

Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska

MELISSA A. FLEMING*† and JOSEPH A. COOK*‡

*University of Alaska Museum, 907 Yukon Drive Fairbanks, Alaska 99775–6960, ‡Biological Sciences, Idaho State University, Pocatello, Idaho 83209–8007

Abstract

The North Pacific Coast (NPC) of North America is a region of high mammalian endemism, possibly due to its highly fragmented landscape and complex glacial history. For example, four island and one mainland subspecies of ermine, *Mustela erminea*, have been described as endemic to southeast Alaska alone. To better understand the role of past climatic change in generating diversity in the region, we examined DNA sequence variation in the mitochondrial cytochrome b gene of 210 ermine from across North America, with an emphasis on Alaska and British Columbia. We found three distinct (1.5–3.6% uncorrected 'p') lineages of ermine, all of which occur in southeast Alaska. One lineage includes a southeast Alaska endemic and specimens from Alaska (outside of southeast) and Eurasia. A second lineage includes two southeast Alaskan endemics and ermine from western Canada and the coterminous United States. The close relationships of these purported endemics to ermine outside of southeast Alaska suggest that they colonized the region from Beringian and southern glacial refugia, respectively, following deglaciation of the NPC. The third lineage appears restricted to the Prince of Wales Island complex in southeast Alaska (two subspecies) and Graham Island (Haida Gwaii), British Columbia. This restricted distribution suggests that these populations may be derived from relicts that persisted in a coastal refugium during the Wisconsin glaciation. Studies of nuclear genes and adaptive morphological evolution are necessary to further explore discrepancies between the geographical pattern of differentiation based on mtDNA and the existing subspecific taxonomy based on morphology.

Keywords: cytochrome b, endemism, glacial refugia, mitochondrial DNA, Mustelidae

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Introduction

Controversy has surrounded the possibility of a North Pacific coastal refugium during the Wisconsin glaciation (McCabe & Cowan 1945; Foster 1965; Ogilvie 1989; Byun *et al.* 1997; Demboski *et al.* 1999). Evidence for coastal refugia has been derived primarily from the distribution of endemic taxa in the region (e.g. Ogilvie 1989; MacDonald & Cook 1996). Renewed interest in this question has been stimulated by the examination of phylogeographical patterns of northwest coastal organisms (Zink 1996; Conroy & Cook 2000; Cook *et al.* 2001) and the re-evaluation of

endemic taxa of conservation concern (Cook & MacDonald 2001).

Some 24 mammal species or subspecies are endemic to the stretch of the North Pacific coast encompassing southeast Alaska (MacDonald & Cook 1996). *Sorex monticolus*, *Peromyscus keeni*, *Clethrionomys gapperi*, *Microtus oeconomus*, and *Mustela erminea* account for 67% of these taxa. Twelve other taxa in southeast Alaska have ranges that extend only to coastal British Columbia (Nagorsen 1990). This high level of nominal diversity may be due to the fragmented landscape of the North Pacific coast (Cook & MacDonald 2001). Southeast Alaska includes more than 2000 named islands in the Alexander Archipelago and a topographically complex, narrow strip of coastal mainland that is largely isolated from the remainder of North America by glaciated mountain ranges. In addition, the region has experienced considerable temporal fragmentation, with as

Correspondence: Melissa A. Fleming. †Present address: Human Biology, D4-100, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109 USA. Fax: 206 6672917; E-mail: mfleming@fhcrc.org

many as 20 glacial advances covering all or part of the North Pacific coast during the last two million years (Mann & Hamilton 1995; Barrie & Conway 1999). The most recent glacial event was the Wisconsin glaciation, which began its retreat approximately 15 000 years ago. High levels of intraspecific diversity in southeast Alaska may be the result of postglacial colonization from multiple refugia (Cook *et al.* 2001) and subsequent differentiation via island biogeographical processes.

The most diverse taxon described for southeast Alaska is the ermine, *Mustela erminea*, with five endemic subspecies. One subspecies is widespread on the coastal mainland and on near shore islands while the other four are restricted to particular large islands or island complexes (Hall 1944, 1951). All are listed as 'potentially threatened' by the IUCN Mustelid and Viverrid Research Group because of their limited distributions and the paucity of information about population and taxonomic status (Schreiber *et al.* 1989). Original descriptions of many insular taxa were based on small sample sizes (e.g. a single specimen of Suemez Island ermine, *M. e. seclusa*, six specimens of the Chichagof/Baranof Island ermine, *M. e. initis*). Efforts to reassess the taxonomic status of southeast Alaska endemics and interpret the biogeographical history of the region can provide guidance in mitigating the impact of timber harvests on insular faunas throughout this temperate rainforest (Cook *et al.* 2001).

Ermine originated in Europe in the early Pleistocene and apparently expanded into the coterminous U.S. by ~500 000 years ago (Kurtén & Anderson 1980). During the Wisconsin glacial advances (~300 000–10 000 years ago), North American ermine may have persisted in several distinct refugia including well documented ice-free regions of interior Alaska, Yukon Territory and eastern Russia (Beringia; Hopkins 1967; Sher 1999), south of the ice sheets in the coterminous United States (Pielou 1991) and proposed refugia along the North Pacific coast (Heaton *et al.* 1996; Byun *et al.* 1999). Thus, extant ermine in southeast Alaska may include neoendemics whose ancestors colonized the region during the Holocene ($\leq 10\ 000$ years ago), and palaeoendemics derived from relictual populations that were isolated in one or more ice-age refugia along the North Pacific coast.

To elucidate the evolutionary history of endemic ermine of southeast Alaska, we sequenced the mitochondrial DNA (mtDNA) cytochrome b gene (*cyt-b*), widely used in phylogeographical studies to distinguish Quaternary divergences within vertebrate species (Avice *et al.* 1998; Avice & Walker 1998). Although we focused on insular and mainland ermine in southeast Alaska, we also sampled other locations in North America to explore geographical variation in the species and identify the possible refugial origins of southeast Alaska lineages. We hypothesized that southeast Alaskan endemics represent descendents of refugial populations from north and south of the Cordilleran and Laurentide ice sheets (neoendemics), and

possibly from unglaciated regions of the North Pacific coast itself (palaeoendemics). Neoendemics would be identified by having mtDNA *cyt-b* haplotypes most similar to widespread haplotypes in neighbouring populations in other recolonized areas and in former refugia outside the North Pacific coast region. Palaeoendemics would have mtDNA *cyt-b* haplotypes with restricted distributions quite divergent from neighbouring populations. We also compared the relationships of southeast Alaskan ermine derived from the mtDNA data with the subspecies taxonomy based on morphology to evaluate the extent of overlap between these two measures of geographical variation.

