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**Quaternary Biogeography of Western North America:**
Insights from mtDNA Phylogeography of Endemic Vertebrates from Haida Gwaii

by
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B.Sc., York University, 1992

A Dissertation Submitted in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

We accept this dissertation as conforming
to the required standard

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# Abstract

Population fragmentation and subsequent isolation in different refugia during the glacial advances of the Pleistocene are believed to have had a significant impact on current levels of genetic and morphological diversity. Despite the importance of these glacial refugia for biodiversity, our understanding of their distribution on the northwestern coast of North America and their relative impact on populations remains limited.

As the most isolated group of islands in the Pacific Northwest, Haida Gwaii has been the subject of intense study both from the perspective of its complex glacial history and endemic flora and fauna. The ubiquitous presence of glacial features on this archipelago points to extensive ice cover during the late Wisconsin (Fraser glaciation) and populations which could only have become established postglacially. However, the large assemblage of unique mammalian and avian fauna found on Haida Gwaii has led to suggestions that these divergent vertebrates actually evolved through long isolation by continuously inhabiting these islands or nearby regions throughout the last glacial maximum.

To assess Haida Gwaii's role as a glacial refugium and the relictual status of its endemic black bear (*Ursus americanus*), marten (*Martes americana*), short-tailed weasel (*Mustela erminea*), caribou (*Rangifer tarandus*) and Saw-whet Owl (*Aegolius acadicus*) , a broad phylogeographic study using sequence comparisons of the mitochondrial gene cytochrome b was undertaken. Phylogeographic structure was observed in the black bear (n= 33), marten (n= 18) and short-tailed weasel (n= 32).

Based on parsimony, maximum likelihood, and neighbour-joining analyses of 719 bp of cytochrome b, two geographically structured black bear lineages were

unambiguously identified: 1) a continental lineage found in the Yukon, Alberta, Alaska, Montana and Pennsylvania (*americanus*) and mainland BC (*americanus* and *cinnamomum*) and 2) a coastal lineage found on Haida Gwaii (*carlottae*), Vancouver Island (*vancouveri*) and the Olympic Peninsula (*altifrontalis*). The two lineages were defined by 24 synapomorphies and an average sequence divergence of 3.6%. Average intralineage divergence was 0.1%. Similarly, two geographically structured lineages, continental and coastal, were also identified in marten using the same types of analyses on 311 bp of cytochrome b. The continental lineage included marten from mainland BC (*caurina* and *abietinoides*) and Newfoundland (*atrata*) whereas the coastal lineage included marten from Haida Gwaii (*nesophila*) and Vancouver Island (*vancouverensis*). The two lineages were defined by three synapomorphies and an average sequence divergence of 1.2%. Average intralineage divergence was 1%. Phylogeographic structure was also observed in the short-tailed weasel using 148 to 673 bp of cytochrome b. Three major lineages were identified and named according to their putative refugial source areas: Beringia, which included weasels from Japan (*orientalis*) and the Yukon (*arctica*), a continental or southern source, which encompassed weasels from mainland BC (*richardsonii*, *invicta*, *fallenda*), Manitoba (*bangsi*), and Ontario (*cicognanii*), and Haida Gwaii which included only those weasels from Haida Gwaii (*haidarum*). Short-tailed weasels from Vancouver Island (*anguinae*) and some areas along the coast demonstrated an affinity to both southern and Haida Gwaii weasels. Relative to the continental lineage, the coastal lineage was defined by 13 synapomorphies; the Beringian lineage was defined by 10 synapomorphies. Average sequence divergence was 2.5 % and 2.2% respectively. Divergence between the coastal weasels and Beringian weasels was 2.4%. There was little mtDNA diversity within the coastal lineage as the average intralineage divergence was 0.8%.

Little or no phylogeographic structure was observed in the caribou and Saw-whet Owl. Of the 313 bp examined in two barren ground caribou (*granti*) and seven

woodland caribou (four *tarandus* and three *dawsoni*), three *tarandus* and two *dawsoni* formed a lineage defined by one synapomorphy. The two barren ground, one *tarandus*, and one *dawsoni* were excluded from this lineage by one to three substitutions. Similarly, little phylogeographic structure was observed in the Saw-whet Owl. Analyses of a 241 bp of cytochrome b sequenced from this species indicated no genetic divergence between individuals as far apart as Haida Gwaii (*brooksi*) and Manitoba (*acadicus*). The maximum divergence observed between individuals was 0.4%.

The phylogeographic patterns from these five species have two major implications with regard to the issue of glacial refugia and the relictual status of the Haida Gwaii endemics: 1) With the possible exception of *haidarum*, the suite of morphological features characterizing the endemics *carlottae*, *nesophila*, *dawsoni* and *brooksi* appear to have been derived postglacially. In fact close genetic affinity of these endemic subspecies with adjacent conspecifics suggest that population fragmentation caused by glaciers has had little effect on morphological differentiation and that adaptation to local ecological environments has played a more influential role in their evolution. 2) Emerging data of a mid-Pleistocene split of many vertebrate taxa and the geographic distribution of these various genetic lineages, including the black bear, marten and short-tailed weasel in this region cumulatively suggests that a refugium existed on the continental shelf off the central coast of British Columbia and was possibly part of a larger (or series of refugia) refugium which extended further north and south along this coast. Given the broad assemblage of taxa which might have persisted here during the last glaciation, this refugium was probably ecologically productive and as such, was likely to have been an important alternate source area for the postglacial recolonization of northwestern North America.

Keywords: Haida Gwaii, Queen Charlotte Islands, Pacific Northwest, refugia, Wisconsin glaciation, Fraser glaciation, Cordilleran Ice Sheet, black bear, marten, short-tailed weasel, caribou, Dawson caribou, Saw-whet Owl, endemism, mtDNA, cytochrome b, phylogeography, postglacial dispersal routes, biogeography

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## Dedication

For
my parents,
Jae, Sean,
and Sheldon,
Who always showed up when you were needed most.

## Frontispiece

The sun and earth describe orbital changes
which drive climate cycles and modify ranges.
The shape of the land forms a number of places
which allow the survival of different races.
When enclaves advance with the ice in retreat
some form hybrid zones where the two ranges meet.
Such regions are common and not very wide
so the mixing of genes affects neither side.
They divide up the range in a patchwork of pieces
with echoes and glimpses on the nature of species.
A brief rendez-vous and the ice comes again.

When the glaciers melt so that ranges expand
some plants will spread quickly where there's suitable land.
Those insects which eat them will follow this lead
some flying, some walking to establish their breed.
Those that try later meet a resident band
they must somehow be better to make their own stand.
But the mixture will change as more types arrive
and warming conditions allow more species to thrive.
Some will move on to fresh places ahead
those that remain must adapt or are dead.
And then the tide turns and the ice comes again.

Each refuge could foster a deviant form
  new neighbours, chance changes and drift from the norm.
When the warm breakout comes, those few in the van
  disperse from the edge and breed where they can.
Pioneer pockets grow to large populations
a very good place to strike new variations.
Some may not work well with their parental kind
  so stopping the spread of those from behind.
Continental theatres provide plenty of chances
  to establish new morphs in both retreats and advances.
New species may form when the ice comes again.

G. M. Hewitt, 1993

# Chapter One

# Introduction

Islands have always been of central importance to the development of evolutionary and ecological ideas. From the development of natural selection by Darwin and Wallace to uncovering the fundamentals of biological diversity, islands, by virtue of their unique selective regimes and impoverished flora and fauna, have allowed insight into processes that are obscured in more complex continental systems.

Species diversity on islands is fundamentally a consequence of age, climate, richness of adjacent sources, geographical distance from other land masses and absolute size (Carlquist 1974; Cox and Moore 1985). The combination of these factors often results in a peculiar assemblage of organisms such that islands typically exhibit disharmony and biotic impoverishment (Cox and Moore 1985). Biotic impoverishment usually results in a reduction in predation as well as competition, thereby relaxing selective constraints and permitting niche expansion and development of unusual morphological characteristics. Such adaptive radiation and endemism is exemplified by the diversity of finches (*Geospiza*) on the Galapagos Islands, the nearly 400 species of Drosphilidae on the Hawaiian Archipelago, and the evolution of four endemic genera of cyprinid fish in Lake Lanao (Philippines) over the last 10,000 years (Myers 1960).

Endemic organisms are a common attribute of island flora and fauna. The extent of endemism, which can occur at all taxonomic levels, varies as a function of geographical distance from continents, duration of isolation as well as distinctiveness of selective regimes. However, endemism on north temperate islands is further affected by

the extirpation of large terrestrial habitats which occurred during the latest Pleistocene (Wisconsin) glaciation. As disruption by glaciers prevented long term continuity of populations and reduced opportunities for endemism, the presence of endemics in northern archipelagos is often assumed to be indirect evidence of long habitat continuity and as such, glacial refugia.

One of the largest and most remote of north temperate archipelagos is Haida Gwaii (previously the Queen Charlotte Islands) found 60 kilometres off the western coast of Canada (Fig 1a and Fig 1b). As a consequence of its relatively remote location, Haida Gwaii has a predictably impoverished but unique biota. The assemblage of endemic taxa from these northern islands which include most resident mammals (Foster 1965), birds (Foster 1965), fish (Moodie and Reimchen 1976a, b), beetles (Kavanaugh 1992), angiosperms (Calder and Taylor 1968), and bryophytes (Schofield 1984, 1989), have cumulatively provided support for a glacial refugium on this archipelago (summary in Scudder and Gessler 1989), despite overwhelming physical evidence that it was extensively glaciated during the Wisconsin (Sutherland and Nasmith 1962).

Over the past fifty years, there has been tremendous interest in resolving this controversy, not only to understand the evolution of the Haida Gwaii endemics but also to assess the importance of this putative refugial source area in the recolonization of northwestern North America. It is the intention of this research to clarify the status of Haida Gwaii as a glacial refugium and also the relictual status of some of its endemics.

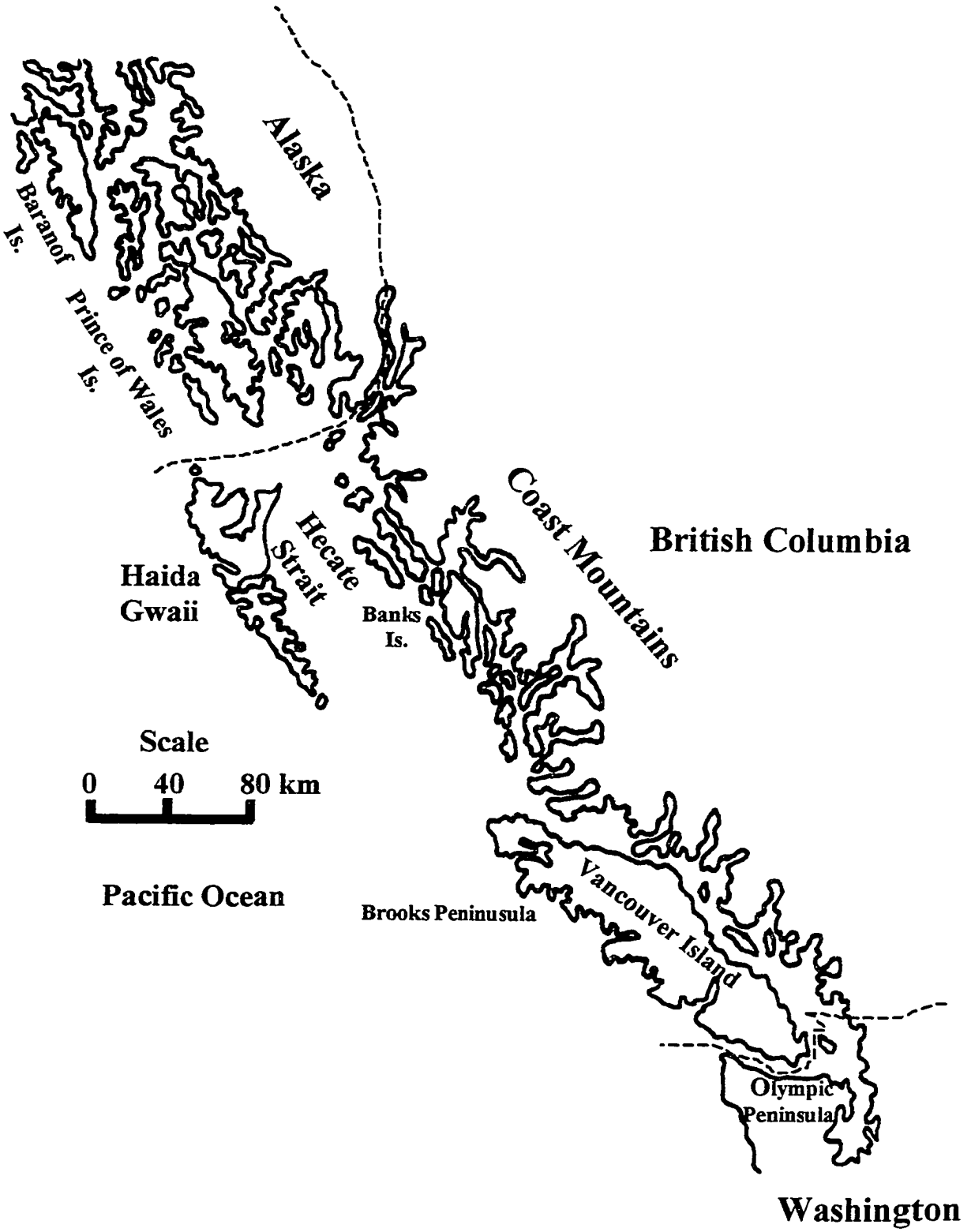



**Figure 1a. Map of coastal British Columbia and southern Alaska.** Dotted lines (- - - ) indicate the border between Canada and the U.S.A..

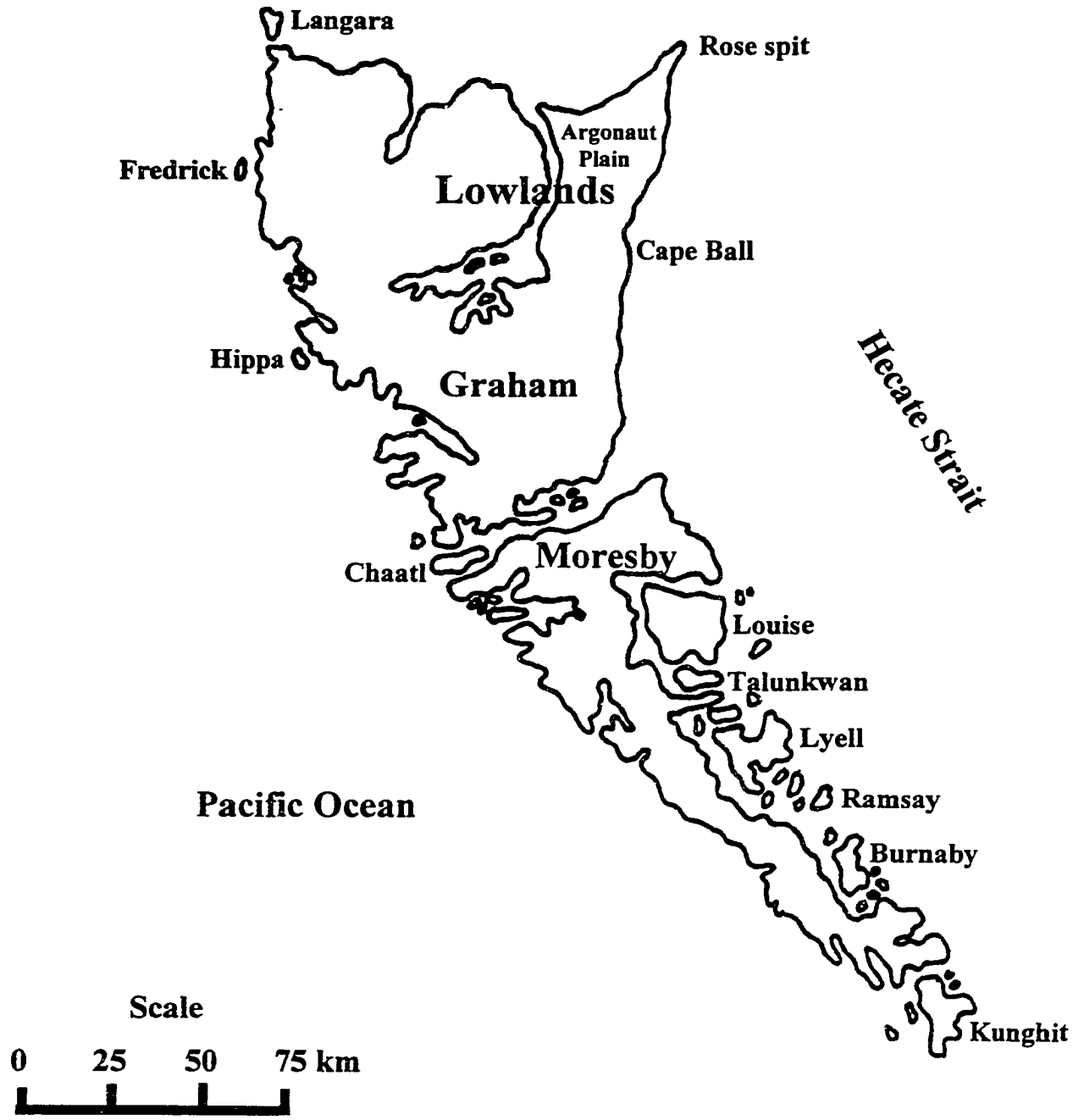



**Figure 1b**

Haida Gwaii consists of approximately 150 islands lying on the edge of the continental shelf. The landscape is extremely diverse, ranging from mountains, broad sandy beaches, to muskeg lowlands on northern Graham Island. The Queen Charlotte Ranges, which form a divide along the western edge of Moresby and Graham Island, rise over 900 metres, eventually falling to just over 700 metres to form the Skidegate Plateau immediately west of the Lowlands

## Glacial History of North America

Population fragmentation by Pleistocene glaciers had an immense impact on the evolution of northern North American taxa (Haffer 1969). Isolation in various refugia during these glacial advances resulted in genetic divergence as well as changes in morphology, behavior and ecological requirements as species adapted to new habitats and climates (Hewitt 1996). These changes occurred recently enough that many taxa affected by these multiple global glacial advances still reflect the ranges contractions and subsequent expansions experienced throughout this glacial age. Therefore, understanding the glacial history of a region is important for interpreting the current distributions of flora and fauna and uncovering the reasons for their genetic and morphological differences. The following is a general review of the environmental circumstances in the Pacific Northwest during the time of the most recent glacial advance and retreat, the Wisconsin.

### *Wisconsin Glaciation*

Our knowledge and understanding is most complete for the last glacial cycle and the transition from these ice age conditions to our present interglacial, the Holocene. The last Wisconsin glaciation (Dawson 1992), which in the Pacific Northwest is known as the Fraser Glaciation, lasted from 35,000 to 10,000 years BP (before present). During this time, North America was covered by two major ice masses (Fig 2). The Laurentide ice sheet, the largest of all Quaternary ice sheets, covered more than 16 million $km^2$ of eastern North America at its glacial maximum 18,000 years BP. The western part of North America was covered by a glacier complex known as the Cordilleran Ice Sheet

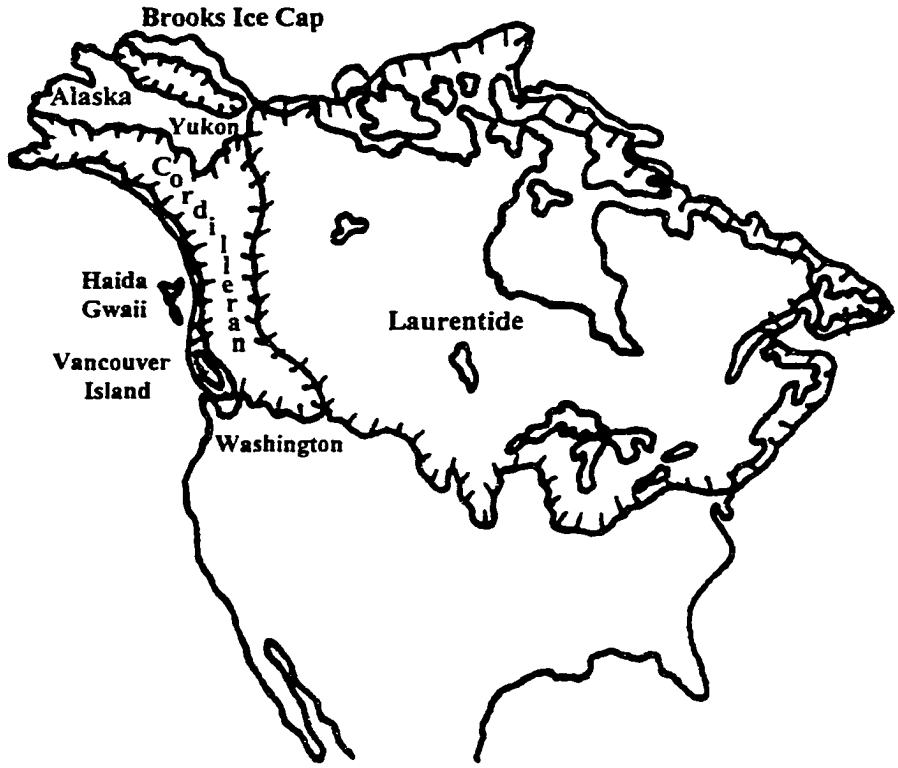



**Figure 2**
Extent of ice cover in Canada at 18,000 years BP. Haida Gwaii was not overrun by the Cordilleran ice sheet but was covered by local glaciers which developed in the Queen Charlotte Ranges (adapted from Matsch 1976)

which originated in the Coastal Range, the Cascades and the Rocky mountains (Dawson 1992) (Fig 3 a, b, c). This complex of valley glaciers (Clague 1989) extended eastward to meet the Laurentide ice sheet, covering an area of over two million $km^2$ (Matsch 1976). At its maximum 15,000 years BP (Pielou 1992), the Cordilleran ice sheet ranged from 1800 to 2100 metres thick (Heusser 1989) and inundated most of British Columbia, southern Yukon Territory, southern Alaska, and northwestern United States. Expansion of the Cordilleran sheet east of the Rockies was presumably limited because this area was effectively sheltered from the precipitation-bearing westerlies.

The maximal southern extension of the Cordilleran ice sheet occurred about 14,000 to 14,500 years ago during the Vashon stade of the last major glacial advance in British Columbia (Hicock and Armstrong 1985). Coastal mountain glaciers coalesced with glaciers from Vancouver Island to form piedmont glaciers which covered the Puget Lowlands and flowed west into Juan de Fuca Strait. At the southern margin of this ice sheet, topographic restrictions resulted in multiple ice lobes, the major ones being the Juan de Fuca, Puget and Okanogan lobes (Easterbrook 1992). These ice lobes caused periodic damming of major river valleys, creating enormous lakes around the southern edge of the ice sheet (Matsch 1976; Ryder et al. 1991).

### *Glaciation of Haida Gwaii*

According to core data and stratigraphic evidence from Quaternary exposures from Hecate Strait and Dixon Entrance, piedmont glaciers of the Cordilleran Ice Sheet reached the northeastern shores of Haida Gwaii between 21,000 to 23,000 years BP.

