likelihood scores from constrained trees using the Kishino-Hasegawa test (Kishino and Hasegawa, 1989).

Constraint - monophyly of:	Best tree score	*P-value*
genus *Martes*	-4524.31494	0.5704
"caurina" and *M. zibellina*	-4606.76812	<0.0001*
"caurina", *M. zibellina*, and *martes*	-4568.31108	0.0005*
M. melampus, zibellina, and *martes*	-4525.87504	0.5803
M. melampus and *americana*	-4526.71190	0.4605

* $P < 0.01$ (α adjusted for multiple comparisons)

Table 5. Condensed dot matrix assembled using aldolase C sequence (corresponding to sites 2756-2996 of *Rattus norvegicus*; Mukai *et al.*, 1991). Number of individuals with identical sequences is in parentheses. C/T represents heterozygous individuals. Characters symbolizing positions are referred to in Fig. 2.

Specimens:	Position 2763 ‡	Position 2946 §	Position 2972 *
Martes americana			
"americana" group			
Interior Alaska, USA (2)	T	C	T
British Columbia, Canada (2)	.	.	.
Southeast Alaska, USA (6)	.	.	.
Kuiu Island, Southeast Alaska, USA (3)	.	.	.
Kuiu Island, Southeast Alaska, USA (1)	C	.	.
Kuiu Island, Southeast Alaska, USA (1)	C/T	.	.
Montana, USA (2)	.	.	.
"caurina" group			
Southeast Alaska, USA (5)	C	.	.
Kuiu Island, Southeast Alaska, USA (1)	C	.	.
Kuiu Island, Southeast Alaska, USA (1)	.	.	.
Kuiu Island, Southeast Alaska, USA (3)	C/T	.	.
Haida Gwaii, British Columbia, Canada (3)	C	.	.
Oregon, USA (2)	C	.	.
Montana, USA (2)	C	.	.
Wyoming, USA (1)	C	.	.
Martes zibellina (2)	C	.	.
Martes martes (2)	C	.	.
Martes foina (2)	C	G	.
Martes pennanti (1)	C	G	C

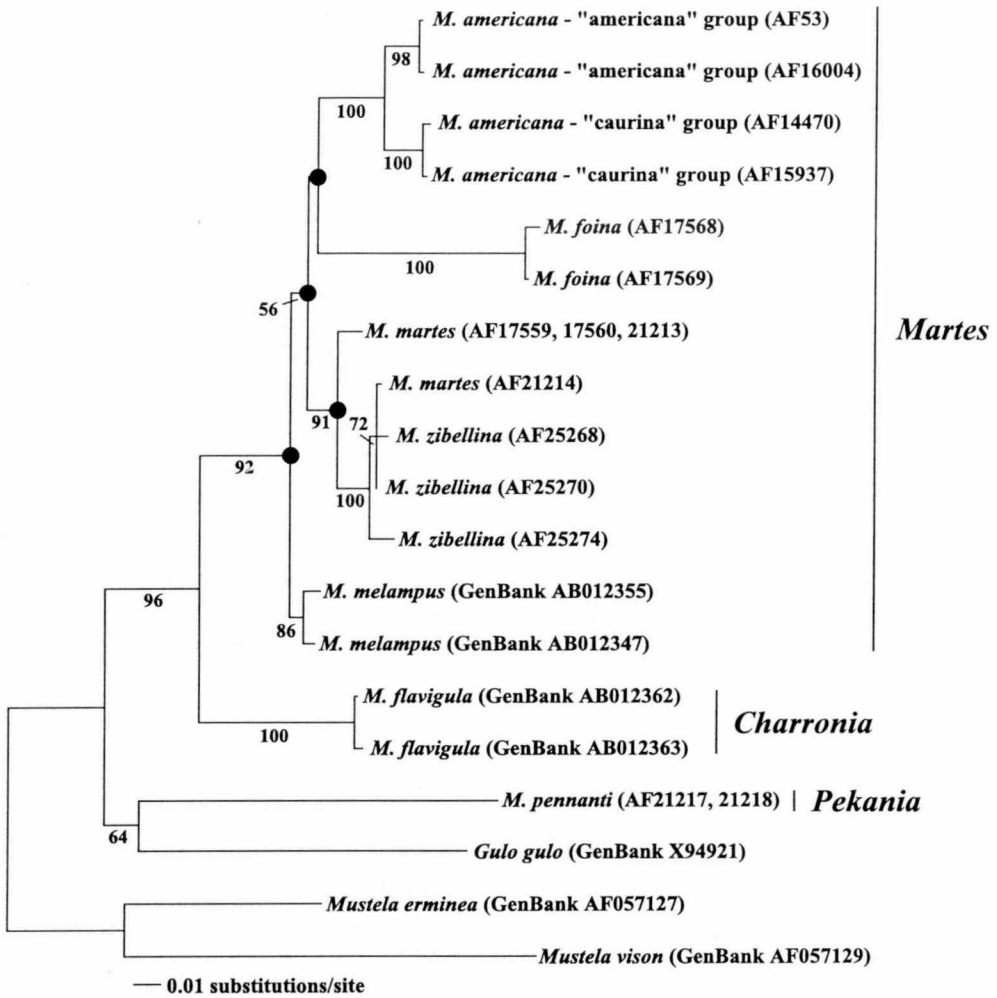

Fig. 1. Maximum likelihood tree (–ln = 4523.20501) generated from cytochrome *b* gene sequences of *Martes* (this study; Kurose *et al.*, 1999) and *Gulo* (Ledje and Arnason, 1996) individuals. The tree was generated using a general time-reversible – 6 rates of substitution (Yang, 1994) + proportion of invariable sites + gamma-distribution shape parameter (GTR+I+Γ) model with the GTR rmat = 9.9032 (A-C), 164.6027 (A-G), 8.7640 (A-T), 2.7711 (C-G), 142.0685 (C-T), I = 0.5614, and α = 2.0783. Bootstrap values are shown below bars, voucher (AF) or GenBank numbers are in parentheses after taxon names, and subgenera are listed along the right margin. *Mustela erminea* and *M. vison* were used as outgroups (Koepfli and Wayne, 1998). ● = nodes non-clock-like according to the two cluster test (Takezaki *et al.*, 1995).

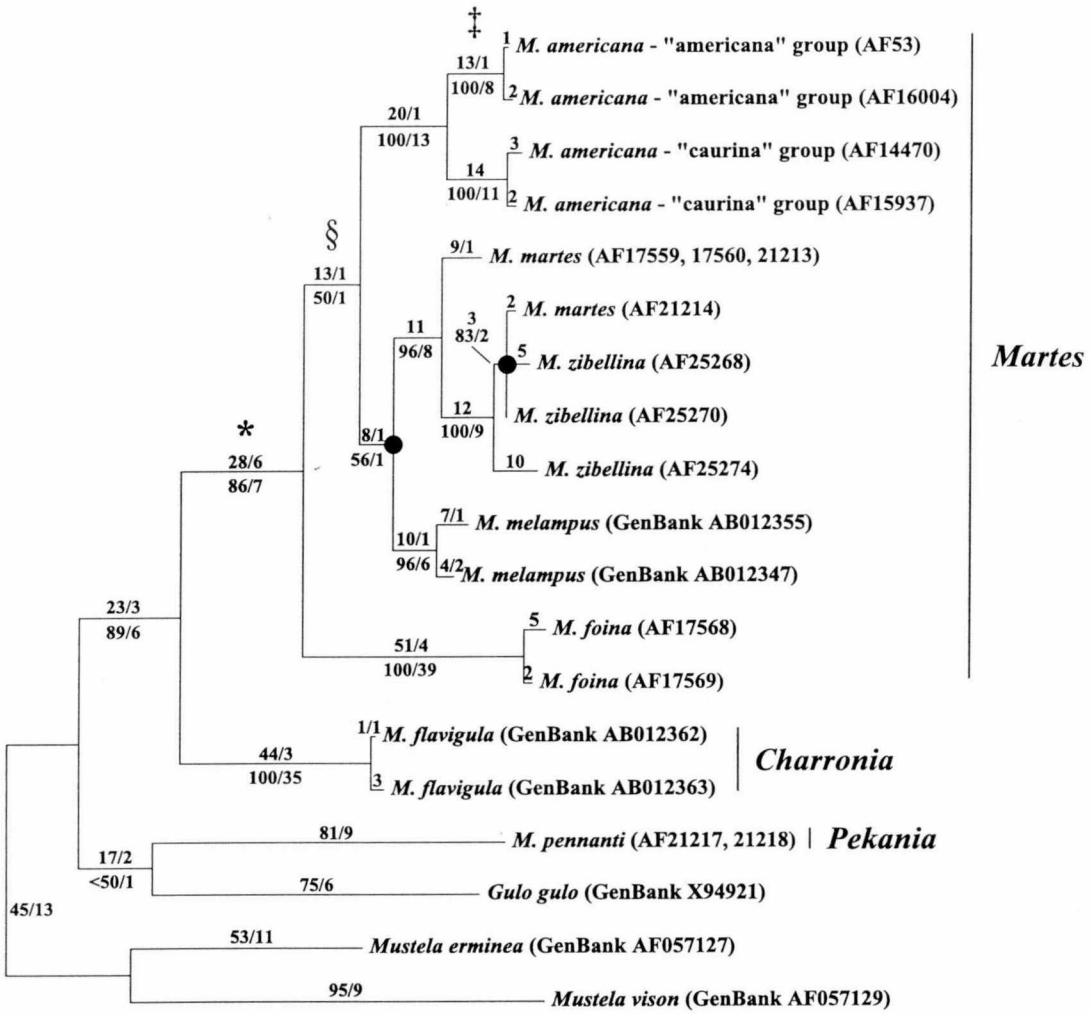

Fig. 2. Strict consensus tree of two equally parsimonious trees (length = 672 steps; CI = 0.629; RI = 0.681) generated with a branch-and-bound search from complete cytochrome *b* gene sequences of *Martes* (this study; Kurose *et al.*, 1999) and *Gulo* (Ledje and Arnason, 1996) individuals. Branch lengths/number of third position transversions are shown above branches, bootstrap values/Bremer decay indices are below branches, voucher (AF) or GenBank numbers are in parentheses after taxa names, and subgenera are listed along the right margin. *Mustela erminea* and *M. vison* were used as outgroups (Koepfli and Wayne, 1998). Symbols (‡, §, *) refer to base substitutions in the aldolase C gene (see Table 5). ● = nodes non-clock-like according to the two cluster test (Takezaki *et al.*, 1995).

IX. Chapter 2

Post-glacial colonization of northwestern North America by the forest associated American marten (*Martes americana*)[2]

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 distinctiveness between them has been debated. I examined mitochondrial cytochrome *b* gene haplotypes from 680 martens to explore 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. My data indicated two colonization events for the Pacific Northwest (one by each clade) and were consistent with the persistence of populations throughout past glacial periods in eastern and western refugia. Due to patterns of genetic variation, I hypothesize the

[2]Stone, K. D., R. W. Flynn, and J. A. Cook. (submitted). Post-glacial colonization of northwestern North America by the forest associated American marten (*Martes americana*). Molecular Ecology.

caurina clade spread along the North Pacific Coast (including southeastern Alaska) earlier than the *americana* clade. These clades are distinctive (inter-clade divergence ranged from 2.5-3.0% (uncorrected *p*), and intra-clade divergence was < 0.7%), and narrow zones of contact have been identified. Genetic signatures of past admixture in hybrid zones may have been extinguished during subsequent glacial periods when the range of the species contracted.

Introduction

Our understanding of the dynamics of past movements and colonization of organisms traditionally has relied on the fossil record. However, molecular analyses applied within the framework of phylogeography (Avise 1994; Avise and Hamrick 1996) are beginning to provide insight into the history of range expansions and contractions of many species (*e.g.*, Wooding and Ward 1997; Bernatchez and Wilson 1998; Conroy and Cook 2000). Information on distributions gleaned from mammalian fossils generally is limited to taxonomic units at or above the level of species because sample sizes are seldom large enough to characterize geographic variation within species. DNA sequences have 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 (*e.g.*, Wooding and Ward 1997), and even exploring temporal variation in effective population size (Rogers and Harpending 1992; Rogers 1995; Schneider and Excoffier 1999). Molecular (and morphological) studies of extant species,

in combination with paleoecology, may provide opportunities to test hypotheses related to the genetic effects of Pleistocene ice ages (Hewitt 1996).