Materials and Methods

Sampling

We examined a total of 210 ermine from 45 localities, including 199 specimens representing 38 localities from Alaska (including all five southeast Alaskan endemics) and British Columbia, 10 specimens from six localities across the remainder of the species' range in North America, and a single specimen from eastern Russia (Fig. 1, Appendix 1). We sequenced *cyt-b* ($n = 67$ specimens) for individuals from 37 localities, including at least two individuals from 21 of the localities. After we identified diagnostic sequences, we used restriction fragment length polymorphisms (RFLP) in *cyt-b* to screen 143 additional specimens from 34 localities.

DNA extraction

We extracted DNA from frozen tissue (usually spleen or heart) using a NaCl extraction method (Miller *et al.* 1988) or a phenol-chloroform extraction protocol (Sambrook *et al.* 1989). The Miller *et al.* (1988) method was modified for use with < 50 mg of frozen tissue in 1.5 mL microcentrifuge tubes. Tissue was rapidly washed (2x) in 4 °C 1x STE buffer and digested at 55–60 °C for 4 h to overnight in 550 μ L lysis buffer (50 mM Tris-HCL, pH 8.0, 50 mM EDTA pH 8.0, 100 mM NaCl, 1% SDS and 1% 2-mercaptoethanol) with 11 μ L of proteinase K (10 mg/mL). After digestion, 5.5 μ L of RNase A (10 mg/mL) and 350 μ L of 5 M NaCl were added. Tubes were centrifuged at 16 000 *g* for 30 min. The supernatant was transferred to two new 1.5 mL tubes (~450 μ L per tube) for ethanol precipitation. The DNA was pelleted by centrifugation, desalted with two 70% ethanol washes (centrifuged for 5 min at 2940 *g* after each) and resuspended in 10 mM Tris or T(1/10)E buffer.

Sequencing

The mtDNA *cyt-b* gene was sequenced in both directions in two or three overlapping segments using the following

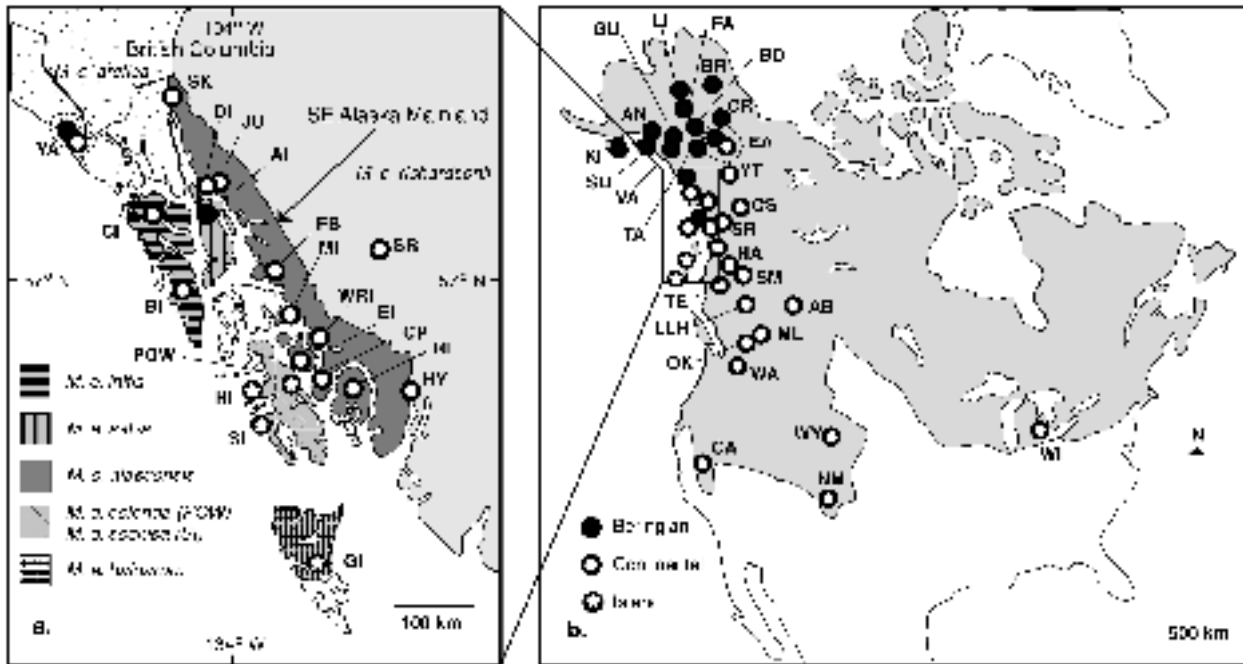


Fig. 1 Distributions of the ermine subspecies sampled from Alaska and British Columbia and of the Beringian, Continental and Island clades in: (a) southeast Alaska and (b) North America from mtDNA *cyt-b* sequencing and RFLP. Shading in (a) indicates the ranges of eight subspecies; symbols (a and b) indicate clades identified in each sampling location. Two and three letter codes indicate sampling locations as described in Appendix 1.

primers MVZ04, MVZ05, MVZ16, MVZ14, MVZ23 (Smith & Patton 1993), Marten37 (Demboski *et al.* 1999), L14609 (Koepfli & Wayne 1998), Mustela06 (5'-GTGGAATGGGATTTTGTTCAGAGTCGGA-3') and Mustela07 (5'-TTCATCATTTTCAGCACTAGCAGCAGTC-3'). DNA was amplified by double-stranded polymerase chain reaction (PCR) using an initial denaturation of 45 s at 95 °C followed by 35 cycles of 10 s at 94 °C, 15 s at 45 °C, 45 s at 72 °C, with a 3-min final extension at 72 °C. Negative controls were run with each set of PCR reactions. PCR products were purified by polyethylene glycol (PEG) precipitation, resuspended in 10 mM Tris, and cycle-sequenced using the *Taq* DyeDeoxy terminator cycle sequencing kit (Perkin Elmer/ABI). Cycle-sequencing products were cleaned using Sephadex G-50 columns and run on an Applied Biosystems 373 automated sequencer. Sequences were aligned by eye using Sequence Navigator, Version 1.01 (ABI).

