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Molecular evolution of Holarctic martens (genus *Martes*, Mammalia: Carnivora: Mustelidae)

Karen D. Stone* and Joseph A. Cook[1]

University of Alaska Museum, Fairbanks, AK 99775, USA
Alaska Cooperative Fish and Wildlife Research Unit, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

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Abstract

The Bering Land Bridge has served as a major corridor of interchange between the northern continents for many organisms. We investigated the phylogeny of all extant species of *Martes* (except for *Martes gwatkinsi* from India) to infer evolutionary relationships and characterize the extent of trans-Beringian movements. Analyses of complete sequences of the mitochondrial cytochrome *b* gene and partial sequences of the nuclear aldolase C gene (241 bp) suggested that the genus *Martes* may be paraphyletic with respect to *Gulo gulo*. These data 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 member of the subgenus *Martes*. Contrary to hypotheses based on morphological evidence, the ''americana'' and ''caurina'' subspecies groups of *Martes americana* are not the result of independent colonizations of North America. The phylogenetic analyses of cytochrome *b* data were consistent with the recognition of these subspecies groups as monophyletic clades; however, variation in the aldolase C sequences indicated that these generally parapatric groups may interbreed in a region of limited geographic overlap.

Keywords: Aldolase C; Bering Land Bridge; Cytochrome *b*; Martens; *Martes*; Phylogeny

1. 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 across the Bering Land Bridge (Hoffmann, 1981; Repenning, 1967). This 1800 km wide connection was repeatedly exposed prior to and during the Pleistocene (Elias, 1995; Hopkins, 1959, 1967). Although the habitats of the region apparently were variable during its existence (Colinvaux, 1964; Elias et al., 1996; Hoffmann, 1985), the land bridge played a major role in filtering the exchange of boreal mammals between Siberia and Alaska (Hoffmann, 1985). Molecular techniques (see Avise, 1994) have provided the opportunity to re-evaluate and further investigate hypotheses related to movements of northern mammals. Of these studies, most have concentrated on rodents (e.g., Conroy and Cook, 2000; Fedorov and Goropashnaya, 1999; Lance and Cook, 1998; Steppan et al., 1999). We focus on the biogeographic history of a clade of medium-sized carnivores, the Holarctic genus of martens (*Martes*) to further explore the generality of organismal patterns of differentiation at northern latitudes.

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 *Pekania* and *Charronia*; whereas, marten fossils of the subgenus *Martes* do not appear until the

* Corresponding author. Present address: Department of Biology, Southern Oregon University, 1250 Siskiyou Boulevard, Ashland, OR 97520-5071, USA. Fax: +1-541-552-6415.

E-mail addresses: stonek@sou.edu (K.D. Stone), cookjose@isu.edu (J.A. Cook).

[1] Present address: Department of Biological Sciences, Idaho State University, Pocatello, ID 83209-8007, USA.

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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

middle Pliocene from deposits in Poland (Anderson, 1970). The fossil record also indicates a mid-Pleistocene colonization by ancestors of *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 $\geqslant 780,000$ years ago from Porcupine Cave, Colorado (Anderson, 1997). This specimen apparently documents an earlier colonization of North America across the Bering Land Bridge than previously recognized. If ancestors of the subgenera *Pekania* and *Martes* represent independent colonizations of the New World, then we would predict that these taxa would be distantly related. 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). Recent analyses of mitochondrial cytochrome *b* sequences (Hosoda et al., 2000) suggest, however, that *Martes* may possibly be paraphyletic with respect to *Gulo*.

All members of the subgenus *Martes*, with the exception of *M. foina*, are morphologically similar and maintain allopatric or parapatric distributions (Anderson, 1970). Anderson (1970) also suggests that these species possibly form a superspecies—"a monophyletic group of closely related and entirely or largely allopatric species that are too distinct to be included in a single species or that demonstrate their reproductive isolation in a zone of contact" (Mayr and Ashlock, 1991, p. 430). Possibly due to these close relationships, the placement of *M. melampus* and two subspecies groups within *M. americana* has been troublesome. *Martes melampus* is thought to be closely related to *M. zibellina* (Anderson, 1970); however, Carr and Hicks (1997) were unable to resolve the relationship of *M. melampus* to *M. zibellina*, *M. martes* or *M. americana*.