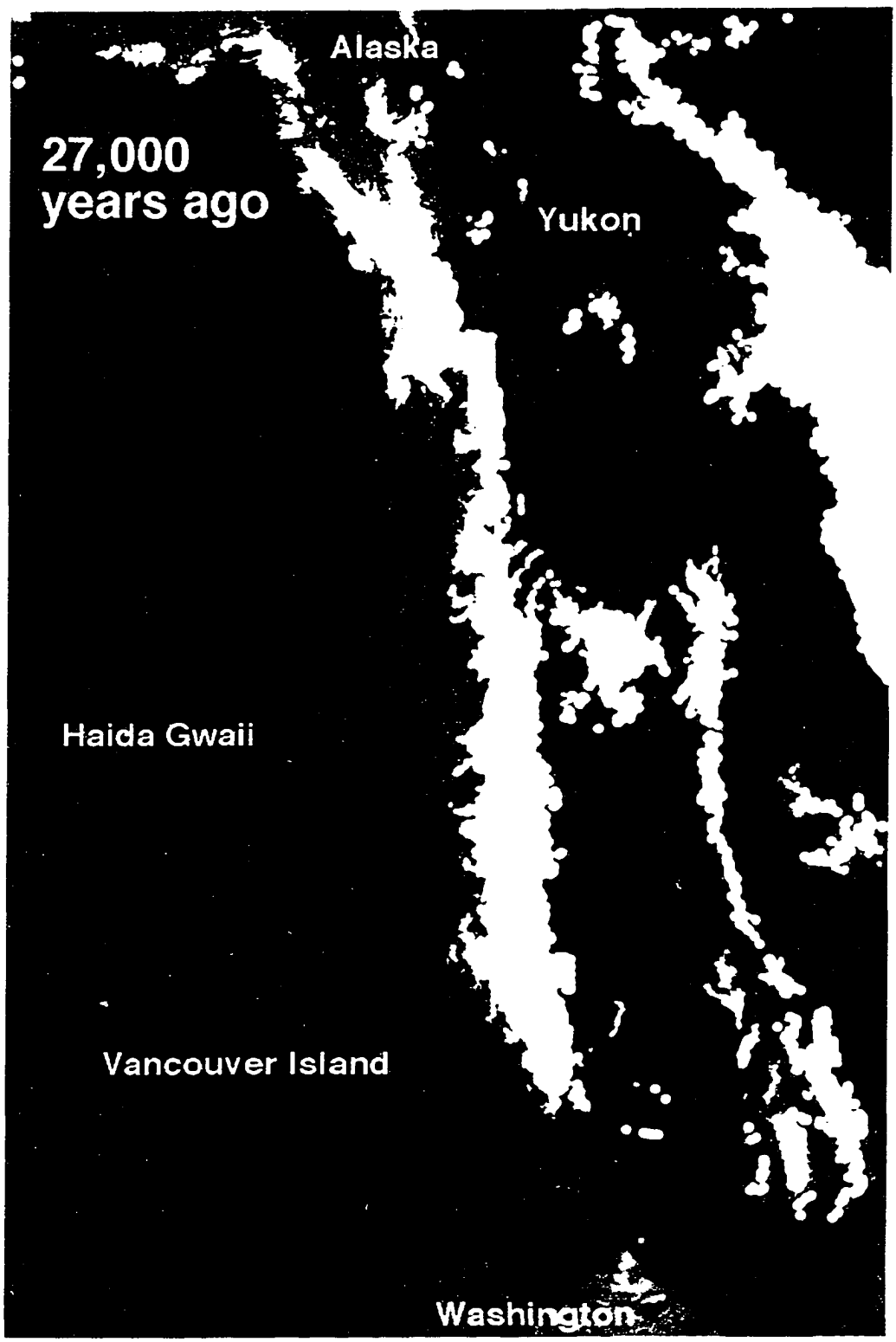

Alaska
27,000
years ago
Yukon
Haida Gwaii
Vancouver Island
Washington

fig 3a


fig 3b

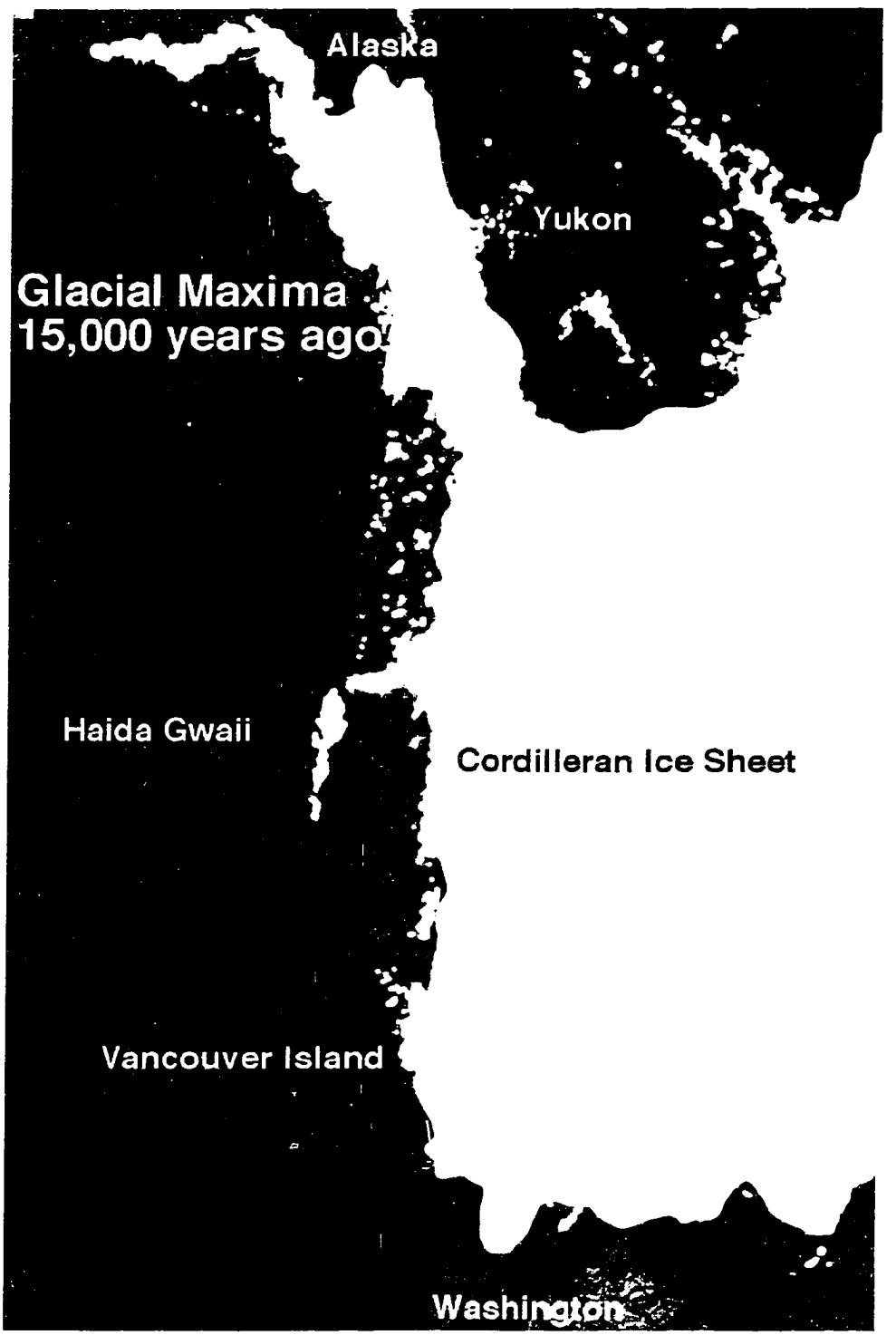

Alaska
Yukon
Glacial Maxima
15,000 years ago
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washington

fig 3c

**Figures 3 a, b, c Glaciation of the Pacific Northwest.**
The three figures depict the overall advancement of the Cordilleran ice sheet based on current literature compiled by the author. About 27,000 years ago, glaciers began growing in the Coastal Range, the Cascades, and Rocky Mountains. Coalescence of valley and piedmont glaciers resulted in the Cordilleran glacier complex which reached its maximum about 15,000 years BP. The formation of glaciers had enormous effects on sea levels. During the height of the Wisconsin, portions of the continental shelf, outlined in white, were above sea level.

Although this continental ice sheet did not override this archipelago, Haida Gwaii was nonetheless heavily inundated by an independent complex of valley and piedmont glaciers (Sutherland Brown and Nasmith 1962) which reached their maxima about 1000 years earlier than on the mainland (Blaise et al. 1990). Striations, flutings and cirques oriented in every direction suggest that Haida Gwaii was covered by local ice which originated in the Queen Charlotte Ranges and formed an ice cap over 900 metres thick. Bold topographic features such as U-shaped valleys, striated and polished bedrock, roches moutonnées and erractics are evidence of an intensive period of glaciation which extended from Kunghit to Langara Island (Sutherland Brown and Nasmith 1962; Clague 1989). Because of the small size of mountain source areas, the proximity of deep water, and the sharp land decline from west to east, glaciers on Haida Gwaii were not as extensive as those on the adjacent mainland. These topographic conditions restricted thickening and lateral spreading of the glaciers and allowed some mountain peaks to remain unglaciated as they were too high to be overrun by ice. These unglaciated peaks, otherwise known as nunataks, were probably subject to severe weather conditions like frequent gales and snowslides and were not likely to have supported productive ecosystems at this time.

***Refugia***

Although the Cordilleran ice sheet was quite extensive, several regions along the North Pacific coast from Kodiak Island to the Olympic Peninsula were free of ice (Heusser 1989; Hebda and Haggerty 1997). Areas not covered by ice are referred to as

glacial refugia and are extremely important because these areas supported the source populations which eventually recolonized North America following deglaciation.

During the last glacial maximum, there were two well established refugia on the nearby mainland (Fig 2). North of the Cordilleran Ice Sheet, a large ice-free area otherwise known as eastern Beringia existed in the interior of Alaska and the Yukon. Pollen sequences taken from Isabella Basin, Birch Lake, and Antifreeze Pond suggest that this refugium consisted largely of herb tundra during the height of the last Wisconsin advance (Matthews 1974; Rampton 1971), and that the climatic conditions were colder and drier than they are at present (Hare and Hay 1974). The area south of the ice margin on the western coast was dominated by subalpine parkland, characterized by spruce, mountain hemlock, grasses and various herbs. This was eventually replaced by tundra or parkland during the height of glaciation. This area, known as the Washington refugium was a major centre of postglacial dispersal and probably had a greater influence in recolonizing coastal and south/central BC than the Alaska/Yukon centre (Heusser 1989).

Although coastal refugia are presumed to have existed, these were generally nunataks or small coastal areas which became free of ice through dynamic fluctuations along the ice margins. However, these are currently believed to be to have had little impact on species diversity in the Pacific Northwest. Populations in British Columbia are presently assumed to be principally derived from the two mainland refugia found north and south of the Cordilleran ice sheet.

*Deglaciation*

The Cordilleran ice sheet began retreating about 15,000 years BP (Fig 3 d, e, f) (Matsch 1979). However, glacial retreat was not simply a reverse of glacial expansion. While coastal glaciers retreated rapidly because of calving, in the interior deglaciation was dominated by thinning rather than actual glacier retreat (Fulton 1967; Ryder et al. 1991). As a consequence, the Cordilleran ice sheet broke up into several remnant masses, which stagnated and eventually shrank in the valleys and lowlands (Fulton 1991).

Glacial retreat on Haida Gwaii occurred much earlier than it did on the mainland. Late glacial grass/herb pollen remains from the northeastern coast (Cape Ball) radiocarbon dated to be about 15,400 ± 190 to 16,000 ± 570 years old indicate that there was tundra-like vegetation on Haida Gwaii about this time (Warner et al. 1982).

At the end of the Fraser glaciation, valleys in the southern interior of British Columbia became inundated by a complex of recessional lakes (Fulton 1969). In mountainous areas, tributary valleys filled with glacial meltwater while trunk valleys were still occupied by ice (Clague 1975). Although minor readvances like the Sumas in the Fraser Lowland about 11,500 years BP (Saunders et al. 1987) occurred throughout the recession, overall retreat of the Cordilleran Ice Sheet was relatively rapid and continuous.

Although deglaciation had begun, northward migration from southern refugia was seriously impeded as the Puget Lowland quickly flooded with marine water from 11,500 to 13,500 years BP as a consequence of isostatic depression (Easterbrook 1992). Enormous proglacial lakes, the largest of which were Glacial Lake Missoula and Glacial Lake Columbia, formed about 15,000 years BP immediately south of the Cordilleran

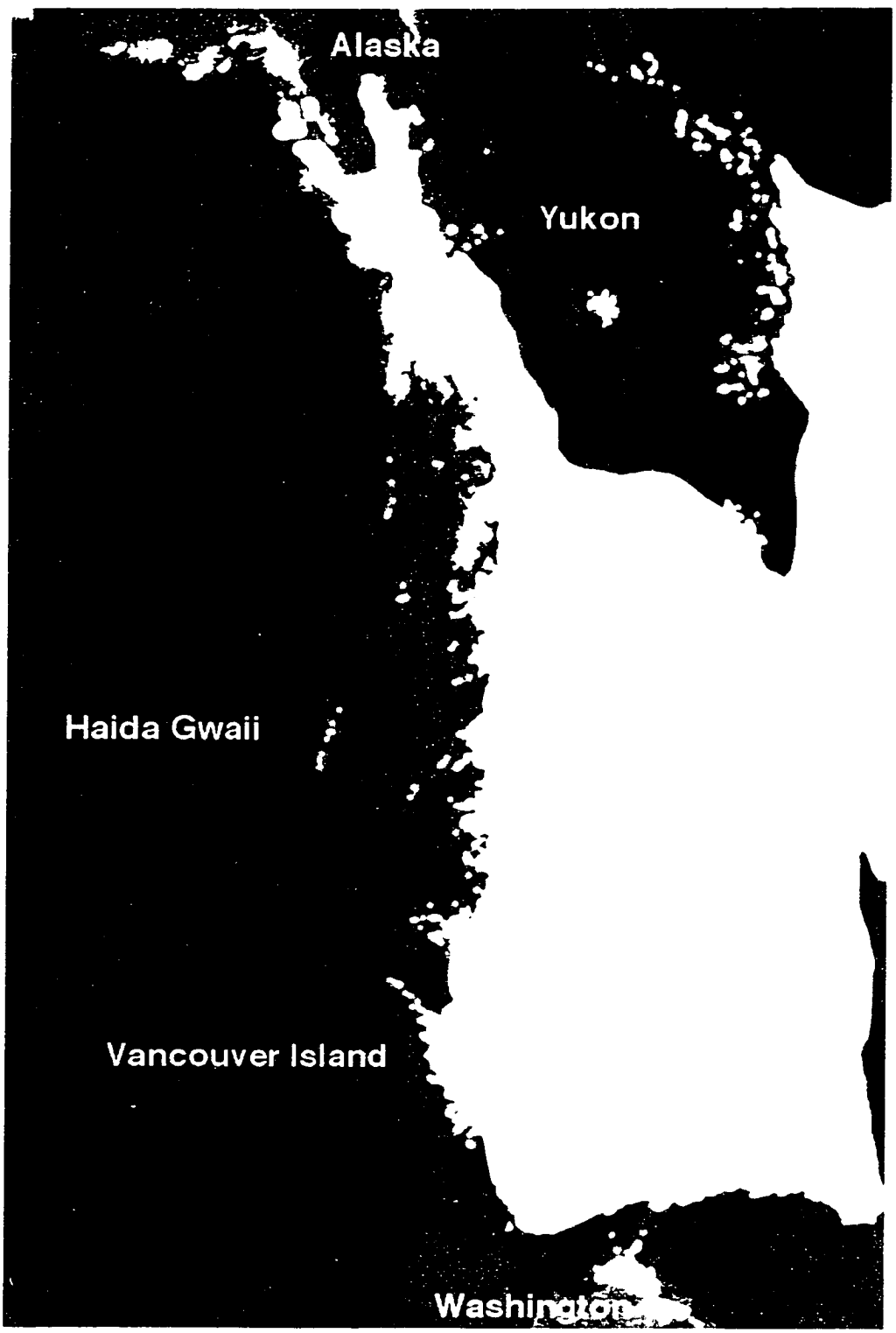



fig 3d

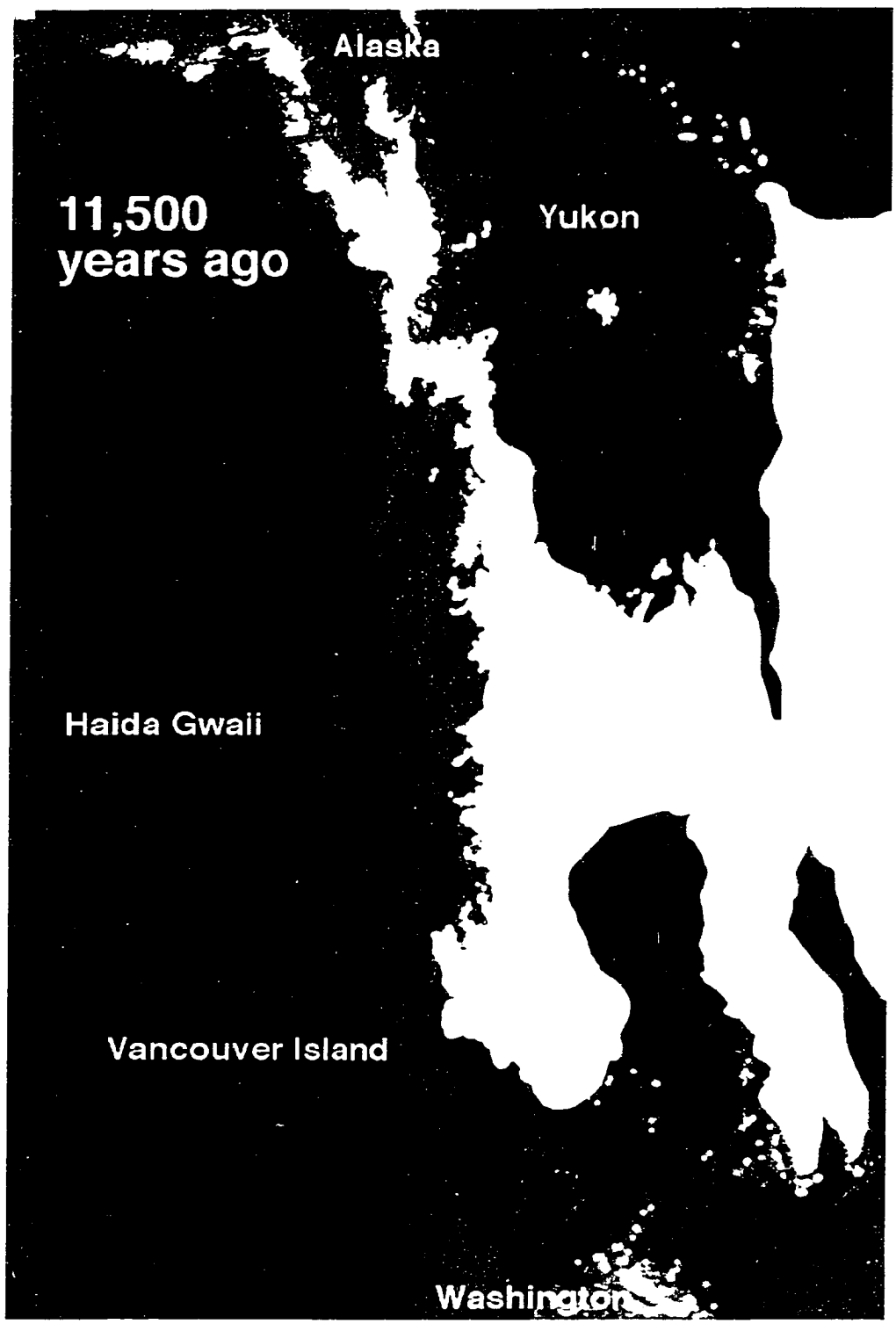

Alaska
11,500
years ago
Yukon
Haida Gwaii
Vancouver Island
Washington

fig 3e

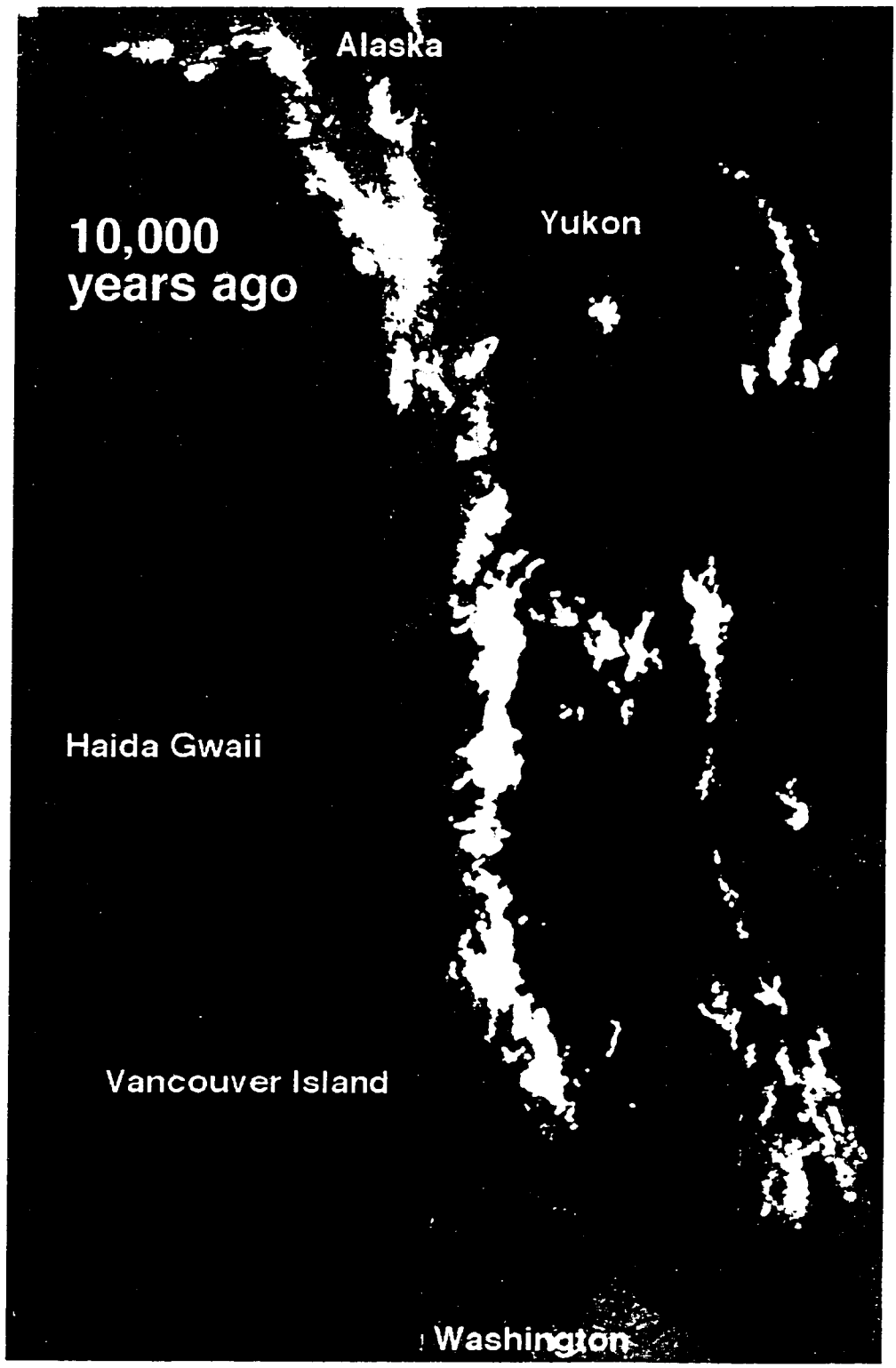

Alaska
10,000
years ago
Yukon
Haida Gwaii
Vancouver Island
Washington

fig 3f

**Figure 3 d, e, f Deglaciation of the Pacific Northwest.**
The three figures depict the overall retreat of the Cordilleran ice sheet. Glaciers began retreating soon after the glacial maximum. The Cordilleran ice sheet retreated rapidly as a consequence of downwasting. Combinations of eustatic and isostatic pressures caused significant changes in sea level. At the ice margins, large recessional lakes formed which were the source of catastrophic floods which occurred intermittently for several thousands of years. By 10,000 years BP, the glacier had fully retreated and dispersal from adjacent refugia was already underway.