We sequenced the complete gene (1140 bases) for 19 ermine and 792 bases from the 3'-end for another 48 (Appendix 1). A complete sequence from a California ermine was obtained from GenBank (Koepfli & Wayne 1998; GenBank AF057128) resulting in a total sample of 68 sequenced individuals. Complete sequences of *Mustela putorius* (Koepfli & Wayne 1998; GenBank AF057127) and *M. nivalis* (GenBank accession no: AF457461) were used as outgroups. Sequence statistics and phylogenetic analyses were generated using MacClade 4.0 (Maddison & Maddison 2000) and PAUP*, VERSION 4.0b8a (Swofford 2000).

The 68 ermine sequences represented 28 different *cyt-b* haplotypes (deposited in GenBank with accession numbers: AF271060-8 and AF457441-60). These nonredundant sequences were analysed using unweighted maximum parsimony (MP) and a heuristic search with 10 replicates of random taxon addition. Tree bisection and reconnection (TBR) branch swapping was limited to 1 000 000 rearrangements for each replicate. One hundred bootstrap replicates were generated under the same conditions, except that the search was constrained to generate a maximum of 1000 trees. A maximum likelihood (ML) analysis was performed using 13 complete nonredundant ermine sequences, the two outgroups, and partial ermine sequences from Britain (337 bp from the 5' end of the gene; Davison *et al.* 1998; GenBank AF068546) and Japan (375 bp from the 5' end of the gene; Masuda & Yoshida 1994; GenBank D26515). The appropriate ML model was GTR + I + G as determined by MODELTEST v3.04 (Posada *et al.* 2000). The ML tree search and bootstrapping were conducted as described for MP. To determine whether lineages were evolving in a clock-like manner, we tested for rate heterogeneity by evaluating ML trees constructed with and without a molecular clock constraint (Felsenstein 1988).

Restriction fragment length polymorphisms

Three distinct lineages identified from sequence analysis had diagnostic RFLPs when digested with the restriction

enzyme *DdeI* (selected by DNA STRIDER 1.2, Dr C. Marck, Service de Biochimie et de Genetique Moleculaire, Bat. 142 Center d'Etudes de Saclay, 91191 GIF-SUR-YVETTE CEDEX, France). We screened 143 additional specimens from 26 localities with sequenced individuals and from eight other localities (Appendix 1). As positive controls, RFLPs were also analysed for 24 specimens that were previously sequenced and represented the three identified lineages. In most cases, specimens first analysed by RFLP were also sequenced when they represented previously unsequenced localities or when the RFLP results suggested more than one haplotype in a locality or region.

PCR products (495 bp) were obtained using primers MVZ23 and MVZ14. Nine μ L of PCR product were digested with 0.2 μ L of *DdeI* and 1 μ L of 10 \times NE Buffer (New England Biosystems) at 37 °C for 2.5–3 h and separated on 4% NuSieve gels at 80 V. *DdeI* digestion produced fragment lengths of 163, 128, 79, 70 and 55 bp for Beringian haplotypes, 233, 128, 79 and 55 bp for Continental haplotypes and 233, 183 and 79 bp for Island haplotypes. On each gel, sample bands were scored by comparison to bands from a set of samples of known sequence.

Nested Clade Analysis

We conducted a nested clade analysis to determine to what extent the divergences observed in southeast Alaskan ermine are due to historical fragmentation and range expansion vs. current restrictions on gene flow. We used the program *tcs* (Clement *et al.* 2000) to generate a haplotype cladogram with 95% parsimoniously plausible connections between haplotypes (as described by Templeton *et al.* 1992). To minimize ambiguity in the haplotype cladogram, only sequence data available for all specimens (349–1140 bp of *cyt-b*) were used. The program *GEODIS* (Posada *et al.* 2000) was then used to calculate clade distances (D_c), nested clade distances (D_n), and interior-tip distances for both ($I-T_c$ and $I-T_n$, respectively) using the methods of Templeton *et al.* (1995). One thousand random permutations of the procedure (Roff & Bentzen 1989) were run to establish whether any distances were significantly small or large at the 5% level. Results were interpreted using the inference key in Templeton (1998).

Results

MtDNA sequences

Base composition and distribution of variable sites were as expected for mammalian (Irwin *et al.* 1991; Johns & Avise 1998) and mustelid cytochrome b sequences (Koepfli & Wayne 1998). There was a deficit of guanines (G: 12.2%) relative to other bases (A: 30.0%; C: 30.2%; T: 27.6%). Across the three species of *Mustela*, there were 159 variable

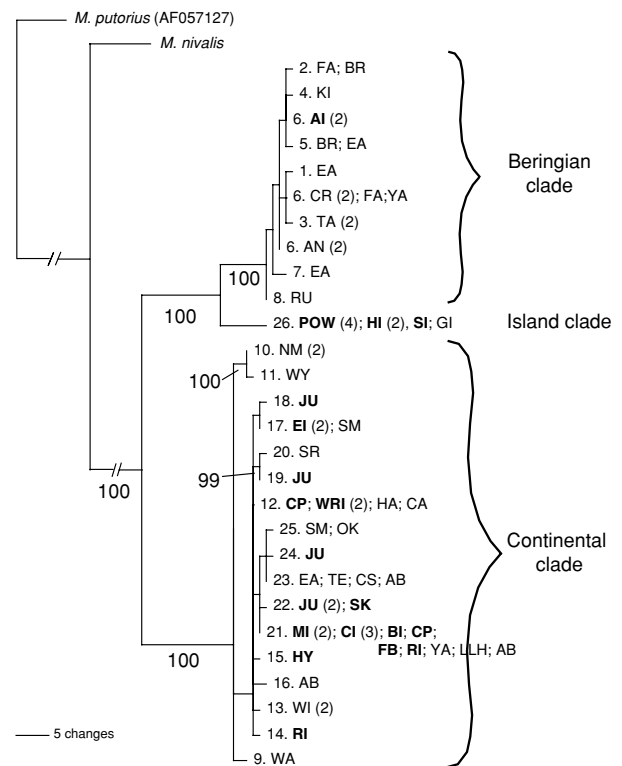


Fig. 2 One of 34 153 equally parsimonious trees (length = 202, CI = 0.842, RI = 0.920) generated by a heuristic search with 10 replicates of random taxon addition for 30 nonredundant sequences of mtDNA *cyt-b*. Haplotypes are numbered to correspond to Fig. 4 and individuals with identical sequences from different locations (two and three letter codes defined in Appendix 1) are included. Southeast Alaska locations are bold. Bootstrap percentages greater than 70% for 100 replicates are indicated below the branches.

sites; 81.1% occurred at the third position (116 transitions: 13 transversions), 14.5% first position (22:1), and 4.4% second position (seven transitions). Nonsynonymous changes (12) were consistent with amino acid sites previously identified as variable for the cytochrome b protein (Irwin *et al.* 1991). Within ermine, there were 65 variable sites (49 at third, seven at first and three at second positions), 45 of which were parsimony informative, and five variable amino acids. All five transversions were at third positions.