With respect to *M. americana*, eight subspecies are recognized (Clark et al., 1987), and these have been placed into two distinct subspecies groups ("americana" and "caurina") based on morphology (Merriam, 1890) and mitochondrial DNA sequences (Carr and Hicks, 1997; Stone et al., in press). 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. (in press) did not find support for these hypotheses based on sequence of the mitochondrial cytochrome *b* gene (cyt *b*). Both studies report that the "americana" and "caurina" groups form a sister clade apart from other extant species. 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%).

We investigated the phylogeny of *Martes* using sequences from mitochondrial and nuclear genes. In particular, we 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 extant populations of North American *M. americana* are the result of multiple colonizations from Eurasia.

2. Materials and methods

2.1. 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. Methods for extracting, amplifying, and sequencing DNA and aligning sequences were carried out according to Lessa and Cook (1998) unless and otherwise noted. Amplifications were in 50-μl volumes containing 1.5 mM $MgCl_2$, 0.02 mM of each dNTP, 50 pmoles of each primer, 1.25 U of Perkin–Elmer AmpliTaq DNA polymerase, Perkin–Elmer 1× PCR buffer, and 1–100 ng whole genomic DNA. The mitochondrial (mt) marker, cyt *b*, was amplified using a Perkin–Elmer GeneAmp PCR System 2400 with the following PCR conditions: one cycle of 94 °C for 45 s, then 35 cycles of denaturation at 94 °C for 10 s, annealing at 45 °C for 15 s, and

an extension at 72 °C for 45 s, followed by one cycle of 72 °C for 3 min. Negative controls were included in each PCR experiment. The following primer pairs amplified cyt *b* (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.

2.2. Phylogenetic analyses of cytochrome b

The complete cyt *b* gene (1140 bp) 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* (Appendix A). DNA sequences correspond to GenBank Accession Nos. AF154964, AF154968, AF154975, and AF448237-448248. *Martes americana* samples were drawn from a larger data set of 680 individuals that represented two distinctive clades (Stone et al., in press). 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 Accession Nos. AB012347 and AB012355; Kurose et al., 1999), two *M. flavigula* (GenBank Accession No. AB012362-3; Kurose et al., 1999), and *Gulo gulo* (GenBank Accession No. X94921; Ledje and Arnason, 1996). *Mustela erminea* and *M. vison* were used as outgroups for all analyses (GenBank Accession Nos. AF057127 and AF057129, respectively; Koepfli and Wayne, 1998).

The data set was analyzed first using Modeltest (Version 3.0; Posada and Crandall, 1998) to determine an appropriate model of DNA substitution. The program Modeltest calculated a neighbor-joining (NJ) tree, employing the JC69 model of evolution (Jukes and Cantor, 1969) to approximate tree topology. This tree was used to evaluate 56 different nested likelihood models, ranging from JC to GTR + I + Γ. Likelihood ratio test statistics and associated *P*-values were calculated using a χ^2 distribution (see Modeltest documentation for further details, http://zoology.byu.edu/crandall_lab/modeltest.htm). The least complex model that was significantly better than other simpler models, according to the Akaike information criterion (Akaike, 1974) and likelihood ratio test statistics, was chosen for further analyses.

Relationships among *Martes* and *Gulo* cyt *b* sequences were examined using PAUP* (Version 4.0b8; Swofford, 1999). A successive approximation approach was used for the maximum likelihood (ML) search, whereby the substitution model, base frequencies, proportion of invariable sites (I), and gamma-distribution shape parameter (α) were estimated from the NJ tree. These values were used in the original ML search (TrN + I + Γ model; heuristic search with TBR branch swapping). After completion of the search, values for the substitution model, base frequencies, I, and α were re-estimated. The subsequent ML search used the new estimates. This procedure was continued until the likelihood score did not change. Statistical support for the nodes of the optimal tree was assessed using the bootstrap (100 replicates; Felsenstein, 1985), with ML heuristic searches and a maximum of one tree per replicate (MAXTREES = 1).

Phylogenetic trees were also constructed using maximum parsimony methods (unweighted and transition/transversion weighting of 1/2, 1/5, 1/10, and 1/20). 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) for 100 bootstrap replicates, with maximum parsimony heuristic searches. Statistical support for the nodes of the strict consensus tree was assessed using the bootstrap (1000 replicates; Felsenstein, 1985), with heuristic searches and a maximum of 100 trees per replicate (MAXTREES = 100).