Ice Sheet. These lakes repeatedly emptied and filled over 40 times. The catastrophic floods which accompanied these cycles were so powerful that they created the channeled scablands of eastern Washington (Pielou 1992). Other major eustatic (transfer of water between ocean and ice) and isostatic (loading and unloading of the crust by glaciers) changes were occurring around the coast at this time. During the glacial period, the region was subjected to severe fluctuations in sea level. These eustatic changes exposed areas around the continental shelf creating land bridges like the one across the Bering Strait. Presence of submerged valleys and cliffs up to 42 metres below sea level suggests that lowlands once extended onto other parts of the continental shelf, including the western Hecate Strait and South Dixon Entrance about 11,000 years BP (Clague 1989). With the retreat of the glaciers, these areas were subsequently submerged. Relict shorelines on Haida Gwaii, as shown by wave cut scarps and forminiferous marine deposits, reveal that sea level was about 15 metres above present levels about 8000 years BP. Shorelines reached their present position about 2000 years BP (Clague 1989).

## Haida Gwaii as a Glacial Refugium

Because of widespread physical evidence that Haida Gwaii was extensively glaciated during the Wisconsin, it is presumed that significant biological refugia were absent on these islands. However, over the past fifty years indirect biological evidence has been accumulating suggesting that Haida Gwaii may have in fact supported a variety of interglacial plant and animal populations from which part of the North Pacific flora and fauna was derived. The following sections briefly review this evidence, providing the rationale and context in which this thesis was originally conceived.

***Endemic and Disjunct Plants***

*Bryophytes*

One of the earliest indications for glacial refugia on Haida Gwaii is the unusually high proportion of endemic and disjunct bryophytes found on this archipelago. One of the first reports of phytogeographically interesting bryophytes was the description a new moss species *Hypopterygium canadense* (Kindberg 1899). This species was later discovered in Alaska, coastal British Columbia (Schofield 1989) and southeastern Asia (Schofield 1965) and was the first of many disjunct bryophytes later identified. There are currently eighteen disjunct hepatics and twelve disjunct mosses known in the Western Hemisphere only on Haida Gwaii, the Pacific Coast of British Columbia and adjacent Alaska. In addition to this, there are seven disjunct bryophytes (*Dendrobazzania griffithiana*, *Radula auriculata, Daltonia splachnoides*, *Dicranodontium subporodictyon*, *Leptodontium recurvifolium*, *Sphagnum junghuhnianum*, and *Zygodon gracilis*) found only in North America on Haida Gwaii.

Strong affinity of many of these disjunct bryophytes with bryophytes found in western Europe or southeastern Asia is suggestive that they may be relicts of ancient flora, possibly dating back to the Tertiary. Persistence of suspected Tertiary relicts on Haida Gwaii despite extensive glacial cover during the Pleistocene was suggested by Schofield (1984) to be evidence that suitable habitat continued to exist during multiple glacial advances in the Pacific Northwest. Although it is possible that the present distribution is due to recent dispersal events from refugia south or north of the Cordilleran ice sheet, it is considered unlikely for two reasons: 1) Asexual reproduction is

relatively insignificant in these disjuncts and as such, they are limited to local populations. All of the endemic and disjunct bryophytes on Haida Gwaii lack readily dispersable diaspores and are unlikely to have come from the Washington refugia over 600 miles away. Foster (1965) suggested that because pollen profiles from Langara Island (the most northern island of Haida Gwaii) are much older than putative source areas in Prince Rupert and Ketchikan, these diaspores were unlikely to have colonized from even further north in the Alaska/ Yukon refugium 2) Many of these disjuncts are intimate components of closed forest communities on Haida Gwaii. Schofield (1989) claimed that these plant species were unlikely to be recent immigrants to Haida Gwaii since new arrivals typically occupy disturbed sites.

There are five endemic bryophytes (*Ctenidium schofieldii, Seligeria careyana*, *Sphagnum schofieldii*, *Sphagnum wilfii*, and *Wijkia carlottae*) known to Haida Gwaii. Like most of the disjuncts, the endemics are terrestrial or epilithic and generally found in well established communities along cliff ledges or bases. They are found at sea level and in the sub-alpine, demonstrating the potential ecological tolerance of these species which would allow them to survive in treeless refugia. These bryophytes were probably able to survive quite well in these areas because as inhabitants of microenvironments, they tend to be less affected by macroenvironmental changes. As such, they are valuable indicators of refugia (Schofield 1989). However, the occurrence of these disjunct bryophytes is not indisputable evidence of large, ecologically rich refugia as survival in nunataks and postglacial spore dispersal from Asia via ocean currents could also account for these distributions.

*Vascular Plants*

Of the 611 known taxa of vascular plants on Haida Gwaii, thirteen were originally identified as endemic. Although further explorations have located nine of these species (*Calamagrostis purpurascens tasuensis*, *Cassiope lycopodioides cristapilosa*, *Geum schofieldii*, *Isopyrum savilei*, *Ligusticum calderi*, *Lloydia serotina flava*, *Saxifraga taylori*, *Senecio moresbiensis*, and *Viola biflora carlottae*) in the Brooks Peninsula on Vancouver Island, *Senecio newcombei* (now referred to as *Sinisenecio newcombei* see Janovec and Barkley 1996) is one species which still remains endemic to Haida Gwaii (Ogilvie 1989; Taylor 1989; Ogilvie 1997). Based on the concentration of endemic and disjunct vascular plant taxa (Ogilvie and Roemer 1984), Calder and Taylor (1968) proposed that Haida Gwaii likely provided refugia for these species and that these refugia were likely to have been found on the west coast, mountain summits, as well as ridges and chasm walls.

Although the occurrence of endemic and disjunct plant taxa from Haida Gwaii is suggestive that refugia did exist on this archipelago during the late Wisconsin, the evidence is ambiguous. With more extensive botanical surveys, previous claims of endemism have been refuted (see above) and it is possible that current distributions of many of these disjunct plants, especially the vascular plants, are to random postglacial dispersal. The occurrence of many of these taxa in well established communities does suggest that their arrival is not recent (Schoefield 1989). However, it is conceivable that these taxa arrived during the early postglacial period migrating up the coast as these regions gradually became deglaciated. Isolation for 12,000 years could have been enough time for substantial differentiation and floral development. Furthermore, even if

a refugium that could have supported these bryophytes and vascular plants existed throughout the late Wisconsin, the extreme environments in which they could have persisted were not likely to have supported a wide diversity of taxa. As such, this refugium probably would have had a limited influence on the faunal diversity currently observed on this archipelago and the rest of the Pacific Northwest.

***Paleobotany***

Particularly compelling evidence for early deglaication on Haida Gwaii comes from late glacial grass/herb pollen remains from Cape Ball (Fig 1b) (Warner et al. 1982). The upper surface of glacial sediments contain plant material radiocarbon dated to be about 15,400 ± 190 to 16,000 ± 570 years old, providing a minimum age for deglaciation around the northeastern coast. The vegetation found at Cape Ball 16,000 years BP was typically tundra-like. Over 50 % of the total pollen count are from grasses. However, pollen from sedge, sage, *Asteraceae*, and Ericales have also been found, along with seeds and pollen from Caryophyllaceae, dock, rushes, and pondweeds. Up to 20 % of the pollen is arboreal. The most abundant type of arboreal pollen is spruce (Warner et al. 1982; Mathewes 1989), represented by an influx of 40 spruce grains/cm$^2$/year. The source of this pollen is problematic as no source areas are known to have existed on adjacent coastal regions. Mathewes (1989) suggested that although these pollen grains were likely reworked from older sediments or melting ice, there remains the possibility that they originated from stunted, infrequently pollinating trees. The existence of such trees would be strong evidence for the existence of a refugium near or on Haida Gwaii.

The sudden and great abundance of plant varieties found at Cape Ball contrasts with the gradual increase that would be expected as a consequence of migration and succession of newly deglaciated surfaces. This points to well-established plant communities somewhere in the area. Because surrounding areas were simultaneously experiencing a glacial maximum, it was suggested that a nearby coastal refugium might have served as a source for plant dispersal.

Although the data from Cape Ball are compelling, the sudden abundance of plant taxa could alternatively be attributed to rapid range expansions. Such rapid expansions have been reported for Norway spruce (*Picea abies*) across northern Europe during the Holocene (Bradshaw and Zackrisson 1990). Rapid migration over great dispersal barriers has been documented (Woods and Davis 1989; Kullman 1996) and has been theorized to have been accomplished by long distance jumps (Clark et al. 1998). Such long jump dispersal would produce outlier populations which would be too sparse to be detected in pollen records. Once conditions became more favorable, as in the case of newly deglaciated surfaces, these outliers would become the source areas for rapid invasion (Pitelka et al. 1997).

Despite the wealth of information which has been gathered from examining Quaternary exposures and cores, little is published about the plant life on Haida Gwaii between 21,000 and 16,000 years BP. This hiatus in the fossil record, which coincides with the Wisconsin glacial maximum, (Mathewes 1989) makes it difficult to state with confidence that plants or animals inhabited these islands during the most intense periods of the late Wisconsin.

### *Endemic Fauna*

Haida Gwaii's fauna was first documented by Osgood in 1901 and since then has been the subject of much study. Despite having a typically impoverished fauna, it has an exceptional assemblage of endemic taxa, ranging from mammals, birds, fish, and invertebrates (Appendix 1). Extensive glaciation during the Wisconsin led to the assumption that Haida Gwaii's fauna was postglacially derived from either northern or southern mainland refugia about 12,000 years BP. As the morphological traits characterizing Haida Gwaii's endemic fauna were recognized as typical adaptations of insular populations, they were assumed to be the result of rapid postglacial differentiation caused by these selective regimes (Foster 1969).

#### *Invertebrates*

Three endemic species of carabid beetles are known to occur on Haida Gwaii: *Nebria carlottae*, *N. louisae*, and *N. haida* (Kavanaugh 1989). These beetles occur in upper sea beach and alpine habitats, and were believed to be relictual organisms. However, a recent mtDNA and morphometric analysis suggests that these three endemics are not relictual at all but in fact, are likely to be products of rapid postglacial radiation (Clarke 1998). Morphological measurements of body length and pronotal shape led Clarke (1998) to suggest that *carlottae*, *lousiae* and *haida* were part of a morphological continuum which include the species *N. lituyea* and *N. gregaria* which are found in the Alaskan Panhandle and Aleutian Islands. Although this study provided insight into carabid morphology and phylogeny, it did not directly address the issue of whether significant biological refugia existed on the coast. These endemic carabids occur in areas

which are already known to have been free of ice such as nunataks and small beach areas. These small areas were unlikely to have supported a diversity of organisms and as such were not likely to have been major source areas for postglacial recolonization.

*Avifauna*

Considering the potential for gene flow, it is surprising that there are any endemic birds on Haida Gwaii, especially if they are postglacial migrants. There are four endemic land birds: Saw-whet Owl (*Aegolius acadicus brooksi*), Hairy Woodpecker (*Picoides villosus picoideus*), Steller's Jay (*Cyanocitta stelleri carlottae*), and Pine Grosbeak (*Pinicola enucleator carlottae*) known to this archipelago. Their differences from nearby conspecifics tend to include darker plumage, longer tarsi and variable beak size (Foster 1965; Cowan 1989). These variations are typical of insular birds (Murphy 1938) and may also be the result of selection-mediated trends dictated by ecogeographic rules (Foster 1965).

Interestingly, all of the endemic birds are non-migratory. Although it is possible, especially for birds that travel in flocks like the Pine Grosbeak and Steller's Jay, to have been blown to Haida Gwaii during fierce storms, this is unlikely to be the case for solitary birds like the Saw-whet Owl and Hairy Woodpecker (Cowan 1989).

Only about 58% of the potentially available bird species from the mainland have successfully migrated and established themselves on Haida Gwaii. Cowan considered the absence of the Gray Jay, Mountain Chickadee, and ptarmigans, species typically associated with alpine and sub-alpine habitats, quite curious, especially if such habitats existed on Haida Gwaii during the Wisconsin in the form nunataks or other coastal

mountain refugia. However, as such habitats are not widely available on Haida Gwaii today, their absence is not particularly remarkable nor insightful.

*Land Mammals*

Osgood (1901) described 11 species of indigenous land mammals: dusky shrew (*Sorex monticolus elassodon* and *S. m. prevostensis*), deer mice *(Peromyscus maniculatus keeni* and *P. sitkensis prevostensis*), bats (*Myotis californicus caurinus*, *M. keeni, keeni*, *M. lucufugus alascensis*, and *Lasionycteris noctivagans*), river otter *(Lutra canadensis periclyzomae*), marten (*Martes americana nesophilia*), short-tailed weasel (*Mustela erminea haidarum*), and black bear (*Ursus americanus carlottae*). Though not included on Osgood's list of indigenous mammals, Haida Gwaii also possessed a unique subspecies of caribou, *Rangifer tarandus dawsoni*, which apparently went extinct in the early 1900's (Cowan and Guiguet 1956; Banfield 1961).

In addition to the curious morphology of Haida Gwaii's indigenous mammals, Osgood (1901) also noted the odd absence of deer (*Odocoileus*), squirrels (*Sciurus*) and voles (*Microtus*). As these absent genera were common on the adjacent mainland and well adapted to conditions on Haida Gwaii, Osgood attributed their absence to the effectiveness of Hecate Strait as a barrier to dispersal. Not only did Osgood consider the strait too wide to swim, but also suggested that the strait's lengthwise currents would probably sweep small animals carried on driftwood either north or south of the archipelago instead of across to it. Osgood concluded that the degree of insular differentiation exhibited by the mammals of Haida Gwaii, especially larger bodied ones like *Ursus* and *Martes* was the strongest evidence of isolation. In addition to the extent

of differentiation of Haida Gwaii's endemic fauna with the mainland, Osgood made exceptional note of the morphological differences between *Peromyscus* and *Sorex* of Prevost (now Kunghit) Island and the rest of the archipelago.

Further investigation into the derivation of these various insular forms led Cowan (1935) and McCabe and Cowan (1945) to speculate that *Peromyscus sitkensis prevostensis* (previously *P. prevostensis*) of Kunghit Island was the original inhabitant of Haida Gwaii and unable to compete with the more recent arrival *P. maniculatus keeni* which eventually restricted the former species' range. Based upon this supposition, Cowan (1935) and McCabe and Cowan (1945) advanced the controversial theory that *P. s. provestensis* was a relict of a former interglacial population which persisted to the present time in small ice free areas on Haida Gwaii during the Wisconsin glaciation.

As part of a wider study of Haida Gwaii's endemic fauna, Foster (1965) analyzed a total of 515 specimens of *P. sitkensis prevostensis* and *P. m. keeni* from 29 islands and concluded that the differences in size and proportion between the two species were clinal and that *P. s. prevostensis* and *P.m. keeni* were actually conspecifics. Observations by Foster (1965) that *Peromyscus* collected from Kunghit Island in 1900 and later in the 1960's had significantly different body proportions attests to the highly plastic nature of their morphology and the uncertainty of using these characters to elucidate their true relationships.

Although Foster did not regard *P. s. prevostensis* as a refugial relict, he did conclude that the dusky shrew, *S. m. elassodon*, was likely to have existed on Haida Gwaii since pre-glacial times. The other subspecies of dusky shrew found on Haida Gwaii is *S. m. prevostensis*. Morphological differences between these conspecifics were

assessed by Foster (1965) using a total of five to eight measurements including total length, tail length, length of the hind condylobasal length, palate length, maximum skull width and length of tooth row. Based on these measurements, Foster (1965) concluded that *S. m. prevostensis* was significantly larger than *S. m. elassodon* and more similar to the species of dusky shrew (*S. longicauda* and *S. insularis*) on the adjacent mainland. Foster surmised that *S. m. prevostensis* was a post-glacial arrival to Haida Gwaii and a descendent of *S. m. longicauda*. However, the distribution of *elassodon* and *prevostensis* in south-eastern Alaska is curiously discontinuous and not adequately explained by the glacial relict hypothesis put forward by Foster (Cowan 1989).

Morphological analyses of river otters and the four species of indigenous bats from Haida Gwaii, revealed no significant differentiation from conspecifics on the mainland. Foster attributed this to the high mobility and great gene flow potential within these species and did not consider them in any further detail. The remaining four indigenous mammals however, the black bear, marten, short-tailed weasel and Dawson caribou, show remarkable morphological differentiation typically characterized by changes in body size, pelage color and skull size.

Both McCabe and Cowan (1945) and Foster (1965) considered the extent of morphological divergence of Haida Gwaii's endemic mammals too great to be accounted for by rapid postglacial evolution and suggested that at least a proportion of Haida Gwaii's endemic fauna derived their suite of morphological characteristics through long isolation. However, such long isolation requires population continuity and the existence of glacial refugia capable of maintaining stable populations of such high trophic level species. There is currently no evidence of such refugia on the coast.

Although the morphological divergence exhibited by the endemics on Haida Gwaii is considered extreme given their supposedly short history on the islands, it is difficult to say with certainty that such divergence could not have occurred within the last 10,000 years.

For example, threespine stickleback (*Gasterosteus aculeatus*) populations from Haida Gwaii exhibit remarkable morphological differentiation, not only from mainland populations but from lake to lake within the archipelago. Sticklebacks from the northeastern corner of Graham Island have been reported to lack pelvic girdles (Boulton Lake), be large and melanistic (Mayer Lake) or lack lateral scutes (Skonun Lake) (Moodie and Reimchen 1976b). Considering the suite of biological evidence suggesting that parts of Haida Gwaii were ice free during the Wisconsin Glaciation, and the highly divergent sticklebacks found on the islands, it was not unreasonable to speculate a pre-glacial origin for these endemics. However, based upon the absence of an adequate dispersal pattern that could account for the variation among populations and the presence of adaptations suited for selective regimes particular to these various lakes, Moodie and Reimchen (1976a) concluded that the highly derived features characterizing these endemic sticklebacks probably evolved postglacially and rapidly *in situ* in response to varying predation pressure.

Variable rates of morpholgical evolution caused by diverse selective regimes can confound biogeographical analysis. As such, the plasticity of these morphological features often renders them unreliable indicators of a species' biogeographical history. Because much of the evidence for refugia on Haida Gwaii hinges on its morphologically divergent biota, this issue has remained controversial for the last fifty years.

## Using Molecular Markers

Recently, analyses of intraspecific genetic variation has permitted a greater understanding of population processes by minimizing confounding factors such as environmental plasticity, dispersal, and unpredictable rates of evolution (see Avise 1994 for review). By using mitochondrial DNA (mtDNA) as a molecular marker to examine biogeographical questions, many of the inherent limitations present in morphological analyses can be circumvented and may actually provide higher resolution analyses of intraspecific relationships.

### MtDNA: Useful Features for Examining Biogeographical History

In comparison with nuclear DNA, mtDNA evolves at a higher rate (Brown et al. 1979). The average rate of synonymous substitution (substitutions which do not alter the amino acid sequence) is estimated to be $5.7X10^{-8}$ (Brown et al. 1982), about 10 times the synonymous substitution rate in nuclear DNA. Such a high substitution rate makes mtDNA an ideal marker for examining relationships of closely related taxa. The rate of evolution is neither constant nor linear for all parts of the mitochondrial genome over long periods of time. However, over shorter periods (less than 15% overall divergence) the number of substitutions is believed to be an approximately linear function of time (Avise et al. 1987; Moritz et al. 1987). Although such a molecular clock is highly probabilistic and not universal for all genes in all lineages, it is possible to get a crude approximation of divergence time between lineages if properly calibrated using the fossil record (Wilson et al. 1987; Li and Graur 1991).

Intraspecific phylogenies are concerned with geographic population structure. However, at the population level, complex reticulate patterns caused by introgression result in relationships which are often not correctly inferred by cladistic methods. Because of the importance of identifying monophyletic groups in studies of historical biogeography, cladistic methods are often preferred (Wiley 1988; Sober 1988). Unfortunately, populations which experience reticulation are not monophyletic by definition. Davis and Nixon (1992) suggested that in order for cladistic analysis to approximate evolutionary history, two conditions must be fulfilled: 1) hierarchy between the terminals and 2) descendents do not carry a recombined form. MtDNA fulfills both of these conditions; mtDNA haplotypes can be ordered and polarized and mtDNA does not recombine.

Because mtDNA does not recombine, it can be seen as having a discrete origin. The occurrence of a lineage in an area is unambiguous as it must have originated there or dispersed there. MtDNA is maternally inherited (Wolstenholme 1992). The importance of this feature to historical biogeography is that in those species where females tend to disperse less than males (which is the case for most mammals), mtDNA may more closely reflect the original biogeographical distribution by limiting obscuring effects of dispersal.

Recently, the use of mtDNA as an alternative approach for investigating biogeographical questions has permitted reevaluations of a number of controversial hypotheses (see Klein and Brown 1994; Hedges et al. 1992). One of these reevaluations was a reexamination of the issue of stickleback colonization and the historical relationships of these divergent forms on Haida Gwaii. Gach and Reimchen (1987)

analyzed the mtDNA diversity between two morphologically divergent stickleback populations from Boulton Lake and Drizzle Lake based upon restriction fragment length polymorphisms (RFLPs). The discovery of a unique restriction site shared between these two geographically isolated populations implied that these endemics might have diverged from a common ancestor that inhabited periglacial freshwater habitats rather than arising independently from a marine ancestor as was previously assumed. To further investigate this possibility O'Reilly et al. (1993) used mtDNA RFLPs and discovered two lineages of sticklebacks on Haida Gwaii, the adjacent mainland and surrounding marine waters. These were referred to as the marine lineage and Argonaut lineage. The marine lineage consisted of nine haplotypes and was found in marine water, mainland freshwater and freshwater localities on Haida Gwaii. The Argonaut lineage, composed of the remaining two haplotypes, differed from the marine lineage by at least seven site changes. This lineage was so named because of its restriction to freshwater lakes on the northeastern corner of Graham Island known as the Argonaut Plain (Fig 1b). Based upon a rate of mtDNA sequence divergence calibrated for mammals of about 2% per million years, O'Reilly et al. (1993) estimated that these lineages diverged about 1.2 million years ago. Even considering differential rates of mtDNA evolution, the divergence of these lineages still occurred well before the beginning of the Wisconsin. The occurrence of a divergent lineage (Argonaut lineage) that presumably diverged pre-glacially, and managed to persist in a restricted locale near Cape Ball, provided the first molecular evidence of a glacial refugium on Haida Gwaii.