Morphological analyses of recent specimens (Wright 1953; Anderson 1970; Giannico and Nagorsen 1989) and fossils (Graham and Graham 1994) have investigated the history and taxonomy of American martens, *Martes americana*. Although 8 subspecies of *Martes americana* have been described (Clark *et al.* 1987), these are traditionally placed in two morphologically distinct groups (*americana* and *caurina*). The *americana* group is distributed in Montana/Idaho northward to Alaska and eastward to the Atlantic Coast, and the *caurina* group is described from parts of the West Coast (California to British Columbia), Wyoming, Montana, and Idaho (Fig. 3, inset map; Wright 1953; Hall 1981; Carr and Hicks 1997). Although many studies (*e.g.*, Merriam 1890; Anderson 1970; Hall 1981; Clark *et al.* 1987) have corroborated the separation of *Martes americana* into two groups, the level of distinctiveness between them has been debated. These groups were described as different species based on morphology (Merriam 1890), a conclusion supported by a limited number of mitochondrial DNA sequences (Carr and Hicks 1997). Wright (1953), however, reports intergradation between the groups and suggests they belong to the same species, *M. americana*. I document the extent of geographic variation in the mitochondrial cytochrome *b* (cyt *b*) gene across populations of this species from the Pacific Northwest with a particular focus on southeastern Alaska, where secondary contact between these groups is probable (Giannico and Nagorsen 1989).

Southeastern Alaska is a heterogeneous landscape that includes the 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 and Cook 1996). Phylogeographic investigations have revealed distinct evolutionary lineages of dusky shrew (*Sorex monticolus*; Demboski *et al.* 1999), brown bear (*Ursus arctos*; Talbot and Shields 1996), black bear (*U. americanus*; Stone and Cook 2000), and long-tailed voles (*Microtus longicaudus*; Conroy and Cook 2000). This high degree of endemism and diversity of lineages suggests a complex colonization history for deglaciated areas within the Pacific Northwest.

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). Current distribution patterns and molecular and fossil data from 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; Heusser 1989; Warner *et al.* 1982; O'Reilly *et al.* 1993; Heaton *et al.* 1996; Byun *et al.* 1997). However, fossil evidence of extant endemics that spans the periods of glacial maxima have not been revealed (Heusser 1989).

The high degree of endemism and intraspecific lineage diversity in southeastern Alaska may be due to refugial populations, secondary contact of previously isolated populations, or a combination of these and other factors. I examined genetic differentiation of martens from the Pacific Northwest to elucidate colonization history of a forest associated medium-sized carnivore and to test ideas concerning the post-glacial colonization of the region.

Materials and Methods

DNA was extracted from marten tissues (heart, kidney, liver, spleen, skeletal muscle, skin, or blood) and 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 and Cook (1998) unless otherwise noted. Amplifications were in 50 µl volumes containing 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 1.0 µM of each primer, 1.25 units of Perkin-Elmer AmpliTaq DNA polymerase, Perkin-Elmer 1X 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: 1 cycle of 94°C for 45 sec, followed by 35 cycles of denaturation at 94°C for 10 sec, annealing at 45°C for 15 sec, and an extension at 72°C for 45 sec, followed by 1 cycle of 72°C for 3 min. Negative controls were included in each PCR experiment. The following primer pairs

amplified cyt *b*: MVZ4 and MVZ5, MVZ14 and MVZ23, MVZ16 and Marten37 (Table 6). Both forward and reverse strands were sequenced for each individual.

A total of 680 American martens were examined. Complete cyt *b* sequences (1140 base pairs; corresponding to sites 14139-15282 of *Mus musculus*; Bibb *et al.* 1981) were generated from 30 *M. americana*, partial cyt b sequences (441 base pairs using primers Marten37/MVZ16; corresponding to sites 14498-14938 of *Mus musculus*; Bibb *et al.* 1981) were generated from an additional 151 *M. americana*, and restriction fragment length polymorphism (RFLP) profiles were determined for the remaining 499 individuals (Appendix I). All DNA sequences will be deposited in GenBank. 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. Complete cyt *b* sequences also were generated from one European pine marten (*M. martes*) and one sable (*M. zibellina*) and used as outgroups.

Relationships among sequences were examined using 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 neighbor-joining (Kimura 2-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) with 100 replicates and maximum parsimony heuristic searches. The strict consensus tree was then subjected to 1000 bootstrap replicates.

I used RFLP analysis to document the geographic extent of the *americana* and *caurina* groups in the Pacific Northwest. A restriction enzyme (Nla III) that differentially digested PCR products from each mtDNA lineage was determined using DNA Strider 1.2 (written by C. Marck). A portion of the cyt *b* gene and flanking region (using primers Marten37/MVZ14) was amplified from 16 martens of known mtDNA lineages. A mixture of 9.0 µl PCR product, 1.0 µl New England Biolabs 10X buffer4, 0.10 µl BSA (10 mg/mL), and 0.2 µl Nla III restriction enzyme (2 units) was placed in a 37°C incubator for 2-3 hours. DNA fragments were visualized on a 1.5% agarose gel stained with ethidium bromide. After RFLP banding patterns were established for the divergent lineages, I screened an additional 499 martens to determine lineage profiles (Appendix I).

Results

Base composition (A = 28.0%, C = 31.0%, G = 14.5%, T = 26.5%) for cyt *b* was consistent with other mammals (*e.g.*, Irwin *et al.* 1991; Talbot and Shields 1996; Stone and 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 saturation has not been reached (Lara *et al.* 1996). Four equally parsimonious trees (112

steps with 65 informative characters) displayed two reciprocally monophyletic clades corresponding to the *americana* and *caurina* groups. Two subclades within the *caurina* clade (Fig. 4) were also apparent. Divergence between clades ranged from 2.5-3.0% (uncorrected *p*); whereas, intra-clade 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) differed between the *americana* and *caurina* clades (five first position, two second position, and 20 third position changes). 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 the *caurina* clade (one first position transitions and three third position transitions). The three third position transitions were synonymous; whereas, the first position transition resulted in an amino acid change. This non-synonymous 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).

A condensed dot matrix (Table 7) was generated to display variation among the 151 partial cyt *b* sequences, plus the corresponding portion for the 30 full cyt *b* sequences. The partial cyt *b* sequences (N = 151) from various locations revealed low levels of intra-clade variation (Table 7). RFLP analyses (Fig. 3) identified 413 *americana* and 86 *caurina* haplotypes (Appendix I). The widespread *americana* group

extends from interior Alaska south to Montana and eastward to Newfoundland and New England. The *caurina* group minimally extends from Admiralty Island, southeastern Alaska south to Oregon and Wyoming (Fig. 3, inset map).

Discussion

Glacial refugia and intraspecific differentiation

Wooding and 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, *Ursus americanus*. During much of the last 120,000 years, they contend these segregated forests formed a barrier to dispersal for some species. Following segregation while ice sheets were receding, eastern forests apparently expanded more rapidly than western forests (Williams *et al.* 1993).

American martens show a pattern of geographic diversification similar to black bears (Stone and Cook 2000), with relatively large inter-clade differences (inter-clade variation = 2.5-3.0%; intra-clade variation < 0.7%). The two marten morphological groups correspond to these reciprocally monophyletic clades. Broad correspondence in geographic distribution of eastern and western clades in martens and black bears implicates a similar set of vicariant events and is consistent with Hoffmann's idea that "taxa from the large glacial refugium in southeastern North America reoccupied a larger area" than taxa from smaller western refugia (Hoffmann 1985, p. 470-1). Similarly, the distribution of late Pleistocene – late Holocene fossil records of martens also supports the hypothesis of separate forest refugia since the last (Wisconsinan) glaciation (Fig. 5).

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 $\pm$ 0.8 million years since divergence, Wooding and Ward 1997; Stone and Cook 2000) and many other taxa (Klicka and Zink 1997; Avise *et al.* 1998). Estimates of divergence may be impacted, however, by a variety of factors such as levels of ancestral polymorphisms. Divergent clades may have come into secondary contact multiple times over the past million years during inter-glacial periods (*e.g.*, Leonard *et al.* 2000), but I detected no genetic signature of past contact (*e.g.*, divergent *americana* individuals located in western United States). Genetic admixture, occurring during the potentially repeated northward expansions of inter-glacial periods, may have been eliminated by subsequent glacial advances (Hewitt 1996). In other words, "hybrid zones may protect the integrity of two genomes until the next ice age reduces the species to its refugia; and this may recur over several ice ages" (Hewitt 1996, p. 259).

Within the *americana* clade, little geographic structure was present, possibly suggesting these individuals came from a recently expanded population. An association between the central island cluster of the Alexander Archipelago (*i.e.*, Mitkof and Kuiu islands) and British Columbia samples (Fig. 4) suggested gene flow between these disjunct areas, possibly through river corridors that transect the Coast Mountain Range. This aspect should be addressed using more rapidly evolving nuclear markers (Bruford and Wayne 1993; Queller *et al.* 1993).

Within the *caurina* clade (Fig. 4), subclades may have resulted from separation into distinctive refugial populations in western North America during more recent glaciation events. Populations in the upper subclade of the *caurina* clade (including southeastern Alaska, Haida Gwaii, Vancouver Island, and southern Montana; Fig. 4) probably diverged since the past glaciation, as did populations within the lower subclade (including Oregon, southern Montana, and Wyoming). My data were consistent with a North Pacific Coast glacial refugium; 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.

Colonization history of a forest associated mammal and contact zones

I hypothesize that the *caurina* clade colonized the North Pacific Coast (including southeastern Alaska) earlier than the *americana* clade (see Fig. 5C). Lodgepole pine, *Pinus contorta*, was established as early as 10,500 years before present (BP) along the southeastern Alaskan coast. Establishment of the same species on the inland (eastern) side of the Coast Mountain Range did not occur until about 2,300 BP (Peteet 1991). Additional research (Mathewes 1989; Fedje and Josenhans 2000) suggests 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). Because I expected a general correlation between range expansion of vegetation and associated animals (Hewitt 1996), the ice-free, forested corridor may have served as a route for forest-associated species, such as

martens, to colonize the coast from a southerly refugial population (MacDonald and Cook 1996). It is doubtful that martens would have colonized areas before the existence of suitable forested regions because of their strong association with such habitats as seen throughout their current distributional range and fossil history (Clark *et al.* 1987).

Admiralty, Kuiu, Haida Gwaii, and Vancouver island populations maintained unique *caurina* haplotypes (Table 7 and Fig. 4) suggesting these populations have been isolated. Although haplotypes were unique, differentiation was minimal (1-2 mutations) suggesting the effects of post-glacial events and supporting Giannico and Nagorsen's (1989) idea that the distinct phenotype of martens from Haida Gwaii (Queen Charlotte islands) evolved very recently. Haplotypes of the *americana* clade were distributed across a much larger geographic area (Fig. 3, inset map) and may be indicative of a more rapidly expanding population (Hewitt 1993). Populations in this clade apparently followed the westward progression of the eastern refugial forest. The North Pacific Coast apparently was colonized by the *americana* clade subsequent to the opening of ice-free corridors through the Coast Mountain Range. This colonization may have resulted in the shared occurrence of *americana* haplotypes on the mainland and near-shore islands (Table 7 and Fig. 4).

The current disjunct distribution of the *caurina* clade along the coast may be partially the result of genetic swamping of this clade by the *americana* clade. When gene flow is relatively high between two taxa, outbreeding depression and extinction via hybridization or genetic assimilation may occur (Ellstrand 1992). Furthermore, alleles with a slight advantage can spread rapidly through a population (Barton 1986; Barton and

Hewitt 1989). Extensive sampling in southeastern Alaska revealed only one region of contact (Kuiu Island). I suspect that Kuiu Island was colonized recently by the *americana* clade as a result of island hopping across Mitkof and Kupreanof islands (peninsular effect) and shallow water channels (Fig. 3). Possible introgression and/or genetic swamping of the *caurina* by the *americana* clade should be investigated. The *caurina* clade may persist on islands such as Admiralty, Haida Gwaii, and Vancouver because these islands were sufficiently isolated to eliminate potential colonization by the *americana* clade.

