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American marten (*Martes americana*) in the Pacific Northwest: population differentiation across a landscape fragmented in time and space

MAUREEN P. SMALL,* KAREN D. STONE† and JOSEPH A. COOK‡
*WDFW, Genetics Division, 600 Capitol Way N., Olympia, WA 98501–1091, USA, †Department of Biology, Southern Oregon University, 1250 Siskiyou Blvd, Ashland, OR 97520, USA, ‡Biological Science, Idaho State University, Pocatello, ID 83209, USA

Abstract

American marten (*Martes americana*) have a close association with mature temperate forests, a habitat that expanded throughout the Pacific Northwest as glaciers receded at the end of the Pleistocene. Similar to several other forest-associated mammals in North America (e.g. black bear), genetic analysis of the marten shows a deep phylogeographical subdivision that reflects populations with distinctive evolutionary histories. Using a suite of 14 microsatellite markers, we explored the genetic structure of marten populations in two reciprocally monophyletic clades in the Pacific Northwest identified previously as *M. caurina* and *M. americana* by mitochondrial haplotypes and morphology. Microsatellite phylogeographical patterns were congruent with mitochondrial analyses. These independent data sets shed light upon hybridization patterns, population structure and evolutionary histories. Hybridization between *M. caurina* and *M. americana* individuals was documented in two regions of sympatry (Kuiu Island in southeastern Alaska and southern Montana). Northern insular populations of *M. caurina* exhibited higher differentiation and lower variability relative to northern populations of *M. americana*. Greater divergence among *M. caurina* populations may reflect longer isolation and persistence in coastal forest habitat that was fragmented by rising sea level in the early Holocene. Lower differentiation among northern *M. americana* populations and close relationship to other continental *M. americana* populations may reflect more recent expansion into the Pacific Northwest and/or continued gene flow among populations. Differentiation among *M. caurina* populations was attributed to habitat fragmentation (i.e. rising sea level), as opposed to isolation-by-distance; oceanic straits pose significant barriers to gene flow among *M. caurina* populations and between populations of *M. caurina* and *M. americana*.

Keywords: hybridization, *Martes americana*, microsatellites, Pacific Northwest, phylogeography, southeastern Alaska

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Introduction

The retreat of the Wisconsin glaciers, beginning about 12 000 years ago, was followed by dynamic northward colonization of boreal forests and associated species into high latitude regions of North America. This biome is becoming a focus of investigations related to the impact of current deforestation and habitat fragmentation on wildlife. In the last 100 years, this region has experienced intensive logging that has changed habitat (Strickland & Douglas 1987) and prey base (Thompson 1994) for forest-associated carnivores such as marten (*Martes americana*). Genetic-based investigations of population structure (Mitton & Raphael 1990; McGowan *et al*. 1999; Kyle *et al*. 2000) have begun to explore dispersal patterns, gene flow and genetic diversity in marten. A genetic framework provides an opportunity to explore the dynamic historical biogeography of this medium-sized carnivore and to predict the organism's response to habitat change, thus further delimiting units for more effective conservation and management in regions experiencing current

Correspondence: Maureen P. Small. Fax: 360 902 2943; E-mail: mosmall@islandnet.com

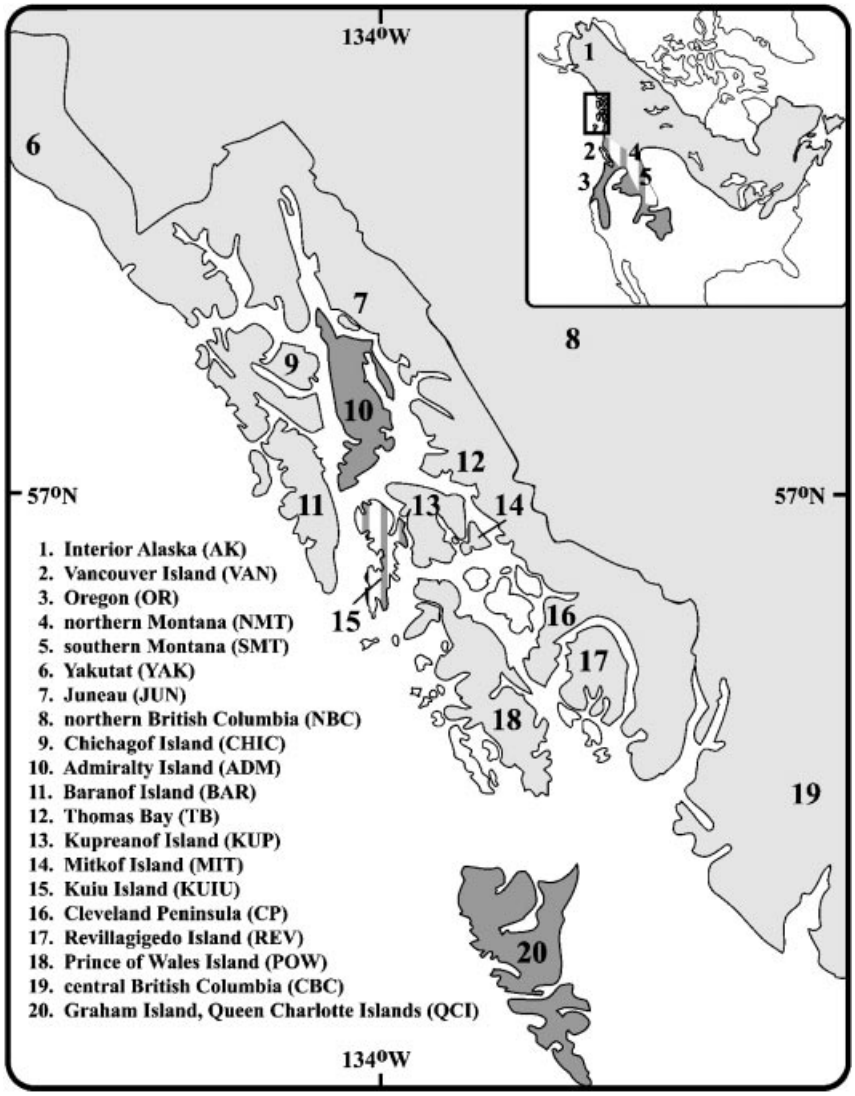

Fig. 1 Map of sampling locations also showing distribution of mitochondrial clades in American martens (*Martes americana*). Light and dark grey shading represents regions inhabited by members of the *americana* and *caurina* clades, respectively, with striped regions indicating contact zones. Inset map shows the North American distribution of martens modified from Hall (1981).

widespread anthropogenic disturbance (Crozier 1992; Ryder 1986; Vane-Wright *et al*. 1991; Faith 1992; Moritz 1994).

Although eight subspecies of *M. americana* have been described (Clark *et al*. 1987), these are traditionally placed into two morphological groups, *caurina* and *americana* (Merriam 1890; Clark *et al*. 1987). Molecular investigations corroborate the distinction of *caurina* and *americana* as two reciprocally monophyletic mitochondrial clades (Carr & Hicks 1997; Stone *et al*. 2002). These forms appear to have diverged due to isolation in distinct southern glacial refugia (*caurina* populations isolated in western and *americana* populations isolated in eastern United States, respectively; Carr & Hicks 1997; Stone & Cook 2002; Stone *et al*. 2002), because there is little evidence of a high latitude refugium during the Wisconsin that was forested (Pielou 1991). Youngman (1993) suggests the persistence of *americana* in Beringia during the Pleistocene, but material that he examined was not dated either stratigraphically or through isotope analysis and may have been deposited during the Holocene.