The existence of a refugium large enough to support freshwater fish throughout the Wisconsin implies that other aquatic and terrestrial biota might have also

been able to persist. Vicariant events, like those caused by glacier activity, should be identifiable through comparative searches for congruent patterns in other species (Wiley 1988; Avise 1994). As an extension of the molecular work done by O'Reilly et al. (1993), this study was initiated as a broad genetic survey of the endemic fauna of Haida Gwaii to uncover any such congruency. The study was not designed to provide a comprehensive investigation of any one particular species, but rather through comparison of phylogeographic patterns, to ascertain the affinities of putative relict species and uncover any divergent haplotypes in the endemic populations of these species on Haida Gwaii. The rest of this thesis deals with each of these potential relicts, black bear, marten, short-tailed weasel, Dawson caribou and Saw-whet Owl, and comments on the role of Haida Gwaii as a glacial refugium and postglacial source area based on a synthesis of this phylogeographic information.

# Chapter 2

## Black Bear (*Ursus americanus*)

### Introduction

Of the three bear species found in North America, black bears are the most numerous with an estimated population of 450,000 (Servheen 1990). Their success is largely due to their highly adaptable nature which gives them great latitude in diet and habitat. Black bears are most commonly associated with old growth forests, although they are also found in such diverse habitats as the deserts of Arizona and subtropical forests of Florida and Georgia (Powell et al. 1997). Black bears are also known to occur in alpine meadows, estuaries, inter/subtidal zones and swamps (BC Ministry of Forests

1991) and are only absent from these areas when excluded by humans or brown bears (*Ursus arctos*). Though brown bears are one of the few natural enemies of the black bear, encounters may be rare due to the latter's nocturnal feeding habits and preference for heavily forested areas.

Black bears are highly opportunistic, consuming anything from insects to mammals, fruits, vegetables, grasses, a wide variety of seasonal wild plants and carrion when available (Cowan and Guiget 1956; Banfield 1974). The annual diet of a continental black bear consists of approximately 76.7 per cent vegetable matter, 7.4 percent insects, 15.2 per cent carrion and 0.7 per cent small mammals (Banfield 1974). However, this diet varies greatly from one location to another.

On the coast, black bears consume primarily marine invertebrates, berries, and fish (Cowan and Guiget 1956). Coastal habitats are much more productive than habitats found further inland due to the presence of salmon. The salmon runs during the fall are of utmost importance to the black bear in its preparation for winter denning; during this time salmon can account for more than 50% of the bear's total protein intake and it can gain as much as 2-4 pounds a day. Such productivity permits bear densities on the coast to be much greater because smaller home ranges are needed to maintain individual nutritional needs (Gilbert and Lanner 1995). Variable diets differing from one region to another and from season to season, results in black bears with a diversity of body sizes. Although the average male black bear weighs about 60-140 kg and females 40-70 kg, black bears comparable in size to interior brown bears (approximately 360 kg) have occasionally been reported from southern Manitoba.

## Evolution of *Ursus*

The family Ursidae first appeared in the fossil record approximately 35 million years ago during the Miocene (Powell et al. 1997) and currently consists of seven extant species: black bears (*Ursus americanus*), brown bears (*U. arctos*), polar bears (*U. maritimus*), Asiatic black bears (*U. thibetanus*), Malayasian sun bears (*Helarctos malayanus*), sloth bears (*Melursus ursinus*), and spectacled bears (*Tremarctos ornatus*) (Zhang and Ryder 1995).

The first bear to arrive in North America was the ancestral form of the black bear (Thenius 1990). Its arrival during the Pliocene was followed shortly thereafter by the appearance of the brown bear sometime during the mid-Pleistocene and the origin of the polar bear sometime during the last 200,000 years (Kurtén 1964).

During the Wisconsin glaciation, black bears are believed to have persisted in southern refugia (Kurtén and Anderson 1980) from which they recolonized the Pacific Northwest and its offshore islands. During the last 12,000 years since black bear dispersed from this refugium, it has differentiated into seven subspecies in northwestern North America: *americanus*, *cinnamomum*, *altifrontalis*, *pugnax*, *kermodei*, *vancouveri*, and *carlottae* (Hall 1981).

### The Haida Gwaii Black Bear (*Ursus americanus carlottae*)

According to Hall (1981) there are 16 subspecies of black bear over all North America, identified principally by cranial and dental morphology. The subspecies distributions are shown in Figure 4. A brief description of the subspecies pertinent to this study is given in Appendix II.

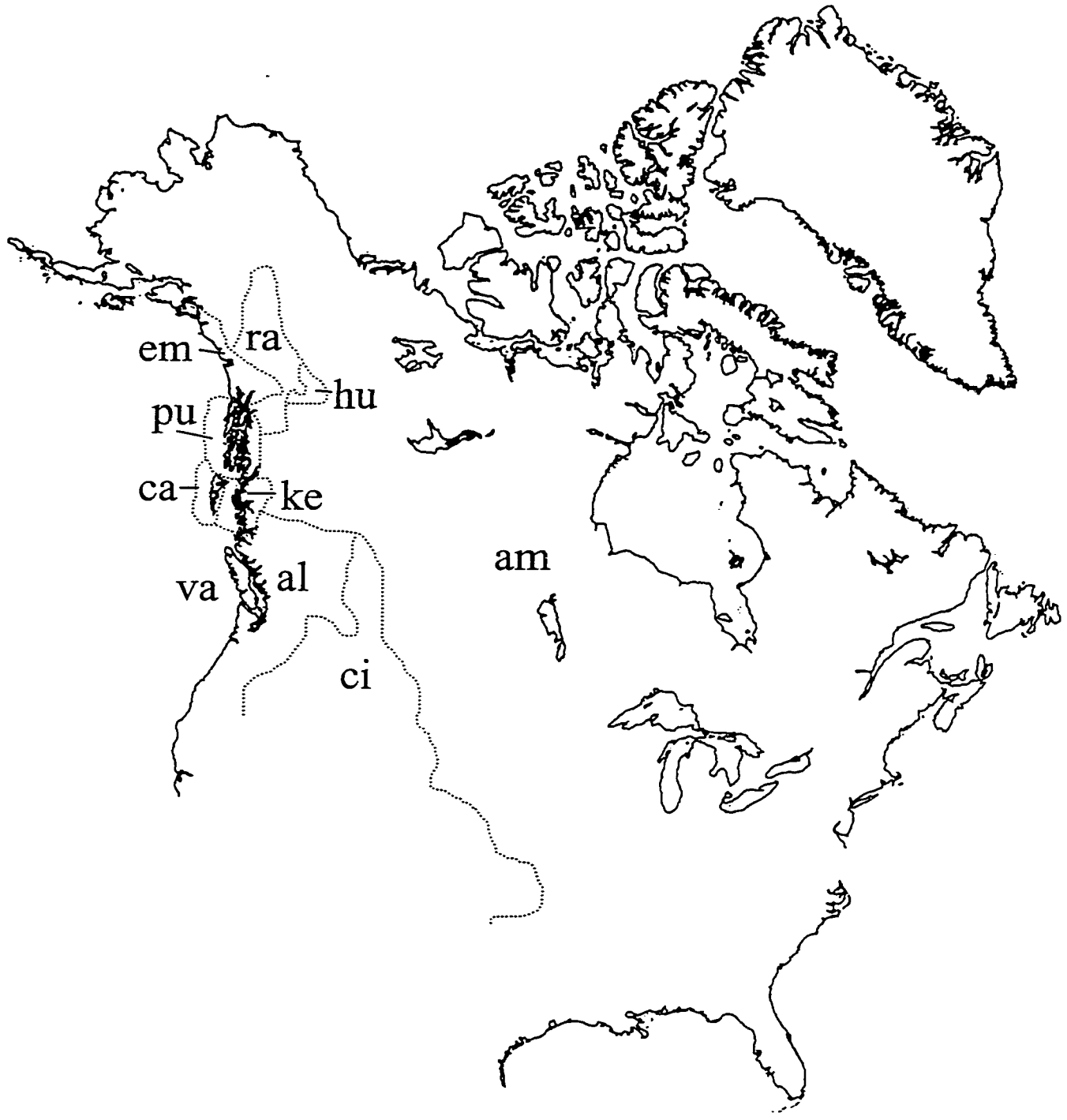



**Figure 4 Subspecies distribution map for *Ursus americanus*.**
Subspecies abbreviations: ca - *carlottae*, va - *vancouveri*, ke - *kermodei*, al - *altifrontalis*, am - *americanus*, ci - *cinnamomum*, pu - *pugnax*, em - *emonsii*, ra - *randi*, hu - *hunteri*. Adapted from Hall 1981.

The Haida Gwaii black bear was originally described as a distinct species by Osgood (1901) based on its robust skull, heavy dentition, and exceptionally large body size. In fact these characteristics are so pronounced that *carlottae* is known as the largest black bear in the world. Although the Haida Gwaii black bear was eventually reduced to the level of subspecies (Hall, 1928), it still remains the most morphologically distinct of all coastal bears including other insular subspecies found on the Alexander Archipelago (*pugnax*) and Vancouver Island (*vancouveri*).

One of the features that distinguish *carlottae* from adjacent coastal conspecifics like *pugnax*, *vancouveri* and *altifrontalis* is the length of its last molar (Foster 1965). Whereas Foster (1965) acknowledged that the unique attributes of the Haida Gwaii bear, such as its robust dentition and skull, were likely to be functional adaptations for foraging on marine resources, he also noted the inadequacy of this explanation to account for some of these divergent characteristics. Foster (1965) considered the discrepancy of last molar length between *carlottae* and *pugnax* unusual given the similarity of their habitats and he concluded that *carlottae* was unique either because of its more complete isolation or because it had been isolated for a much longer period of time. However, limited access to intertidal regions on the Alexander Archipelago could also account for the discrepancy in dental morphology between these two subspecies and *carlottae's* more robust molars may simply have been a response to this resource.

There is considerable uncertainty in trying to use divergence of morphological characters as a measure of isolation time. This is especially true of insular forms because of a number of confounding factors such as relaxation of selective constraints due to

biotic impoverishment. Differences between the Haida Gwaii black bear and adjacent subspecies could equally be attributed to its more extreme isolation, longer isolation, unique selective regimes, or combinations thereof, and as such, does not truly address the issue of *carlottae*'s status as a glacial relict.

An alternative way to examine this issue is to assess the genetic divergence and phylogenetic affinity of *carlottae* to other black bear subspecies. As black bear are believed to have persisted south of the Cordilleran ice sheet during the Wisconsin (Kurtén and Anderson 1980), all of the subspecies including *carlottae* are assumed to have originated post-glacially from a southern source. As such, the subspecies, *carlottae*, *americanus*, *altifrontalis*, *cinnamomum*, *kermodei* and *pugnax* should all demonstrate close genetic affinity. However, if *carlottae* is a glacial relict and diverged from other black bears prior to the Fraser glaciation, then it should be genetically divergent and reflect a pre-glacial separation of at least 27,500 years BP. From a mtDNA study done by Cronin et al. (1991), North American black bear on the mainland was found to be genetically homogenous and presumably members of a single mtDNA lineage. If black bears persisted and recolonized North America from more than one refugium, then more than one mtDNA lineage would be expected. The distribution of these lineages would suggest where they dispersed from and as such where they persisted during the late Wisconsin.

## Materials and Methods

### Samples

A total of 33 black bears were examined in this study including the subspecies *carlottae* (n=11), *vancouveri* (n=5), *kermodei* (n=9), *altifrontalis* (n=1), *cinnamomum* (n=1), and *americanus* (n=2). An additional four individuals from Khutzymateen, Tweedsmuir, and Jasper were also sequenced but as these locales were near subspecies boundaries, they could not be unambiguously identified. DNA from these black bears was obtained from frozen muscle, blood and a preserved hide. I also used two DNA extracts for the individuals from the Olympic Peninsula and Jasper sent by S. Wasserman and D. Paetkau respectively. Sample details are given in Table 1.

### DNA Isolation

*Muscle/Preserved Skin*

DNA was obtained from tissue according to a modification of the protocol originally developed by Doyle and Doyle (1987), Small pieces of frozen muscle were immersed in enough cetyltrimethylammoniumbromide (CTAB) buffer (100 mM Tris-HCL pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% 2-ß mercaptoethanol to completely saturate the tissue. The mixture was vigorously shaken for 1 minute and incubated at 60°C for 45 minutes. DNA was extracted using chloroform:isoayml alcohol (24:1), and precipitated with 70% ethanol. The DNA was resuspended in 0.1XTE (1 mM Tris, 0.01 mM EDTA) buffer pH 7.5, and stored at -20 °C. DNA from preserved museum hide was similarly extracted with the following modifications:1) after the addition of CTAB buffer, the tissue was mixed with a small amount of quartz sand and ground with a

**Table 1**
**Subspecies, geographical location and sample sizes for black bear.**

| Subspecies | Location | Sample size | Sample type | Source |
|---|---|---|---|---|
| *carlottae* | Haida Gwaii | 11 | muscle | author |
| *vancouveri* | Vancouver Island | 5* | muscle/blood | author |
| *kermodei* | Terrrace, BC | 3 | muscle/preserved skin | author |
| *kermodei* | Prince Rupert, BC | 3 | muscle | author |
| *kermodei* | Lakelse, BC | 1* | muscle | author |
| *kermodei* | Moricetown,BC | 1 | muscle | author |
| *kermodei* | Rosswood, BC | 1 | muscle | author |
| ssp? | Khutzamyteen, BC | 1 | muscle | author |
| *altifrontalis* | Olympic Peninsula | 1 | DNA | author |
| ssp? | Tweedsmuir, BC | 2* | muscle | author |
| *cinnamomum* | Williston Lake, BC | 1 | muscle | author |
| ssp? | Jasper, AB | 1 | DNA | author |
| *americanus* | Yukon | 1 | muscle | author |
| ssp? | south coastal BC | 1 | muscle | author |
| *americanus* | New Jersey/Pennsylvania | 1* | n/a | Vrana et al. 1994 |
| ssp? | Montana | 1* | n/a | Zhang and Ryder 1995 |
| *americanus* | Alaska | 2 | muscle, n/a | author, Shields and Kocher 1995 |

*Indicates only partial sequences were used. The region H15149 to L14841 was used for one individual from Vancouver Island, one individual from Tweedsmuir, one individual from Alaska (Shields and Kocher 1991) and the individuals from Montana (Zhang and Ryder 1995) and Pennsylvania (Vrana et al. 1994). The region L15086 to H15560 was used for the individual from Lakeisle.

ssp?- these individuals were located on subspecies boundaries

disposable pestle 2) preserved hide was incubated at 65 °C for approximately four hours instead of 45 minutes.

An additional modification of this protocol was used to extract DNA from the *kermodei* hide. The reason for this was that it was not preserved as the museum hides were but preserved with salt. The hide had to be washed several times in sterile, distilled water to remove the salt before being cut into pieces. The rest of the protocol was identical to the protocol used for the other preserved hides.

*Blood*

DNA from whole blood was obtained by using a modified DTAB/CTAB protocol from Gustincich et al. (1992) and chelex (Walsh et al. 1991). Extracting DNA using DTAB/CTAB required 300 µl of whole blood with 600 µl of blood lysis buffer (8% DTAB, 1.5 M NaCl, 100 mM Tris-HCL pH 8.6, 50 mM EDTA) incubated at 68 °C for 5 minutes. Chloroform (750 µl) was added to the DNA treated with CTAB (5% in 4M NaCl) to a final CTAB concentration of 0.3%, After centrifuging, the DNA/CTAB pellet was resuspended in 250 µl of 1.2 M NaCl. DNA was ethanol precipitated and dissolved in 50 µl of 1XTE pH 7.5 (10 mM Tris-HCL, 1 mM EDTA).

Extracting DNA using chelex required 3 µl of whole blood dissolved in 1 ml of sterile distilled water. This was incubated at room temperature for 30 minutes and then centrifuged for three minutes at 15,000 g. All but 30 µl of supernatant was remove and 5% chelex was added to a final volume of 200 µl. The resulting mixture was incubated at 56 °C for 30 minutes and then vortexed at high speed for 10 seconds. After incubating in a boiling water bath for 8 minutes, the solution was vortexed again and centrifuged for 3

minutes at 15,000 g.

**Amplification**

*Cytochrome b*

A 719 bp region from the cytochrome b gene was amplified using primers H15149 and L14841 (Kocher et al. 1989) and H15560 (Kocher et al. 1989) and L15086 (TAC TAT GGC TCA TAC CTA CTC) designed by the author. One of two types of PCR conditions was used. 1 µl of DNA extracted with CTAB or 20 µl of DNA extracted with chelex, 0.2 mM dNTPs, 0.1 mM of each primer, 0.5 units of Taq polymerase (Perkin Elmer) and 1/10th volume of TNK buffer (100 mM Tris-HCL pH 8.3, 50 mM NH4Cl, 15 mM $MgCl_2$ and various amounts of KCL (250 mM KCL, 500 mM KCL, 1M KCL) to optimize stringency (Blanchard et al. 1993). When 0.5 units of an `in house' Taq polymerase was used instead of the commercial Taq, the PCR conditions were identical with the exception of the buffer which was a standard 10X PCR buffer (100 mM Tris-HCL pH 8.3, 500 mM KCL, 25 mM $MgCl_2$, 0.01% gelatin) or Promega PCR buffer (500 mM KCL, 100 µM Tris-HCL pH 8.8, 1% Triton X-100). $MgCl_2$ was added to a final concentration of 2.5 µM. PCR was carried out for 30-35 cycles using the following steps: denaturation at 94 °C for 1 minute; annealing at 50-60 °C for 2 minutes; and extension at 72 °C for two minutes. Each reaction was initially denatured for 2 minutes at 94 °C, after which $MgCl_2$ was added (hot start). For preserved skin, PCR was run for 40 cycles.

*D-loop*

Amplification of the D-loop was also attempted using primers H15980 (GCT GGT ACC ACC ATC AGC ACC), L16500 (CTG GGT ACC ATC GAG ATG TCT TAT TTA AGG GGA ACG), H16500 (AYA GGT ACC CCT TAA ATA AGA CAT CTC GAT GG), L650 (RYT GGT ACC AAG GCT RGG ACC AAA CCT) L1067 (ATA GTG GGG TAC CTA ATC CCA GTT) and H15560 (CAC ATG GTA CCC GAA TGA TAT TTC CTA TTY GC). Various PCR conditions were used. However, the best results were achieved using the following steps: 94 °C for 3 minutes; 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 3 minutes; 7 minute extension at 72 °C.

**Purification of PCR Products**

PCR products were purified in two ways depending upon which sequencing (manual or automated) protocol was used. For manual sequencing, PCR products were purified using Wizard PCR purification columns (Promega). For automated sequencing, PCR products were cut out of a 2% Nusieve (FMC Biochemicals) stained with 0.25 μg/ml ethidium bromide and then purified through Promega Wizard purification columns. Initially PCR products were purified directly with these columns but primer sequences were not being properly eliminated leading to lower efficiency of subsequent ligations. Running products through the gel solved the problem. Purifying PCR products by standard phenol, chloroform:isoamyl (24:1) procedure was also attempted. However, yield was low and did not solve the problem of contamination by smaller fragments.

**Restriction Analysis**

The possibility of using RFLP analysis to differentiate between black bear lineages was investigated. A restriction site was found 59bp away from the 3' end which would differentiate the two lineages. Since this fragment was too small to visualize using agarose or nusieve, Metaphor (FMC Biochemicals) gels were made to detect it.

**Cloning**

*Ligations*

Ligations were performed using Invitrogen plasmid vector pCR II. Because ligations are based upon poly A overhangs on PCR products, ligations were usually done within 48 hours to minimize the loss of these overhangs. A slight modification of the suggested protocol was used: 2 µl (~50ng) purified DNA, 1 µl ligation buffer, 2 µl pCR II vector, 4 µl dH2O, and 1 µl of ligase. These were incubated overnight at 12-14 °C. Ligations using a Kpn I digested M13 vector with Kpn I digested PCR fragments were also attempted (15 µl PCR product, 30 µl sterile double distilled water, 5 µl one-phor all buffer, 3 µl Kpn I incubated at 37 °C for 2 hours followed by 30 minute incubation at 80 °C). However, the system was unreliable and eventually abandoned.

*Transformation*

Transformations were done using the Invitrogen One Shot Kit. Into each vial of prepared competent cells (INV(F')), 2 µl of ß-mercaptoethanol and 1 µl of the ligate was added. This was incubated for 30 minutes on ice. Cells were heat shocked for 30

seconds at 41-43°C and immediately placed on ice for 2 minutes. These were placed in a 37 °C incubator and shaken at 225 rpm for 1 hour (caps were unscrewed slightly for aeration). Cells (50 - 100 µl) were grown on LB (Luria Bertani) plates with 50 µg/ml of ampicillin and 40 µl of a 40 mg/ml X-Gal. Colonies were allowed to grow for 12-16 hours and stored at 4 °C for several hours. Colonies were grown in 6 mls of terrific broth (TB) (12 g bacto-tryptone, 24 g bacto-yeast extract, 4 ml glycerol per litre). Added to this was 100 ml of 0.17 M $KH_2PO_4$ and 0.72 M $K_2HPO_4$ and 0.02 mg/ml of ampicillin for a minimum of 12 hours at 37°C. One ml of overnight culture was taken off and stored in 250 µl of sterile glycerol at -70 °C for long-term storage. The remaining overnight culture was then purified.

*Plasmid purification*

Cells were lysed using a lysis buffer (25 mM Tris-HCL pH 8.0, 10 mM EDTA, 50 mM glucose) and the remaining protein removed using a 3M ice cold potassium acetate solution pH 4.8 and 0.2 N NaOH/1% SDS. The preparation was treated with 20 µg/ml RNAase for at least 1 hour at 37 °C. One phenol/chloroform:isoamyl (25:24:1) and one chloroform:isoamyl (24:1) extraction was done. DNA was precipitated using 95% ethanol. The pellet was further purified using 13% polyethylene glycol (PEG) and rinsed with 70% ethanol. The pellet was thoroughly dried and then dissolved in 13 µl of sterile distilled water. One µl of this preparation was used to check for the presence of the correct insert and 2 µl was used to determine DNA concentrations using the spectrometer.

**Automated Sequencing**

Automated cycle sequencing of cloned PCR fragments were done using the 21 M13 primer kit (PRISM) on the ABI 373A according to conditions suggested by ABI (Applied Biosystems Inc.). For each dideoxy reactions, adenine (A) and Cytosine (C), 4 µl of PRISM mix was added to 250 ng of DNA. For guanine (G) and thymine (T), 8 µl of PRISM mix was added to 500 ng of DNA. These were run through the following sequencing reaction: 94 °C for 2 minutes; 15 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 70 °C for 1 minute; 15 cycles of 95 °C for 30 seconds, 70 °C for 1 minute; 20 °C for 10 minutes; 4 °C to store. The four reactions were subsequently mixed and the DNA ethanol precipitated. DNA was dissolved in 4.1 µl of formamide/EDTA (5 parts formamide to 1 part 50 mM EDTA) and run for 12 hours on a 4.8 % acrylamide gel.

**Manual Sequencing**

Manual cycle sequencing (Sanger et al. 1977) was done using the Promega fmol cycle sequencing kit on purified PCR products. Reaction conditions suggested by Promega (Madison, WI) was used (5 µl of 5X buffer, 3 µg of primer L14841, 1 µl of dideoxy, 1 µl [$\alpha$-$^{35}$S] dATP (1000ci/nmol) and 9 µl of PCR product). $^{35}$S was directly incorporated using the following sequencing reaction conditions: 94 °C for 3 minutes, 30 cycles of 94 °C, 68-70 °C for 2 minutes; 4 °C to store. Bands were run on 8% acrylamide gels and then exposed for 72 hours. Sequencing using [$\alpha$-$^{32}$P] dATP (400ci/nmol) end labeled primers was also attempted. However, this was abandoned because results were not significantly better than direct incorporation and the technique was more technically demanding. Single stranded sequencing was also attempted. However, great difficulty

was experienced trying to generate single stranded PCR products consistently. Two methods were attempted: asymmetric PCR and a H15149 biotinylated primer.