Phylogenetic analysis

Both MP and ML methods revealed three distinct clades of ermine (Figs 2 and 3) in southeast Alaska which we designated 'Beringian', 'Continental' and 'Island' clades (Fig. 1). We could not reject the molecular clock hypothesis ($\chi^2 = 10.70$, d.f. = 15, $P > 0.1$). The 18 ermine in the Beringian clade included 10 haplotypes from 10 localities in Russia, interior and southcentral Alaska (including the Kodiak Island endemic, *M. e. kadiacensis*), and the Admiralty Island subspecies, *M. e. salva*, in southeast Alaska. Ermine

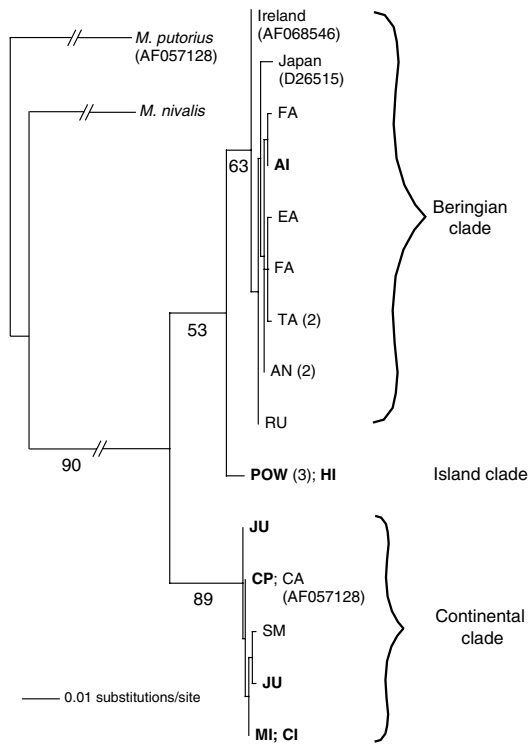


Fig. 3 A maximum likelihood tree ($-\ln L = 2429.3215$) generated by a heuristic search with 10 replicates of random taxon addition from 13 complete, nonredundant mtDNA *cyt-b* sequences for ermine (this study) and partial sequences from Ireland and Japan (followed by GenBank accession numbers). Bootstrap percentages greater than 70% for 100 replicates are indicated below the branches. Bootstrap percentages less than 70% are included for major clades from Fig. 2.

from Ireland and Japan were also in this clade (Fig. 3). The 42 ermine in the Continental clade comprised 17 haplotypes from 26 localities including New Mexico, Wyoming, Washington, California, Wisconsin, Alberta and mainland British Columbia. In southeast Alaska, the Continental clade included the Chichagof and Baranof Island endemic, *M. e. initis*, and *M. e. alascensis*, a subspecies found on the mainland and on Mitkof, Etolin, Wrangell, and Revillagigedo islands. The eight ermine in the Island clade shared a single haplotype in four localities: Prince of Wales Island (*M. e. celenda*); two small islands farther west in the 'POW complex' (Suemez, *M. e. seclusa*, and Heceta islands); and on Graham Island, British Columbia (Haida Gwaii, *M. e. haidarum*). The two specimens from Heceta Island represent a new island record for the species.

RFLP screening of 143 additional ermine from these and eight other localities supported the geographical distribution of the clades identified by sequencing (Appendix 1). We also detected two localities where the Beringian and Continental clades overlap (Figs 1 and 2). Of eight ermine from Yakutat in southcentral Alaska, seven were identified

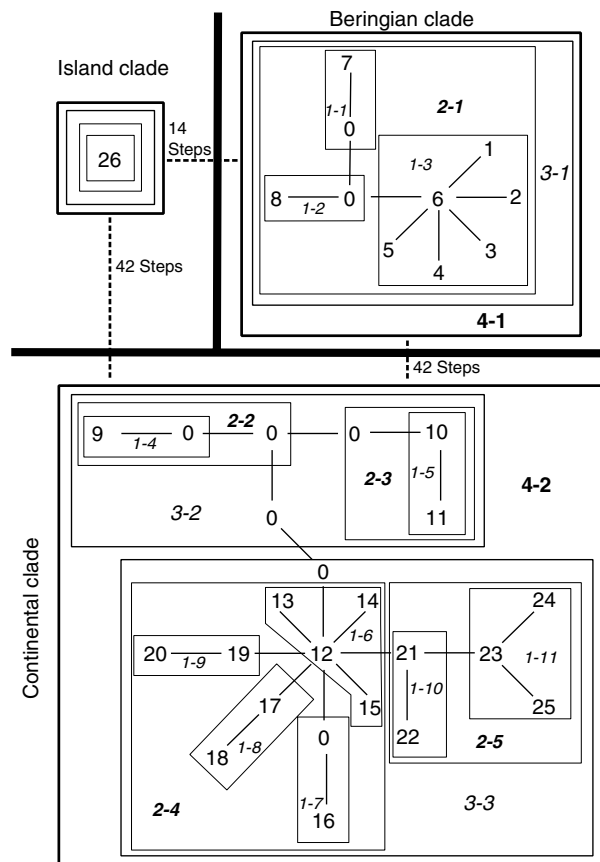


Fig. 4 The estimated 95% parsimoniously plausible set of cladograms and the nested design for the mtDNA *cyt-b* haplotypes (based on 792 bp) found in *Mustela erminea*. Haplotypes are indicated by numbers that correspond to those in Fig. 2. Solid lines between haplotypes represent single mutational changes; dashed lines represent more than one mutational change as indicated. Zeros represent intermediate haplotypes not observed in the sample. Boxes enclose 1- to 4-step clades as indicated by '1-x', '2-x', etc., where 'x' is the clade number. The heavy solid lines denote clades separated by a larger number of mutational steps than can be shown.

as members of the Beringian clade while the eighth was Continental. Of 10 ermine from Eagle in eastern interior Alaska, nine exhibited the Beringian haplotype and the tenth was Continental. The haplotypes of specimens with minority representation in these localities were confirmed by sequencing (Appendix 1).