The two forms are largely allopatric, with *caurina* populations documented along the West Coast (California to southeastern Alaska), and then eastward to Wyoming, Montana and Idaho (Fig. 1; Wright 1953; Hall 1981; Carr & Hicks 1997; Stone *et al*. 2002). Currently, *americana* populations are more widespread, distributed from Montana and Idaho northward to Alaska and eastward to the Atlantic Coast. Two zones of contact have been identified; one extending through southern and western Montana and the other on Kuiu Island in southeastern Alaska (Stone *et al*. 2002; Wright 1953; Stone & Cook 2002). Although Merriam (1890) describes these groups as distinctive species, Wright (1953) reports morphological intergradation between the groups in Montana and suggested that they belong to a single species, *M. americana*, a conclusion reflected by the current taxonomy (Wilson & Reeder 1993). Carr &

Hicks (1997), however, questioned whether gene flow exists between these groups and concluded that the *caurina* and *americana* clades represent distinct species.

We investigated genetic differentiation among marten from 20 localities throughout the Pacific Northwest of North America using 14 nuclear microsatellite markers. We focus on southeastern Alaska because both *caurina* and *americana* populations occur in this region of high endemism (Cook & MacDonald 2001). Southeastern Alaska has long been recognized as a unique biogeographical region (Swarth 1936; Klein 1965) with numerous species and subspecies identified nominally as endemics (MacDonald & Cook 1996). This region is an important natural laboratory for investigating the effects of dispersal and habitat fragmentation during the Holocene, information useful for predicting the effects of habitat fragmentation on population structure and viability (McCauley 1993). For example, molecular phylogeographical studies have demonstrated that several mammalian species are represented by distinct evolutionary lineages in southeastern Alaska (Talbot & Shields 1996; Demboski *et al*. 1999; Conroy & Cook 2000; Stone & Cook 2000; Cook *et al*. 2001; Fleming & Cook 2002; Stone *et al*. 2002). This common pattern indicates that each species colonized the region on multiple occasions and from multiple source populations following glacial retreat. Stone *et al*. (2002) hypothesized that *M. caurina* populations represent an early Holocene colonization northward along the coast as coastal ice receded at the end of the last glaciation, whereas *americana* populations represent a later colonization from continental source populations that expanded through river corridors traversing the coastal mountains. Coastal mountains were deglaciated much later than the Alexander Archipelago (Pielou 1991). Several sympatric species show similar patterns of differentiation (Cook *et al*. 2001).

This study explores the distinctive histories of *caurina* and *americana* populations using nuclear microsatellite markers. We investigated differentiation of marten populations in the Pacific Northwest in light of potentially ephemeral corridors and barriers to colonization of more recently deglaciated areas. We consider the dynamic history of the area and the role of glaciers, changing sea levels and geographical distance in isolating populations or in promoting contact between these distinctive forms of American marten.

Materials and methods

Sampling

Twenty localities (hereafter referred to as populations, 10 island, 10 mainland; Fig. 1), represented by 413 individuals, were chosen centring on the Pacific Northwest region (40–52° N, 113–126° W) of North America (12 populations from southeastern Alaska, four from British Columbia, one from interior Alaska, two from Montana and one from Oregon; Fig. 1, Table 1). With the exception of northern British Columbia ($n = 5$), each population was represented by 11–26 individuals (Table 1). Previously described areas of sympatry between *caurina* and *americana* populations (Stone *et al*. 2002) were represented by southern Montana ($n = 11$) and Kuiu Island, southeastern Alaska ($n = 25$). Three island populations from southeastern Alaska (Chichagof, Baranof and Prince of Wales Islands) were the result of human introductions in the mid-1900s by the Alaska Game Commission (Elkins & Nelson 1954; Burris & McKnight 1973).

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 NaCl extraction followed those of Fleming & Cook (2002). All samples had been screened previously to determine mtDNA clade profiles (*americana* or *caurina*) using automated sequencing or restriction fragment length analysis (Stone *et al*. 2002). Amplifications of microsatellite DNA contained 0.23 mM of each primer, 154 mM each dNTP, 1.4, 2.5 or 4.3 mM $MgCl_2$, 25 mg/mL Bovine Serum Albumin (BSA), 0.25 units of DNA polymerase, Perkin-Elmer 1× PCR buffer II and 50–100 ng genomic DNA. We used a Perkin-Elmer GeneAmp PCR System 9700 with the following PCR conditions: one cycle of 94 °C for 1 min (for AmpliTaq) or one cycle of 95 °C for 12 min (for AmpliTaq Gold), followed by two 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 one cycle (30 min at 72 °C).

The following primers were used: MA1, MA2, MA3, MA5, MA8, MA11, MA14, MA15, MA18, MA19 (Davis & Strobeck 1998), MER041, MVIS020, MVIS072 and MVIS075 (Fleming *et al*. 1999). Perkin-Elmer AmpliTaq DNA polymerase was used with most primers, with the exception of MA3, MA19, MER041, MVIS020, MVIS072 and MVIS075 where AmpliTaq Gold DNA polymerase was used. The final concentration of $MgCl_2$ for most reactions was 4.3 mM, with the exception of reactions using primers MA8, MA15, and MVIS020 (1.4 mM $MgCl_2$) and MER041, MVIS072 and MVIS075 primers (2.5 mM $MgCl_2$). Samples were run on an ABI 373 automated sequencer and alleles were sized (basepairs, bp) using an internal lane size standard (GS350 by Perkin-Elmer), GeneScan Analysis 3.1, and Genotyper 1.1 computer programs.

Data analysis

FSTAT 2.9.3 (http://www.unil.ch/izea/softwares/fstat.html) (Goudet 2001) was used to calculate descriptive statistics

Table 1 Descriptive statistics for *americana* and *caurina* populations of American martens, including population locations and abbreviations (abbr.), mtDNA clade profile (mtDNA), sample size (n), mean number of alleles (alleles), mean allelic richness (richness), expected heterozygosity (H_E), observed heterozygosity (H_O), F_{IS}, (bold out of HWE) and F_{ST} for the clade comparison

Populations	abbr.	mtDNA	n	alleles	richness	H_E	H_O	F_{IS}	F_{ST}
Baranof Island, SE AK	BAR	*americana*	26	3.64	2.5	0.39	0.45	**0.26**	
Northern British Columbia	NBC	*americana*	5	3.86	3.9	0.66	0.66	0.07	
central British Columbia	CBC	*americana*	17	5.29	4.04	0.68	0.70	**0.16**	
Chichagof Island, SE AK	CHIC	*americana*	25	4.29	3.2	0.53	0.57	**0.16**	
Cleveland Penninsula, SE AK	CP	*americana*	25	5.36	3.65	0.65	0.65	**0.12**	
Juneau, SE AK	JUN	*americana*	25	5.07	3.45	0.58	0.61	**0.19**	
Kupreanof Island, SE AK	KUP	*americana*	25	4.43	3.1	0.55	0.56	**0.15**	
Mitkof Island, SE AK	MIT	*americana*	25	4.71	3.3	0.52	0.58	**0.23**	
northern Montana	NMT	*americana*	11	4.56	3.7	0.62	0.65	**0.19**	
Prince of Wales Island, SE AK	POW	*americana*	25	4.43	3.2	0.55	0.61	**0.26**	
Revillagigedo Island, SE AK	REV	*americana*	25	3.64	2.8	0.47	0.49	**0.24**	
Thomas Bay, SE AK	TB	*americana*	20	4.29	3.3	0.58	0.62	**0.18**	
Yakutat, SE AK	YAK	*americana*	22	4.0	2.8	0.48	0.51	0.13	
Yukon Flats, interior AK	AK	*americana*	25	5.57	3.5	0.62	0.62	**0.14**	
Kuiu Island, SE AK	KUIU	*caurina***	25	4.86	3.15	0.50	0.52	**0.19**	
southern Montana	SMT	*caurina***	11	5.29	4.2	0.59	0.68	**0.26**	
Admiralty Island, SE AK	ADM	*caurina*	25	1.29	1.22	0.06	0.07	0.08	
Queen Charlotte Islands, BC	QCI	*caurina*	11	1.93	1.8	0.24	0.26	0.04	
Vancouver Island, BC	VAN	*caurina*	23	2.93	2.1	0.32	0.32	0.13	
Oregon	OR	*caurina*	17	3.36	2.7	0.48	0.49	–0.02	
americana clade			301		3.3	0.58	0.47	0.196	0.14
caurina clade			112		2.5	0.37	0.31	0.144	0.42