Introduced populations of martens

In the 1930's, 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 1949-1952. These transplants were made without knowledge of the underlying morphologic and genetic variation that exists across the region (Elkins and Nelson 1954; Burris and McKnight 1973; MacDonald and 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 and Nelson 1954). Extensive sampling of these introduced populations (*e.g.*, Chichagof Island, N = 117) suggested that the *americana* clade had been the sole source of populations for these introductions. Giannico and Nagorsen's (1989) morphological assessment of samples from Baranof and Chichagof islands indicated that these populations belong to the *americana* clade.

However, contrary to their conclusion that *americana* was found exclusively throughout the region, my data indicated some individuals (martens from Admiralty Island and some martens from Kuiu Island) also belong to the *caurina* clade.

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 islands of the Alexander Archipelago were naturally colonized first by the *caurina* clade, then the persistent populations of *caurina* on Admiralty and Kuiu islands may be remnants of a once much more widespread clade across the archipelago. Although the disjunct distribution of the *caurina* clade (*i.e.*, *caurina* on Admiralty and Kuiu islands) may also appear to be the result of introductions, I doubt this is the case because both populations maintain unique haplotypes. Marten populations along the North Pacific Coast displayed significant genetic substructure apparently due to multiple colonizations of the region by divergent lineages (both natural and human-induced). These data further exemplify the need to develop a historical framework for biota of a region through extensive sampling if we are to hope to effectively understand the complexities associated with environmental change (Wilson 2000).

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Table 6. Sequences and associated references for primers used to amplify the mitochondrial cytochrome *b* gene.

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

Table 7. Condensed dot matrix displaying cytochrome *b* haplotypes and positions of substitutions.

Location	4	4	4	4	4	4	5	5	5	5	5	5	6	6	7	7	7	7	7	7	7
	3	4	4	6	7	8	2	6	6	7	7	8	0	1	1	2	3	7	7	8	9
	8	5	7	8	7	0	8	5	9	6	7	5	3	8	3	2	2	0	7	3	2
Chichagof Island, SE AK (1)	T	T	G	C	T	T	G	A	C	A	G	T	C	T	T	T	A	C	C	A	G
Chichagof Island, SE AK (10)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Chichagof Island, SE AK (4)	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Baranof Island, SE AK (10)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
Kruzof Island, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
Partofshikof Island, SE AK (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
Kupreanof Island, SE AK (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Mitkof Island, SE AK (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Mitkof Island, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Woewodski Island, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kuiu Island, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Kuiu Island, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
Kuiu Island, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Prince of Wales Island, SE AK (8)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Prince of Wales Island, SE AK (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Kosciusko Island, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Revillagigedo Island, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Yakutat, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Glacier Bay, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Glacier Bay, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
Katzehin River, SE AK (5)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Juneau, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Juneau, SE AK (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Thomas Bay, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Thomas Bay, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
Thomas Bay, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cleveland Peninsula, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Cleveland Peninsula, SE AK (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 7 continued.

Cleveland Peninsula, SE AK (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.
Interior Alaska (7)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Interior Alaska (1)	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
Interior Alaska (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
South-central Alaska (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A
South-central Alaska (1)	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
South-central Alaska (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
northern British Columbia (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
northern British Columbia (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
northern British Columbia (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.
central British Columbia (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
central British Columbia (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
northern Montana (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
southern Montana (1)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Admiralty Island, SE AK (21)	.	C	A	T	.	C	.	.	T	G	.	A	T	.	C	.	.	T	.	G	A
Kuiu Island, SE AK (12)	.	C	A	.	C	C	.	.	T	G	.	A	T	.	C	.	.	T	.	G	A
Haida Gwaii, BC (5)	.	C	A	.	.	C	.	.	T	G	.	A	T	.	C	.	.	.	.	G	A
Vancouver Island, BC (2)	.	C	A	.	.	C	.	.	T	G	.	A	T	.	C	.	.	T	.	G	A
southern Montana (1)	.	C	A	.	.	C	A	G	T	G	.	A	T	.	C	.	G	T	.	G	A
southern Montana (1)	.	C	A	.	.	C	.	.	T	G	.	A	T	.	C	.	.	T	.	G	A
Oregon (6)	.	C	A	.	.	C	A	G	T	G	.	A	T	.	C	.	.	T	.	G	A
Wyoming (4)	.	C	A	.	.	C	A	G	T	G	.	A	T	.	C	.	.	T	.	G	A
Wyoming (1)	.	C	A	.	.	C	A	G	T	G	.	A	T	C	C	.	.	T	.	G	A

Appendix I. Collection locations, lineage profiles, molecular methods used, and voucher numbers for *Martes americana* specimens.

Locality	Lineage	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*	RFLP	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*	RFLP	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*	RFLP	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*	RFLP	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*	RFLP	14476-90, 14492-4, 16028-32, 16034-53, 16057, 19937-40, 19947-61

Appendix I continued.

Woewodski Island, SE AK	*americana*	441 bp cyt b	10822
Woewodski Island, SE AK	*americana*	RFLP	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*	RFLP	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*	RFLP	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*	RFLP	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*	RFLP	10774-89, 24454
Glacier Bay, SE AK	*americana*	441 bp cyt b	10848-51, 14628

Appendix I continued.

Glacier Bay, SE AK	*americana*	RFLP	19990
Katzehin River, SE AK	*americana*	441 bp cyt b	14591-5
Katzehin River, SE AK	*americana*	RFLP	14592
Juneau, SE AK	*americana*	full cyt b	14952
Juneau, SE AK	*americana*	441 bp cyt b	14954, 14956-7
Juneau, SE AK	*americana*	RFLP	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*	RFLP	19946, 20071-3, 20077-80, 24500-6
Cleveland Peninsula, SE AK	*americana*	441 bp cyt b	10695-9
Cleveland Peninsula, SE AK	*americana*	RFLP	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*	RFLP	24601-15, 24627, 24629, 24631-2, 24636-46
South-central Alaska	*americana*	441 bp cyt b	14111-2, 14114-6
South-central Alaska	*americana*	RFLP	13559
northern BC	*americana*	full cyt b	16004
northern BC	*americana*	441 bp cyt b	16005-8

Appendix I continued.

northern BC	*americana*	RFLP	16007
central BC	*americana*	full cyt b	16010, 16020
central BC	*americana*	441 bp cyt b	16009, 16014-5, 16019
central BC	*americana*	RFLP	16011-3, 16016-8, 16021-3, 16026, 16033, 20612
northern Montana	*americana*	full cyt b	23185
northern Montana	*americana*	RFLP	23180-2, 23185-92
southern Montana	*americana*	full cyt b	23183
southern Montana	*americana*	RFLP	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*	RFLP	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*	RFLP	24472, 25301, 25303, 25307, 25309, 25316, 25319, 25321, 25327, 25330
Graham Island, Haida Gwaii, BC	*caurina*	full cyt b	20601, 20604

Appendix I continued.

Graham Island, Haida Gwaii, BC	*caurina*	441 bp cyt b	20603, 20605-6
Graham Island, Haida Gwaii, BC	*caurina*	RFLP	20602, 20607-11
Vancouver Island, BC	*caurina*	full cyt b	24477-8
Vancouver Island, BC	*caurina*	RFLP	24475-8, 24479-97
southern Montana	*caurina*	full cyt b	23169, 23171
southern Montana	*caurina*	RFLP	23168-71, 23172-9
Oregon	*caurina*	full cyt b	15936-7
Oregon	*caurina*	441 bp cyt b	15931, 15935, 15938-9
Oregon	*caurina*	RFLP	15941, 19543, 15945-7, 15950-5
Wyoming	*caurina*	full cyt b	20613-4
Wyoming	*caurina*	441 bp cyt b	20615-7

* 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 (RFLP)

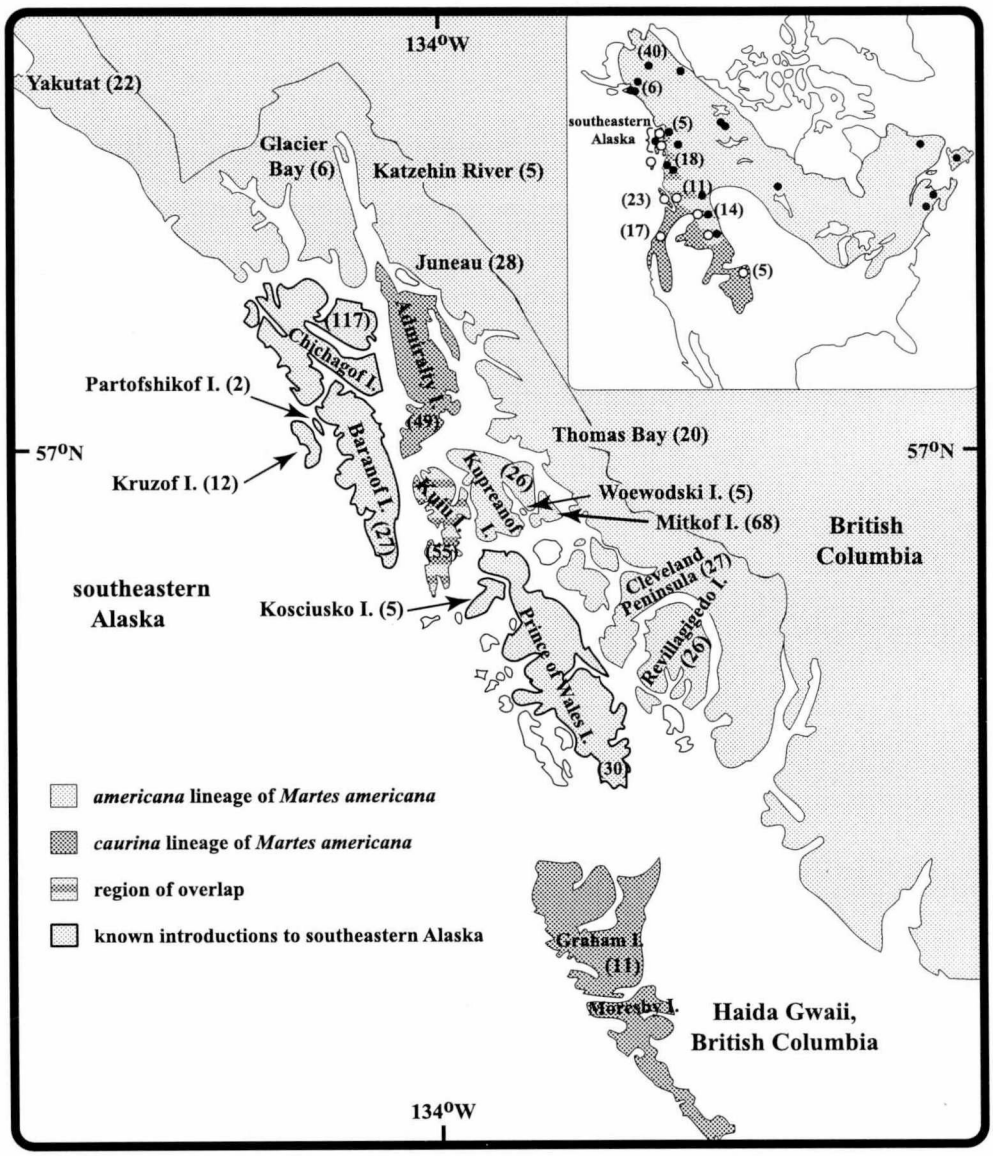

Fig. 3. Distribution of mitochondrial clades of American martens (*Martes americana*) in southeastern Alaska. Numbers in parentheses indicate sample sizes for locations analyzed. Inset map shows the North American distribution of martens modified from Hall (1981) and plots sample localities from this study, Carr and Hicks (1997), and Hosoda *et al.* (1997). Black dots and open circles represent marten samples belonging to the *americana* and *caurina* clades, respectively.