**Phylogenetic Analyses**

Sequence assembly and alignments were performed using Lasergene Navigator (DNASTAR, Madison, WI). A minimum of three sequences were used to generate the consensus.

*Parsimony*

Weighted and unweighted parsimony analyses (Eck and Dayoff 1966; Fitch 1977) were performed using PAUP 3.1 (Swofford 1993). Weights were determined by the frequency of substitutions at each of the three codon base positions and the ratio of transitions and transversions in our data set. Rooted majority rule trees were produced by a heuristic search of character state matrices using the Asiatic black bear (*Ursus thibetanus*) as an outgroup for all analyses. Taxa were either added randomly or in the order in which they were presented in the data matrix. Heuristic parsimony searches were conducted using tree bisection and reconnection (TBR) branch swapping and with the steepest descent option in effect. All equally parsimonious trees were retained during branch swapping using the MULPARS option. Data was resampled using 100 heuristic bootstrap replicates.

*Maximum Likelihood*

Maximum likelihood analysis was performed using the HKY 85 (Hasegawa et al. 1985) model and a $T_i/T_v$ ratio of 2 on PAUP* (Swofford in press). The HKY model of substitution accounts for two substitution types (transitions and transversions) as well as allowing for unequal equilibrium base frequencies. Empirical base frequencies used were A=0.28, C=0.29, G=0.15, and T=0.28. Starting branch lengths were generated used Rogers-Swofford approximation method. One hundred trees were obtained from a heuristic search. Branch swapping on these trees was implemented using tree bisection reconnection and steepest descent. The trees were evaluated using 2000 heuristic bootstrap replicates.

*Distance Analyses*

Distance trees were generated using the neighbor-joining (Saitou and Nei 1987) method under PAUP* (Swofford in press). A distance matrix was generated using a two parameter (Kimura 1980) model. The resulting matrix was used to construct the neighbour-joining tree and to calculate average sequence divergence. In addition to two parameter distances, synonymous ($K_s$) (substitutions which do not change the final amino acid sequence) and nonsynonymous ($K_a$) (substitutions which do change the final amino acid sequence) variation corrected for multiple hits was calculated using MEGA 1.02 (Kumar et al. 1993).

*Relative Rate Test*

A relative rate test (Sarich and Wilson 1973) was done to determine whether either coastal or continental lineage exhibited a notable increase in the rate of evolution. Distances calculated using Kimura's two parameter model were used. The Asiatic black bear was used as the outgroup.

## Results

A total of 719 bp from 29 individuals and 307 bp from four individuals were sequenced (Table 2) (see Appendix III for GenBank accession numbers). Phylogenetic analyses of these cytochrome b sequences (the bear from south coastal BC was not included due to ambiguity with regards to its origin) and three additional black bear sequences from GenBank revealed the existence of two geographically structured, monophyletic black bear lineages on the northwestern coast of North America (Fig 5, 6, 7). A continental lineage grouped samples from British Columbia, Yukon, Alberta, Montana (Zhang and Ryder 1995), Pennsylvania (Vrana et al. 1994) and Alaska (Shields and Kocher 1991) and encompassed the distribution of two subspecies, *americanus* and *cinnamomum*. The second coastal lineage was found exclusively in bears from Haida Gwaii (*carlottae*), Vancouver Island (*vancouveri*), the Olympic Peninsula (*altifrontalis*) and most bears from the coastal fringe of British Columbia (*kermodei*) (Fig 8).

## Table 2 Black bear character matrix.

The following table shows The variable sites in reference to the sequence shown below. Position numbers are shown above the table, nucleotides are shown below position number. ? indicates missing data.

### Kermode

```
1                                                  40                                                  80
GAAACTTCGGATCCCTCCTCGGAGTATGTTTAGTACTACAAATTCTAACGGGCCTATTTCTAGCCATACACTACACATCA
81                                                 120                                                 160
GATACAACTACAGCCTTTTCATCAATCACCCATATTTGCCGAGATGTTCACTACGGATGAATTATCCGATACATACATGC
161                                                200                                                 240
TAACGGAGCTTCCATGTTCTTTATCTGCCTGTTCATGCACGTAGGACGGGGTCTGTACTATGGCTCATACCTACTCTCAG
241                                                280                                                 320
AAACATGAAACATTGGCATTATCCTCCTATTTACAGTTATAGCCACCGCATTCATAGGATATGTCCTGCCCTGAGGCCAA
321                                                360                                                 400
ATATCCTTCTGAGGAGCAACTGTTATCACCAACCTCCTATCAGCCATCCCCTATATTGGAACAGACCTAGTAGAATGGAT
401                                                440                                                 480
CTGAGGGGGCTTTTCTGTGAATAAGGCAACTCTGACACGATTCTTTGCCTTCCACTTTATTCTTCCATTCATCATCTTGA
481                                                520                                                 560
CACTAGCAGCAGTCCACCTATTATTCCTACACGAAACAGGATCTAATAACCCCTCTGGAATCCCATCTGACTCAGACAAA
561                                                600                                                 640
ATCCCATTTCATCCATATTATACAATTAAAGACGCCCTAGGCGCCCTACTTTTCATCCTAGCCCTAGCAACTCTAGTCCT
641                                                681                                                 719
ATTCTCGCCTGACCTACTAGGAGATCCCGATAACTACACCCCCGCAAACCCACTGAGCACCCCACCCCACATCAAACCT
```

**Position Number**

| Taxon | 1111111111112222222222222233333333333333333333333344<br>1222333444556788890022356778990111355667778990001112223344568888899911<br>8104903501709543462585813106217523682302018836278147126894708013571568 69 |
|---|---|
| Jasper | CGCGTTGAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTACACATCAATCCCAGAATTATGTG |
| Olympic | CACGTTGAAAAGYYYYAAAAAYTCRTTGTGGGTCTCGKTTTTTGCATTGCACATCAATCCCRAAACTAKGTG |
| Kermode | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Khutzymateen | CGCGTTGAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTACAAATCAATCCCAGAATTATGTG |
| Lakelse | ??????????????????????????????????????CATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Prince Rupert 1 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Prince Rupert 2 | ??????????????????????????????????????CATTWTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Prince Rupert 3 | ??????????????????????????????????????????????GTTGCACATCATTCACAAATCTATGTG |
| Rosswood | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Terrace 1 | C?CGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Terrace 2 | C?CGTT?WAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTT?GTATTGCACATCAATCCCAAAACCATGTG |
| Tweedsmuir 1 | CGCGTTGAAAAGCTTCAAGAATTCATTATGGGTCTCATTTTTCGTATTACACATCAACCCCAGAATTATGTG |
| Tweedsmuir 2 | CACGTTGAAAAATCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGC?????????????????????? |
| Williston Lake | CGCGTTGAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTACACATCAACCCCAGAATTATGTG |
| south coastal BC | ??????GAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTAT?????????????????????????? |
| Moricetown | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTTATTTCTTGCATTGCACATCAATCCCAAATCTATGTG |
| Montana | CGCGTTAAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATAA?????????????????????? |
| NJ/Pennsylvania | TGAGTTGAAAAGTTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTAT?????????????????????? |
| Yukon | CGCGTTGAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTACAAATCAACCCCAGAATTATGTG |
| Alaska 1 | CGCGTTGAAAAGCTTCAAAAATTCATTATGGGTCTCATTTTTCGTATTACACATCAACCCCAGAATTATGTG |
| Alaska 2 | ???GTTGAAAAGTTTCAAAAATTCATTAAGGGTCTCATTTTTCGTACTA?????????????????????? |
| QC 1 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| QC 2 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCAAATAAATCCCAAAACTATGTG |
| QC 3 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| QC 4 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| QC 5 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| QC 6 | CACGTTGAWAAGTCCTAAAAATTCATTGTGGCTCTCATTTTTTGCATTGCGCATCAATCCCAAAACTATGTG |
| QC 7 | ?????TTGAAAAGTCCTAAAAATTCATTGTGGGTCACATTTTTTGCA?????????????????????????? |

# Table 2 Black bear character matrix continued.

| | |
|---|---|
| QC 8 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTA?GTG |
| QC 9 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTCTTGCATTGCACWWCAATCCCAAAACTATGTG |
| QC 10 | ????????AAAGTTCTAAAAATTCATTGTGGGTCTCATTTTGTGCATTGCACATCAATCCCAAAACTATGTG |
| QC 11 | CACGTTGAAAAGCCCTGAAAATTCATTGTGGGTCTCATTTCTTACATTGCACWWCAATCCCAAAACTATGTG |
| VI 1 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| VI 2 | GACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| VI 3 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCAAATCAATCCCAAAACTATGTG |
| VI 4 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGC?????????????????????? |
| VI 5 | CACGTTGAAAAGTCCTAAAAATTCATTGTGGGTCTCATTTTTTGCATTGCACATCAATCCCAAAACTATGTG |
| Asiatic black bear | TACACCACAGGATTCCGGAGGCCTAACGCAAGCTTCATCTTTTGCATTACACATTGACTCTAAGACTGTACA |

| | |
|---|---|
| | 44444444444444555555555555555566666666666666667777777 |
| | 22334566777899001222455566778911223355566777800011111 |
| **Taxon** | 05149114079007038147824869280027250804958179514536 89 |

| | |
|---|---|
| Jasper | AATACTCTCTGACCGAAATTTTAAAT?CAACCTTGCTCACCCCT?TACCACT |
| Olympic | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCANT |
| Kermode | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Khutzymateen | AATACTCTCTGAACGAGATTTTAAATTCAACCTCGCTCACCCCTCTATCACT |
| Lakelse | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Prince Rupert 1 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCTGCACCACT |
| Prince Rupert 2 | AGTGCTTTCTGACCAAAGTTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Prince Rupert 3 | AGTGCWTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Rosswood | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Terrace 1 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| Terrace 2 | AGTGCTTCCTGACCAAAATCTTAAATTTAATCCTACTCATCTCCSCACCACT |
| Tweedsmuir 1 | AATACTCTCTGACCGAAATTTTAAATTCAACCTTGCTCACCCCTSTACSACT |
| Tweedsmuir 2 | ?????????????????????????????????????????????????? |
| Williston Lake | AATACTCTCTGACCGAAATTTTAAATTCAACCTTGCTCACCCCTCTACTACT |
| south coastal BC | ?????????????????????????????????????????????????? |
| Moricetown | AGTGCTTTCTGAGCAAAATTTTAGATTTAATCCTACTCATCTCCGCACCACT |
| Montana | ?????????????????????????????????????????????????? |
| NJ/Pennsylvania | ?????????????????????????????????????????????????? |
| Yukon | AATACTCTCTGACCGAAATTTTAAATTCAACCTTGCTCACCCCTCTACCACT |
| Alaska 1 | AATACTCTCTGACCGAAATTTTAAATTCAACCTTGCTCACCCCTCTACCACT |
| Alaska 2 | ?????????????????????????????????????????????????? |
| QC 1 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 2 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 3 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 4 | AGTGCTTTCTGACCAAAATTTTAAATTTTATCCTACTCATCTCCCCACCACT |
| QC 5 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 6 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 7 | ?????????????????????????????????????????????????? |
| QC 8 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 9 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| QC 10 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCGCACCACT |
| QC 11 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCCCACCACT |
| VI 1 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCSCACCACT |
| VI 2 | AGTGCTTTCTGACCAAAATTTTAAGTTTAATCCTACTCATCTCCCCACCACT |
| VI 3 | AGTGCTTTCTGACCAAAATTTCAAATTTAATCCTACTCATCTCCCCACCACT |
| VI 4 | ?????????????????????????????????????????????????? |
| VI 5 | AGTGCTTTCTGACCAAAATTTTAAATTTAATCCTACTCATCTCCSCACCACT |
| Asiatic black bear | GACATTCTTCAGCTAGAACCCTGAACCCAGCTCTATCTGCTCTCCCGCCGCC |

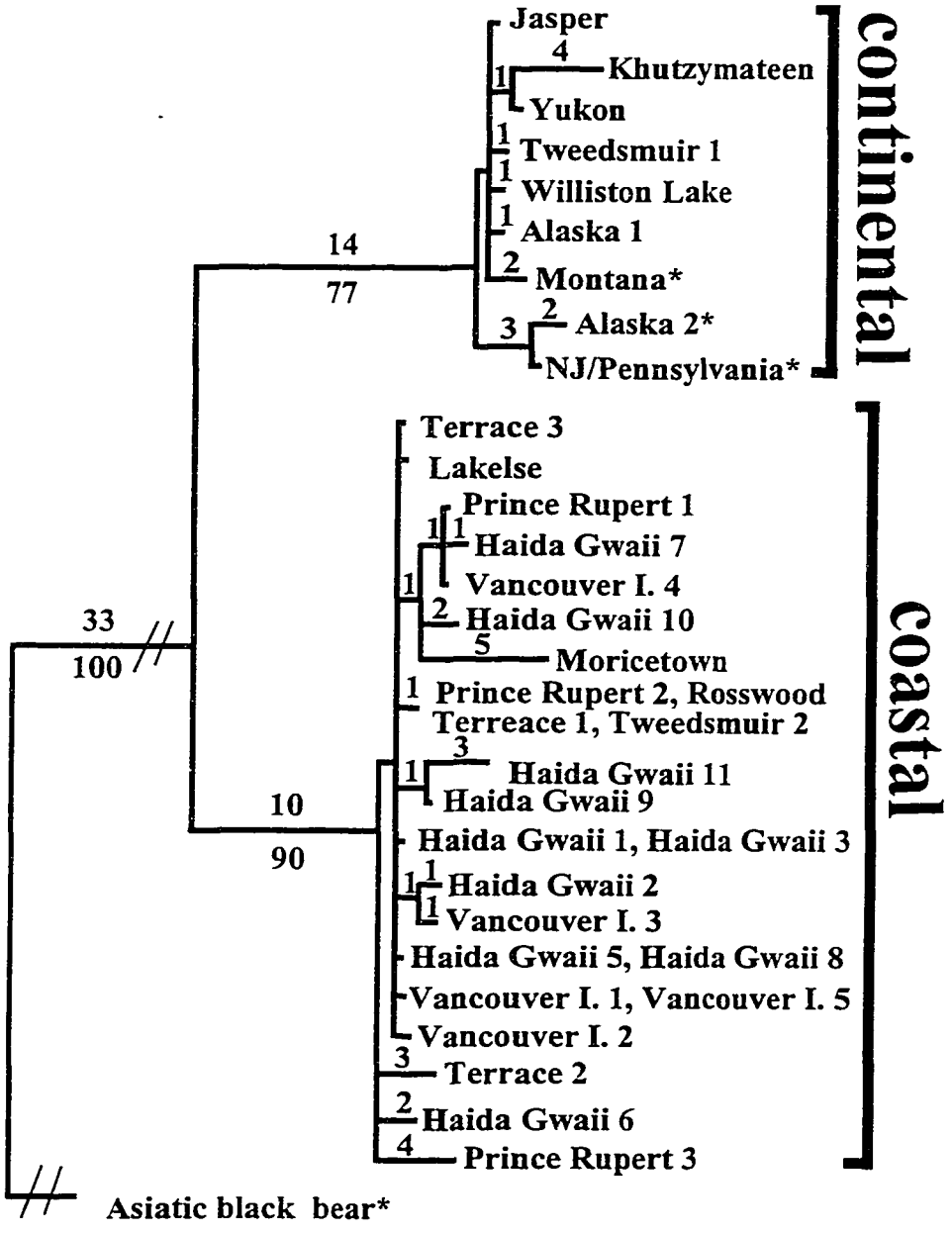



**Figure 5 Black bear 50% majority rule tree.**
Tree topology is based on a 50% majority rule of 100 equally parsimonious trees. Two major black bear lineages are evident from this tree: 1) continental and 2) coastal. Individuals comprising the continental lineage are generally from east of the continental divide, whereas those individuals comprising the coastal lineage are found west of the divide including the islands of British Columbia. The tree was drawn to reflect the number of synapomorphies as shown by varying branch lengths. Branch lengths are shown above the branches while bootstrap values are reported below. Total tree length is 176 and consistency index (CI) is 0.87. Hatch marks indicate that branches are not to scale. *These sequences were taken from Genbank.

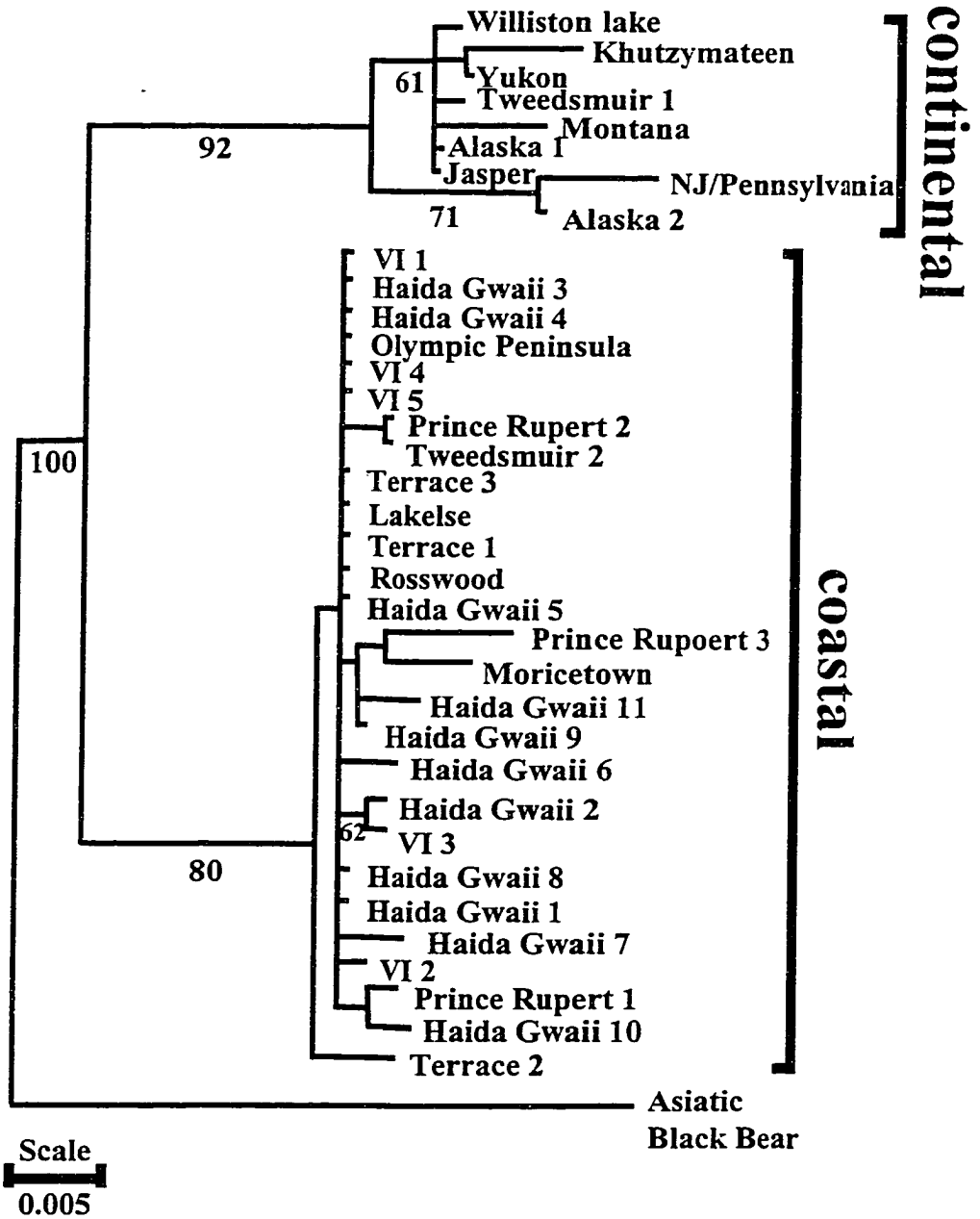



**Figure 6 Maximum likelihood tree for black bear.**

This tree demonstrates the topology from a strict consensus of 100 trees of equal likelihood (ln likelihood -1694.03). Two black bear lineages were found. These lineages correspond to those bears found on the coastal regions of the Pacific Northwest and to those bears found in continental areas. Bootstrap values from 2000 heuristic replicates are shown below branches. Branches are scaled to indicate the ln likelihood of each branch.

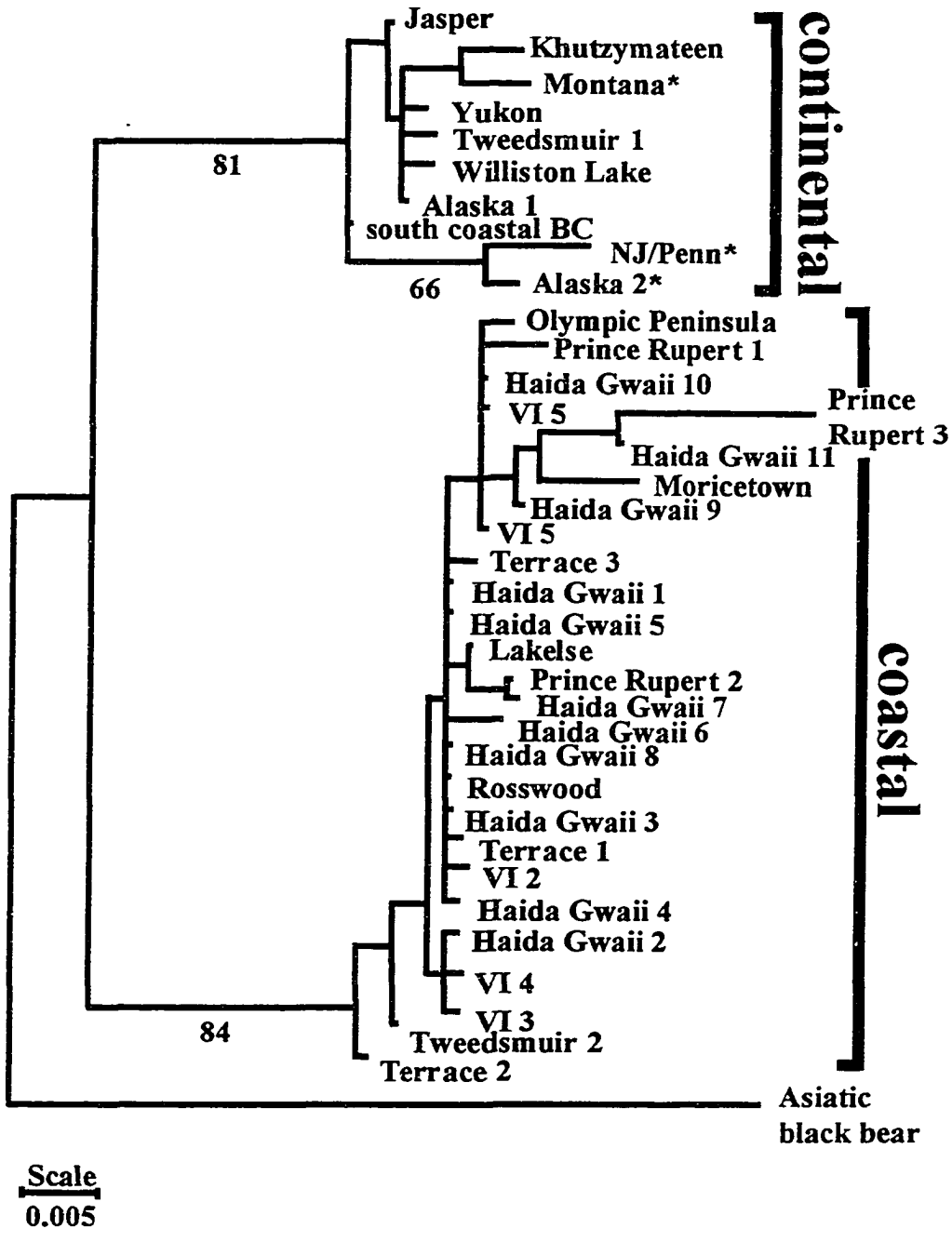



**Figure 7 Neighbour-joining tree for black bear.**
As with parsimony and maximum likelihood, two lineages were uncovered by this analysis: coastal and continental. The coastal lineages included all black bears from Haida Gwaii, Vancouver Island, and bears from various locations west of the continental divide. Bootstraps are shown below branches. Branches are scaled to indicate transformed distances.