*SE AK = southeastern Alaska, USA; BC = British Columbia, Canada.
**Mixed = both M. americana and M. caurina mitochondrial DNA haplotypes.

for populations including mean number of alleles, allelic richness (number of alleles corrected for sample size), observed and expected heterozygosity and F_{IS} values. Significance of population F_{IS} values was tested with 28 000 randomizations. GENEPOP version 3.3 (http://www.cefe.cnrs-mop.fr/) (Raymond & Rousset 1995) was used to test for Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium. Deviations from HWE and linkage disequilibrium were tested per locus and between each pair of loci. For loci with four or fewer alleles, exact tests (Louis & Dempster 1987) were used to estimate P-values to test for deviations from HWE. Loci with greater than four alleles had P-values estimated by the Markov chain method (Guo & Thompson 1992). Genotypic linkage disequilibrium was tested using the Markov chain method with 10 000 dememorization, 5000 batches and 10 000 iterations. Results were corrected using a sequential Bonferroni adjustment (initial $\alpha = 0.05$) for multiple comparisons.

An unrooted network of genetic relationships among individuals was inferred from allele-sharing distances (Bowcock *et al*. 1994). Pairwise distances were calculated using SHAREDST (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 used to construct a Fitch & Margoliash (1967) tree from the allele-sharing distance matrix.

Pairwise chord distances (Cavalli-Sforza & Edwards 1967) among populations and population networks were generated from allele frequency data using PHYLIP. Chord distances were calculated in GENDIST and used to construct a neighbour-joining (NJ) tree in the program NEIGHBOUR. To test the robustness of tree topologies, 1000 bootstrap replicates of the allele frequency file were generated in SEQBOOT and analysed in GENDIST. Tree topologies were created for all replicates using NEIGHBOUR and a consensus tree was generated in CONSENSE. In addition, Nei's genetic distance (Nei 1972) was calculated and used in a NJ analysis. A maximum likelihood analysis was also performed using SEQBOOT, CONTML and CONSENSE to generate a bootstrapped maximum likelihood (ML) tree. A principal-components analysis (PCA) was performed upon populations using PCAGEN (http://www.unil.ch/izea/softwares/pcagen.html). Populations were ordinated according to the first, second and third PCA axes.

A Bayesian clustering method, STRUCTURE (Pritchard *et al*. 2000), was used to examine population structure and assign individuals to inferred population clusters based

upon multilocus genotype data. We calculated the probability of individual assignments to population clusters (K) without prior information of the origin of individuals. A series of tests was conducted using different numbers of population clusters (MAXPOPS 1–30) to guide an empirical estimate of the number of identifiable populations with burn in and replication values set at 100 000–1000 000. Each test yielded a log likelihood value of the data (Ln probability), the highest of which would indicate which test was closest to the actual number of genetically distinct populations. These tests also provided an alpha value, the measure of admixed individuals in the data set. Individuals were assigned a probability of assignment to a population, or jointly to two or more populations if their genotype profile indicated that they were admixed.

FSTAT 2.9.3 was used to calculate Weir & Cockerham's (1984) estimator of F_{ST} (θ, hereafter referred to as F_{ST}) and R_{ST} values (Slatkin 1995) for each locus and globally. Confidence intervals (99%) were calculated for F_{ST} values to determine if they differed significantly from zero using 15 000 bootstrap replicates over loci. Pairwise F_{ST} values for all pairs of populations were calculated using ARLEQUIN (Schneider *et al*. 2000). Isolation-by-distance was examined (with the Mantel test and 1000 permutations) in GENEPOP using the pairwise F_{ST} as a measure of genetic differentiation. Isolation-by-distance was assessed for the complete data set and two subsets: (1) populations belonging to the *M. caurina* clade and (2) mainland populations belonging to the *M. americana* clade (excluding introduced island populations). The Mantel test was used to test for correlation between pairwise F_{ST} values and Ln geographical distance (in km).

An analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) was performed using ARLEQUIN (Schneider *et al*. 2000). The analysis calculated the partitioning of the molecular variance among groups, among populations within groups and among individuals, using both allele frequency data (F_{ST}) and allele size and frequency data (R_{ST}). Populations were divided into two groups, *M. caurina* or *M. americana* (see Table 1 for population clade identities), identified by our neighbour-joining dendrogram and previously by mitochondrial DNA analysis (Stone & Cook 2002; Stone *et al*. 2002). The two groups were also tested for differences in allelic richness, observed and expected heterozygosity, F_{ST} and F_{IS} values using FSTAT 2.9.3.

Results

Allele frequencies and heterozygosity

Considerable variability was observed across the 14 microsatellite loci (data available upon request). Numbers of alleles per locus ranged from 2 (locus MA15) to 23 (locus MA1) and values of H_E ranged from 0.06 (Admiralty Island) to 0.68 (central British Columbia, Table 1). Mean number of alleles was highest in interior Alaska (5.57) and lowest in Admiralty Island (1.29), a population that was fixed for a single allele at most loci. Allelic richness was highest in southern Montana (4.2) and lowest in Admiralty Island (1.2). *Caurina* populations had a higher frequency of unique alleles than *americana* populations (2.33 vs. 0.78 alleles per population, respectively, allele frequency data available upon request) and significantly lower H_E and H_O (Table 1). Allele frequencies differed among populations and allele size classes and allele frequencies differed between the two mtDNA clades (data available upon request). The allele size class divergence was most noticeable at MA1 where allele sizes for the *americana* clade ranged from 207 to 223 bp and allele sizes for the *M. caurina* clade ranged from 195 to 205 bp. At MVIS020, MER041, MVIS072 and MVIS075, *caurina* populations had a subset of the alleles present in the *americana* clade. *Caurina* populations were fixed for allele 195 at MA15, and *americana* populations had two alleles, 195 and 197, with mainly a higher frequency of allele 197. Kuiu Island and southern Montana, populations from areas of sympatry between *caurina* and *americana* clades, had low frequencies of allele 197 at MA15.

Hardy–Weinberg and genotypic equilibrium

Significant F_{IS} values ($F_{IS} > 0.12$, Table 1) in most populations suggest that populations experience some inbreeding. In locus by population tests, excess homozygosity caused departures from HWE at MA5 (Mitkof Island), MA15 (Baranof, Kupreanof, Mitkof and Prince of Wales Islands), MA14 (Baranof Island), MVIS020 (Baranof, Chichagof, Mitkof, Revillagigedo Islands, Juneau and southern British Columbia), MVIS072 (Kuiu Island) and MVIS075 (Baranof, Cleveland, Kuiu, Kupreanof, Prince of Wales, Revillagigedo Islands and Thomas Bay). Excess heterozygosity caused departure from HWE at MA11 (Cleveland Island). A total of 21 tests of 560 were out of HWE for homozygote or heterozygote excess with 19 departures in *americana* populations and two in Kuiu Island, a mixed *caurina*/*americana* population. Tests for genotypic linkage disequilibrium indicated that MA2 and MA8 were associated in Chichagof and Mitkof Island populations, further suggesting that these populations have experienced some inbreeding.