Additional analyses were conducted to confirm the placement of *M. pennanti* and *Gulo*. An ML tree was generated, as previously described, using the data set but with the *Gulo* sequence removed. Another ML tree was

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)

generated using the complete data set (*Gulo* included) but with the exclusion of third position transitions to eliminate potentially confounding homoplasies. Finally, a parsimony tree was generated; whereby, nucleotides were replaced with amino acids.

2.3. Molecular clock and other constraints

Using the same parameters as the final ML search, a maximum likelihood score was calculated when enforcing a molecular clock. Likelihood scores of trees generated with and without the molecular clock enforced 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 (Voelker, 1999) for both ML and parsimony topologies. The two-cluster test requires fully resolved trees, so we generated new trees using the same parameters with one *M. zibellina* sequence (AF25270) removed from the data set. For the ML topology, the two-cluster test used the Tamura–Nei+Γ distance option (with $\alpha = 2.5374$) to determine whether two daughter lineages at a node have evolved at significantly different rates. Tamura–Nei distance was used for the two-cluster assessment of the parsimony topology.

In addition, constrained trees were generated to test alternative hypotheses already established in the literature and identify well-resolved nodes as opposed to unresolved relationships. Because alternative hypotheses were specified a priori (Goldman et al., 2000), ML scores for constrained trees were compared to the optimal ML score using the Kishino–Hasegawa test (Kishino and Hasegawa, 1989). Due to multiple comparisons ($N = 5$), we 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*.

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

A portion (241 bp) 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 bp at the 5′ end. An annealing temperature of 60 °C was used for these amplifications with all other conditions the same as previously described. 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* (Appendix A). Several *M. americana* were sequenced to assess possible hybridization between individuals of the two subspecies groups. Individuals detected as possibly heterozygous (through sequence analysis) were subjected to single-strand conformation polymorphism (SSCP) to confirm the automated sequencing results (Pfau et al., 1999). Variable sites were mapped onto the phylogeny constructed under maximum parsimony.

3. Results

3.1. 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). After evaluating different likelihood models, the TrN (Tamura and Nei, 1993) + proportion of invariable sites + gamma-distribution shape parameter ($\mathrm{TrN} + \mathrm{I} + \Gamma$) was determined to be the significantly best model tested with the least complexity. The ML search, employing $\mathrm{TrN} + \mathrm{I} + \Gamma$ and run to termination with preset values, resulted in one tree (Fig. 1). Additional analyses conducted to confirm the placement of *M. pennanti* and *Gulo* indicated that the deep position of *Pekania* was not an artifact of long-branch attraction toward *Gulo*, and that *M. pennanti* and *Gulo* are sister taxa and remain basal even if third position transitions are removed from the data set.

Constraining the monophyly of (a) a clade of "caurina" and *M. zibellina*, and (b) a clade of "caurina," *M. zibellina* and *martes* significantly decreased the likelihood score (Table 3). However, all other constrained trees were not significantly different from the initial ML tree (Table 3). Other constraints tested included the monophyly of (a) the genus *Martes*, (b) *M. melampus*, *zibellina*, and *martes*, and (c) *M. melampus* and *americana*. Enforcing a molecular clock also significantly decreased the likelihood score ($= -4539.3198$; $P = 0.0013$); therefore, we assumed these taxa did not evolve at equal rates. The two-cluster test identified non-clock-like nodes (Figs. 1 and 2).

A strict consensus tree of two equally parsimonious trees (672 steps with 265 informative characters) was constructed (Fig. 2). Well-resolved nodes corresponded across both methods (ML and parsimony) of tree reconstruction.

Several features were evident from our phylogenetic analyses: (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 placement of *M. foina* and *melampus* was unresolved; (4) *M. martes* and *zibellina* formed a monophyletic group with *M. martes* paraphyletic with respect to *M. zibellina*; (5) the "americana" and "caurina" groups of *M. americana*