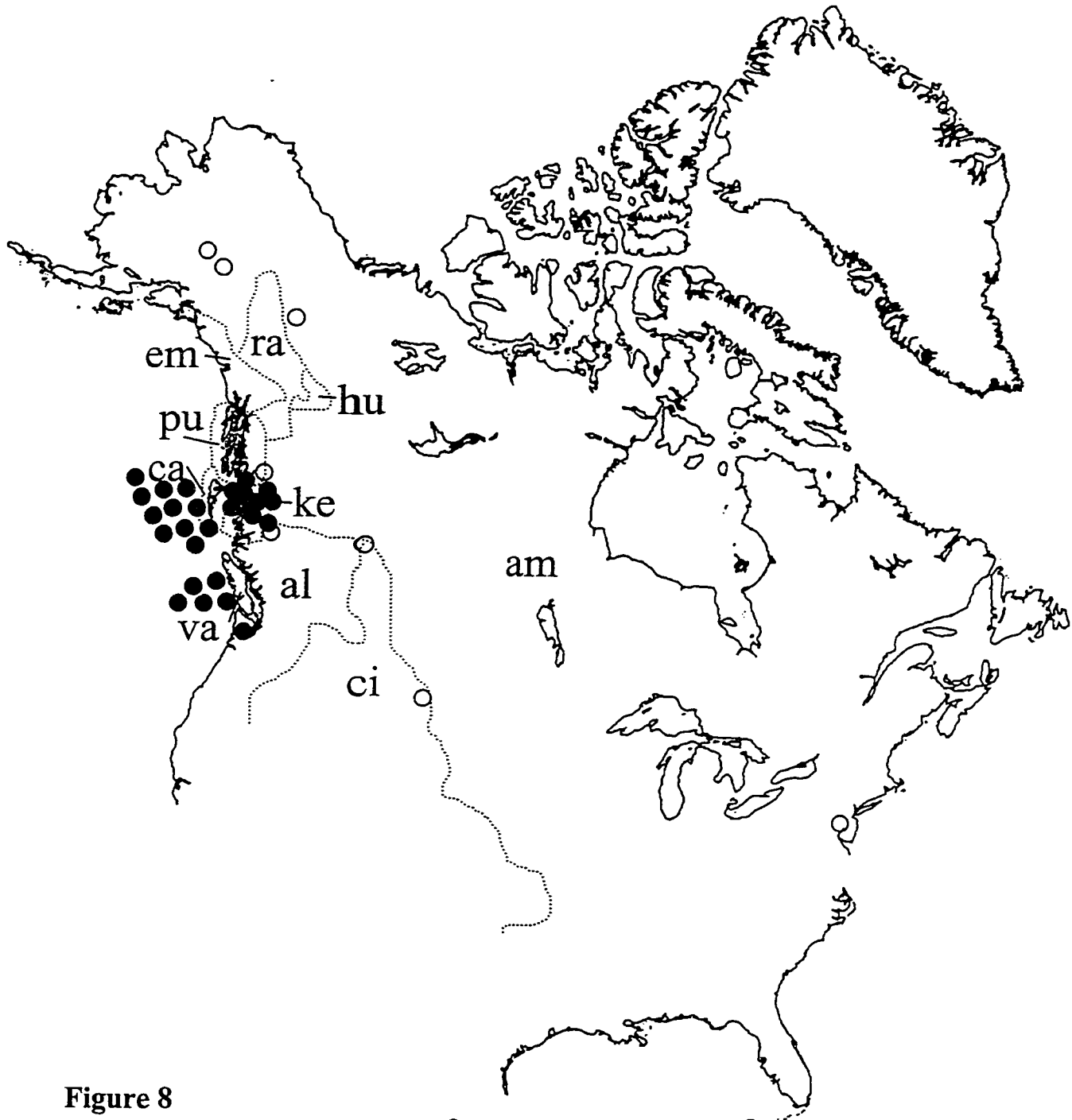



**Figure 8**

Geographic distribution of coastal (●) (n=26) and continental (○) (n=8) mtDNA lineages in black bear. The coastal lineage was found in four coastal subspecies: *carlottae* (ca) (n=11), *vancouveri* (va) (n=5), *kermodei* (ke) (n=9) and *altifrontalis* (al) (n=1). The continenetal lineage was found dispersed throughout the continent in subspecies *americanus* (am) (n=4) and on subspecies boundaries between *americanus* and *cinnamomum* (ci),between *kermodei* and *altifrontalis*, and between *americanus* and *altifrontalis*.

*Maximum Parsimony*

Out of a total of 719 characters, there were 64 parsimony informative sites. Maximum parsimony recovered 100 equally parsimonious trees, all with a tree length of 178, a consistency index (CI) of 0.87 and retention index (RI) of 0.90. The topology from the 50% majority rule tree is shown (Fig 5). The tree produced by strict consensus was similar to the 50% majority rule tree in that the continental and coastal lineages were clearly defined. Branch lengths were introduced to the 50% majority rule tree to indicate the number of synapomorphies along each branch.

Continental and coastal lineages differed by 24 synapomorphies. All 24 synapomorphies were transitions, 79.2% occurring at the third base, 12.5% occurring at the first-base and 8.3% occurring at the second base position, a substitution rate consistent with previous observations (Brown 1985). Substitutions were observed in the transmembrane, outer ($Q_o$-redox centre), and inner ($Q_i$) segments in the ratio 9:14:1 respectively. Although about 3.5 % of the unambiguous changes occurred in the $Q_o$ redox reaction centre, none of these occurred in the most conserved region. Most of the substitutions (71%) were C-T transitions. Intraspecific variability for this region was low; 3.3% of the sites were variable, 25% of which were transversions, 62.5% of variable sites were third base changes, and 16.7% were third base changes.

*Maximum Likelihood*

A strict consensus and 50% majority rule tree were constructed from 100 trees of equal likelihood uncovered by PAUP*. The ln likelihood was -1694.03. Figure 6 shows a maximum likelihood tree which not only reflects the topology of the strict consensus

trees, but also shows branch lengths from one of 100 trees uncovered by a heuristic search. Topology of the strict consensus and 50% majority rule tree were not identical. Because intralineage relationships were different between the two trees, I have shown the more conservative topology. Despite intralineage differences between the two trees, both coastal and continental lineages were clearly identifiable in both strict and 50% majority rule consensus. Branch lengths of $10^{-9}$ or less were collapsed to 0. Bootstrap analysis showed strong support for the coastal and continental lineages with a bootstrap of 80 and 92 respectively.

*Distance*

Pairwise distances based on Kimura's (1980) two parameter model was used to generate a neighbour-joining tree (Fig 7). As with maximum parsimony, neighbour-joining analysis revealed coastal and continental black bear lineages each strongly supported by bootstrap values of 84 and 81 respectively. Based on pairwise distances (Table 3), average sequence divergence between the two lineages was about 3.6%. Within lineages the average divergence was about 0.1%.

Synonymous variation ($K_s$) between continental and coastal black bear lineages was 0.12 whereas within lineages it was 0.021 and 0.016 respectively. Nonsynonymous variation ($K_a$) between continental and coastal bear lineages was 0.012. $K_a$ within these lineages was 0.00038 and 0.0041 respectively. A divergence rate of 10% per million years for silent changes (Brown et al. 1982; Irwin et al. 1991) was used to estimate a separation time ranging from 360,000 years (average sequence divergence) to 1.2 million (synonymous $K_s$) for coastal and continental mtDNA lineages. Divergence within each

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus*.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 Jasper | - | | | | | | |
| 2 Olympic | 0.02902 | - | | | | | |
| 3 Terrace 3 | 0.03317 | 0.00141 | - | | | | |
| 4 Khutzamateen | 0.00700 | 0.03631 | 0.04043 | - | | | |
| 5 Lakelse | 0.03685 | 0.00222 | 0.00000 | 0.04777 | - | | |
| 6 Prince Rupert 1 | 0.03169 | 0.00422 | 0.00279 | 0.04038 | 0.00437 | - | |
| 7 Prince Rupert 2 | 0.03907 | 0.00426 | 0.00203 | 0.05003 | 0.00207 | 0.00642 | - |
| 8 Prince Rupert 3 | 0.04682 | 0.00929 | 0.00930 | 0.05944 | 0.00958 | 0.01436 | 0.01195 |
| 9 Rosswood | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00279 | 0.00203 |
| 10 Terrace 1 | 0.03172 | 0.00141 | 0.00000 | 0.03899 | 0.00000 | 0.00279 | 0.00202 |
| 11 Terrace 2 | 0.03337 | 0.00716 | 0.00564 | 0.04068 | 0.00821 | 0.00706 | 0.01027 |
| 12 Tweedsmuir 1 | 0.00280 | 0.03195 | 0.03609 | 0.00982 | 0.03896 | 0.03459 | 0.04118 |
| 13 Tweedsmuir 2 | 0.03326 | 0.00595 | 0.00295 | 0.03325 | 0.00000 | 0.00298 | 0.00000 |
| 14 Williston Lake | 0.00281 | 0.03199 | 0.03611 | 0.00982 | 0.04127 | 0.03606 | 0.04351 |
| 15 south coastal BC | 0.00000 | 0.01532 | 0.02732 | 0.00000 | 0.03496 | 0.02729 | 0.03566 |
| 16 Moricetown | 0.04059 | 0.00986 | 0.00839 | 0.04795 | 0.01294 | 0.00840 | 0.01504 |
| 17 Montana | 0.00598 | 0.02565 | 0.03637 | 0.00600 | 0.05683 | 0.03640 | 0.05791 |
| 18 NJ/Penn | 0.01462 | 0.03106 | 0.03816 | 0.01451 | 0.05814 | 0.03804 | 0.05909 |
| 19 Yukon | 0.00279 | 0.03187 | 0.03599 | 0.00699 | 0.04091 | 0.03594 | 0.04314 |
| 20 Alaska 1 | 0.00140 | 0.03043 | 0.03455 | 0.00840 | 0.03879 | 0.03450 | 0.04102 |
| 21 Alaska 2 | 0.01046 | 0.02386 | 0.03216 | 0.01044 | 0.05823 | 0.03216 | 0.05936 |
| 22 QC 1 | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00279 | 0.00203 |
| 23 QC 2 | 0.03612 | 0.00421 | 0.00280 | 0.04050 | 0.00406 | 0.00560 | 0.00609 |
| 24 QC 3 | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00279 | 0.00203 |
| 25 QC 4 | 0.03461 | 0.00280 | 0.00139 | 0.04188 | 0.00217 | 0.00418 | 0.00422 |
| 26 QC 5 | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00279 | 0.00203 |
| 27 QC 6 | 0.03616 | 0.00421 | 0.00279 | 0.04346 | 0.00218 | 0.00558 | 0.00422 |
| 28 QC 7 | 0.03001 | 0.00685 | 0.00325 | 0.02995 | 0.00000 | 0.00326 | 0.00000 |
| 29 QC 8 | 0.03322 | 0.00141 | 0.00000 | 0.04049 | 0.00000 | 0.00279 | 0.00203 |

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 30 | QC 9 | 0.03472 | 0.00283 | 0.00140 | 0.04201 | 0.00198 | 0.00420 | 0.00402 |
| 31 | QC 10 | 0.03351 | 0.00446 | 0.00440 | 0.04268 | 0.00429 | 0.00443 | 0.00635 |
| 32 | QC 11 | 0.03615 | 0.00565 | 0.00559 | 0.04345 | 0.00400 | 0.00841 | 0.00604 |
| 33 | VI 1 | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00139 | 0.00203 |
| 34 | VI 2 | 0.03466 | 0.00280 | 0.00139 | 0.04194 | 0.00220 | 0.00419 | 0.00424 |
| 35 | VI 3 | 0.03617 | 0.00423 | 0.00279 | 0.04056 | 0.00400 | 0.00560 | 0.00604 |
| 36 | VI 4 | 0.03005 | 0.00297 | 0.00000 | 0.03002 | 0.00000 | 0.00000 | 0.00000 |
| 37 | VI 5 | 0.03317 | 0.00141 | 0.00000 | 0.04043 | 0.00000 | 0.00139 | 0.00203 |
| 38 | Brown bear | 0.13630 | 0.12667 | 0.13224 | 0.14458 | 0.13038 | 0.13566 | 0.13311 |
| 39 | Asiatic black bear | 0.11253 | 0.10643 | 0.10868 | 0.12236 | 0.10464 | 0.11199 | 0.10726 |

| | | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|
| 8 | Prince Rupert 3 | - | | | | | | |
| 9 | Rosswood | 0.00930 | - | | | | | |
| 10 | Terrace 1 | 0.00928 | 0.00000 | - | | | | |
| 11 | Terrace 2 | 0.01592 | 0.00564 | 0.00565 | - | | | |
| 12 | Tweedsmuir 1 | 0.04915 | 0.03609 | 0.03465 | 0.03634 | - | | |
| 13 | Tweedsmuir 2 | 0.05856 | 0.00295 | 0.00297 | 0.00597 | 0.03646 | - | |
| 14 | Williston Lake | 0.05194 | 0.03611 | 0.03466 | 0.03632 | 0.00279 | 0.03327 | - |
| 15 | south coastal BC | 0.07133 | 0.02732 | 0.02736 | 0.01960 | 0.00365 | 0.03087 | 0.00000 |
| 16 | Moricetown | 0.01431 | 0.00839 | 0.00839 | 0.01276 | 0.04352 | 0.00967 | 0.04499 |
| 17 | Montana | 0.15789 | 0.03637 | 0.03286 | 0.02299 | 0.00899 | 0.04042 | 0.00597 |
| 18 | NJ/Penn | 0.18465 | 0.03816 | 0.03461 | 0.02816 | 0.01776 | 0.04025 | 0.01467 |
| 19 | Yukon | 0.05144 | 0.03599 | 0.03454 | 0.03620 | 0.00279 | 0.03325 | 0.00279 |
| 20 | Alaska 1 | 0.04898 | 0.03455 | 0.03310 | 0.03475 | 0.00139 | 0.03327 | 0.00139 |
| 21 | Alaska 2 | 0.19945 | 0.03216 | 0.03220 | 0.02481 | 0.01377 | 0.03609 | 0.01047 |
| 22 | QC 1 | 0.00930 | 0.00000 | 0.00000 | 0.00564 | 0.03609 | 0.00295 | 0.03611 |
| 23 | QC 2 | 0.01404 | 0.00280 | 0.00281 | 0.00849 | 0.03906 | 0.00296 | 0.03908 |

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 QC 3 | 0.00930 | 0.00000 | 0.00000 | 0.00564 | 0.03609 | 0.00295 | 0.03611 |
| 25 QC 4 | 0.01190 | 0.00139 | 0.00139 | 0.00705 | 0.03753 | 0.00295 | 0.03756 |
| 26 QC 5 | 0.00930 | 0.00000 | 0.00000 | 0.00564 | 0.03609 | 0.00295 | 0.03611 |
| 27 QC 6 | 0.01178 | 0.00279 | 0.00279 | 0.00849 | 0.03912 | 0.00660 | 0.03911 |
| 28 QC 7 | 0.09332 | 0.00325 | 0.00325 | 0.00648 | 0.03378 | 0.00701 | 0.03003 |
| 29 QC 8 | 0.00933 | 0.00000 | 0.00000 | 0.00565 | 0.03614 | 0.00296 | 0.03617 |
| 30 QC 9 | 0.00939 | 0.00140 | 0.00140 | 0.00706 | 0.03765 | 0.00657 | 0.03767 |
| 31 QC 10 | 0.01183 | 0.00440 | 0.00440 | 0.00890 | 0.03660 | 0.01026 | 0.03810 |
| 32 QC 11 | 0.00937 | 0.00559 | 0.00560 | 0.01132 | 0.03909 | 0.01654 | 0.03910 |
| 33 VI 1 | 0.00931 | 0.00000 | 0.00000 | 0.00564 | 0.03609 | 0.00296 | 0.03611 |
| 34 VI 2 | 0.01181 | 0.00139 | 0.00139 | 0.00706 | 0.03759 | 0.00298 | 0.03760 |
| 35 VI 3 | 0.01389 | 0.00279 | 0.00280 | 0.00845 | 0.03910 | 0.00295 | 0.03912 |
| 36 VI 4 | 0.05804 | 0.00000 | 0.00000 | 0.00294 | 0.03325 | 0.00323 | 0.03006 |
| 37 VI 5 | 0.00931 | 0.00000 | 0.00000 | 0.00564 | 0.03609 | 0.00296 | 0.03611 |
| 38 Brown bear | 0.14897 | 0.13224 | 0.13237 | 0.12593 | 0.13607 | 0.12245 | 0.13608 |
| 39 Asiatic black bear | 0.12876 | 0.10868 | 0.10879 | 0.11112 | 0.11408 | 0.09263 | 0.11405 |

| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|
| 15 south coastal BC | - | | | | | | |
| 16 Moricetown | 0.03550 | - | | | | | |
| 17 Montana | 0.00359 | 0.04376 | - | | | | |
| 18 NJ/Penn | 0.00380 | 0.04521 | 0.01645 | - | | | |
| 19 Yukon | 0.00000 | 0.04485 | 0.00596 | 0.01465 | - | | |
| 20 Alaska 1 | 0.00000 | 0.04339 | 0.00596 | 0.01472 | 0.00139 | - | |
| 21 Alaska 2 | 0.01109 | 0.04007 | 0.01742 | 0.00702 | 0.01045 | 0.01047 | - |
| 22 QC 1 | 0.02732 | 0.00839 | 0.03637 | 0.03816 | 0.03599 | 0.03455 | 0.03216 |
| 23 QC 2 | 0.02717 | 0.01123 | 0.03629 | 0.03793 | 0.03606 | 0.03751 | 0.03202 |
| 24 QC 3 | 0.02732 | 0.00839 | 0.03637 | 0.03816 | 0.03599 | 0.03455 | 0.03216 |

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 QC 4 | 0.02738 | 0.00980 | 0.03641 | 0.03818 | 0.03743 | 0.03599 | 0.03222 |
| 26 QC 5 | 0.02732 | 0.00839 | 0.03637 | 0.03816 | 0.03599 | 0.03455 | 0.03216 |
| 27 QC 6 | 0.03161 | 0.01122 | 0.04034 | 0.04180 | 0.03899 | 0.03755 | 0.03644 |
| 28 QC 7 | 0.03069 | 0.01083 | 0.03323 | 0.02592 | 0.03000 | 0.03005 | 0.02934 |
| 29 QC 8 | 0.02731 | 0.00841 | 0.03635 | 0.03818 | 0.03604 | 0.03460 | 0.03214 |
| 30 QC 9 | 0.03175 | 0.00701 | 0.04033 | 0.04192 | 0.03754 | 0.03610 | 0.03650 |
| 31 QC 10 | 0.02692 | 0.01042 | 0.03308 | 0.03002 | 0.03794 | 0.03643 | 0.03302 |
| 32 QC 11 | 0.03564 | 0.01123 | 0.04379 | 0.05254 | 0.03898 | 0.03754 | 0.04800 |
| 33 VI 1 | 0.02731 | 0.00700 | 0.03638 | 0.03814 | 0.03599 | 0.03455 | 0.03216 |
| 34 VI 2 | 0.02736 | 0.00980 | 0.03644 | 0.03820 | 0.03748 | 0.03604 | 0.03222 |
| 35 VI 3 | 0.02740 | 0.01123 | 0.03644 | 0.03826 | 0.03610 | 0.03754 | 0.03223 |
| 36 VI 4 | 0.02714 | 0.00659 | 0.03696 | 0.03675 | 0.03005 | 0.03007 | 0.03248 |
| 37 VI 5 | 0.02731 | 0.00700 | 0.03638 | 0.03814 | 0.03599 | 0.03455 | 0.03216 |
| 38 Brown bear | 0.12597 | 0.14254 | 0.11923 | 0.12420 | 0.13566 | 0.13405 | 0.12095 |
| 39 Asiatic black bear | 0.10396 | 0.11865 | 0.10927 | 0.11006 | 0.11372 | 0.11214 | 0.11013 |

| | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|
| 22 QC 1 | - | | | | | | |
| 23 QC 2 | 0.00280 | - | | | | | |
| 24 QC 3 | 0.00000 | 0.00280 | - | | | | |
| 25 QC 4 | 0.00139 | 0.00420 | 0.00139 | - | | | |
| 26 QC 5 | 0.00000 | 0.00280 | 0.00000 | 0.00139 | - | | |
| 27 QC 6 | 0.00279 | 0.00559 | 0.00279 | 0.00418 | 0.00279 | - | |
| 28 QC 7 | 0.00325 | 0.00325 | 0.00325 | 0.00326 | 0.00325 | 0.00752 | - |
| 29 QC 8 | 0.00000 | 0.00280 | 0.00000 | 0.00140 | 0.00000 | 0.00279 | 0.00324 |
| 30 QC 9 | 0.00140 | 0.00421 | 0.00140 | 0.00281 | 0.00140 | 0.00420 | 0.00753 |
| 31 QC 10 | 0.00440 | 0.00735 | 0.00440 | 0.00589 | 0.00440 | 0.00737 | 0.01066 |

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 32 QC 11 | 0.00559 | 0.00842 | 0.00559 | 0.00700 | 0.00559 | 0.00842 | 0.01899 |
| 33 VI 1 | 0.00000 | 0.00280 | 0.00000 | 0.00139 | 0.00000 | 0.00279 | 0.00325 |
| 34 VI 2 | 0.00139 | 0.00420 | 0.00139 | 0.00279 | 0.00139 | 0.00419 | 0.00325 |
| 35 VI 3 | 0.00279 | 0.00281 | 0.00279 | 0.00420 | 0.00279 | 0.00560 | 0.00325 |
| 36 VI 4 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00357 | 0.00353 |
| 37 VI 5 | 0.00000 | 0.00280 | 0.00000 | 0.00139 | 0.00000 | 0.00279 | 0.00325 |
| 38 Brown bear | 0.13224 | 0.13570 | 0.13224 | 0.13385 | 0.13224 | 0.13228 | 0.13145 |
| 39 Asiatic black bear | 0.10868 | 0.11030 | 0.10868 | 0.11026 | 0.10868 | 0.11219 | 0.10172 |

| | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
|---|---|---|---|---|---|---|---|
| 29 QC 8 | - | | | | | | |
| 30 QC 9 | 0.00140 | - | | | | | |
| 31 QC 10 | 0.00440 | 0.00587 | - | | | | |
| 32 QC 11 | 0.00559 | 0.00419 | 0.01033 | - | | | |
| 33 VI 1 | 0.00000 | 0.00140 | 0.00294 | 0.00559 | - | | |
| 34 VI 2 | 0.00139 | 0.00280 | 0.00591 | 0.00700 | 0.00139 | - | |
| 35 VI 3 | 0.00279 | 0.00420 | 0.00733 | 0.00841 | 0.00279 | 0.00419 | - |
| 36 VI 4 | 0.00000 | 0.00359 | 0.00682 | 0.01351 | 0.00000 | 0.00000 | 0.00000 |
| 37 VI 5 | 0.00000 | 0.00140 | 0.00294 | 0.00559 | 0.00000 | 0.00139 | 0.00279 |
| 38 Brown bear | 0.13247 | 0.13430 | 0.13865 | 0.13588 | 0.13225 | 0.13405 | 0.13589 |
| 39 Asiatic black bear | 0.10883 | 0.11058 | 0.10610 | 0.11217 | 0.10868 | 0.11041 | 0.11215 |

**Table 3 Kimura 2-parameter distance matrix for *Ursus americanus* cont.**

| | 36 | 37 | 38 | 39 |
|---|---|---|---|---|
| 36 VI 4 | - | | | |
| 37 VI 5 | 0.00000 | - | | |
| 38 Brown bear | 0.12655 | 0.13225 | - | |
| 39 Asiatic black bear | 0.09644 | 0.10868 | 0.11555 | - |

lineage was estimated at 10,000 years (average divergence) and 150,000 (synonymous). I applied the rate of 10% per million years to average sequence divergence as the number of synonymous changes was far greater than the number of nonsynonymous changes. If the standard rate of mtDNA evolution (2% per million years) is used on average sequence divergence, the divergence of the two black bear lineages is 1.8 million years. Using a molecular clock calibrated on an estimated divergence between black bear and brown bear of 5 million years and an observed average sequence divergence of 11% (Taberlet and Bouvet 1994) suggests that the coastal and continental lineages diverged about 1.6 million years ago. Regardless of which rate or distance estimate is used, the two black bear lineages apparently diverged well before the late Wisconsin glaciation began about 27,500 years BP.