Clustering of individuals

Genetic relationships among individuals, determined by the allele-sharing distance, define two groups (Fig. 2). Group I contained individuals from mtDNA *caurina* populations and the contact populations with both mtDNA *caurina* and mtDNA *americana* (Kuiu Island and southern

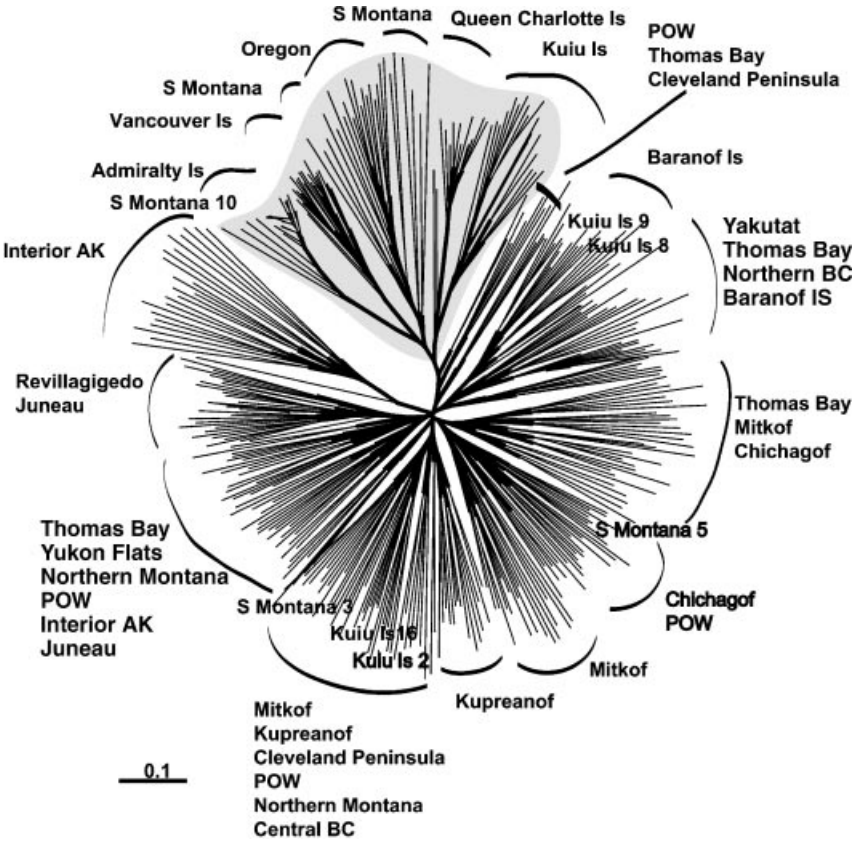

Fig. 2 Unrooted network of genetic relationships among 413 American martens (*Martes americana*) inferred from allele-sharing distances constructed using FITCH in PHYLIP 3.5c (Felsenstein 1993). All unique genotypes are represented by a line. Group I (shaded) includes individuals from *caurina*-type populations and contact zone populations. Group II (unshaded) includes individuals from the *americana*-type populations and contact zone. Population clusters are indicated by semicircles and labelled with population names. Individuals from Kuiu Island and southern Montana clustering within the *americana* clade are labelled (see text).

Montana), while Group II contained individuals from all mtDNA *americana* populations. Most *americana* individuals grouped in multipopulation clusters. In contrast, pure mtDNA *caurina* populations (Admiralty Island, Queen Charlotte Island, Vancouver Island and Oregon) were highly distinctive with all individuals clustering within their respective population groups. Kuiu Island formed a distinct cluster but four individuals were within Group II. Two of these were within the Baranof Island cluster, and two were within a highly mixed cluster (Fig. 2, see Table 2 for mtDNA haplotypes of Kuiu Island and southern Montana individuals). Most southern Montana individuals clustered with the Oregon samples, although one placed near the Admiralty Island population and two were within Group II (one within Chichagof Island and one within a highly mixed cluster).

Phylogenetic analyses and PCA

Some same-sized microsatellite alleles in *caurina* and *americana* may be homoplastic and the result of convergent evolution (Queney *et al*. 2001), because mtDNA analysis suggests a deep separation between these groups (Stone & Cook 2002). Thus, we present chord distances in the NJ analysis. A NJ assessment of pairwise distances using Nei's infinite allele model revealed the same well-supported nodes as the chord distance tree, with minor differences occurring at unsupported nodes. NJ and ML performed similarly and provided a view of the evolutionary history and geographical structure of these populations. These data also provided insight into the history of human-mediated introductions of marten to several islands in the archipelago. The unrooted network of chord distances among populations in NJ and ML analyses identified two well-defined groups supported with bootstrap values of 100% (NJ) and 99.5% (ML) (Fig. 3). Group I consisted of *caurina* populations and the two populations with both *americana* and *caurina* mtDNA haplotypes (Kuiu Island and southern Montana) identified previously by Stone *et al*. (2002). Group II contained *americana* populations. Branch lengths within each group indicated different evolutionary histories; within *caurina*, populations were highly divergent and separated by large chord distances suggesting longer periods of isolation for each population. Kuiu Island, a contact population, appeared between Groups I and II but was included in Group I with 100% support (Fig. 3). Human-introduced populations are reflected by the grouping of Baranof Island with its source population Thomas Bay, and Prince of Wales Island with its source population, Cleveland Peninsula (Elkins &

Table 2 Bayesian assignments of martens from Kuiu Island and southern Montana using STRUCTURE (Pritchard *et al.* 2000). Individual mtDNA identity (a = *americana*, c = *caurina*) is in mtDNA column. The archive number for individual's tissue stored at the Alaska Frozen Tissue Collection, Fairbanks, AK museum is given in the AF column. Model was given no prior information of the origin of individuals and asked to assign them to two or 20 clusters based upon their genotype. Under two clusters, we list the probability of membership in *americana*-dominated cluster or *caurina*-dominated cluster. Under 20 clusters we list the probability of each individual's membership in the majority cluster for their population (79% KUIU, 57% SMT respectively, see Table 4). If membership in the cluster of origin is below 85%, we list probabilities of membership(s) in other clusters identified by the dominant population(s) in those clusters, (abbreviations follow Table 1). When individual is assigned membership to undefined clusters (clusters with less than 10% of any single population), we give the number of clusters and approximate membership percentage in each

			Two clusters		20 clusters Probable membership	20 clusters Probable membership
mtDNA	AF no.	Individual	*americana*	*caurina*	in KUIU cluster	in other cluster
c	19887	KUIU1	0.24	0.76	0.888	
a	19888	KUIU2	0.693	0.307	0.447	KUP, MIT 0.3; CHIC AK 0.1
a	17533	KUIU3	0.01	0.99	0.947	
c	17542	KUIU4	0.011	0.989	0.961	
a	17543	KUIU5	0.006	0.994	0.948	
c	17544	KUIU6	0.004	0.996	0.967	
a	17545	KUIU7	0.002	0.998	0.968	
c	17549	KUIU8	0.991	0.009	0.006	TB, BAR 0.91
a	17534	KUIU9	0.997	0.003	0.003	TB, BAR, 0.934
c	17552	KUIU10	0.308	0.692	0.7	KUP, MIT 0.09; QCI 0.045
c	17553	KUIU11	0.155	0.845	0.935	
a	17535	KUIU12	0.002	0.998	0.971	
a	17536	KUIU13	0.101	0.899	0.945	
a	17537	KUIU14	0.03	0.97	0.945	
a	17538	KUIU15	0.226	0.774	0.893	
c	17539	KUIU16	0.486	0.514	0.465	KUP, MIT 0.42
c	17540	KUIU17	0.034	0.966	0.871	
c	17541	KUIU18	0.355	0.645	0.818	KUP, MIT 0.037
c	25301	KUIU19	0.109	0.891	0.936	
a	25310	KUIU20	0.025	0.975	0.931	
c	25302	KUIU21	0.269	0.731	0.91	
a	25303	KUIU22	0.519	0.481	0.532	KUP, MIT 0.176; CP, POW 0.075
a	25305	KUIU23	0.003	0.997	0.951	
c	25306	KUIU24	0.04	0.96	0.966	
a	25309	KUIU25	0.003	0.997	0.949	
					Probable membership in SMT cluster	
c	23185	SMT1	0.045	0.955	0.755	7 clusters, 0.022
c	23186	SMT2	0.007	0.993	0.866	
c	23187	SMT3	0.99	0.009	0.005	CHIC AK 0.34; 9 clusters 0.05
c	23192	SMT4	0.02	0.978	0.371	ADM 0.25, KUIU 0.12
c	23180	SMT5	0.99	0.003	0.002	CHIC AK 0.87
c	23181	SMT6	0.02	0.977	0.33	8 clusters 0.07
a	23182	SMT7	0.005	0.995	0.909	
a	23188	SMT8	0.008	0.992	0.802	
c	23189	SMT9	0.008	0.992	0.867	
c	23190	SMT10	0.016	0.984	0.595	ADM 0.24
c	23191	SMT11	0.003	0.997	0.769	8 clusters 0.02