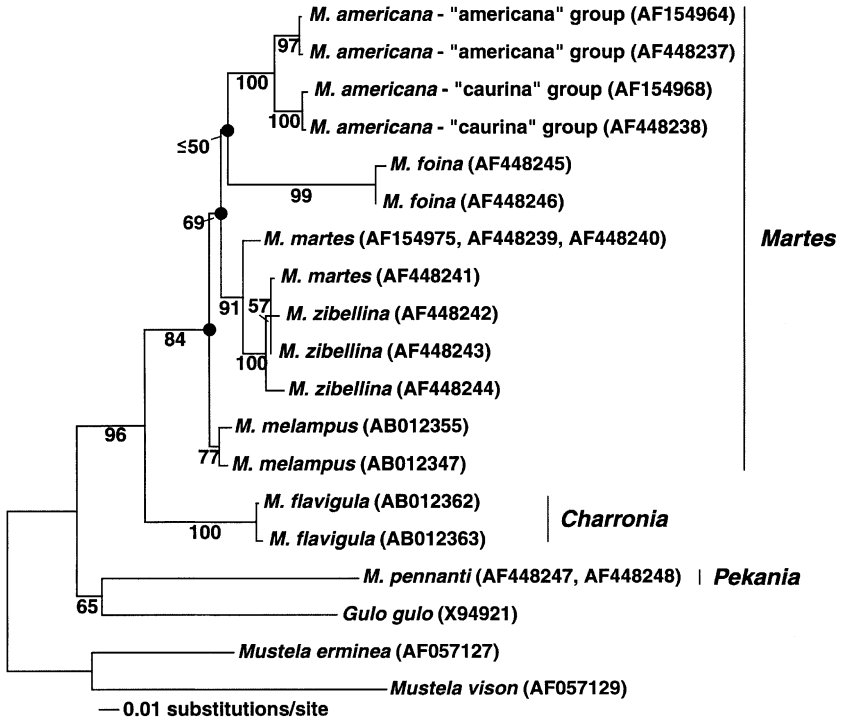

Fig. 1. Maximum likelihood tree ($-\ln L = 4519.2815$) generated from cytochrome *b* gene sequences of *Martes* (this study; Kurose et al., 1999) and *Gulo* (Ledje and Arnason, 1996) individuals. The tree was generated using an TrN (Tamura and Nei, 1993) + proportion of invariable sites + gamma-distribution shape parameter (TrN + I + Γ) model with a substitution model of $A - G = 34.8973$ and $C - T = 20.4705$, base frequencies of $A = 0.3117$, $C = 0.3277$, $G = 0.1081$, $T = 0.2525$, $I = 0.5953$, and $\alpha = 2.5374$. Bootstrap values are shown below bars, GenBank numbers are in parentheses after taxon names, and subgenera are listed along the right margin. *Mustela erminea* and *Mustela vison* were used as outgroups (Koepfli and Wayne, 1998). • = nodes non-clock-like according to the two cluster test (Takezaki et al., 1995).

Table 3
Comparison of optimal (unconstrained) maximum likelihood tree score (= −4519.2815) 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*	−4520.4008	0.5841
"caurina" and *M. zibellina*	−4597.7287	< 0.0001*
"caurina", *M. zibellina*, and *martes*	−4562.8780	0.0005*
M. melampus, zibellina, and *martes*	−4522.7783	0.4065
M. melampus and *americana*	−4522.7279	0.4201

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

were sister taxa; and (6) several daughter lineages have not evolved in a clock-like manner.

3.2. Nuclear aldolase C

Less variation was found in ald C sequence relative to cyt *b* (Table 4), 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 4) found in exon 5 distinguished the "americana" and "caurina" clades of *M. americana*, and heterozygous individuals were detected in a region of sympatry on Kuiu Island, southeastern Alaska. Eight individuals from Kuiu Island, analyzed with SSCP, revealed four homozygotes (two of each genotype) and confirmed the four presumed heterozygotes determined via automated sequencing.

4. Discussion

The Bering Land Bridge has intermittently permitted the interchange of many organisms between the northern continents over the past several million years. The Bering Strait first opened 4.8–7.4 million years ago (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; Elias et al., 1996; Guthrie, 1985, 1990). Undoubtedly, the land bridge was 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).