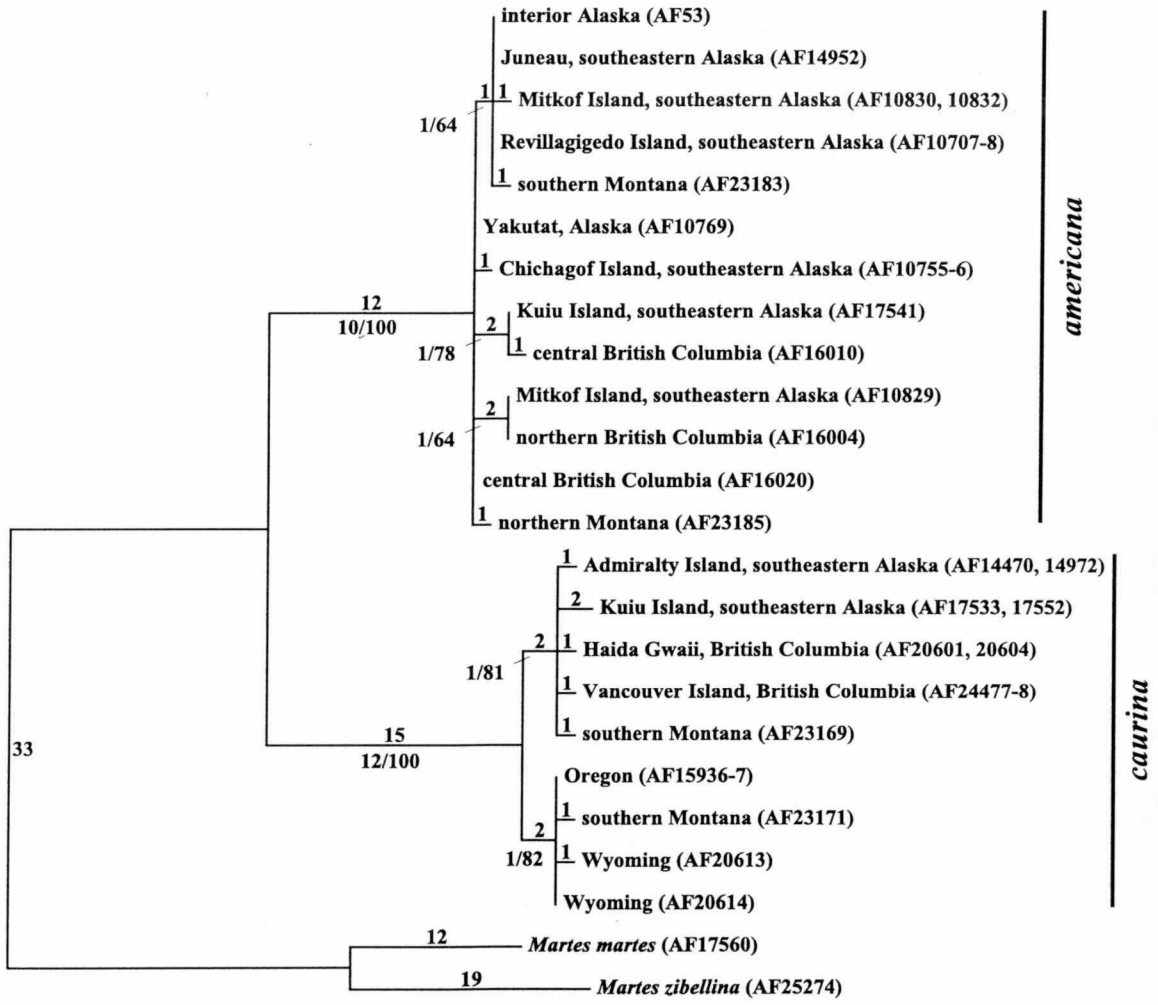

Fig. 4. Strict consensus tree of four equally parsimonious trees (length = 112 steps; CI = 0.9107; RI = 0.9669) generated from complete cytochrome *b* gene sequences of American martens (*Martes americana*) with a branch-and-bound search. Branch lengths are shown above branches, and Bremer decay indices/bootstrap values are below branches.

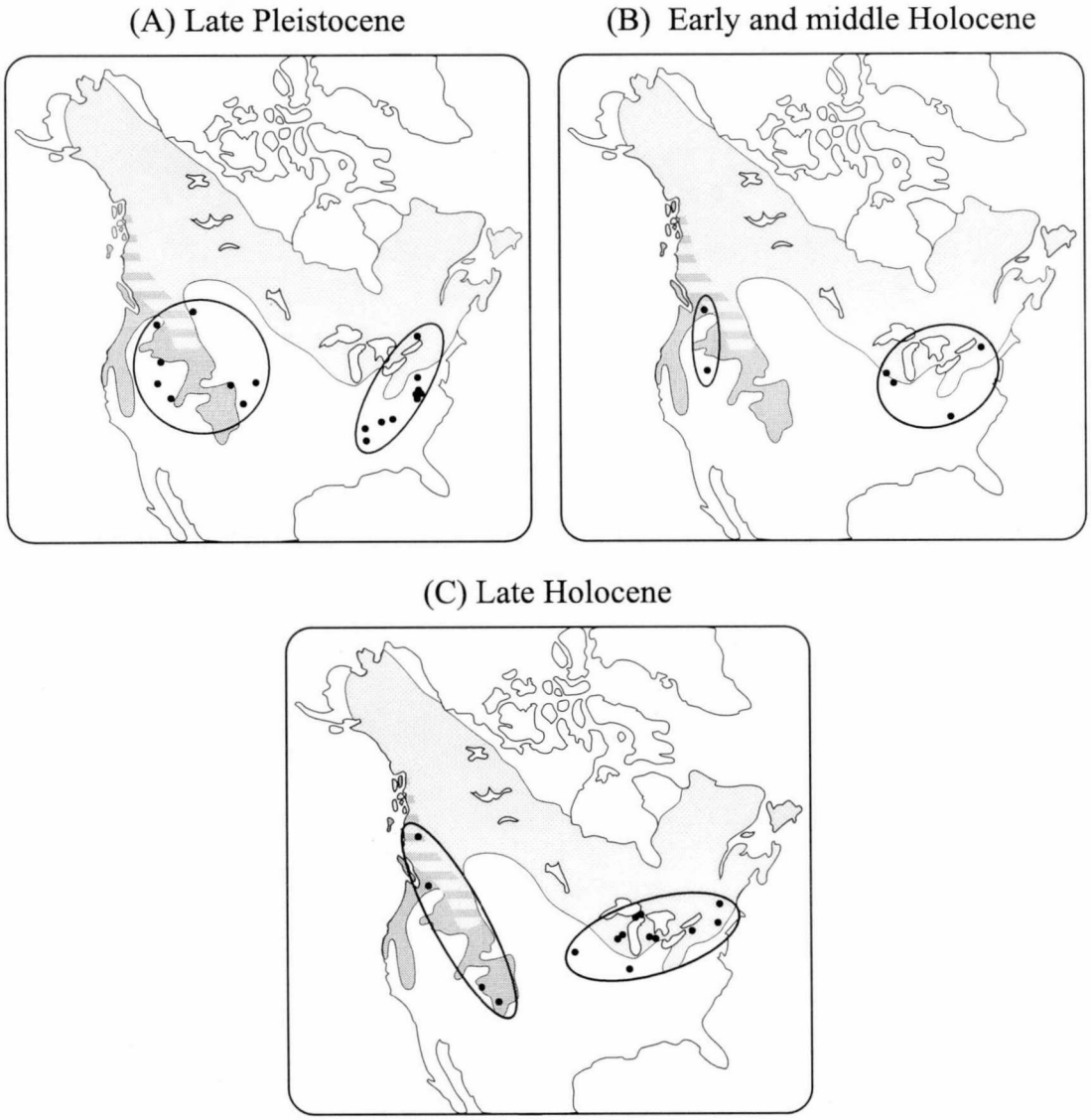

Fig. 5. Figure modified from Graham and Graham (1994) of fossil records of American martens, *Martes americana*, from (A) late Pleistocene, (B) early/middle Holocene, and (C) late Holocene. Fossil records are overlaid upon the current distribution of marten (Hall 1981) with light and dark gray representing 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.

X. Chapter 3

Differentiation of American marten (*Martes americana*) populations across a fragmented landscape[3]

Abstract

Nuclear microsatellites have been used extensively to assess population differentiation and gene flow. I examined microsatellite variation across 7 loci for 211 American martens (*Martes americana*) to investigate population genetics in the Pacific Northwest. Within American martens, two reciprocally monophyletic clades (*americana* and *caurina*) have been identified based on morphology and mitochondrial DNA sequences. I determined that 1 of 7 microsatellite loci was diagnostic for the two clades, corroborating their distinctiveness. This locus revealed that hybridization has occurred in a limited region of sympatry (Kuiu Island, southeastern Alaska); however, asymmetrical introgression may exist and play a major role in the dynamics of the *americana – caurina* hybrid zone. In addition, analyses indicated that *caurina* populations have been isolated and differentiation has occurred; whereas, the *americana* clade may represent recently expanding populations. Population differentiation was attributed to habitat fragmentation, as opposed to isolation-by-distance, with oceanic straits and other physiographic features posing potential barriers to gene flow.

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[3]Stone, K. D., and J. A. Cook. (in preparation). Differentiation of American marten (*Martes americana*) populations across a fragmented landscape. Journal of Mammalogy.

Introduction

Nuclear microsatellite loci have been used extensively to assess population genetic structure (for review, see Bruford and Wayne 1993; Queller et al. 1993; Schlötterer and Pemberton 1994). Because of their relatively high mutation rates (Ellegren 1995; Schlötterer and Tautz 1992; Tautz 1989), microsatellites are valued as tools for genome mapping, characterizing populations, and identifying individuals (Bruford and Wayne 1993; Freimer and Slatkin 1996; Weissenbach et al. 1992). However, microsatellites have questionable utility for deeper phylogenetic reconstruction (FitzSimmons et al. 1995) because too many mutations may accumulate between species creating a vast amount of homoplasy (Garza and Freimer 1996).

I used microsatellite markers to assess preliminary characteristics of population structure and to explore phylogeographic patterns previously identified (Carr and Hicks 1997; Stone et al. submitted) for a medium-sized carnivore, American marten (*Martes americana*). This species is valued as a furbearer throughout most of its distribution in northern North America (Fig. 6, inset map; Hall 1981). Numerous studies have investigated habitat requirements and limitations of the species (*e.g.*, Buskirk et al. 1994; Clark et al. 1987; Proulx et al. 1997); however, investigations of population genetic structure are few (Kyle and Strobeck in press; McGowan et al. 1999; Mitton and Raphael 1990). Information on gene flow and genetic diversity can help delimit units for more effective conservation and management of the species (Crozier 1992; Faith 1992; Moritz 1994; Ryder 1986; Vane-Wright et al. 1991).

Although 8 subspecies of *Martes americana* have been described (Clark et al. 1987), these are traditionally placed in two morphologically and genetically distinct groups, *americana* and *caurina* (Carr and Hicks 1997; Clark et al. 1987; Merriam 1890; Stone et al. submitted). The *americana* subspecies group contains the following subspecies (Clark et al. 1987): *M. a. abietinoides*, *actuosa*, *americana*, *atrata*, and *kenaiensis*. The remaining three subspecies (*M. a. caurina*, *humboldtensis*, and *nesophila*) are included in the *caurina* subspecies group. These subspecies groups may have diverged during the Pleistocene as a result of isolation in distinct southern refugia (*americana* isolated in eastern and *caurina* isolated in western United States, respectively; Carr and Hicks 1997; Stone et al. submitted; Stone and Cook in preparation).

Currently, the *americana* group is more widespread, distributed from Montana and Idaho northward to Alaska and eastward to the Atlantic Coast. The *caurina* group is described from along the West Coast (California to southeastern Alaska), eastward to Wyoming, Montana, and Idaho (Fig. 6, inset map; Carr and Hicks 1997; Hall 1981; Stone et al. submitted; Wright 1953). Zones of sympatry between the two groups have been identified from southern and western Montana and southeastern Alaska (Kuiu Island; Stone et al. submitted; Stone and Cook in preparation; Wright 1953). Wright (1953) reports intergradation between the groups and suggests that they belong to a single species, *M. americana*, which reflects the current taxonomy (Wilson and Reeder 1993). However, Carr and Hicks (1997) question whether unidirectional gene flow exists between these groups and suggest the *caurina* group represents a distinct species.