Ermine in the Continental clade were highly distinct from those in the Beringian (mean \pm SE uncorrected 'p', $3.50 \pm 0.02\%$) and Island ($3.57 \pm 0.06\%$) clades, while the distinction between the Beringian and Island clades was less ($1.50 \pm 0.04\%$). Within each clade, mean divergence was low ($0.26 \pm 0.02\%$ within the Beringian clade; $0.51 \pm 0.02\%$ within the Continental clade) or nonexistent in the case of the Island clade in which all eight sequences were identical.

The haplotype cladogram is shown in Fig. 4 and

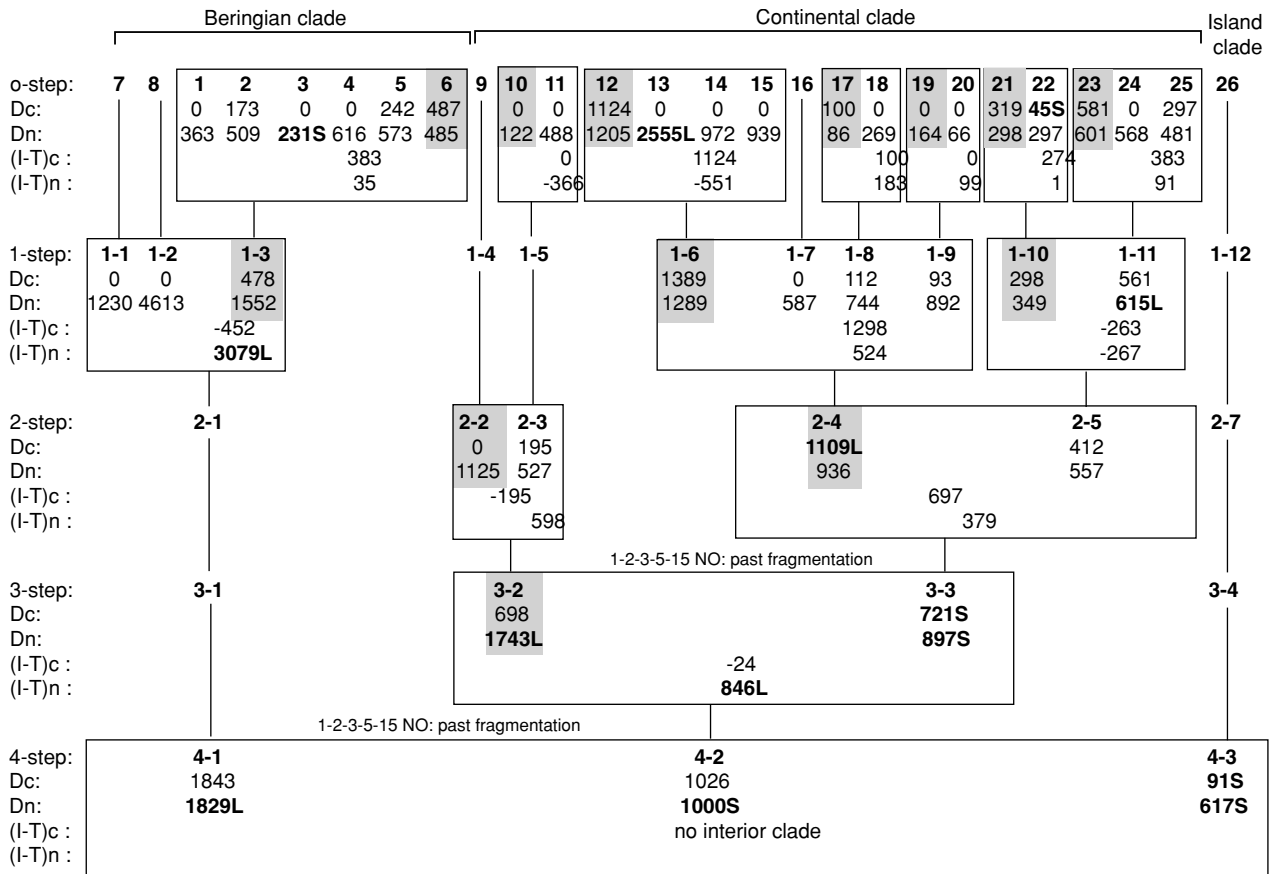


Fig. 5 Results of the nested clad analysis of the geographical distances for mtDNA *cyt-b* haplotypes of *Mustela erminea*. Haplotypes are in bold on the top line and clades structure is shown by boxing clades from lower to higher nested (top to bottom of figure). Where tip/interior status of a clade is known, interior clade names and distances are shaded. Distances that are significantly small or large at a 5% level are also in bold and followed by an 'S' or 'L', respectively. The inference chains and resulting inferences are shown above nested set of clades that have significant geographical structure as reported in Table 1.

Table 1 The permutational chi-square statistics and probabilities for the nesting clades of *Mustela erminea* in Figs 4 and 5. A probability of less than 0.05 indicates significant geographical structure. Clades with no genetic or geographical structure are excluded

Clade	Chi-square statistic	Probability
1-3	49.11	0.092
1-5	3.00	0.345
1-6	27.00	0.057
1-8	4.00	0.492
1-9	2.00	1.000
1-10	15.00	0.144
1-11	11.38	0.627
2-1	24.59	0.191
2-4	39.11	0.113
2-5	14.32	0.401
3-2	4.00	0.501
3-3	20.09	0.326
4-2	42.00	0.000
Entire Cladogram	129.45	0.000

results of the nested clad analysis are shown in Fig. 5. Significant geographical structure was observed only for nested clade 4-2 and at the > 4-step clade level (Table 1). Clade 4-2 includes two 3-step clades: WA-WY-NM and the rest of the Continental clade (Figs 2 and 4). The > 4-step clade includes the Beringian, Continental and Island clades (Figs 2 and 4). The non or minimally overlapping distributions of nested clades and the relatively large numbers of missing haplotypes between them (particularly at the > 4-step level) suggest past fragmentation is responsible for their geographical structure (Fig. 5). We did not detect the postglacial range expansion that must have occurred, particularly for the Continental clade, although significantly large clade, nested clade or interior-tip nested clade distances were identified for clades that included sample locations in presumed refugia [e.g. nested clade 1-11 includes haplotype 12 from California and 13 from Wisconsin (Fig. 5)].