*Relative Rate Test*

Based on the relative rate test, there was no significant increase in the rate of evolution along either coastal or continental lineages using both the Asiatic black bear and brown bear as an outgroup. From the brown bear, average distance of the continental bears was 0.13±0.0028 (s (standard deviation) = 0.0085); average distance of coastal bears was 0.13±0.0010 (s = 0.0054). From the Asiatic black bear, average distance of the continental bears was 0.11±0.0013 (s= 0.0039); average distance of the coastal bears was 0.11±0.00067 (s= 0.0034).

## Discussion

The Haida Gwaii black bear (*U. a. carlottae*) is the most morphologically distinct coastal form. Its suite of divergent characteristics has been used to indicate that it might be the result of long isolation that commenced before the last glacial advance. However, the combined effects of evolutionary processes which are known to take place on islands may be largely responsible for *carlottae*'s unique morphology. In such case, there would be no need to assume that its evolution required isolated persistence in a coastal refugium.

Whether the Haida Gwaii black bear diverged from its conspecifics prior to or after the last glaciation may be ascertained through mtDNA sequence comparisons. Although it is common practice to use gene trees to infer processes at the species level (see Avise 1994 for a review), all such extrapolations must be viewed with caution. Gene trees, such as those produced here are not necessarily congruent with species trees (Maddison 1997; Doyle 1997). With this note of caution, I will discuss the implications of the results with respect to morphology and the issue of coastal glacial refugia.

A single source population of black bears south of the ice sheet is currently believed to be responsible for the recolonization of northwestern North America (Kurtén and Anderson 1980). If true, black bears of this region should exhibit genetic similarity. However, if *carlottae* originated from a different source population, then these black bears should exhibit substantial genetic differentiation and at least two mtDNA groups of black bear should exist in the Pacific Northwest.

Based on cytochrome b sequence data, two mtDNA lineages, coastal and continental, were discovered. Although these two lineages apparently diverged at least

360,000 years BP, low average intralineage sequence divergences of less than 0.1% suggest a recent, possibly postglacial divergence within coastal (*carlottae*, *vancouveri*, *altifrontalis*, *kermodei*) and continental forms (*americanus* and *cinnamomum*).

## Implications for Morphology

### *Body Size*

As a determinant for various biological functions, body size is extremely responsive to selective pressure (Purdue and Reitz 1993). Large body size of coastal bears may be a consequence of history, greater caloric intake in coastal habitats, a function of latitude (Bergmann's rule) or combinations of these factors. One of the attributes of the Haida Gwaii black bear that distinguish it from other coastal subspecies is its large body size. Although black bears from Vancouver Island are not as large, they do demonstrate the same insular trend.

Whether the large body size of the Haida Gwaii bear is ancestral or derived is unknown. However, recent discovery of bear skeletal remains from three individuals (carbon dated at 10,000 years BP) on northwestern Vancouver Island suggest that early postglacial black bear colonists were significantly larger than modern black bears on both Vancouver Island and adjacent continental regions (Nagorsen et al. 1995). If this is true, then large body size may have been a response to the cold periglacial environment of refugia or an abundance of high energy foods like salmon.

### *Dentition and Cranial Features*

Foster (1965) suggested that the heavy molars characteristic of Haida Gwaii bears

might have evolved as an adaptation to coarse beach food which they may have relied upon during the Wisconsin glacial advance. However, as there is no direct evidence that refugia existed on Haida Gwaii that was productive enough to maintain a relict coastal population of black bears, the robust molars and skull may be a post-glacial adaptation to foraging on marine crustaceans. Though Haida Gwaii bears and Vancouver Island bears are similar with regards to their large body size, the dentition of *vancouveri* is much less robust than that found in *carlottae*. If *vancouveri*, *kermodei*, *altifrontalis* and *carlottae* are all derived from the same refugial population as suggested by mtDNA data, then changes in dentition occurred postglacially.

The Haida Gwaii black bear has a massive skull. Although *vancouveri* has a similarly robust skull, it does not appear to be a conserved feature within the coastal mtDNA lineage. Consequently, variations of this feature are probably postglacially derived as well and may be of some adaptive value. Increased skull surface area increases the area for muscle attachment and may help *carlottae* feed in the intertidal zone by giving them greater crushing power.

*Color Variations*

Black bears have the most variable pelage color of any carnivore on earth (Hall 1928; Rounds 1987). Depending upon geographical location, the pelage can vary from jet black, cinnamon, dark brown, yellowish white, to blue. Most of the color variations appear to be concentrated in northwestern North America and have been attributed to sympatry with brown bears, protective camouflage, as well as variations in litter size, sex and weight. A study of black bear colormorph distribution suggests that this feature is

correlated with habitat; black coloration is associated with dense forest canopies such as those found in deciduous and boreal forests of eastern Canada and the temperate rainforests of the Pacific Coast of North America (Rounds 1987). However, there appears to be no association of color ratios with either subspecific taxonomic classifications (Rounds 1987) or the mtDNA lineages found in this study. As such, evolution of these variable pelage colors in black bear may have occurred postglacially and cannot be used to make any assumptions regarding biogeographical history.

Sequence data suggest that *carlottae*, *vancouveri*, *kermodei* and *altifrontalis* shared a recent common ancestor, as did *cinnamomum* and *americanus*. Given the plasticity of morphological characters, it is not unreasonable that the multiple morphological attributes such as cranial and dental features, pelage colour and body size that characterize the coastal and continental subspecies of black bear, including *carlottae* arose in post-glacial periods.

**Implications for Refugia**

The Pacific Northwest is currently believed to have been recolonized from two major refugia: southern Washington and Alaska/Yukon. All black bears in the Pacific Northwest are believed to have been derived from southern Washington, largely because the Alaska/Yukon refugium was still ice locked when southern areas were deglaciated and also because there is no fossil evidence of black bears in northern areas (Kurtén and Anderson 1980). Northern dispersal from Washington probably occurred during the early Holocene, though somewhat impeded by changing sea levels on the coast and instability of ecosystems at the edge of retreating glaciers. Dispersal of these black bears

to coastal and offshore islands may have taken place by walking over ice or newly deglaciated surfaces, but rapidly rising sea levels during the early stages of deglaciation probably made this difficult.

The occurrence of two highly divergent mtDNA lineages in North American black bear differentiating coastal bears (Haida Gwaii, Vancouver Island, north coastal BC and the Olympic Peninsula) from continental bears (Alaska, continental BC, Alberta, central and eastern US) contradicts the supposition that all black bears in the Pacific Northwest were derived from a single southern refugial population. The occurrence of a divergent lineage restricted to Haida Gwaii, Vancouver Island, and coastal regions of mainland British Columbia suggest that these areas were recolonized by the same source population, but not the source population which recolonized continental regions and eventually differentiated into subspecies *americanus* and *cinnamomum*.

Although two refugia (Alaska/Yukon and southern Washington) in the Pacific Northwest are currently known (Pielou 1992), various lines of evidence (see previous chapter) suggest that a third mid-coastal glacial refugium persisted on the now submerged continental shelf separating Haida Gwaii from the mainland. Cores taken midway between Haida Gwaii and the mainland indicate that large portions of the Hecate Strait were terrestrial and ice free during the late Wisconsin (see Josenhans et al. 1993; Barrie et al. 1993; Josenhans et al. 1995). The coastal plain which was uncovered on the shelf would have connected Haida Gwaii and the coastal mainland, and perhaps the northern tip of Vancouver Island as well. The distribution of the coastal mtDNA lineage could be explained if black bear persisted in the Hecate refugium and during the early stages of deglaciation recolonized Haida Gwaii, the coastal mainland and Vancouver Island. The

coastal lineage is probably not seen in high proportions further inland since movement into the interior of British Columbia would have been impeded by the Cordilleran ice sheet and the rapid rise in sea level in early post-glacial periods would have isolated the mainland from Vancouver Island and Haida Gwaii resulting in the present lineage distribution. The continental lineage, which probably resided south of the Cordilleran ice sheet, may have been able to repopulate the interior regions more effectively due to greater accessibility to the mid-continental corridor which became more habitable by the Holocene (Pielou 1992) (Fig 9). Black bear populations in the interior of British Columbia which contain both lineages may represent easterly dispersal of the coastal lineage and a westerly or northerly dispersal of the continental lineage. Although the data suggests a lineage separation largely congruent with the boundary between *kermodei* and *cinnamomum*, individuals with the coastal lineage may extend as far as the Rockies if the outliers reported by both Cronin et al. (1991) and Paetkau and Strobeck (1996) in their molecular studies are equivalent to our coastal lineage. Furthermore, reports of a divergent lineage extending as far down as the most southern extent of *altifrontalis*'s range into California has been reported (Wooding and Ward 1997).

The 3.6 % average sequence divergence (and putative minimum separation time of 360,000 years) between black bear mtDNA lineages indicate that they have persisted through multiple glacial and interglacial periods. This is surprising given the numerous population bottlenecks and opportunities for lineage sorting (see Avise 1994). These two lineages could have been maintained if they were reproductively isolated, but as of yet, there is no evidence to suggest that there are any reproductive barriers. These lineages could have also persisted if they had been geographically isolated for the past 360,000

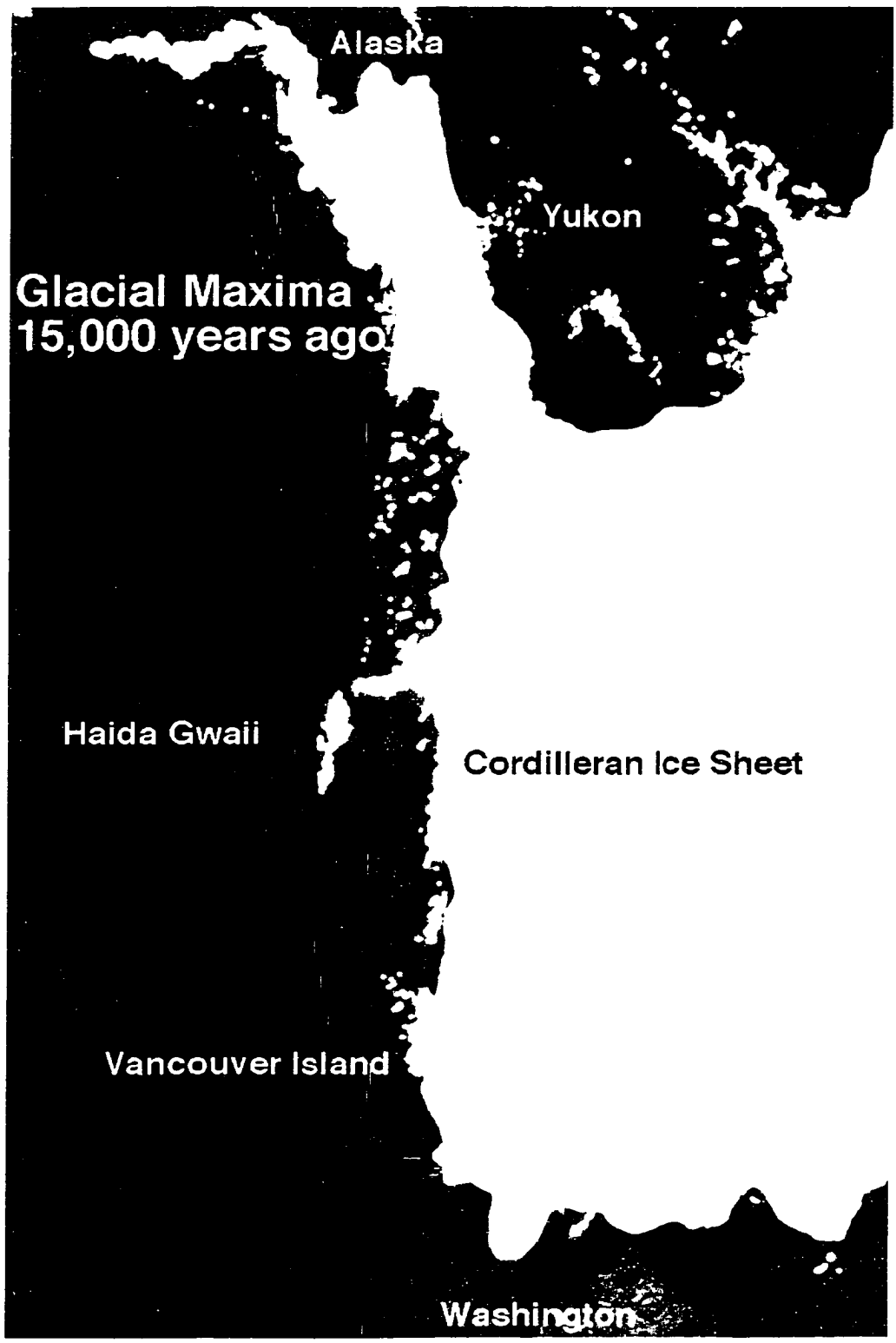

Alaska
Yukon
Glacial Maxima
15,000 years ago
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washington

fig 9

**Figure 9 Putative postglacial dispersal route of black bear from the Hecate Refugium.**

Black bears on Haida Gwaii, Vancouver Island, and coastal mainland of British Columbia and Washington may be derived from a coastal source population which existed on the continental shelf during the late Wisconsin. Given the possibility that the coastal and continental lineages have been in existence since the mid-Pleistocene, this coastal lineage can probably be found in more southern and inland locales. However, it should appear with decreasing frequency in locales further from the northwestern coast.

years. Given the cyclical nature of glacier formation, it is reasonable to suggest that during the last two glacial periods, the coastal lineage was segregated from the continental lineage by being restricted to the Hecate refugium and during the last two interglacials isolated on Haida Gwaii. In other words, Haida Gwaii may be the ultimate source area for the black bear coastal lineage in the Pacific Northwest (Byun et al. 1997).

# Chapter Three

## Marten (*Martes americana*)

### Introduction

The North American marten is both cursorial and arboreal. Marten are generally associated with mixed and mature coniferous forests (Giannico 1986) but are also known to inhabit talus fields (Streeter and Braun 1968) and early post-fire areas where adequate overhead cover is provided for by dense herbaceous growth or a few fallen trees (Johnson and Paragi 1993). In fact, marten can inhabit a variety of habitats so long as food and overhead cover are available (Henault and Renaud 1993). Marten have been referred to as "wilderness animals" because of their strong association with late-successional coniferous stands and their ability to elude detection. Their strong avoidance of open areas is presumably a behavioural adaptation to evade predators like coyotes, great-horned owls and red foxes (Hawley and Newby 1957; Buskirk 1994).

Human activities such as logging and trapping have had devastating effects on marten, particularly in the Pacific Northwest and around their southern-most range

(Buskirk 1994). Despite their ability to use a variety of habitats, it is generally agreed that older coniferous forests are essential for survival and reproductive success (Bissonette and Sherburne 1993) and the loss of such habitats are of pressing concern in marten conservation.

Marten are highly opportunistic, taking anything from small mammals, bird eggs, insects, fish, berries, fruits, carrion (Giannico 1986; Buskirk 1994) and on the coast, marine crustaceans (Cowan and Guiguet 1956). Home range size is quite diverse and averages about 16-24 km $^2$ for male marten in the Pacific Northwest during the winter months. As with most mammals, females are more sedentary. This flexibility in diet and home range size also extends to their activity patterns such that marten can be active both day and night (Cowan and Guiget 1956).

## Evolution of *Martes*

*Martes* first appears in the fossil record during the early Miocene shortly after the appearance of the family Mustelidae. *M. americana* first appears in the fossil record during the late Pleistocene and are believed to have originally populated North America via Beringia sometime during the early Wisconsin about 65,000 to 122,000 years ago (Anderson 1994). These marten apparently spread eastward and were subsequently isolated in eastern North America by the Laurentide ice sheet. Following the retreat of the glaciers, they expanded westward, but were largely excluded from the northwestern coast of North America by a second group of marten which had already colonized this region (Anderson 1994).

These two waves of marten dispersal are currently recognized as two

morphotypes: *americana* and *caurina* (Grinnell and Dixon 1926; Hagmeier 1961). They are distinguished from one another based upon their respective skull and auditory bullae shapes as well as the relative size of their upper molar. Due to *caurina's* greater morphological affinity to the Eurasian sable, they are believed to be the result of the second, more recent dispersal. *Caurina* is restricted to the west coast of Canada, the Sierra Nevada, and Rocky Mountains while *americana* occupies the rest of the species' range.

Differentiation of subspecies within the two morphotypes is believed to have taken place after the continental glaciers retreated (Hagmeier 1955; Foster 1965). Within the group *americana*, there are a total of seven marten subspecies: *M. a. americana*, *M. a brumalis*, *M. a. atrata*, *M. a. abieticola*, *M. a. actuosa*, *M. a. kenaiensis*, *M. a. abietinoides*. There are also seven subspecies within the *caurina* subspecies group: *M. a. caurina*, *M. a. humboldtensis*, *M. a. vancouverensis*, *M. a. nesophila*, *M. a. vulpina*, *M. a. origenes*, and *M. a. sierrae*.

## The Haida Gwaii Marten (*Martes americana nesophila*)

Marten range from about 500 to 680 mm in length and weigh about 500-1400 grams. Their weight and size varies widely as a function of geographical location, age and sex (Giannico 1986; Buskirk 1994). Similarly, the colour of their pelage and their throat/chest patch varies with location and season and also exhibits a substantial amount of individual variation.

The great diversity in pelage color, body size, and throat/chest patches has

presented enormous difficulty in understanding the taxonomy of *M. americana*. Although 14 subspecies of *M. americana* are currently recognized (Hall 1981), these subspecies designations are considered to be arbitrary by some due to the discordant and clinal nature of the variations (Hagmeier 1958; Hagmeier 1961; Anderson 1994). Figure 10 shows the distribution of these subspecies. A brief description of subspecies relevant to this study is given in Appendix II.

Of all the subspecies in North America, the Haida Gwaii marten (*M. a. nesophila*) is the most distinct. Among its most distinguishing characteristics are its thick, heavy rostrum and robust molar teeth (Osgood 1901). It is the largest race of North American marten and demonstrates marked sexual dimorphism (Giannico and Nagorsen 1989). Although this short-haired, pale colored race is considered to be restricted to Haida Gwaii and the Alexander Archipelago (Hall 1981), Giannico and Nagorsen (1989) suggested that *nesophila* should be restricted to Haida Gwaii based on their multivariate analysis of cranial characteristics which demonstrated a significant difference between the marten of these two archipelagos.

Despite the fact that much of the diversity within the North American marten is likely to be clinal (Hagmeier 1961) *nesophila* appears to be a outlier. Hagmeier (1955) showed that although condylobasal length varies clinally according to Bergmann's rule, condylobasal length in *nesophila* was significantly larger than that of any adjacent subspecies to the north, south and to the east. Pelage color, which also varies clinally with precipitation is difficult to explain in *nesophila* which has a pale colored pelage despite its humid environment. Both Hagmeier (1955) and Foster (1965) considered *nesophila* to be significantly different from all other North American marten and

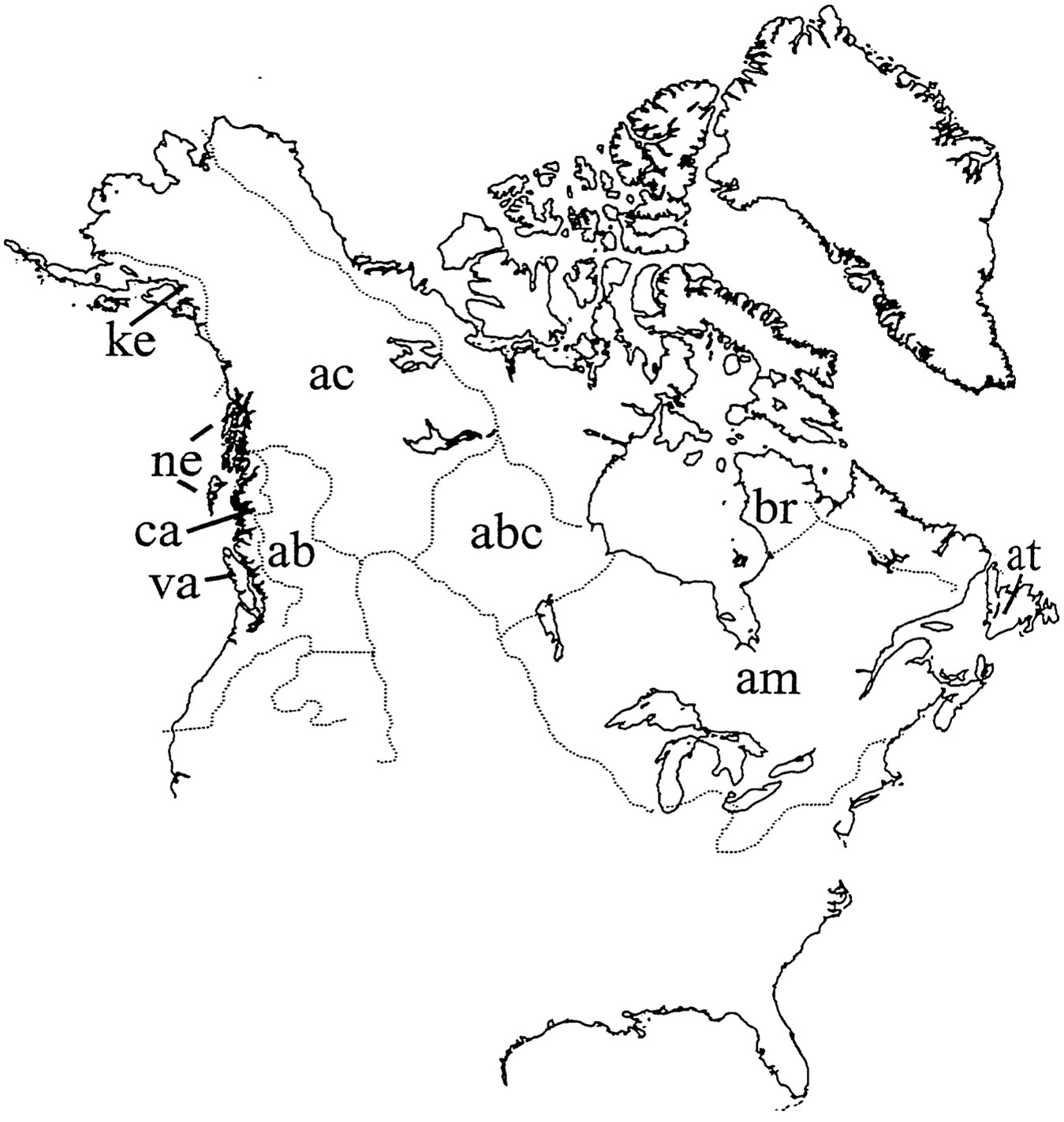



**Figure 10 Subspecies distribution of *Martes americana*.**
Subspecies abbreviations: ne - *nesophila*, va - *vancouverensis*, ca - *caurina*, ab- *abetinoides*, at - *atrata*, ac - *actuosa*, abc - *abietcola*, ke - *kenaiensis*, br - *brumalis*. Adapted from Hall 1981.

suggested that its particular characteristics were too distinct to have evolved in postglacial times. They suggested that *nesophila* evolved in isolation in a refugium on Haida Gwaii, with the added supposition by Hagmeier (1955) that the entire subspecies group *caurina*, was isolated from *americana* during the Wisconsin by persisting in coastal refugia. However, because morphological divergence cannot be equated with divergence time, this issue was left unresolved.