Nelson 1954; Burris & McKnight 1973). Several sources were used to stock Chichagof Island, which has an unsupported central position (Fig. 3). Geographic structure is more evident in Group II where proximal populations formed well-supported clusters (Mitkof Island and Kupreanof Island at 84%; Revillagigedo Island and Cleveland Peninsula at 87%). Within Group II much shorter branches defined most populations (except Baranof and Revillagigedo islands and Yakutat).

PCA (figure not shown) was similar to ML and NJ analyses with *caurina* and *americana* divided into two distinct population clusters along the first axis. Similarly,

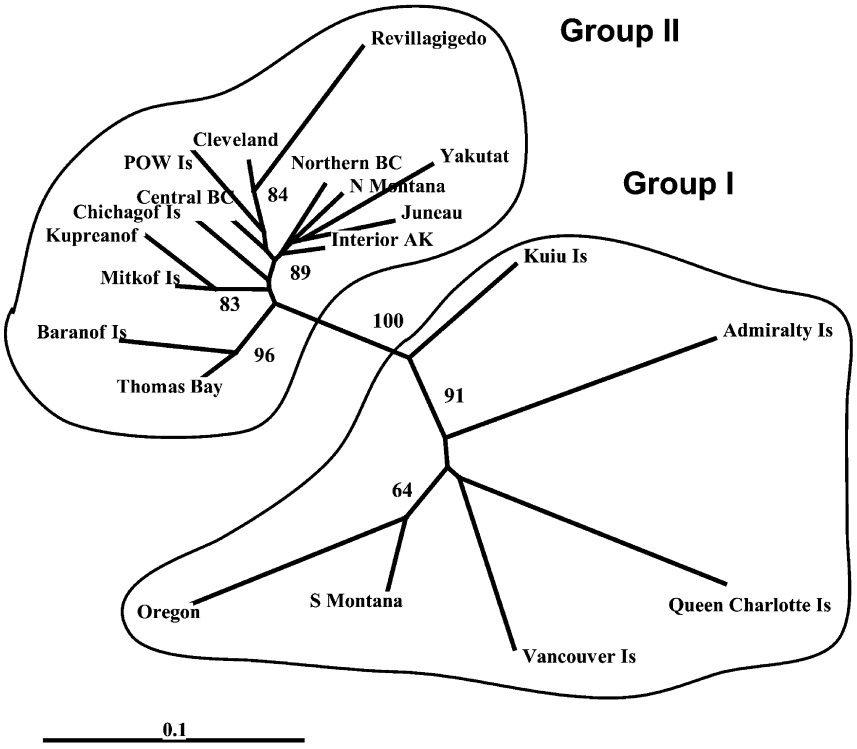

Fig. 3 An unrooted network of chord distances among 20 American marten (*Martes americana*) populations inferred from the neighbour-joining analysis. Abbreviations include 'Cleveland' for 'Cleveland Peninsula, 'Is' for island, and 'Interior AK' for Yukon Flats, Alaska, and all others follow Table 1. Bootstrap values above 60% are placed at the nodes. Bootstrap values, derived from a consensus tree, represent the percentage of 1000 trees where populations beyond the node grouped together. Group I included *caurina* populations and mixed *caurina*/*americana* populations and Group II included *americana* populations.

caurina populations were widely divergent and *americana* populations clustered more tightly. Only the first axis was significant, explaining 32.4% of the variation ($P < 0.001$). The first two axes explain cumulatively nearly half of the total genetic diversity (43.6%). Populations of *caurina* were separated along the second axis (11.2% variation, not significant) with Admiralty Island most distinct. The *americana* populations separated along the third axis (8.5% variation, not significant).

Admixture analysis and assignment tests

In the admixture analysis, we tested substructuring of the data by assuming different numbers of clusters or populations, ranging from all data belonging to a single population to the data belonging to 30 populations (Table 3). The probability of the number of populations (K) was estimated in each case (Ln probability of the data) without using any prior population information so that individuals were assigned to a cluster based upon their multilocus genotype profile. The admixture parameter, α, detected with each K-value was also estimated. Our data were sampled from 20 locations, but the highest probability of the data (Ln = –13 397.8, $\alpha = 0.0291$) was found with clusters set at 15. However, the lowest α value was found with populations set at 10 (Ln = –13, 489.7, $\alpha = 0.007$). This suggests the number of clusters defined by allele frequencies is lower than 20 but greater than 10. With more than 10 clusters, some individuals were more difficult to assign rather than more admixed. These results support the population and allele-sharing analyses indicating several well-defined populations and some rather indistinct populations.

Table 3 Bayesian clustering analyses for pooled *Martes americana* data. 413 individuals analysed at 14 microsatellite loci were assigned to clusters using STRUCTURE (Pritchard *et al*. 2000) without using prior information of population origin. The number of clusters is indicated by *K*. Probability of the data is in the Ln probability of *K* clusters column and the variance of the probability is presented in the variance Ln column. Alpha values indicate the admixture value. Lowest *K* and alpha values are in bold type

K	Ln probability of *K* clusters	Variance Ln	Alpha
1	–17 697.1	65.7	6.933
2	–15 474.9	238.0	0.035
5	–14 359.0	667.0	0.032
10	–13 488.3	1257.0	**0.007**
12	–13 493.5	1342.6	0.030
15	**–13 397.8**	1434.5	0.029
20	–13 665.5	1982.0	0.028
22	–13 721.9	2114.2	0.028
25	–13 675.5	1520.3	0.028
30	–13 641.4	1667.0	0.028

In the admixture analysis, individual assignments to clusters indicated which population allele frequency profile was closest to the individual's genotypic profile.

Table 4 Bayesian assignment test results. Proportion of membership (above 0.01) of each predefined population in each of the 20 clusters, given no prior information of population origin using STRUCTURE (Pritchard *et al*. 2000). Program used 100 000 burnins and repetitions. Highest proportion of membership assigned to original population is in bold type. Population (Pop) abbreviations follow abbreviations in Table 1