Discussion

Origins of southeast Alaskan ermine

Three distinct clades of ermine occur in western North America and all are found in southeast Alaska. Two of these lineages are widely distributed beyond this coastal region. The Beringian lineage, represented in southeast Alaska by *Mustela erminea salva* of Admiralty Island, has an Alaska and Palearctic distribution, including eastern Russia, Japan and Ireland. The Continental lineage, represented by *M. e. initis* of Chichagof and Baranof Islands and *M. e. alascensis* of the southeast Alaska mainland and near shore islands, is distributed throughout western Canada and across the coterminous U.S. at least as far east as Wisconsin and south into California and north-western New Mexico. The distribution of the Island clade (*M. e. celenda*, *M. e. seclusa* and *M. e. haidarum*) appears to be more restricted; we documented it on three islands at the southern end of the Alexander Archipelago and on Graham Island, British Columbia. Nested clade analysis suggests that the divergence of these clades is due to the historical fragmentation of ermine populations rather than restricted contemporary gene flow. Our inability to detect postglacial range expansion may be due to inadequate sampling in refugial areas (Templeton *et al.* 1995), particularly south of the ice sheets — the presumed refuge for the Continental clade.

Southeast Alaskan populations from the two widely distributed clades are minimally diverged from populations in these same clades sampled outside the region, as expected for neoendemics. Low divergence among members of the Beringian clade (including Eurasian ermine; Figs 2 and 3) suggests gene flow across the Bering Land Bridge during the Wisconsin glaciation and the colonization of Admiralty Island (*M. e. salva*) from the north following deglaciation of coastal regions. The Continental clade also exhibits low levels of intraclade divergence. Haplotypes within this lineage are shared from southeast Alaska, British Columbia, Alberta, and south to California. Chichagof and Baranof Island ermine (*M. e. initis*) share haplotypes with ermine from near shore islands and southeast mainland (*M. e. alascensis*), and other continental sites, indicating a recent common ancestry for these nominal endemics.

Because the northern and southern limits of the ranges of various mammalian species are found in southeast Alaska, investigators (Klein 1965; MacDonald & Cook 1996; Conroy *et al.* 1999) have proposed that southeast Alaska was recolonized from both Beringian and southern refugia. MtDNA analyses for several species of mammals (Demboski *et al.* 1999; Cook *et al.* 2001), fish (Deagle *et al.* 1996) and plants (Soltis *et al.* 1997) reveal patterns of divergence and spatial distributions of lineages along the North Pacific coast that support this view. Those patterns further suggest

that most species currently in the region colonized from southern refugia. The fossil record for ermine extends from 500 000 years ago in the coterminous U.S. (Kurtén & Anderson 1980). The broad distribution of the Continental clade in the formerly glaciated regions of western Canada and southeast Alaska is consistent with the postglacial colonization of ermine from southern refugia.

In contrast, the distribution of the Beringian clade of ermine in Alaska follows a proposed colonization route of boreal species into southeast Alaska from Beringia. Colonization routes into southeast Alaska from the north were glaciated longer than those from the south (Mandryk 1996), delaying the expansion of Beringian taxa along the North Pacific coast. However, the presence of *M. e. salva* on Admiralty Island and the Beringian clade in Yakutat suggests colonization of southeast Alaska from a Beringian refugium may have occurred via the coast when sea levels were lower (Mann & Hamilton 1995). Other coastal species, such as the tundra vole (*Microtus oeconomus*) on Chichagof and Baranof Islands, apparently colonized from Beringia also and a coastal route has been suspected (Lance & Cook 1998).

The three island endemic subspecies included in the Island clade, *M. e. celenda*, *M. e. seclusa*, and *M. e. haidarum*, exhibit several characteristics predicted for palaeoendemics from a coastal refugium. First, the limited distribution of the clade includes Prince of Wales Island and Graham Island of the Queen Charlotte Islands ('Haida Gwaii') of British Columbia, both of which have been proposed as glacial refugia from fossil and geological evidence (Scudder & Gessler 1989; Josenhans *et al.* 1995; Heaton *et al.* 1996). Second, the identical haplotypes of the eight ermine from four islands suggest a recent common ancestry stemming from a historically low population size or a population bottleneck as might be expected in a small refugium (Demboski *et al.* 1998; Bidlack & Cook 2001). Third, the depth of divergence of this Island clade from the Beringian and Continental clades indicates prolonged isolation.

None of these characteristics is unassailable evidence for the palaeoendemism of the Island clade, however. The geological and palaeontological evidence for coastal refugia at various times during the last glaciation has not yet demonstrated the long-term persistence of refugia, particularly during the last glacial maximum (Mann & Hamilton 1995). Reduced genetic diversity of the Island clade could be due to population bottlenecks that preceded the colonization of the southern Alexander Archipelago and Haida Gwaii. Serial bottlenecks have been proposed to explain reduced genetic diversity in other postglacial invaders (Hewitt 1996; Soltis *et al.* 1997; Conroy & Cook 2000).

Finally, other southeast Alaskan mammals show two mtDNA lineages with divergences greater than or comparable to ermine and disjunct distributions across North America that argue for their expansion from two distinct

southern refugia rather than from southern and North Pacific coast refugia (Demboski *et al.* 1999; Conroy & Cook 2000; Stone & Cook 2000). Demboski *et al.* (1999) reported two American marten (*Martes americana*, 2.5–2.8% uncorrected 'p') and two dusky shrew (*Sorex monticolus*; 4.7–5.7% uncorrected 'p') clades in southeast Alaska. These clades have distributions that extend south along the west coast of North America ('coastal clade') or east into central or eastern United States and Canada ('continental clade'). The distribution of two black bear clades (*Ursus americanus*, 3.1–3.6% uncorrected 'p'; Stone & Cook 2000) found in southeast Alaska is also consistent with colonization from disjunct west and east coast forest refugia (Wooding & Ward 1997).

The palaeoendemism of divergent clades of sticklebacks (O'Reilly *et al.* 1993) and black bears (Byun *et al.* 1997; Byun *et al.* 1999) on Haida Gwaii and brown bears on Admiralty Island (Talbot & Shields 1996) has been re-evaluated in light of later studies that identified 'endemic' haplotypes over a greater geographical range than originally identified (Wooding & Ward 1997; Stone & Cook 2000; for black bears; Orti *et al.* 1994; Deagle *et al.* 1996 for sticklebacks; Leonard *et al.* 2000 for brown bears). We still have relatively limited specimen representation throughout the proposed western coastal refugium (Hoffmann 1981; Wooding & Ward 1997) in the United States, including only a single ermine from the Cascade Mountains in Washington and another from the Sierra Nevada in California (Koepfli & Wayne 1998). Furthermore, the nested clade analysis revealed historical fragmentation within the Continental clade, which may reflect the isolation of ermine in two or more southern refugia. Other North Pacific coast mammals such as *Microtus longicaudus* (Conroy & Cook 2000) and *S. monticolus* (Demboski & Cook 2001) include multiple clades in southwestern North America. Expanded sampling throughout the coterminous U.S., but particularly on the west coast, is necessary to more firmly establish the geographical distribution of the Island clade. Analysing a suite of nuclear loci may also help determine whether the Island clade is a glacial relict from the North Pacific coast or a postglacial colonizer.