I investigated population differentiation of 20 marten populations from the Pacific Northwest using 7 nuclear microsatellite markers. The Pacific Northwest was chosen as an area of concentration, with particular focus on southeastern Alaska, because the *americana* and *caurina* clades meet in this region. The deep fjords and glaciers of the mainland of southeastern Alaska, combined with the 2000+ named islands of the Alexander Archipelago, form a heterogeneous landscape. These features and a dynamic glacial history during the Pleistocene have contributed to a highly fragmented flora and fauna with many potentially significant barriers to gene flow among populations.

Southeastern Alaska has long been recognized as a unique region (Klein 1965; Swarth 1936) with numerous endemic nominal species and subspecies (MacDonald and Cook 1996) and genetic investigations have begun to reveal distinct evolutionary lineages of several mammalian taxa (Conroy and Cook 2000; Cook et al. in press; Demboski et al. 1999; Stone and Cook 2000; Stone et al. submitted; Talbot and Shields 1996). This high degree of endemism and diversity of lineages suggests a complex colonization history for deglaciated areas within the Pacific Northwest. For example, Stone et al. (submitted) hypothesize that the *caurina* clade of *Martes americana* represents an early colonization of the region as coastal ice receded at the end of the past glaciation whereas *americana* represents a more recent colonizer of the region.

In this study, I examined phylogeographic relationships among populations and evaluated nuclear data to determine if it corroborated mitochondrial (mt) DNA results that identified the *americana* and *caurina* clades. In addition, I investigated

differentiation of marten populations from the Pacific Northwest within the context of barriers to gene flow and colonization into deglaciated areas.

Materials and Methods

Sampling–

Twenty populations (10 island, 10 mainland; Fig. 6), represented by 211 individuals, were chosen and centered on the Pacific Northwest region of North America (12 populations from southeastern Alaska, 4 from British Columbia, 1 from interior Alaska, 2 from Montana, and 1 from Oregon; Fig. 6, Table 8). Each population was represented by 10 individuals with 3 exceptions (Table 8): 2 previously described areas of sympatry between the *americana* and *caurina* clades (Stone et al. submitted) were represented by larger sample sizes (southern Montana, N = 11; Kuiu Island, southeastern Alaska, N = 25), and only 5 samples were available for northern British Columbia. Three island populations from southeastern Alaska (Chichagof, Baranof, and Prince of Wales islands) were the result of human introductions in the mid 1900's by the Alaska Game Commission (Burris and McKnight 1973; Elkins and Nelson 1954).

DNA extraction and microsatellite amplification–

DNA was extracted from marten tissues (heart, spleen, skeletal muscle, skin, or blood) archived in the Alaska Frozen Tissue Collection of the University of Alaska Museum (AFTC). Methods of extraction followed those of Lessa and Cook (1998). All samples had previously been screened to determine mtDNA clade profiles (*americana* or

caurina) using automated sequencing or restriction fragment length analysis (Stone et al. submitted). Amplification of microsatellite markers was done in 13 µl volumes containing 0.23 µM of each primer, 154 µM dNTPs, 1.4 or 4.3 mM $MgCl_2$, 25 µg/mL BSA, 0.25 units of Perkin-Elmer AmpliTaq or AmpliTaq Gold DNA polymerase, Perkin-Elmer 1X PCR buffer II, and 50-100 ng whole genomic DNA. Microsatellites were amplified using a Perkin-Elmer GeneAmp PCR System 9700 with the following PCR conditions: 1 cycle of 94°C for 1 min (for AmpliTaq) or 1 cycle of 95°C for 12 min (for AmpliTaq Gold), followed by 2 cycles (30 s at 94°C, 20 s at 58°C, 5 s at 72°C), 33 cycles (15 s at 94°C, 20 s at 54°C, 5 s at 72°C), and 1 cycle (30 min at 72°C). Negative controls were included in each amplification experiment.

The following primers were used: MA1, MA2, MA3, MA5, MA8, MA15, and MA19 (Davis and Strobeck 1998). AmpliTaq DNA polymerase was used with the preceding primers to amplify DNA fragments with the exception of MA3 and MA19, where AmpliTaq Gold DNA polymerase was used instead. The final concentration of $MgCl_2$ for all reactions was 4.3 mM, with the exception of reactions using primers MA8 and MA15 (in which the final concentration of $MgCl_2$ was 1.4 mM). The 5' end of one primer for each locus was fluorescently labeled with 6-FAM or TET dyes. Samples were run on an ABI 373 automated sequencer. Alleles were sized (bp) using an internal lane size standard (GS350 by Perkin-Elmer), GeneScan Analysis 3.1, and Genotyper 1.1 computer programs.

Data analysis–

Genetic Data Analysis (GDA) version 1.0 (http://alleyn.eeb.uconn.edu/gda/) was used to calculate descriptive statistics, and GENEPOP version 3.2 (ftp://ftp.cefe.cnrs-mop.fr/pub/PC/MSDOS/GENEPOP/; Raymond and Rousset 1995) was used to test for Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium. For each population, HWE and linkage disequilibrium were tested per locus and between each pair of loci, respectively. For loci with 4 or fewer alleles, exact tests (Louis and Dempster 1987) were used to estimate *P*-values to test for deviations from HWE. Loci with greater than 4 alleles had *P*-values estimated by the Markov chain method (Guo and Thompson 1992). Genotypic linkage disequilibrium was tested using the Markov chain method using default parameters for dememorization number, batches, and iterations. These tests used a sequential Bonferroni adjustment (initial $\alpha = 0.0025$) for multiple comparisons (Rice 1989).

An unrooted network of genetic relationships was inferred from allele-sharing distances (Bowcock et al. 1994). Pairwise distances were calculated using SHAREDST (calculator located at http://www.biology.ualberta.ca/jbrzusto/sharedst.html), with the allele-sharing distance defined as one minus half the average number of shared alleles per locus. The FITCH program in PHYLIP version 3.5c (http://evolution.genetics.washington.edu/phylip.html; Felsenstein 1993) was then used to construct a Fitch and Margoliash (1967) tree from the allele-sharing distance matrix.

Pairwise population distances and population networks were also generated using PHYLIP. The maximum-likelihood values and tree file were calculated in the CONTML

program using a random input order. Because the maximum-likelihood analysis assumes that each locus evolves independently by genetic drift, I also calculated Nei's (1972) standard genetic distance (D) for all population pairs in GENDIST. The NEIGHBOR program, employing the neighbor-joining algorithm, was used to generate a tree file. To test the robustness of tree topologies, 1000 bootstrap replicates were generated in SEQBOOT and used as input files for distance programs (CONTML and GENDIST). After tree topologies were created for all replicates, a consensus tree was generated in CONSENSE. Tree files were viewed in TREEVIEW version 1.5 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html; Page 1996).

An assignment test (Paetkau et al. 1995; Paetkau et al. 1997) used the calculator at http://www.biology.ualberta.ca/jbrzusto/Doh.html. This test calculated the probability that an individual came from each of the 20 populations. Individuals were then assigned to a population with the highest probability. The assignment test was run using both Titterington et al.'s (1981) method to adjust all frequencies to avoid zeros and the option in which gene frequencies equal to zero were replaced with 0.01. Genes were shuffled at each locus within populations (with no replacement) using 1000 randomizations.

GDA was used to calculate Weir and Cockerham's (1984) coancestry coefficient (θ), which is analogous to Wright's F_{ST} (Wright 1951). Confidence intervals (99%) were calculated for these values to determine if they differed significantly from zero using 5000 bootstrap replicates. The α level was set to 0.01 because reliable confidence intervals could not be calculated greater than 99% due to the number of polymorphic loci used in this study (N = 6). Isolation-by-distance was examined (with the Mantel test and

1000 permutations) in GENEPOP using θ as a measure of genetic differentiation. Isolation-by-distance was assessed for the complete data set and 3 subsets: (1) populations belonging to the *americana* clade excluding introduced island populations, (2) populations belonging to the *caurina* clade, and (3) *americana* populations from interior Alaska, northern and central British Columbia, and northern Montana. Linear regression assessed the significance of the correlation between θ and geographic distance (in km).

Results

A considerable amount of variability was observed across the 7 microsatellite loci examined (Appendix II). Numbers of alleles per locus across all populations ranged from 2 (locus MA15) to 21 (locus MA1). Locus MA15 deviated from HWE across multiple populations and was therefore removed from further analyses. Similar results are reported in Kyle and Strobeck (in press). Mean number of alleles and variance across 6 loci, percent polymorphic loci, expected heterozygosity, and observed heterozygosity were calculated for each population (Table 8). Only 1 locus (MA1), from 1 population (Juneau), deviated from HWE. Tests for genotypic linkage disequilibrium indicated that all pairwise comparisons of loci were independent.

One locus (MA1) was diagnostic for the mtDNA clades. Allele sizes at this locus for the *americana* clade ranged from 207-223 bp and for the *caurina* clade ranged from 195-205 bp. Individuals from an area of sympatry in southern Montana had nuclear (nDNA) alleles for the MA1 locus that were diagnostic for either the *americana* or

caurina mtDNA clades; whereas, 12 of 25 martens from the other area of sympatry (Kuiu Island, southeastern Alaska) showed nDNA alleles representing both mtDNA clades. All combinations of mtDNA haplotypes and the diagnostic nDNA locus MA1 were found on Kuiu Island with the exception of individuals showing mtDNA characteristic of *caurina* and only nDNA alleles characteristic of *americana*. In addition, few individuals (N = 4) from three "pure" mtDNA *americana* populations (Kupreanof Island, Cleveland Peninsula, and northern Montana) possessed nDNA *caurina* alleles at the MA1 locus.

Genetic relationships among individuals, determined by the allele-sharing distance, define 2 weakly supported clades (Fig. 7). Clade I contained individuals from mtDNA *americana* populations and Queen Charlotte Islands, while Clade II contained individuals from all mtDNA *caurina* populations (with the exception of Queen Charlotte Islands) and populations with both mtDNA *americana* and mtDNA *caurina*. MtDNA *americana* populations throughout Clade I were poorly defined with the exception of Baranof and Revillagigedo islands, and Yakutat. In contrast, mtDNA *caurina* populations (Admiralty, Queen Charlotte, and Vancouver islands, and Oregon) were highly distinctive. In the Admiralty Island population, all 10 individuals were genetically identical across all 6 loci. Likewise, populations (Kuiu Island and southern Montana) from the two areas of sympatry were also well-defined with the exception of a few individuals.

Similarly, the unrooted network of genetic relationships among populations inferred from the maximum-likelihood analysis identified 2 clades (Fig. 8). Clade I consisted of populations of individuals with the mtDNA *americana* haplotype, while

Clade II contained pure mtDNA *caurina* populations and populations with both mtDNA *americana* and mtDNA *caurina* haplotypes. Nei's (1972) standard genetic distance gave similar results with the exception of Queen Charlotte Islands (which was included in Clade I). Bootstrap support was less than 68% for all branches; however, branch lengths were robust across different distance measures (data not shown). Although I could not determine which populations were most closely related, the network indicated that populations in Clade II and populations such as Baranof and Revillagigedo islands, and Yakutat in Clade I had diverged significantly from other populations, probably due to limited gene flow with other populations. Short branches defined most populations of Clade I (except Baranof and Revillagigedo islands, and Yakutat), and these may represent recently expanding populations.

The assignment test (Table 9) used Titterington et al.'s (1981) method to adjust all gene frequencies to avoid zeros and differed minimally from those where zero values were replaced with 0.01. Assignment values corroborated the conclusion that individuals from mtDNA *caurina* populations, populations with both mtDNA *americana* and mtDNA *caurina*, and the mtDNA *americana* populations from Baranof and Revillagigedo islands, and Yakutat were well-defined. Most other populations had several mis-assigned individuals (Table 9).