	Clusters																			
Pop	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ADM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.97**	0
BAR	0	0	0	0.01	0.01	0	0	0	0	0.04	0.01	0	0	0	0	0.01	0	**0.90**	0.00	0
NBC	0.01	0.01	0.01	**0.19**	0.07	0.01	0.01	0.01	0.02	**0.22**	**0.22**	0.02	0.01	0.01	0.01	0.12	0.01	0.02	0.06	0.01
CBC	0.01	0.01	0.01	0.11	0.13	0.01	0.01	0.01	0.08	**0.21**	**0.15**	0.08	0.01	0.01	0.02	0.09	0.01	0.06	0.01	0.01
CHIC	0	0	0	0.01	0.01	0.01	0	0	0.01	**0.85**	0.02	0.01	0	0	0	0.03	0	0.02	0.01	0
CP	0.01	0.01	0.01	0.02	**0.38**	0.01	0.01	0.01	0.14	0.11	0.07	0.17	0.01	0.01	0.01	0.03	0.01	0.02	0	0.01
QCI	0	**0.97**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
JUN	0	0	0	0.02	0.01	0	0	0	0.02	0.07	**0.81**	0.01	0	0	0.01	0.01	0	0.01	0	0
KUIU	0	0.01	0	0.01	0.01	**0.79**	0	0	0	0.01	0	0	0	0	0.01	0.05	0	0.08	0.01	0
KUP	0	0.01	0	0.01	0.01	0.01	0	0	0.01	0.02	0.01	0	0	0	0	**0.90**	0	0.01	0	0
MIT	0	0	0	0.03	0.05	0	0	0	0.01	0.02	0.03	0.03	0	0	0	**0.73**	0	0.06	0	0
SMT	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0	0.11	0.01	0.01	0.02	0.02	**0.57**	0.01	0.02	0	0.05	0.02
NMT	0.01	0.01	0.01	0.16	0.07	0.01	0.01	0.01	0.03	0.11	**0.30**	0.04	0.01	0.01	0.01	0.12	0.01	0.02	0.03	0.01
OR	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	**0.93**	0	0	0	0	0
POW	0	0.01	0	0.01	**0.79**	0	0	0	0.01	0.06	0.04	0.01	0	0	0	0.04	0	0.01	0	0
REV	0	0	0	0.01	0.01	0	0	0	**0.94**	0.01	0	0.01	0	0	0	0.01	0	0.01	0	0
TB	0	0	0	0.02	0.06	0.01	0	0	0.04	0.10	0.02	0.05	0	0	0	0.04	0	**0.62**	0.01	0
VAN	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	**0.95**	0	0	0	0	0
YAK	0	0	0	**0.86**	0.05	0	0	0	0.01	0.01	0.02	0.01	0	0	0	0.01	0	0.01	0.01	0
AK	0.01	0	0	0.05	0.04	0.01	0	0	0.02	**0.35**	**0.37**	0.02	0	0	0.01	0.07	0	0.04	0.01	0

Admixed individuals were identified when they were assigned to clusters other than the cluster containing members of their original population or assigned to multiple clusters. If total populations was set at two, all *americana* individuals were assigned to one cluster while all *caurina* individuals were assigned to the other cluster, with the exception of the contact populations, Kuiu Island and southern Montana. In those populations, 23% and 19%, respectively, were assigned to the *americana* cluster. For Kuiu Island and southern Montana individuals, we show their mitochondrial identification (Stone & Cook 2002; Stone *et al*. 2002) and microsatellite profile, and their population assignment based upon microsatellite genotype (Table 2). Several individuals from Kuiu Island showed evidence of various levels of admixture, with combinations of microsatellite profiles (membership in both *caurina* and *americana* clusters) while all southern Montana individuals had either *caurina* or *americana* microsatellite profile types (> 95% membership in either cluster).

The assignment test was conducted without prior information of the population origin of individuals, with clusters set at 20. Eleven populations had 80% or more of their membership assigned to a single cluster with some of these clusters containing members from multiple populations (Table 4). The other populations had members distributed among the clusters. In Kuiu Island, two members were assigned at least 30% membership in the Mitkof Island, Kupreanof Island cluster and two assigned at least 90% membership to the Baranof Island, Thomas Bay cluster (Table 2, see 20 clusters column). In southern Montana, two individuals had highest membership (34% and 87%) in the Chichagof Island, interior Alaska cluster and five individuals had partial membership in several clusters (Table 2, see 20 clusters column).

AMOVA analysis, F_{ST} and R_{ST}

When populations were divided into *caurina* and *americana* groups, the AMOVA tests indicated that a substantial portion of the microsatellite genetic variability exists between the clades (Table 5, F_{ST} = 16.5% and R_{ST} = 33%). Microsatellite genetic structure thus parallels mtDNA clade identification as suggested by other analyses in this study. The R_{ST} value was more than twice the F_{ST} value, indicating that *caurina* and *americana* clades differ in allele size distributions as well as allele frequencies. Overall F_{ST} values (Table 1) show high divergence among *caurina* populations (0.42) and moderate divergence among *americana* populations (0.14). The two clades also differ significantly in allelic richness, and observed and expected

Table 5 AMOVA results for *Martes americana* data using ARLEQUIN (Schneider *et al.* 2000). Populations were assigned into two groups identified by the neighbour-joining analysis and clade association (*americana* and *caurina*). F_{ST} AMOVA used allele frequency differences as the distance measure. R_{ST} AMOVA used the sum of the squared allele size difference as the distance measure

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
F_{ST} AMOVA	Among groups	1	316.139	0.840	16.49	F_{CT} = 0.1649	< 0.01
	Among populations within groups	18	726.124	0.902	17.7	F_{ST} = 0.2120	< 0.01
	Within populations	806	2701.883	3.352	65.81	F_{SC} = 0.1649	< 0.01
	Total	825	3744.145	5.094			
R_{ST} AMOVA	Among groups	1	67441.523	190.454	33.01	F_{CT} = 0.3301	< 0.01
	Among populations within groups	18	91142.744	116.879	20.26	F_{ST} = 0.5326	< 0.01
	Within populations	806	217354.854	269.671	46.74	F_{SC} = 0.3024	< 0.01
	Total	825	375939.121	577.004			

heterozygosity (Table 1). The *caurina* clade had significantly lower allelic richness ($P < 0.05$) and heterozygosity ($P < 0.01$) and significantly higher genetic subdivision ($P < 0.01$) than the *americana* clade; in sum suggesting longer isolation times of *caurina* populations and ongoing gene flow and more recent expansion of *americana* into southeastern Alaska.

Pairwise F_{ST}

Pairwise F_{ST} values (Table 6) showed varying population differentiation, with 175/190 comparisons significantly greater than zero. Pairwise F_{ST} values in *caurina* population comparisons were mainly above 0.3, indicating little overlap in allele frequencies and great divergence; a finding also reflected by the NJ tree. Lower pairwise F_{ST} values in *americana* population comparisons indicate more overlap in allele frequencies, also seen in the distance relationships and lack of bootstrap support for some nodes in the NJ tree. Northern British Columbia was undifferentiated from other *americana* populations in 11 comparisons, probably a result of small sample size ($n = 5$). Northern Montana was undifferentiated from other populations in four comparisons, also due possibly to small sample size ($n = 11$) or to gene flow with southern Montana. The Mantel test for isolation-by-distance indicated that genetic and geographical distances were independent ($P > 0.05$), even when the two mtDNA clades were analysed separately. In many cases within the Pacific Northwest, oceanic straits appear to be greater barriers to gene flow than distance.

Discussion

The advance and retreat of glaciers during the Pleistocene created a dynamic history of isolation, fragmentation and secondary contact among forest-associated species in the Pacific Northwest (Pielou 1991; Hewitt 1996). A number of species represented by deeply diverged lineages have responded independently to this dynamic geological history (e.g. Cook *et al.* 2001). Genetic structure in coastal marten populations reflects a very distinctive history from that found in continental populations. Microsatellite data, in conjunction with mtDNA and morphological data, suggest that coastal *caurina* populations have been isolated for extended periods. *M. caurina* individuals may have colonized northward along the coast following the establishment of mature forests in this region. Coastal islands then were formed along the continental shelf when landmasses were isolated by rising sea level in the early Holocene, and *caurina* populations may have been fragmented and isolated. In contrast, *americana* individuals appear to be still expanding out of Pleistocene-delimited ranges and now are contacting and hybridizing with *caurina* individuals. Although the rapid evolution of microsatellites may have created homoplasy when comparing alleles across these distinctive clades (Garza & Freimer 1996), this nuclear analysis parallels results from mtDNA and morphological analyses. This new perspective on fine-scale genetic structure within *caurina* and *americana* populations allowed further assessment of hybridization, in addition to providing insight into distinctive colonization patterns of *caurina* and *americana* populations in the Pacific Northwest.