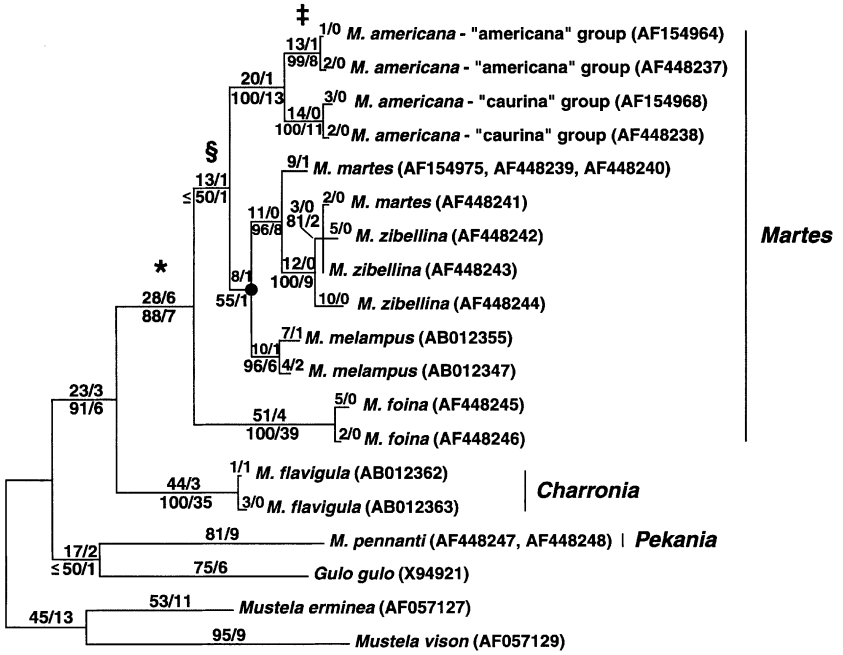

Fig. 2. Strict consensus tree of two equally parsimonious trees (length = 672 steps; CI = 0.629; RI = 0.681) generated with a branch-and-bound search from complete cytochrome *b* gene sequences of *Martes* (this study; Kurose et al., 1999) and *Gulo* (Ledje and Arnason, 1996) individuals. Branch lengths/number of third position transversions are shown above branches, bootstrap values/Bremer decay indices are below branches, 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). Symbols (‡, §, *) refer to base substitutions in the aldolase C gene (see Table 4). • = nodes non-clock-like according to the two cluster test (Takezaki et al., 1995).

Table 4
Condensed dot matrix assembled using aldolase C sequence (corresponding to sites 2756–2996 of *Rattus norvegicus*; Mukai et al., 1991)

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

Number of individuals with identical sequences is in parentheses. C/T represents heterozygous individuals. Characters symbolizing positions are referred in Fig. 2.

Investigations, based on fossils (e.g., Anderson, 1970; Hunt, 1996; Martin, 1989) and comparative morphology (e.g., Anderson, 1970; Rausch, 1994), provide a framework for understanding the timing of interchange of carnivores among continents. 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., Conroy and Cook, 2000; Fedorov and Goropashnaya, 1999; Talbot and Shields, 1996b; Wayne et al., 1989).

4.1. Relative timing of divergence

Because of difficulties associated with calibration points, the dangers of estimating absolute times of divergence under the assumption of a molecular clock (Hillis et al., 1996), and the finding that several nodes on our trees (Figs. 1 and 2) have daughter lineages that are not evolving clock-like, we discuss relative times of divergences rather than absolute times of divergence. Our 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 3). Paraphyly would indicate the need for taxonomic revision with the inclusion of all marten species within genus *Gulo*. Additional nuclear genes should be explored to test the validity of this result. Cyt *b* sequences supported fossil data (Anderson, 1970) indicating early radiations gave rise to *Pekania* and *Charronia*, and that a more recent radiation led to species in the subgenus *Martes*. *P. M. pennanti* was consistently the most basal species of the genus, followed by *C. M. flavigula*; whereas, species of the subgenus *Martes* formed a 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 interpretation, the paraphyletic relationship between the endemic North American species *P. M. pennanti* and *M. M. americana* supports two colonizations across the Bering Land Bridge into North America, one by members of *Pekania* and the other by members of the subgenus *Martes*. With the exception of *M. foina*, martens are strongly associated with forests (Clark et al., 1987; Graham and Graham, 1994; Powell, 1981) so continuous forested habitats most likely existed in Beringia during these colonization events.

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 colonization 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 by *M. americana*. The distinctive clades of marten may have arisen subsequently due to separation into different southerly refugia in North America during Pleistocene glacial cycles (Carr and Hicks, 1997; Stone et al., in press) 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 (Green et al., 1996; Templeton et al., 1995), birds (Bermingham et al., 1992; Gill et al., 1993), and mammals (Arbogast, 1999; Cook et al., 2001).