Weir and Cockerham's (1984) θ for all pairs of marten populations (Table 10) showed varying levels of population differentiation, with 165 comparisons being significantly greater than zero. The Mantel test for isolation-by-distance indicated genetic and geographic distances were independent ($P = 0.5960$). This hypothesis was

also accepted after analyzing the two mtDNA clades separately (*americana*, $P = 0.6270$; *caurina*, $P = 0.5920$). All linear regressions produced R^2 values less than 0.0210. When inland populations (interior Alaska, northern and central British Columbia, and northern Montana) were analyzed separately, geographic distance explained 85% of the variation in genetic distance ($R^2 = 0.8469$; $P = 0.0850$).

Discussion

Fast-evolving nuclear microsatellites were useful for assessing population genetic structure among populations of a forest-associated carnivore, the American marten. My nuclear data corroborated previous mtDNA results distinguishing the *americana* and *caurina* clades, allowed assessment of hybridization (and introgression) between members of these clades, and provided insight into the colonization of the North Pacific Coast.

Nuclear versus mitochondrial perspectives–

To converge on a species tree, multiple independent loci representing both nuclear and mitochondrial genomes should be assessed (Pamilo and Nei 1988). I present a nuclear data set from biparentally inherited loci to complement existing mtDNA data. Previous studies investigated differentiation in American martens using mtDNA define two reciprocally monophyletic clades (*americana* and *caurina*; Carr and Hicks 1997; Stone et al. submitted; Stone and Cook in preparation). From a conservation perspective, Moritz (1994) states the need for evolutionarily significant units to not only be

reciprocally monophyletic for mtDNA alleles but to also maintain significantly diverse allele frequencies at nuclear loci. My study identified one diagnostic microsatellite locus (MA1) for the mtDNA *americana* and *caurina* clades, corroborating sequences from the mt cytochrome *b* gene (Carr and Hicks 1997; Stone et al. submitted) and nuclear aldolase C gene (Stone and Cook in preparation), and earlier morphological work (Merriam 1890; Anderson 1970).

Hybridization–

The question of taxonomy remains and may be best investigated in areas of sympatry. Areas of sympatry are of particular interest, because potential hybrid zones allow us "to quantify the genetic differences responsible for speciation [and] to measure the diffusion of genes between diverging taxa" (Barton and Hewitt 1989, p. 497). The American marten clades are reported to hybridize in western Montana on the basis of morphological characteristics (Wright 1953). The dynamics of hybridization are now being further investigated (Stone and Cook in preparation), and these independent sets of molecular markers provide opportunities to characterize hybrid zones.

I began to investigate whether unidirectional gene flow exists between *americana* and *caurina* with the individuals from the purported contact zones of southern Montana and Kuiu Island that were defined by mt cytochrome *b* sequences (Stone et al. submitted). For southern Montana, 2 mtDNA *americana* individuals maintained nDNA *americana* alleles at the MA1 locus, while 9 mtDNA *caurina* individuals possessed nDNA *caurina* alleles. This suggested that hybridization had not occurred; however, my sampling

locations in Montana spanned a >250 km wide region, and Wright (1953) reports skull measurements of intermediate size between *americana* and *caurina*, suggesting that hybridization has occurred in western Montana.

Twelve of 25 martens from Kuiu Island maintained nDNA alleles at the MA1 locus representing both mtDNA clades, which demonstrated hybridization. In addition, all combinations of mtDNA and nDNA haplotypes were found, indicating that introgression had occurred, with the exception of individuals with mtDNA *caurina* and nDNA *americana*. MtDNA *caurina* individuals possessing nDNA *americana* alleles would be possible from crosses of mtDNA *caurina* hybrid females (mtDNA *caurina* – nDNA heterozygous) with males that possessed nDNA *americana* alleles. Because mtDNA *caurina* – nDNA *americana* individuals were not found, this possibly suggested that mtDNA *caurina* hybrid females (i.e., females with nDNA *americana* and *caurina* alleles at the MA1 locus and mtDNA *caurina* mothers) maintained lower reproductive rates, which would indicate that asymmetrical gene flow may exist between *americana* and *caurina*. MtDNA *caurina* hybrid females are presumably not sterile, however, because mtDNA *caurina* – nDNA *americana* individuals were found when examining the mt cytochrome *b* and nuclear aldolase C genes (Stone and Cook, submitted). Additional data from this region is needed, however, to further test this idea.

Captive breeding studies may provide insight into the reproductive biology of these clades. Grakov (1994) reports breeding experiments conducted on European martens (*Martes martes*) and Siberian sables (*M. zibellina*) in which only crosses between male sables and female European martens produced viable young. Most hybrid

zones involve strong selection and are maintained by factors such as hybrid inviability or sterility (Barton and Hewitt 1989); therefore, asymmetrical gene flow may play a major role in the dynamics of the *americana – caurina* hybrid zone.

If asymmetrical gene flow does exist, *americana* may be genetically swamping *caurina* (Stone et al. submitted); a process consistent with the limited number of nuclear *caurina* alleles found in three "pure" mtDNA *americana* populations (Kupreanof Island, Cleveland Peninsula, and northern Montana). These alleles may represent remnant past signatures of the *caurina* clade. Alternatively, these alleles may be the result of male-mediated gene flow (as seen in brown bears, *Ursus arctos*; Paetkau et al. 1998). Asymmetrical gene flow could cause a shift in the zone of sympatry south-westward, though other factors, such as density and dispersal rate, could also affect the zone's location (Barton and Hewitt 1985).

Apparently, *caurina* populations have persisted only on isolated islands such as Admiralty Island (southeastern Alaska) and Graham Island (Queen Charlotte Islands, British Columbia), but may have been vulnerable to competition by *americana* on the mainland and near-shore islands. Human-mediated introductions of martens took place on Chichagof, Baranof, and Prince of Wales islands, where martens were presumed not to exist, during the mid-1900's (Burris and McKnight 1973; Elkins and Nelson 1954; MacDonald and Cook 1996). Microsatellite signatures indicated no prior inhabitation of these islands by *caurina*, similar to patterns found with mtDNA (Stone et al. submitted). However, if human-mediated introductions transplant *americana* individuals to existing *caurina* populations, we may encounter genetic swamping of *caurina*. Most

introductions occur without prior knowledge of genetic structure within species or potential negative ramifications (for examples, see Rhymer and Simberloff 1996), so managers should be cautious of such disturbances.

Colonization history–

My data showed microsatellites had little ability to resolve relationships among populations but gave great insight into population characteristics (related to recent expansions or extended periods of isolation). Networks based on allele-sharing distances (pairwise individuals comparisons) and maximum-likelihood values (pairwise population comparisons), in addition to the assignment test, reflected past isolation of *caurina* populations and possibly the *americana* populations of Baranof Island (introduced population), Revillagigedo Island (natural population), and Yakutat (which is surrounded by glaciers to the north and east, and by the Pacific Ocean to the west). The population from Admiralty Island (*caurina* clade) was distinctive, with all 10 individuals genetically identical for the loci examined even though these loci were polymorphic in other populations. This monomorphic population may have arisen due to repeated bottlenecks, as has been suggested for other monomorphic populations (Eldridge et al. 1999), and/or to the maintenance of a small effective population size over an extended period of time (Avise et al. 1984).

Results also suggested possible gene flow among several mtDNA *americana* populations (*e.g.*, near-shore islands in southeastern Alaska, mainland southeastern Alaska, British Columbia, and interior Alaska). These conclusions should, however, be

further tested with more individuals per population and increased number of loci which would allow more rigorous analyses to detect migrants. Differentiation and isolation of the *caurina* populations and recently expanded *americana* populations support the hypothesis that *caurina* represents an ancient colonization of the region while *americana* is a more recent colonizer (Stone et al. submitted). The one zone of contact for the region, Kuiu Island, is the farthest of a string of 3 islands that extend from the mainland (Fig. 6). Mitkof, Kupreanof, and Kuiu islands may have created a peninsular effect; whereby, martens from the mainland were able to colonize Mitkof and Kupreanof islands (near-shore islands) across narrow oceanic straits. Individuals then gained access to Kuiu Island. If *americana* represents a later colonization, it may be a relatively recent colonizer of this island. The single, original specimen taken from southeastern Alaska in 1909 was collected from Kuiu Island (Swarth 1911) and identified as the *caurina* morph. The subspecies for the region was designated as *M. americana nesophila* (of the *caurina* group); however, both clades are clearly present in the region with *americana* distributed across a larger range and *caurina* restricted to 2 islands.

Isolation-by-distance does not explain the variation seen in genetic distance. It is probably confounded by the presence of both *americana* and *caurina* populations in the data set, and by the highly fragmented landscape in which populations are located. Colonization history, apparently, had a strong effect on present day population structure. However, when populations are analyzed by clade, genetic and geographic distances are still independent. This indicated that oceanic straits posed significant barriers to gene flow, as did possibly other physiographic features (e.g., distinctiveness of Yakutat).

When only inland populations are considered (interior Alaska, northern and central British Columbia, and northern Montana) where there are fewer topographic barriers, values for isolation-by-distance ($R^2 = 0.8469$; $P = 0.0850$) are comparable to those found by Kyle and Strobeck (in press).

Kyle and Strobeck (in press) conclude that limited barriers to gene flow exist in marten populations from the Yukon through central Northwest Territories, Canada based on low levels of genetic structure. They attributed their results to isolation-by-distance rather than population fragmentation. Potential barriers to gene flow included the MacKenzie Mountain Range, Great Slave Lake, and Great Bear Lake; however, these topographical features seemed to have little effect on gene flow (Kyle and Strobeck in press). The highest concentration of populations in my study (southeastern Alaska) was < 5° south in latitude of Kyle and Strobeck's southerly populations; however, I found that structure could be attributed to population fragmentation as opposed to isolation-by-distance. Potential barriers to gene flow along the North Pacific Coast included the Taku and Stikine rivers, the Coast Mountain Range, numerous glaciers, and oceanic straits (ranging from <1 to 80 km in width) isolating islands. Recently, extensive timber harvesting may also be fragmenting populations of this forest associated species.

Southeastern Alaskan's near-shore islands and mainland populations may have formed a metapopulation among which limited gene flow existed. Therefore, if local populations go extinct, dispersers from surrounding populations may re-colonize the areas (source-sink dynamics; Pulliam 1988). In other regions, no gene flow was apparent (*e.g.*, Admiralty Island population), therefore, if local extinctions occur, re-colonization

may not be possible. In either case, the fragmentation I have detected may have led to decreased genetic variability which in turn influences "both the long-term ecological viability and the evolutionary potential of the species" (McCauley 1993, p. 218).

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Table 8.–Descriptive statistics for 6 microsatellite loci, including population locations and abbreviations (abbr.), mitochondrial DNA clade profile (mtDNA), sample size (N), mean number of alleles (no. alleles) and variance (var.), percent polymorphic loci (% poly.), expected heterozygosity (H_e), and observed heterozygosity (H_o).