Nuclear vs. mitochondrial perspectives

Concordance between nuclear and mtDNA perspectives in marten contrasts with phylogeographical patterns found in coastal brown bears, another carnivore inhabiting the islands of southeastern Alaska (Paetkau *et al.* 1998). Paetkau *et al.* (1998) demonstrate tight connectivity among Admiralty, Baranof and Chichagof Island populations and nearby coastal mainland and interior populations of brown

Table 6 Weir and Cockerham's (1984) F_{ST} for all pairs of marten populations calculated using ARLEQUIN (Schneider *et al*. 2000). Population abbreviations follow Table 1

	ADM	BAR	NBC	CBC	CHIC	CP	QCI	JUN	KUIU	KUP
ADM	0.000									
BAR	0.632	0.000								
NBC	0.653	0.214	0.000							
CBC	0.531	0.158	0.001*	0.000						
CHIC	0.582	0.249	0.094	0.077	0.000					
CP	0.528	0.206	0.041*	0.045*	0.091	0.000				
QCI	0.756	0.499	0.438	0.358	0.470	0.380	0.000			
JUN	0.554	0.232	0.054*	0.056	0.130	0.079	0.413	0.000		
KUIU	0.496	0.367	0.217	0.233	0.294	0.268	0.377	0.301	0.000	
KUP	0.545	0.223	0.095*	0.115	0.132	0.120	0.426	0.175	0.281	0.000
MIT	0.557	0.213	0.059*	0.080	0.156	0.082	0.426	0.128	0.288	0.053
SMT	0.543	0.307	0.127	0.117	0.228	0.158	0.298	0.189	0.213	0.212
NMT	0.611	0.198	0.029*	0.023*	0.116	0.058	0.393	0.039*	0.277	0.109
OR	0.610	0.446	0.318	0.280	0.371	0.323	0.440	0.340	0.332	0.360
POW	0.543	0.220	0.070*	0.058	0.152	0.047	0.371	0.133	0.292	0.150
REV	0.646	0.309	0.208	0.158	0.191	0.117	0.513	0.179	0.405	0.231
TB	0.521	0.148	0.076*	0.091	0.123	0.087	0.428	0.142	0.252	0.140
VAN	0.620	0.480	0.383	0.332	0.400	0.353	0.484	0.375	0.339	0.404
YAK	0.598	0.272	0.096*	0.131	0.237	0.185	0.460	0.143	0.354	0.235
AK	0.533	0.206	−0.004*	0.037	0.079	0.081	0.389	0.037	0.254	0.127

	MIT	SMT	NMT	OR	POW	REV	TB	VAN	YAK	AK
MIT	0.000									
SMT	0.187	0.000								
NMT	0.110	0.155	0.000							
OR	0.347	0.117	0.325	0.000						
POW	0.123	0.166	0.083	0.333	0.000					
REV	0.245	0.335	0.162	0.449	0.214	0.000				
TB	0.105	0.187	0.139	0.345	0.122	0.196	0.000			
VAN	0.391	0.283	0.376	0.369	0.395	0.485	0.358	0.000		
YAK	0.233	0.253	0.110	0.398	0.195	0.269	0.208	0.452	0.000	
AK	0.103	0.163	0.029*	0.334	0.103	0.203	0.137	0.357	0.114	0.000

*Not significantly different from zero ($\alpha = 0.05$).

bears using nuclear microsatellites. However, that nuclear pattern differs from the highly distinctive insular mitochondrial lineages identified only in the bears from Admiralty, Baranof and Chichagof islands. Scandinavian brown bears (Waits *et al*. 2000) show a similar pattern of strikingly different nuclear and mitochondrial structure. Male-mediated gene flow is suggested as the cause for the discordant nuclear and mitochondrial patterns in both studies. Differing life history characteristics between male and female brown bears, such as larger home ranges and greater dispersal from natal range in males (Canfield & Harting 1987), and different N_e between microsatellite and mitochondrial loci apparently impact patterns of variation in nuclear vs. mitochondrial DNA. In contrast, nuclear structure in martens paralleled mitochondrial structure, indicating that dispersal of both males and females is inhibited among most islands and between the mainland and most islands.

From a conservation perspective, Moritz (1994) suggests that evolutionarily significant units should not only be reciprocally monophyletic for mtDNA alleles but also maintain significantly diverse allele frequencies at nuclear loci. Others (Crandall *et al*. 2000) emphasize the need to consider adaptive diversity within a species when considering evolutionary processes in conservation biology. We identified significant partitioning between clades both in allele frequencies and allele sizes at 14 microsatellite loci corroborating the patterns in the mitochondrial cytochrome *b* gene (Carr & Hicks 1997; Stone *et al*. 2002), the nuclear aldolase *C* gene (Stone & Cook 2002) and earlier morphological work (Merriam 1890; Anderson 1970; Giannico & Nagorsen 1989). Because *caurina* populations have historically occupied coastal habitats and *americana* populations have occupied inland habitats, genetic and morphological patterns may reflect adaptations associated with different habitats. We suggest that these forms may

well represent distinctive species and that further investigations of ecological, behavioural or physiological characteristics (e.g. Aune & Schladweiler 1997; Ben-David *et al*. 1997) should be completed within this emerging framework. Investigations centred on the two contact zones of marten provide opportunities to explore genetic differences involved in speciation and the potential exchange of genes between *caurina* and *americana* individuals.

During the mid-1900s, human-mediated introductions of martens took place on Chichagof, Baranof and Prince of Wales Islands, where martens were presumed not to exist (Elkins & Nelson 1954; Burris & McKnight 1973; MacDonald & Cook 1996). Microsatellite signatures indicated no prior inhabitation of these islands by *caurina* individuals, a finding consistent with mtDNA surveys (Stone *et al*. 2002). High diversity and rare alleles in these introduced populations probably reflect that the founders consisted of several *americana* individuals or that multiple introductions occurred. If *caurina* individuals had been present on these islands, human-mediated transplants of *americana* individuals to *caurina* populations may have resulted in genetic swamping of *caurina* populations such as might be in progress currently on Kuiu Island. These introductions occurred without prior knowledge of the considerable genetic structure found within species or the potential negative ramifications of hybridization (for examples, see Rhymer & Simberloff 1996). Future management of these furbearer populations should reflect this new framework.

Hybridization

Hybrid zones provide opportunities to measure gene exchange between diverging taxa (Barton & Hewitt 1985). On the basis of morphological characteristics, Wright (1953) reported hybridization between individuals of the American marten clades in western Montana. Microsatellite analysis provided further information on hybridization between *caurina* and *americana* individuals in the contact zones of southern Montana and Kuiu Island; these zones were defined previously by cytochrome *b* haplotypes (Stone *et al*. 2002). In the Bayesian analysis (Table 2), the two individuals in the southern Montana population with *americana*-type mtDNA profiles were assigned membership in the *caurina* microsatellite cluster while two individuals with *caurina*-type mtDNA were assigned to the *americana* microsatellite cluster. This suggests that hybridization has occurred within the southern Montana population, as indicated previously by Wright (1953), who reported skulls of intermediate size between *caurina* and *americana* individuals in western Montana.