The fossil record suggests that isolation between "americana" and "caurina" marten may have occurred in eastern and western refugia south of the ice sheets. A substantial number of Pleistocene 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 be Holocene in age (Youngman, 1993).

4.2. Hard versus soft polytomies

The fossil record indicates an earlier divergence for *M. foina* (also supported by the ald C sequences); however, the remaining four species of the subgenus *Martes* have been described as a superspecies, suggesting close phylogenetic relationships (Hagmeier, 1961; Anderson, 1970). Our analyses found only weak support for the placement of either *M. foina* or *melampus* (Figs. 1 and 2), resulting in a polytomy of four clades (*americana*, *foina*, (*martes*, *zibellina*), and *melampus*). Sequences from independent genes should be used to test whether these species form a hard polytomy (Maddison and Maddison, 1992) with the unresolved pattern reflecting a rapid radiation. Alternatively, a soft polytomy may be later resolved with additional markers. In some cases, unresolved polytomies have been considered to be hard polytomies and attributed to bursts of speciation (e.g., Lessa and Cook, 1998) and such bursts have been implicated for other high latitude organisms such as voles (Conroy and Cook, 1999), ground squirrels (Steppan et al., 1999), and bears (Waits et al., 1999). If these bursts are contemporaneous across co-distributed organisms, they may reflect a generalized diversification response to past extrinsic forces such as climate change. Further investigation, such as that of Hosoda et al. (2000), with comparable sets of loci may provide the opportunity to test these ideas.

4.3. 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 (Davison et al., 1999; Pamilo and Nei, 1988), we suspect hybridization between *zibellina* and *martes* may have occurred as

has been previously documented through an experimental breeding program (Grakov, 1994). The "americana" and "caurina" groups of *M. americana* apparently also hybridize where they contact in Montana (Wright, 1953; Hagmeier, 1961). *Martes americana* of both the "americana" and "caurina" cyt *b* lineages also coexist on Kuiu Island, southeastern Alaska, where sequences of the nuclear ald C gene were consistent with past hybridization events. 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 4). Complete sequences of cyt *b* consistently identified two historical groups within *M. americana*, while sequence from the nuclear ald C gene indicated 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; however, these apparent zones of hybridization in southeastern Alaska and Montana (this study; Wright, 1953) hint that these mitochondrial gene trees should be tested further using nuclear loci (Maddison, 1997; Pamilo and Nei, 1988). Independent genes, such as the preliminary nuclear ald C data noted herein, provide the opportunity to explore concordant phylogenetic partitions (Avise and Ball, 1990). In addition to nuclear loci, the dynamics of speciation and hybridization will require evaluation of ecological, behavioral, or physiological characteristics that may differ between these two clades of *M. americana*. Investigations centered on these contact zones of marten may be particularly illuminating 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, p. 497).

5. Conclusions

The genus *Martes* may be paraphyletic with respect to *Gulo*. Although this relationship should be tested with additional markers, paraphyly would indicate the need for taxonomic revision with the inclusion of all marten species within the genus *Gulo*. Three distinct clades were apparent corresponding to the morphologically defined subgenera *Pekania*, *Charronia*, and *Martes*; however, the placement of two species within the subgenus *Martes*, *M. foina*, and *melampus*, was unresolved. If this pattern reflects a rapid radiation and is contemporaneous across co-distributed organisms, it may reflect a generalized diversification response to past extrinsic forces such as climate change. Further investigation with comparable sets of loci, across other forest associated species, may provide the opportunity to test this idea. *M. martes* and *zibellina* formed a monophyletic group. The paraphyletic relationship of *M. martes* with respect to *M. zibellina* may reflect past hybridization. Current hybridization was also implied between individuals of the "americana" and "caurina" groups of *M. americana*. The phylogenetic analyses of mitochondrial cytochrome *b* data were consistent with the recognition of these subspecies groups as monophyletic clades; however, the nuclear aldolase C sequences indicated that these generally parapatric groups may interbreed in a region of limited geographic overlap. This sister relationship indicated that the "americana" and "caurina" subspecies groups of *Martes americana* are the result of one colonization of North America and contradicted hypotheses based on morphological evidence.

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Appendix A. 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
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 4).

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