Population*	Abbr.	MtDNA**	N	No. alleles	Var.	% poly.	He	Ho
Admiralty Island, SE AK	ADM	*caurina*	10	1.0	0.0	0.0	0.0000	0.0000
Chichagof Island, SE AK	CHIC	*americana*	10	3.8	2.2	83.3	0.5991	0.5500
Baranof Island, SE AK	BAR	*americana*	10	3.0	2.8	83.3	0.4167	0.4000
Kupreanof Island, SE AK	KUP	*americana*	10	4.0	1.6	100.0	0.6342	0.5833
Mitkof Island, SE AK	MIT	*americana*	10	4.0	1.2	100.0	0.5833	0.5833
Kuiu Island, SE AK	KUIU	mixed	25	4.5	3.5	100.0	0.5052	0.4933
Prince of Wales Island, SE AK	POW	*americana*	10	4.0	3.6	83.3	0.5702	0.6000
Revillagigedo Island, SE AK	REV	*americana*	10	3.0	2.0	83.3	0.4605	0.4500
Queen Charlotte Islands, BC	QCI	*caurina*	10	2.0	0.4	83.3	0.3456	0.3167
Vancouver Island, BC	VAN	*caurina*	10	1.8	0.6	66.7	0.2939	0.2833
Yakutat, SE AK	YAK	*americana*	10	3.7	2.3	83.3	0.5456	0.5667
Juneau, SE AK	JUN	*americana*	10	4.2	3.4	83.3	0.6000	0.5167
Thomas Bay, SE AK	TB	*americana*	10	3.8	2.2	100.0	0.6018	0.5167
Cleveland Peninsula, SE AK	CP	*americana*	10	4.8	6.2	83.3	0.6263	0.5500
Yukon Flats, interior Alaska	AK	*americana*	10	4.5	3.1	100.0	0.6456	0.6667
northern British Columbia	NBC	*americana*	5	4.2	2.2	100.0	0.6778	0.6667
central British Columbia	CBC	*americana*	10	4.8	2.6	100.0	0.6667	0.6333
northern Montana	NMT	*americana*	10	5.0	2.4	100.0	0.6737	0.6500
southern Montana	SMT	mixed	11	6.0	3.2	100.0	0.7251	0.6364
Oregon	OR	*caurina*	10	3.3	0.7	100.0	0.5877	0.6667

* SE AK = southeastern Alaska, USA; BC = British Columbia, Canada

** mixed = both *americana* and *caurina* mitochondrial DNA haplotypes

Table 9.–Assignment test results. Abbreviations are as in Table 8 and are followed by sample size in parentheses.

	Assigned population																			
Source population	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	AK	NBC	CBC	NMT	SMT	OR
ADM (10)	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHIC (10)	-	7	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	-
BAR (10)	-	1	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KUP (10)	-	-	1	6	-	-	-	-	-	-	-	-	1	-	-	1	-	1	-	-
MIT (10)	-	-	-	2	4	-	1	-	-	-	-	-	-	1	-	-	1	1	-	-
KUIU (25)	-	-	2	-	-	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POW (10)	-	-	-	-	2	-	6	-	-	-	-	-	-	1	-	-	-	1	-	-
REV (10)	-	-	-	-	-	-	-	9	-	-	-	-	-	1	-	-	-	-	-	-
QCI (10)	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-
VAN (10)	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-
YAK (10)	-	-	-	-	-	-	1	-	-	-	8	-	-	-	-	1	-	-	-	-
JUN (10)	-	2	-	-	1	-	1	-	-	-	-	3	-	-	1	2	-	-	-	-
TB (10)	-	1	-	1	-	-	-	-	-	-	-	-	6	-	-	1	-	1	-	-
CP (10)	-	-	-	-	1	-	-	2	-	-	-	-	-	4	-	1	-	2	-	-
AK (10)	-	-	-	-	-	-	-	-	-	-	-	2	1	1	4	1	1	-	-	-
NBC (5)	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	2	-	-	-
CBC (10)	-	1	-	-	-	-	1	-	-	-	-	-	1	-	3	1	1	2	-	-
NMT (10)	-	2	-	1	1	-	-	-	-	-	-	-	-	-	-	1	3	2	-	-
SMT (11)	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	7	2
OR (10)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10

Table 10.–Weir and Cockerham's (1984) θ for all pairs of marten populations. Abbreviations are as in Table 8.

	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN
ADM	–									
CHIC	0.5310	–								
BAR	0.6369	0.2128	–							
KUP	0.4540	0.1203	0.1893	–						
MIT	0.5270	0.0959	0.1901	0.0244*	–					
KUIU	0.5503	0.2758	0.3831	0.2335	0.3098	–				
POW	0.5262	0.1277*	0.1736	0.0774	0.0692	0.3172	–			
REV	0.6542	0.2155	0.3574	0.1733	0.1986	0.4090	0.2601	–		
QCI	0.7490	0.3910	0.4184	0.3467	0.3615	0.4814	0.2978	0.4171	–	
VAN	0.7363	0.3074	0.4609	0.3366	0.3287	0.3837	0.3732	0.4606	0.5502	–
YAK	0.5758	0.1758	0.2385	0.1318*	0.1499	0.3804	0.1725	0.2373	0.3566	0.4594
JUN	0.5629	0.0593	0.1903	0.1347	0.0534*	0.3278	0.1056	0.2244	0.3786	0.3465
TB	0.4569	0.0892	0.1674	0.0410	0.0741*	0.2749	0.1138	0.2301	0.3913	0.3696
CP	0.5386	0.0803	0.2236	0.0913	0.0448	0.2974	0.0650	0.1677	0.3166	0.3277
AK	0.5606	0.0659*	0.1995	0.1213	0.0780*	0.2991	0.1473	0.1739	0.3786	0.3392
NBC	0.5619	0.0048*	0.1304	-0.0113*	0.0041*	0.2266	0.0441*	0.1425*	0.3374	0.2946
CBC	0.5281	0.0448*	0.1170	0.0923	0.0727	0.2822	0.0921	0.1974	0.3187	0.2937
NMT	0.5291	0.0594	0.1408	0.0972	0.0898	0.3200	0.0907	0.2032	0.3081	0.3562
SMT	0.4685	0.1379	0.2105	0.1291	0.1163	0.2862	0.1176	0.2697	0.2840	0.2717
OR	0.6023	0.2627	0.3859	0.2578	0.2929	0.3026	0.3033	0.3913	0.4147	0.3147

* not significantly different from zero ($\alpha = 0.01$)

Table 10 continued.

	YAK	JUN	TB	CP	AK	NBC	CBC	NMT	SMT	OR
YAK	–									
JUN	0.1572	–								
TB	0.1655	0.0888	–							
CP	0.1788	0.0918	0.1273	–						
AK	0.1139	0.0210*	0.1043	0.0833	–					
NBC	0.0679*	0.0178*	-0.0101*	0.0402*	0.0039	–				
CBC	0.0822	0.0511*	0.1021	0.0715	0.0128	-0.0113*	–			
NMT	0.0866	0.0843	0.1258	0.0562*	0.0525	0.0267*	-0.0108*	–		
SMT	0.1574	0.1199	0.1557	0.1252	0.1114	0.0701*	0.0845*	0.0960	–	
OR	0.3134	0.2988	0.3042	0.2764	0.2502	0.2252	0.2143	0.2454	0.0714	–

* not significantly different from zero ($\alpha = 0.01$)

Appendix II. Percent frequency of occurrence of alleles for 6 microsatellite loci collected from American martens (*Martes americana*). Population abbreviations are as in Table 8.

Locus	Population																			
MA1	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
191																			41	35
193																			5	15
195						66														
201									50											
203	100			10		2				55								5	32	50
205									50	45				5					5	
207			35			2							15							
209															5					
210												10								
211		10		40	30	6	25				20	5	15	25	15	30	25	35	5	
212												10				10				
213			5	5		4	5				60	15	20		35	20	20	15		
215												10		5						
216		45	60	20	15	14	30	15			10	50	45	5	30	30	35	20	5	
217				20	30	6					5						10	10		
218					15		5	10						40	5					
219							20	25						5	5	10	10			
220		15					5	10						5						

Appendix II continued.

Locus Population

MA1	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
221		30		5	10		10								5			15	9	
222								40						10						
223											5		5							

MA2	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
168		30	75	25	25	16	5	35		25	30	15	10	30	30	30	40	50	14	
170		5	5	20	10	2	45				5			30	5		15	15	14	5
172		20		10	45		35	5			10	55	25	35	35	20	15	10	32	
174	100	45	20	45	20	22	15	60			55	25	65	5	30	50	20	20	5	
176										65		5					10	5	32	55
178						34				10									5	10
180						26			10											30
182									10											
184									80											

MA3	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
136				10	5								35		5	10	5	5	14	
138	100	100	100	65	95	32	100	100	100	100	100	100	65	100	95	90	95	95	73	55
142				25		68													14	45

Appendix II continued.

Locus Population

MA5	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
256		25	5	10	15	2	20				15	15	5	5	5	10	10	15	9	
258		5	50		10	4	10				15	35	15	5	45	10	45	40	9	
260	100	55	45	75	70	86	50	40		100	5	45	80	55	30	60	25	15	14	20
262		15		15	5	8	20	55	75		30	5		35		10	15	25		5
264								5	25						20	10			36	70
266											35						5		5	5
268																		5	5	
270																			23	

MA8	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
116			25			2							15						5	
118														5						
120		40	5		20			10			5	30	25	35	30	10	15	25	14	15
122		15	5	15	5	66	20				10	15	10	20	25	20	5	5	9	
124		20	10	30	25	12		10			55	10	15	5	20	20	35	25	27	45
126		20		5		6	20			45				5		10	10	20	9	30
128		5	40	35	50	2	35	40	50	55	30	45	25	15	25	30	20	5	27	10
130			15	15		12	25	40	50				10	15		10	10	20	5	
132	100																5		5	

Appendix II continued.

Locus Population

MA19	ADM	CHIC	BAR	KUP	MIT	KUIU	POW	REV	QCI	VAN	YAK	JUN	TB	CP	YF	NBC	SBC	NMT	SMT	OR
201		5		40	35		5	90			25	15	20	25	40	20	10	10		
203		25			5		10					15	20	25	5		15	25		
205		15			10	2	10				20	35			10	10	5	15	18	
207		40	10		5	64		10	15	90		10	5	10	30	30	40	5	23	60
209	100	15	90	55	45	34	75		85	10	55	15	55	30	5	40	30	35	50	35
211				5										10	10			10	5	5
213												10								
215																			5	

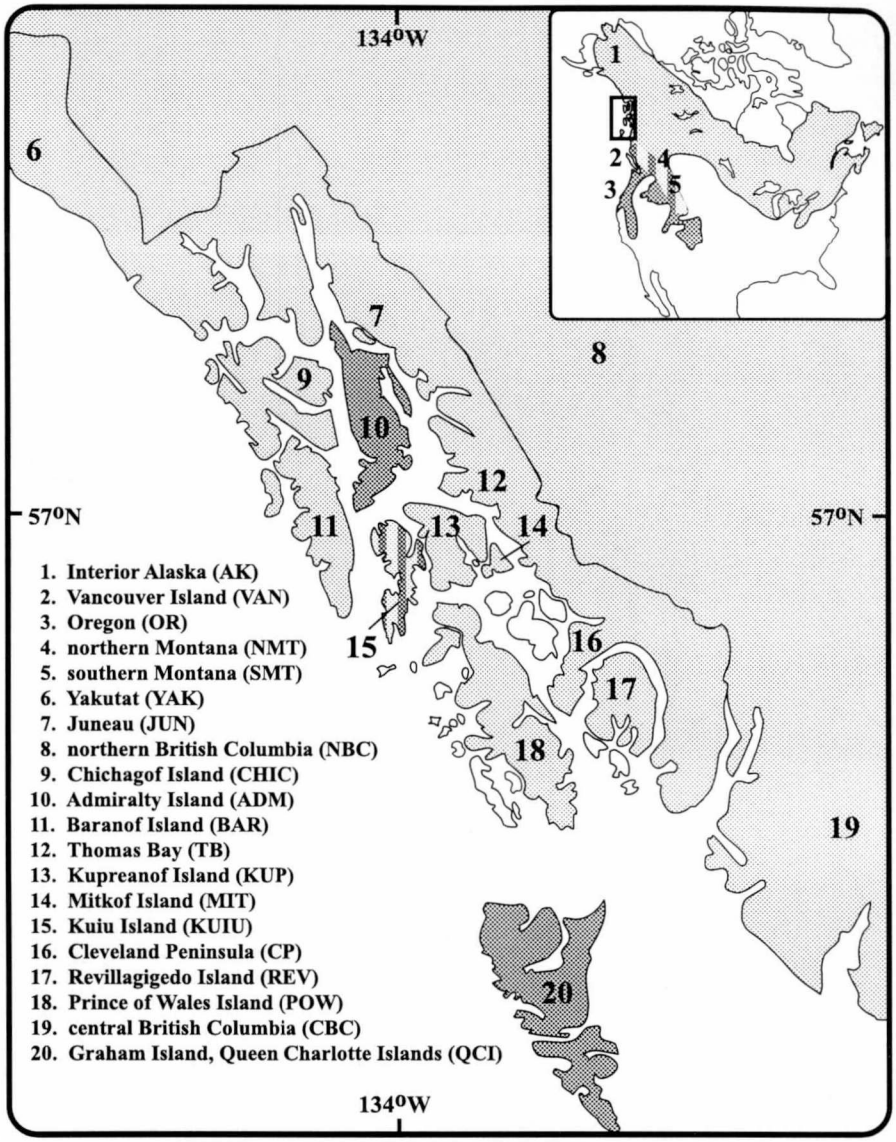

Fig. 6. Map of sampling locations. Distribution of mitochondrial clades of American martens (*Martes americana*) are also shown. Light and dark gray shading represents regions inhabited by members of the *americana* and *caurina* clades, respectively. Inset map shows the North American distribution of martens modified from Hall (1981).