On Kuiu Island, both hybridization and introgression have occurred between *caurina* and *americana* individuals (Table 2). Half of Kuiu Island individuals possessed *americana* haplotypes that were common to nearby island (Mitkof and Kupreanof Islands) and mainland populations. The remaining *caurina* haplotype was unique to Kuiu Island (Stone & Cook 2002). Thus, *americana* haplotypes appear to be recent arrivals from nearby islands, while the distinctive *caurina* haplotype is endemic to Kuiu Island. Twelve martens with *americana*-type mtDNA were assigned 30–99% membership in the *caurina* microsatellite cluster and eight martens with *caurina*-type mtDNA were assigned 10–99% membership in the *americana* microsatellite cluster (Table 2), supporting the hypothesis that *caurina* and *americana* individuals are hybridizing on this island. Microsatellite alleles from mainland *americana* populations may be entering the Kuiu Island nuclear gene pool via immigrant movement across Mitkof and Kupreanof Islands. Because martens on Kuiu Island are hybrids characterized by either *americana* or *caurina* mtDNA haplotypes with a mixture of nuclear genotypes, both male and female *americana* individuals appear to be expanding into this area. We suspect that Kuiu Island was inhabited previously solely by *caurina* individuals. However, *americana* individuals may be competitively superior, such that populations of *caurina* in coastal regions may be persisting only on islands isolated by substantial oceanic straits. If *caurina* populations existed on the mainland or near-shore islands, they may have been vulnerable to competition by *americana* individuals when this group colonized the region.

The zone of contact in southeastern Alaska, Kuiu Island, is the furthest of a string of three islands that extend from the mainland (Fig. 1). Very narrow stretches of water separate Mitkof, Kupreanof and Kuiu Islands, effectively creating a peninsular effect (MacDonald & Cook 1996). Individuals from mainland *americana* populations probably colonized Mitkof, Kupreanof and Kuiu Islands across these narrow oceanic straits. The original specimen taken from southeastern Alaska in 1909 was collected from Kuiu Island (Swarth 1911) and identified as the *caurina* morph. Both morphs are present in southeastern Alaska with the *americana* clade distributed across a larger range (due partially to human introductions to several large islands), while the *caurina* clade is restricted to Admiralty and Kuiu Islands.

Colonization and evolutionary history

Microsatellite data illuminated fine-scale relationships among populations including genetic signatures related to extended periods of isolation and recent expansions. All analyses are consistent with a longer period of isolation for *caurina* populations when compared to the natural (i.e. nonintroduced) *americana* populations. Networks based on allele-sharing distances (pairwise individual comparisons), and assignment tests show similar patterns suggesting past isolation of *caurina* populations and possible expansion and/or extensive gene flow among *americana* populations. Further, significantly lower allelic richness and

heterozygosity and significantly higher division among populations characterized *caurina* in contrast to *americana*. Higher frequency of private alleles and Hardy–Weinberg equilibrium in *caurina* populations indicates that, although these populations have been isolated for an extended time, they are large and stable enough to maintain low-frequency alleles (Queney *et al*. 2001) and avoid inbreeding. In contrast, *americana* populations are modestly divergent, have higher allelic richness and tend to depart from HWE, suggesting more recent expansion into the Pacific Northwest, larger effective population sizes and possibly continuing gene flow. In sum, our results illustrate distinctive evolutionary histories for the coastal *caurina* and continental *americana* forms of marten.

The monomorphic genetic pattern in the Admiralty Island *caurina* population may have arisen due to repeated bottlenecks (Eldridge *et al*. 1999) or from a small effective population size over an extended time (Avise *et al*. 1984). The Admiralty Island population is unlikely to have been founded recently by human introduction due to its unique cytochrome *b* haplotype (Stone & Cook 2002) and microsatellite profiles. Other documented introductions of *americana* individuals resulted in genetically diverse populations. In contrast to the brown bear studies (e.g. Paetkau *et al*. 1998), nuclear and mitochondrial perspectives on Admiralty Island marten are more similar to genetic patterns found in Prince of Wales Island flying squirrels (Bidlack & Cook 2002). Oceanic straits beyond a certain width may thus serve as barriers to gene flow for mid-sized and smaller mammals such as marten and flying squirrels, but not for large mammals such as bears.

Genetic distance among marten populations was independent of geographical distance, even when analysed within clades. Structural patterns are likely confounded by the landscape, highly fragmented both in time and space, in which populations are located. Island populations of *caurina* may have colonized by expanding northward along the coast from a southern refuge roughly 10 000–12 000 years ago after glaciers receded from the coast and as forest habitat was becoming established. Rising sea level may have isolated founders on Admiralty, Kuiu, Queen Charlotte and Vancouver Islands around 10 000 years ago (Warner *et al*. 1982; Pielou 1991; Fedje & Josenhans 2000). In contrast, *americana* populations may be still in the process of expanding from continental refugia, using river corridors through the coastal mountain ranges to access the Pacific coast as these corridors became accessible with the recession of glaciers. Hybridization and introgression on Kuiu Island may represent the furthest front of natural *americana* population expansion into the islands, supporting a hypothesis of recent expansion into southeastern Alaska. Continued gene flow among the expanding *americana* populations and a shorter time since colonization may preclude divergence into distinct populations.

Kyle *et al*. (2000) studied northern continental populations of *americana* and concluded that few barriers to gene flow exist among marten populations from the Yukon through central Northwest Territories, Canada. They attributed the lack of genetic structure to isolation-by-distance and predicted higher population fragmentation should be found in southerly regions where habitat was more disjunct. Indeed, we found that population genetic structure in *caurina*, and to a lesser extent in *americana*, could be attributed to fragmentation. However, in contrast, *americana* population structure in this region was not due to isolation-by-distance and was less fragmented by barriers such as the Coast Mountain Range that parallels coastal southeastern Alaska. Oceanic straits may prevent gene flow among *caurina* populations and may also disrupt gene flow among *americana* populations; Revillagigedo Island, a near-shore island, was the most distinctive *americana* population. Extensive timber harvesting may also be fragmenting populations of this forest-associated species. The persistence of *caurina* populations on larger islands in southeastern Alaska suggests that if habitat islands of similar size are preserved, these insular marten populations can persist. However, we do not know whether *caurina* populations inhabited Alaska islands other than Admiralty and Kuiu Islands in the past and have subsequently gone extinct. New palaeontologic work across the archipelago may shed light upon this (e.g. Heaton & Grady 2003). The *americana* individuals appear to disperse better and outcompete *caurina* individuals when they contact. Thus to maintain *caurina* populations in southeastern Alaska, *caurina* populations may need habitat inaccessible to *americana* individuals.

Conclusions

Microsatellite genetic structure corroborated the division between mitochondrial clades found in American marten, with a pronounced break between *Martes caurina* and *M. americana* populations. Population structure within each clade indicated different evolutionary histories for the coastal *M. caurina* and continental *M. americana* populations. Hybridization between individuals from these distinctive morphs was identified in Kuiu Island and in southern Montana. In light of mtDNA evidence, microsatellite analysis suggests that *M. caurina* populations in southeastern Alaska were founded soon after glacial retreat, and then isolated by rising sea levels. In contrast, individuals of the *M. americana* clade appear to be expanding into southeastern Alaska and hybridizing with *M. caurina* individuals where they contact. The dynamics and viability of the insular *M. caurina* populations, in particular, should be carefully monitored. Given the tremendous habitat modification that has occurred in Pacific Northwest forests over the past century (e.g. Durbin

1999), thoughtful management of these ecosystems will be required. Decisions should be based on the realization that we still have an incomplete taxonomic and evolutionary framework for a significant portion of the biotic diversity of this complex region, including much of the purportedly well-studied mammalian fauna.

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Maureen Small is a biologist in the Genetics Division at the Washington State Department of Fish and Wildlife. This research was a collaborative effort involving Small's postdoctoral project at Idaho State University and Karen Stone's doctoral dissertation work at University of Alaska, Fairbanks, both conducted under the direction of Joseph Cook. Karen Stone is now an Assistant Professor of Biology at Southern Oregon University and Joseph Cook is a Professor of Biology at Idaho State University. Cook's work focuses on how evolutionary and biogeographical histories of organisms have shaped patterns of genetic variation in extant populations.