Comparison of mtDNA and morphological perspectives on endemism

Crandall *et al.* (2000) argued that increasing reliance on molecular phylogenies to determine evolutionary significant units (ESUs) to the exclusion of other data has reduced the relevancy of ESUs for conservation. Adaptive variation in morphology or life history variables can evolve over a time period that may be too short to generate sufficient variation in gene sequences to construct robust molecular phylogenies (e.g. the 10 000 years since southeast Alaska has been deglaciated). Ermine morphology can change quickly in response to environmental conditions (King & Moody

1982; Ralls & Harvey 1985; Eger 1990). Historical distinctiveness and adaptive variation are both legitimate bases for establishing endemic status and subspecific designations. Thus, we were interested in determining the degree to which our mtDNA phylogeny is in agreement with other classifications of North American and southeast Alaska ermine based on morphology.

The spatial distributions of ermine mtDNA clades corroborate the pattern of geographical variation in ermine skull size and shape described by Eger (1990). Eger (1990) reported three basic skull morphologies corresponding to extreme northern North America (interior and south-central Alaska and the Canadian Arctic), the Pacific Northwest (Oregon, Washington, Vancouver Island), and the remaining continental U.S. and Canada. Eger (1990) concluded that variation in skull size correlated well with ecological factors (measured as climatic values) while differences in aspects of skull shape were consistent with the past isolation of North American ermine in separate glacial refugia.

The Beringian mtDNA clade corresponds to Eger's (1990) 'Arctic samples', which she considered representative of a Beringian refugium. In western North America, this correspondence extends to an abrupt change in morphology found in Eagle, Alaska near the border of the Yukon Territory where we also found a contact zone between Beringian and Continental mtDNA clades.

Eger's (1990) southeast Alaska specimens were from Juneau and Prince of Wales Island and these populations represent Continental and Island mtDNA clades, respectively. The Juneau specimen was morphologically similar to specimens from across Canada and the coterminous U.S., a result consistent with the distribution of the Continental mtDNA clade. Eger's (1990) specimens from Prince of Wales Island and Graham Island (Haida Gwaii) were similar in shape to the Pacific Northwest specimens, but considerably larger (possibly due to island effects, Foster 1964). The mtDNA data show a similar distinction between Prince of Wales and Graham Island specimens (Island clade) vs. mainland British Columbia and Alberta specimens (Continental clade), but not specimens from the west coast of the United States (Washington and California, also Continental clade). If skull shape reflects the effects of genetic differentiation in separate glacial refugia, more intensive sampling may reveal a wider distribution of the Island mtDNA clade in the Pacific Northwest, perhaps overlapping the distribution of the Continental clade in Oregon, Washington or coastal British Columbia.

Results from the mtDNA analysis differ from Hall's (1944; 1951) hypothesized relationships among ermine subspecies based on skull morphology. Hall suggested that the Admiralty Island subspecies, *salva*, was by far the least differentiated from the mainland *alascensis*, while the Chichagof/Baranof Island subspecies, *initis*, was

considerably more differentiated. Our data suggest the opposite should be true as *initis* and *alascensis* are both in the Continental clade. Similarly, Hall hypothesized that the Queen Charlotte subspecies, *haidarum*, may be a glacial relict as it was more similar to the interior Alaskan, *arctica*, while all the southeast Alaskan ermine were much more similar to one another. In contrast, mtDNA sequences reveal a much closer relationship between the southeast Alaskan island subspecies *seclusa* and *celenda* and *haidarum*. The observed morphological similarities among the southeast Alaskan subspecies (and differences from *haidarum* and ancestral populations) could be adaptive, but information on the ecology of these populations is lacking. Contradictions between mtDNA and morphological measures of similarity may also be explained by other evolutionary mechanisms, such as contemporary male-mediated gene flow among islands and/or the postglacial colonization of these islands by multiple mtDNA lineages followed by their subsequent loss via lineage sorting, which could be detectable with nuclear markers.

Conclusions

The occurrence of three distinct mtDNA clades of ermine in southeast Alaska supports the hypothesis that the glacial history of the region played a substantial role in shaping intraspecific variation of ermine there. The 'Island clade' (*Mustela erminea celenda*, *seclusa*, and *haidarum*) is perhaps the best candidate so far identified among mammals for a palaeoendemic lineage that persisted in a North Pacific coastal refugium during the last glaciation. Three other endemic subspecies show minimal (*salva*; Beringian clade) or no (*alascensis* and *initis*; Continental clade) divergence from populations outside of the region and these, if they represent valid taxonomic units, may be considered neoendemic to this coastal region.

Both wider sampling across North America and Eurasia and the analysis of nuclear markers are necessary to further examine the palaeo- or neoendemic status of the distinctive lineages of southeast Alaskan ermine. Comparative phylogeographical analyses of other North Pacific mammals (e.g. Cook *et al.* 2001) in combination with the geographical pattern of distinct skull shapes in ermine (Eger 1990) imply the potential for a wider distribution of the Island clade, particularly southward along the coast of western North America. Sampling from across the Holarctic range of ermine would provide a stronger framework for interpreting lineage diversity and might clarify the origin and history of the Island clade. Determining whether the minimally divergent haplotype of *M. e. salva* evolved on Admiralty Island requires further sampling in the former Beringian refugium, and southward along a possible colonization route on the coast of southcentral Alaska. Analysis of more rapidly evolving mtDNA control region

or nuclear markers is necessary to test the neoendemic status for Chichagof/Baranof Island subspecies, *initis*, and southeast Alaska mainland subspecies, *alascensis*, which each share a haplotype with geographically distant populations. Analysis of nuclear markers should also help elucidate whether the peculiar distribution of the Continental and Beringian clades in southeast Alaska (Fig. 1) — east from Yakutat (both clades) to Chichagof and Baranof Islands (Continental clade) to Admiralty Island (Beringian clade) and the southeast Alaska mainland (Continental clade) — is the effect of lineage sorting on ancestral populations consisting of both clades or of differential colonization of islands by these distinctive lineages. Morphological similarities among ermine subspecies that contradict mtDNA relationships may be due to interbreeding before isolation on islands, more contemporary gene flow via male-mediated dispersal, or morphological convergence and should be further investigated through more intensive sampling in both genetic and morphological studies.