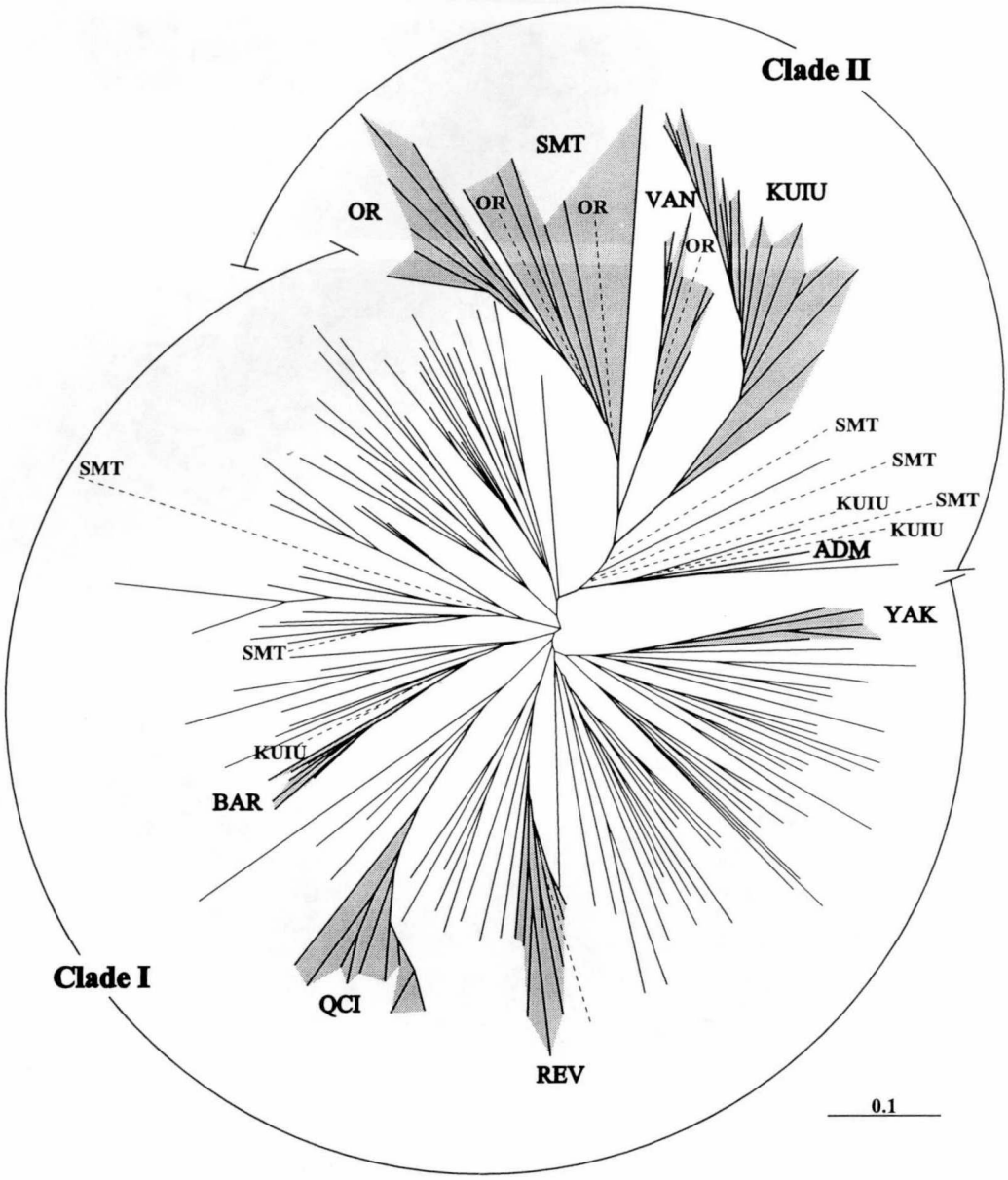

Fig. 7. Unrooted network of genetic relationships among 211 American martens (*Martes americana*) inferred from allele-sharing distances. All unique genotypes are represented by a line in the diagram. Abbreviations are as in Table 8. Well-defined populations are indicated with background shading (dotted lines indicate exceptions). Lines not end-labeled represent individuals from mtDNA *americana* populations.

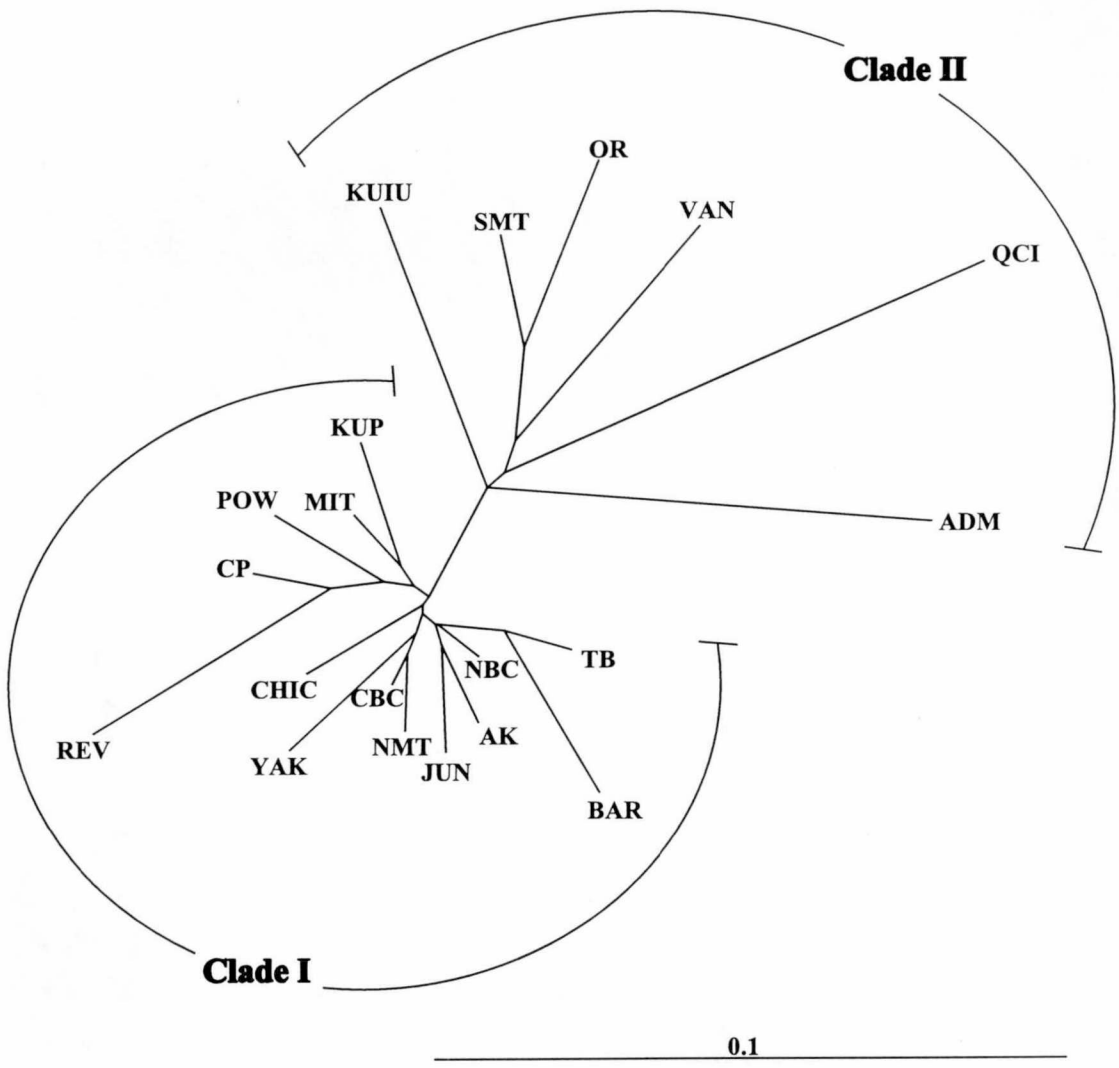

Fig. 8. Unrooted network of genetic relationships among 20 American marten (*Martes americana*) populations inferred from the maximum-likelihood analysis. Abbreviations are as in Table 8.

XI. CONCLUSIONS

This research investigated phylogenetic reconstruction of the genus *Martes* and phylogeography and population differentiation among American martens (*Martes americana*). Nuclear (aldolase C gene and seven microsatellites) and mitochondrial (cytochrome *b* gene) loci were used to examine the evolution, colonization of taxa within the genus, and hybridization between two distinct clades of American martens.

Evolution

The genus *Martes* may be paraphyletic with respect to a closely related species, the wolverine (*Gulo gulo*). Cytochrome *b* sequences supported fossil data (Anderson 1970) indicating early radiations gave rise to subgenera *Pekania* and *Charronia*, and a more recent radiation led to species of the subgenus *Martes*. My data suggested a polytomy containing four clades (*americana*, *foina*, (*martes*, *zibellina*), and *melampus*). These unresolved relationships may be the result of a burst of speciation but should be tested with independent loci.

Colonization across the Bering Land Bridge

Two colonizations across the Bering Land Bridge into North America were apparent for the genus, one by members of the subgenus *Pekania* and the other by subgenus *Martes*. Anderson (1970) suggests that the "americana" clade of *M. americana* represents an earlier colonization of North America than "caurina", because of the

similarity of "caurina" to *M. zibellina*; however, phylogenetic analyses of cytochrome *b* sequence data suggested that "americana" and "caurina" are sister taxa and represent only one colonization across the land bridge.

Cytochrome *b* data were consistent with the recognition of these as monophyletic clades; however, the aldolase C sequences and microsatellite data indicated that these generally parapatric groups have interbred in a region of limited geographic overlap. These clades probably were isolated in eastern ("americana") and western ("caurina") North American refugia south of the ice sheets during the late Pleistocene, but geographic isolation apparently has not led to reproductive isolation.

Colonization of the Pacific Northwest after deglaciation

The widespread "americana" clade presently 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. The current distribution of these clades is consistent with Hoffmann's idea that "taxa from the large glacial refugium in southeastern North America reoccupied a larger area" than taxa from smaller western refugia (Hoffmann 1985, p. 470-1).

Due to patterns of genetic variation in cytochrome *b*, I hypothesize that the "caurina" clade spread along the North Pacific Coast (including southeastern Alaska) earlier than the "americana" clade. Within the "americana" clade, little to no geographic

structure was present indicating these individuals came from a recently expanded population. Because island populations of the "caurina" clade maintained unique cytochrome *b* haplotypes, these populations probably have been isolated. Although haplotypes were unique, differentiation was minimal (1-2 mutations) suggesting the effects of post-glacial events. An analysis of genetic and geographic distances suggested that colonization history had a strong effect on present day population structure and that oceanic straits and possibly other physiographic features posed significant barriers to gene flow.

Hybridization of "americana" and "caurina"

One microsatellite locus diagnostic for the two clades, in combination with aldolase C sequences, revealed that hybridization has occurred in a limited region of sympatry (Kuiu Island, southeastern Alaska); however, asymmetrical introgression may exist and play a major role in the dynamics of the "americana"-"caurina" hybrid zone. Areas of sympatry should be of particular interest in the future, because potential hybrid zones allow us "to quantify the genetic differences responsible for speciation [and] to measure the diffusion of genes between diverging taxa" (Barton and Hewitt 1989, p. 497).

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