By revealing the distinctive histories of the endemic ermine of the three largest islands in southeast Alaska, this mtDNA analysis encourages further study of genetic, morphological and ecological variation to determine whether these populations, and other southeast Alaskan endemics, should be managed as distinctive evolutionary entities. In particular, the mtDNA analyses argue that any taxonomic reassessment of southeast Alaskan endemics should include comparisons with specimens from geographically distant populations in regions likely to have been glacial refugia, as well as neighbouring populations. Disparities between mtDNA haplotypes and subspecific designations could be due in part to selection on morphology, underscoring the potential value of taking adaptive variation as well as genetic isolation into account when defining conservation units in this morphologically variable carnivore.

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Melissa Fleming is interested in the evolution of intraspecific variation at behavioural, physiological and genetic levels and conducted this research as a postdoctoral fellow at the University of Alaska Museum. Joseph Cook's work focuses on how evolutionary and biogeographical histories of northern organisms have shaped patterns of genetic variation in extant populations.

Appendix 1

Source location of specimens, genetic data obtained (i.e. number of base pairs of mtDNA *cyt-b* sequence, RFLP), mtDNA *cytochrome b* clade (B = Beringian, C = Continental, and I = Island), tissue identification numbers and sample sizes (n) for 210 ermine. Bold type indicates sequence data. Two or three letter codes following locations correspond to those on Figs 1–3.

Location	Data	Clade	AF number ¹	n
<i>Russia</i>				
Magadan (RU)	1140	B	6627	1
<i>Interior Alaska</i> ²				
Big Delta (BD)	RFLP	B	22500–1	2
'Brooks Range' (BR)	792; 792 + RFLP; RFLP	B; B; B	22413; 22411; 22600–1	4
Charley River (CR)	792	B	31642–3	2
Eagle (EA)	792; 1140; 792 + RFLP; RFLP	B; B; B/C; B	11755; 11756; 11760/11754; 11751–3, 11757–9	10
Fairbanks (FA)	792; 1140; RFLP	B; B; B	21097; 17863; 5755, 22514–5, 22758, 32175–6, 32187–8	10
Valdez (VA)	RFLP	B	29883–4, 29888	3
Gulkana (GU)	RFLP	B	29881–2	2
Tanacross (TA)	1140; RFLP	B	24375–6; 24773	3
Livengood (LI)	RFLP	B	5756, 22521–2	3
<i>South-central Alaska</i>				
Anchorage (AN)	1140; RFLP	B; B	8829, 8852; 8786	3
Kodiak Island (KI)	792	B	22567	1
Soldotna (SO)	RFLP	B	22602	1
Yakutat (YA)	792 + RFLP; RFLP	B/C; B	22495/22497; 22492–4, 22496, 22498–9	8
<i>Southeast Alaska Mainland</i>				
Skagway (SK)	792	C	8750	1
Juneau (JU)	792; 1140; RFLP	C; C; C	24056, 22469–70; 14967–8; 14966, 14969–71, 17519–20, 24057, 22490–1, 22512–3, 22516–20, 22533–4, 22536–41, 29872–77	35
Farragut Bay (FB)	792	C	24068	1
Cleveland Pen. (CP)	1140; RFLP	C; C	25046, 25049; 24377, 25047–8, 25984	6
Hyder, BC (HY)	792	C	12721	1
<i>Nearshore Islands</i>				
Douglas (DI)	RFLP	C	12641, 14588	2
Mitkof (MI)	792; 1140; RFLP	C; C; C	24061; 24052; 5919–22, 16055–7, 16059–62, 19872–8, 19885–6, 22357, 23781, 23784–5, 23789, 23791–2, 24053–5, 24062, 24064–7	37
Revillagigedo (RI)	792; RFLP	C; C	25603, 29600; 25601	3
Etolin (EI)	792; 792 + RFLP; RFLP	C; C; C	25897; 25888; 25880	3
Wrangell (WRI)	792; RFLP	C; C	22569–70; 22568	3
<i>Island endemics</i>				
Admiralty (AI)	792; 1140 + RFLP; RFLP	B, B; B	16064; 24060; 22468, 24511–5	8
Baranof (BI)	792	C	32474	1
Chichagof (CI)	792; 1140; 792 + RFLP; RFLP	C; C; C; C	24059; 24063; 24058; 1821–2, 16084	6
Prince of Wales (POW)	792; 1140; 1140 + RFLP; RFLP	I; I; I; I	25602; 22865; 16085, 17789; 22872, 25015, 25390, 25392–3	9
Suemez (SI)	792 + RFLP	I	26441	1
Heceta (HI)	1140; 792 + RFLP	I; I	26406; 26405	2
Graham, BC (GI)	792 + RFLP	I	22554	1
<i>Mainland Canada</i>				
Alberta (AB)	792 + RFLP	C	34039–41	3
Cassiar, BC (CS)	792 + RFLP; RFLP	C; C	22704; 22700	2
Hazelton, BC (HA)	792 + RFLP; RFLP	C; C	22544; 29868–70	4
Lac la Hache, BC (LLH)	792 + RFLP; RFLP	C; C	22542; 29854, 29858–61	6
Mabel Lake, BC (ML)	RFLP	C	29862–65, 29871, 29891	6
Okanagan, BC (OK)	792 + RFLP; RFLP	C; C	22543; 29851–2, 29855	4
Smithers, BC (SM)	1140; 792 + RFLP; RFLP	C; C; C	16024; 30228; 16025	3
Stikine R, BC (SR)	792	C	12858	1
Terrace, BC (TE)	792	C	30229	1
Whitehorse, YT (YT)	RFLP	C	29753	1

Appendix 1 *Continued*

Location	Data	Clade	AF number ¹	n
<i>Coterminous U.S.</i>				
Kittitas Co. (WA)	792	C	14920	1
Albany Co. (WY)	792	C	RMS1919	1
Sandoval Co. (NM)	792; 792 + RFLP	C; C	DH3445; KKK351	2
Manitowoc Co. (WI)	792 + RFLP	C	34035-6	2

¹Identification numbers refer to Alaska Frozen Tissue Collection, except for New Mexico and Wyoming samples which are from the New Mexico Museum of Natural History.

²Interior Alaska locations refer to USGS 1: 250 000 quadrangles except for 'Brooks Range'.