Based on the supposition that molecules evolve at a relatively constant rate, sequences comparisons of the mtDNA gene cytochrome b from *nesophila* and its adjacent conspecifics was done in an attempt to verify the possibility that *nesophila* and perhaps the entire *caurina* group might be glacial relicts. Genetic differentiation between morphotypes corresponding to divergence of at least 27,500 years would imply that these two groups of marten separated prior to the late Wisconsin. Substantial genetic divergence of *nesophila* would support pre-glacial separation and persistence of this subspecies in a coastal refugium.

## Materials and Methods

Many of the methods used to compare marten sequence data were described in the previous chapter. Consequently, I include only the differences from the outlined methods.

**Samples**

DNA from marten was obtained from frozen muscle tissue. A total of 18 samples were used in this study and included the subspecies *nesophila* (n= 6), *vancouverensis* (n=4), *caurina* (n=6), and *abietinoides* (n=1). Sample details are given in Table 4.

**DNA Isolation**

DNA was isolated from muscle tissue using the CTAB procedure described in the previous chapter.

**Amplification**

A 311 bp region from the mtDNA gene, cytochrome b, was amplified using primers H15149 to L14841 (Kocher et al. 1989). An additional primer (L14998 TCA ACT ACG GCT GAA TTA TCC GAT ACA TAC ATG CCA ATG GG) was designed as a diagnostic test to determine between an aberrant lineage detected in Alaska marten. PCR conditions were described in the previous chapter.

**Purification of PCR Products**

PCR products were purified using either commercial columns alone (for manual sequencing), or a combination of Nusieve and columns (for automated sequencing) as described previously.

**Cloning**

PCR fragments that were subsequently sequenced using the ABI automated sequencer were ligated and transformed using the Invitrogen Cloning Kit. Clones were

**Table 4**
**Subspecies, geographical location and sample sizes for marten.**

| Subspecies | Location | Sample size | Sample type | Source |
|---|---|---|---|---|
| *nesophila* | Haida Gwaii | 6 | muscle | authors |
| *vancouverensis* | Vancouver Island | 4 | muscle | authors |
| *caurina* | Smithers, BC | 3 | muscle | authors |
| *caurina* | Prince Rupert, BC | 3 | muscle | authors |
| *abietinoides* | Kamloops, BC | 1 | muscle | authors |
| *atrata* | Newfoundland | 1 | n/a | Hicks and Carr 1992 |

purified as described earlier.

**Automated sequencing**

Clones were sequenced according the suggested protocol suggested by ABI.

**Manual sequencing**

PCR products were sequenced using both [$\alpha$-$^{32}$P] dATP end labeling and [$\alpha$-$^{35}$S] dATP direct incorporation. As with the black bear [$\alpha$-$^{35}$S] dATP was the preferred method of manual sequencing.

**Phylogenetic Analysis**

Sequence consensus from ABI and sequence alignments for marten were generated using Lasergene Navigator (DNASTAR, Madison, WI). A minimum of three sequences were used to generate the sequence consensus. Three types of analyses were used: maximum parsimony (Eck and Dayoff 1966; Fitch 1977), maximum likelihood (Cavelli-Sforza and Edwards 1967) and neighbour-joining (Saitou and Nei 1987). All tests were performed using PAUP * (Swofford in press) and with the Japanese marten (*Martes melampus*) as an outgroup.

*Maximum Parsimony*

All taxa were added randomly in an unweighted parsimony analysis. Heuristic searches were conducted using TBR branch swapping and steepest descent. All equally parsimonious trees were retained during branch swapping. Data were resampled using

2000 heuristic bootstrap replicates. Strict consensus and 50% majority rule trees were generated using all most equally parsimonious trees obtained by this analysis.

*Maximum Likelihood*

Maximum likelihood was performed using the HKY 85 (Hasegawa et al. 1985) model and a $T_i/T_v$ ratio of 2. Empirical base frequencies used were A=0.27, C=0.27, G=0.17, and T=0.30. Starting branch lengths were generated used Rogers-Swofford approximation method. One hundred trees were obtained from a heuristic search and branch swapping on these trees was implemented using tree bisection reconnection and with steepest descent. The trees were evaluated using 2000 heuristic bootstrap replicates.

*Distance*

Distance trees were generated using neighbour-joining (Saitou and Nei 1987) on PAUP* (Swofford in press). The neighbour-joining tree was constructed using distances corrected by Kimura's two parameter model and the resulting tree was statistically evaluated using 2000 neighbour-joining bootstrap replicates. Average sequence divergence was calculated directly from these corrected distances. Synonymous ($K_s$) and nonsynonymous ($K_a$) variation corrected for multiple hits was calculated using MEGA 1.02 (Kumar et al. 1993).

*Relative Rate Test*

A relative rate test (Sarich and Wilson 1973) was done to determine whether either coastal or continental lineage exhibited a significant increase in the rate of

evolution. Distances calculated using Kimura's two parameter model were used. The Japanese marten, *Martes melampus*, was used as the outgroup.

## Results

Phylogenetic analyses from maximum parsimony, maximum likelihood, and neighbour-joining using a 311 bp region of marten cytochrome b (H15149-L14841) (Table 5) revealed the existence of two mtDNA lineages (Fig 11, 12, 13). The continental lineage included all of those marten sampled from British Columbia (Prince Rupert, Smithers and Kamloops) and a marten from Newfoundland (Hicks and Carr 1992) encompassing the subspecies *caurina*, *abietinoides* and *atrata*. The coastal lineage included all marten from Haida Gwaii (*nesophila*) and Vancouver Island (*vancouverensis*) (Fig 14).

### *Maximum parsimony*

Maximum parsimony uncovered two equally parsimonious trees, both with a tree length of 21, a CI of 0.95 and RI of 0.96. As an example of the topology that parsimony produced, the topology from a strict consensus tree is shown (Fig 11). Branch lengths were introduced to indicate the number of synapomorphies.

Out of 28 variable characters, there were six parsimony informative sites in this data set. Continental and coastal lineages were identified by three unambiguous third base transitions. Unfortunately, the small number of informative sites in this data set resulted in lineages only marginally supported by bootstrap analysis. However, the

## Table 5 Marten character matrix.

The following table shows the variable sites in reference to the sequence shown below. Position numbers are shown above the table, nucleotides are shown below position number.

? indicates missing data.

### Prince Rupert 1

```
1                                                40                                                80
GAAACTTCGGCTCCCTCCTTGGAATCTGCCTAATCCTACAGATTCTTACAGGTTTATTTCTAGCCATACACTACACATCA
81                                               120                                              160
GATACAGCCACAGCCTTCTCATCAGTTACCCACATTTGCCGAGATGTCAACTACGGCTGAATTATCCGATATATACATGC
161                                              200                                              240
CAATGGGGCTTCCATATTCTTCATCTGCCTGTTCCTGCACGTCGGACGAGGCCTATACTATGGATCTTATATATACCCCG
241                                              280                          311
AAACATGGAATATTGGCATCATCCTATTATTCGCAGTTATAGCAACAGCATTCATAGGTTACGTTCTGCCA
```

**Position Number**

| Taxon | 1111111111122222222233333333333<br>8891345555688903456678802233333344<br>3405985679914695137910343702678901 |
|---|---|
| Prince Rupert 1 | GGTTTTGATTCATTTCATACCGTCTTGTTTCTGC |
| Prince Rupert 2 | GGTTTTGATTCATTTCATACCGTCTTGTTTCTGC |
| Prince Rupert 3 | GGTTTTGATYCACTTCATACCGTCTTGTTTCTGC |
| BC 1 | GGTTTTGATTCACTTCATACCGTCTTGTNNNNNN |
| BC 2 | GGTTTTGATTCACTTCAatCCGTCTTGTTTCTGC |
| BC 3 | GGTTTTGATTCACTTCATACCGTCTTGTTT???? |
| BC 4 | ????TTGATTCACTTCATACCGTCTTNNTTCTG? |
| Haida Gwaii 1 | GGNTTTGATTCACTTTGTACTGGCTTGTTTCTGC |
| Haida Gwaii 2 | GGTTTTGATTCACTTTGTACTGTCTTGTTTC??? |
| Haida Gwaii 3 | GGTTTTGATTCACTTTGTACTGTGANGTTTCTGC |
| Haida Gwaii 4 | GGTTTTGATTCACTTTGTACTGTCTTGTTTCTGC |
| Haida Gwaii 5 | ???TTTGATTCACTTTGTTCNGTCTTGTTTCTGC |
| Haida Gwaii 6 | GGTTTTGATTCACTTTGTACTGTCTTG??????? |
| VI 1 | GGTTTTGATTCACTCNGTACNGTCTT???????? |
| VI 2 | GGTTTTGATTCACTCTGTACTGTCTTG??????? |
| VI 3 | GGTTTTGATTCNCTCTGTACTGTCTTGTTTCTGC |
| VI 4 | NNTTTTGATTCACTCTGTACTGTCTT???????? |
| Newfoundland | GGTTTYTYTTCACTTCATTAARTCTTGT?????? |
| Japanese marten | GGTCCTGACTAACCTCGTACCGCC?????????? |

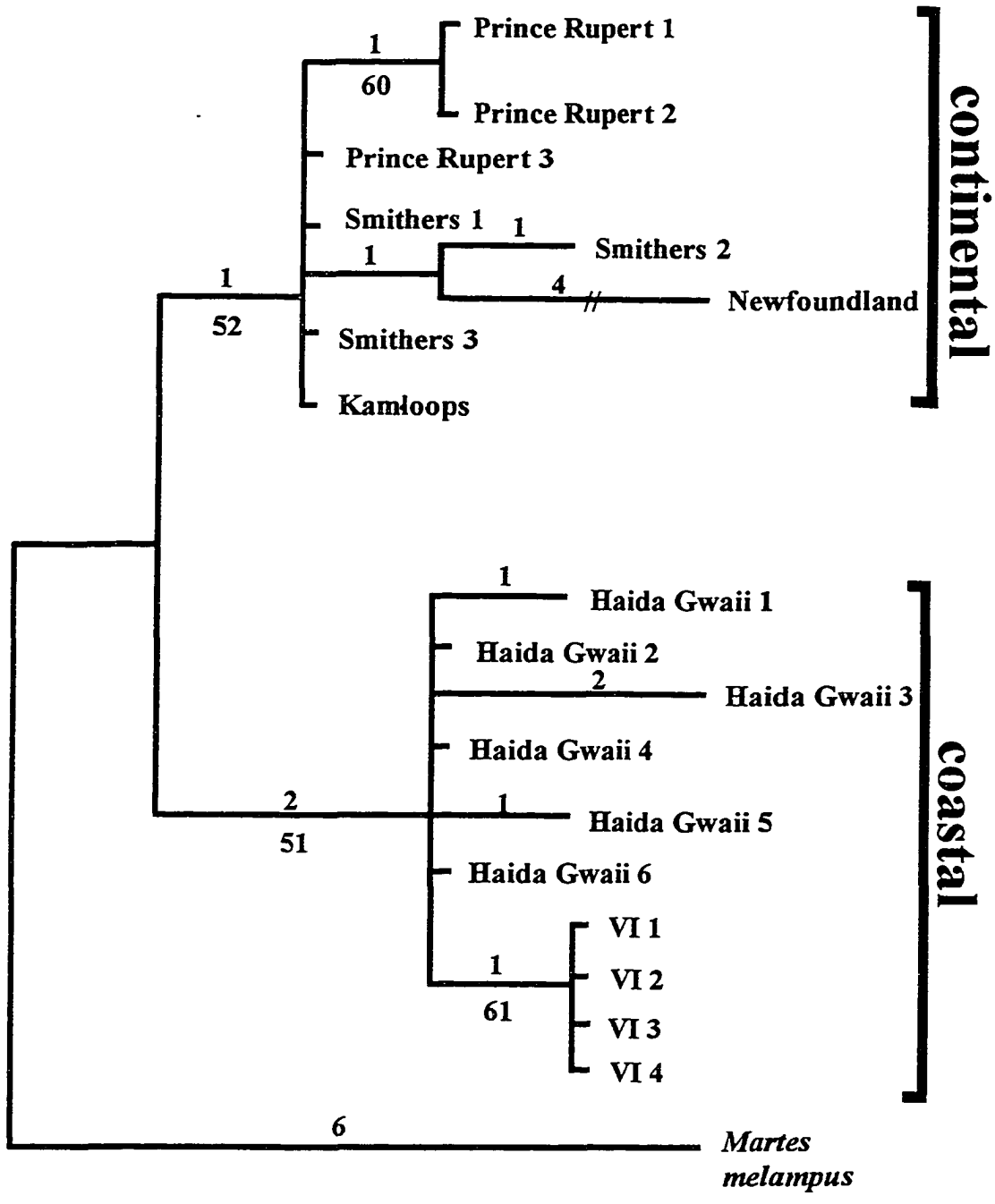



**Figure 11 Marten strict consensus tree.**

Two marten lineages were uncovered by maximum parsimony (strict consensus), continental and coastal. Branch lengths were added to indicate the number of synapomorphies defining each lineage. These, along with autapomorphies are indicated above the branches. Bootstrap values, based on 2000 heuristic replicates, are shown below branches. Tree length was 21, CI =0.95 and RI =0.96. Hatch marks indicate branch lengths which are not to scale.

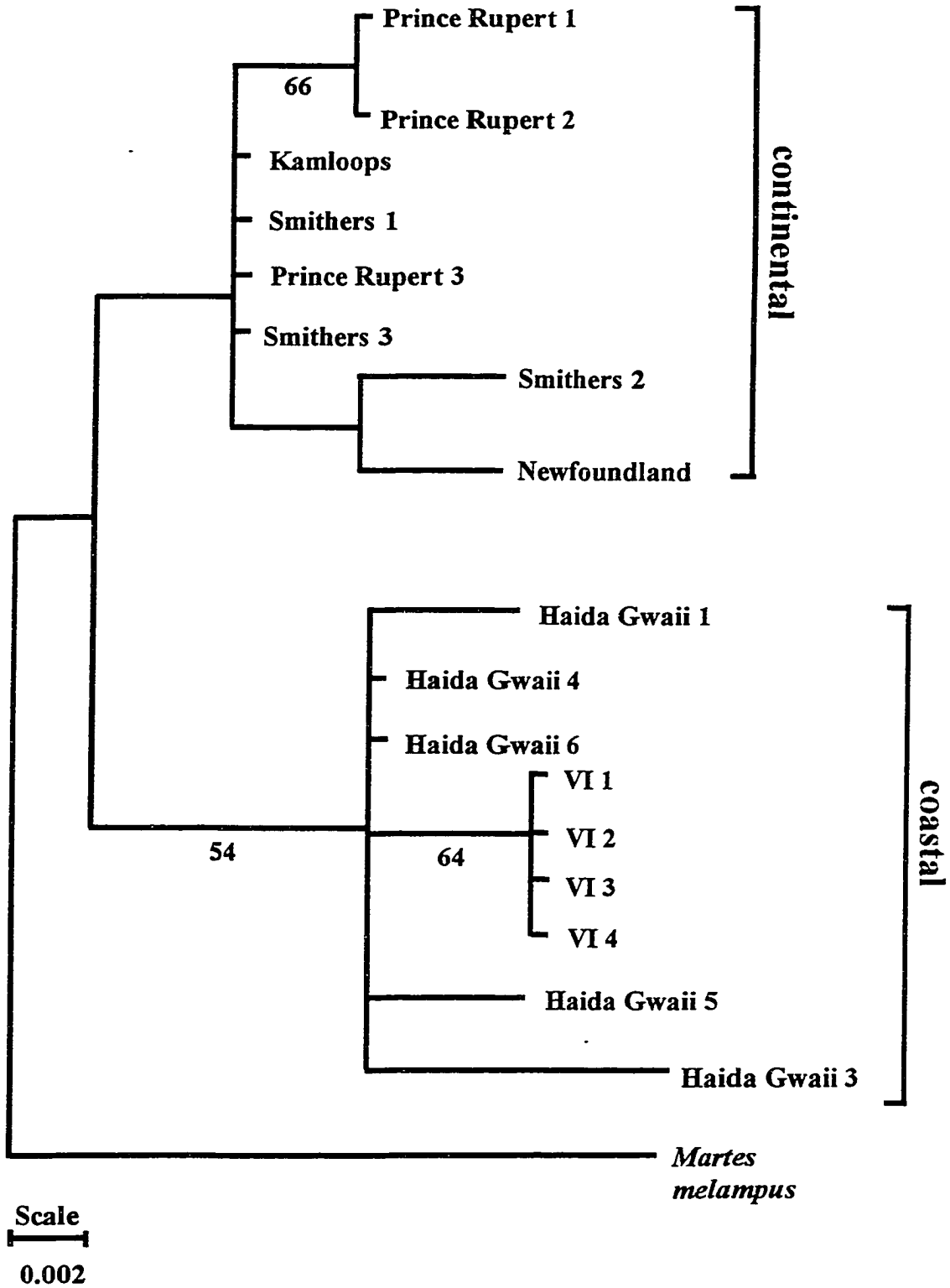



**Figure 12 Maximum likelihood tree for marten.**
This tree demonstrates the topology of a 50% majority rule and strict consensus of 100 trees of equal likelihood (ln likelihood -566.79). Two marten lineages, coastal and continental, are apparent. Branch lengths are scaled to indicate the ln likelihood of each branch. Bootstrap values of 2000 replicates are shown below branches.

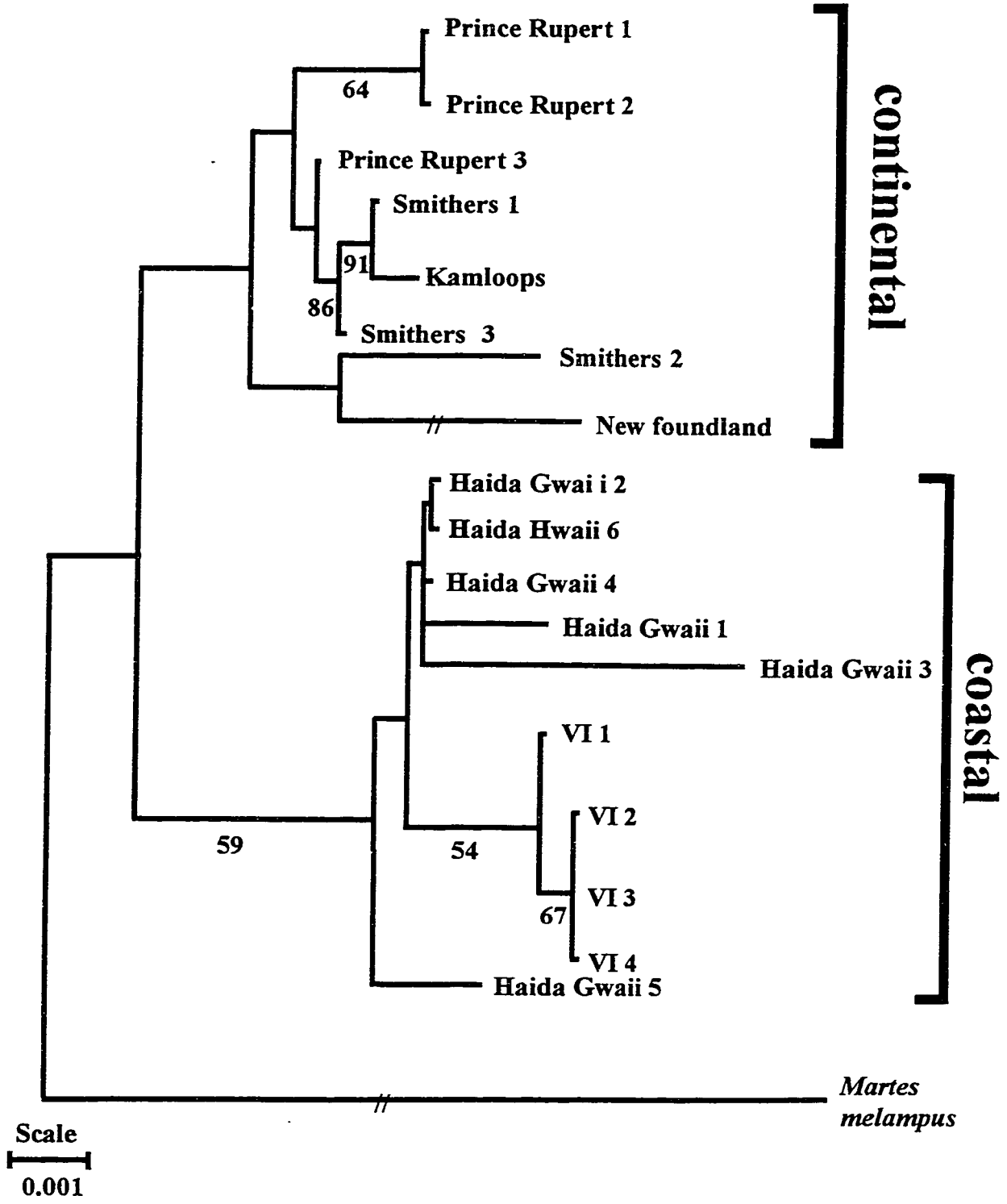



**Figure 13 Neighbour-joining tree for marten.**
Neighbour-joining analysis revealed the existence of the same coastal and continental lineages identified from maximum parsimony and maximum likelihood. However, in this instance, there was no bootstrap support for the continental lineage. Bootstrap values are shown below branches. Branch lengths are drawn to scale and hatch marks indicate those branches which had to be scaled down by 0.01.

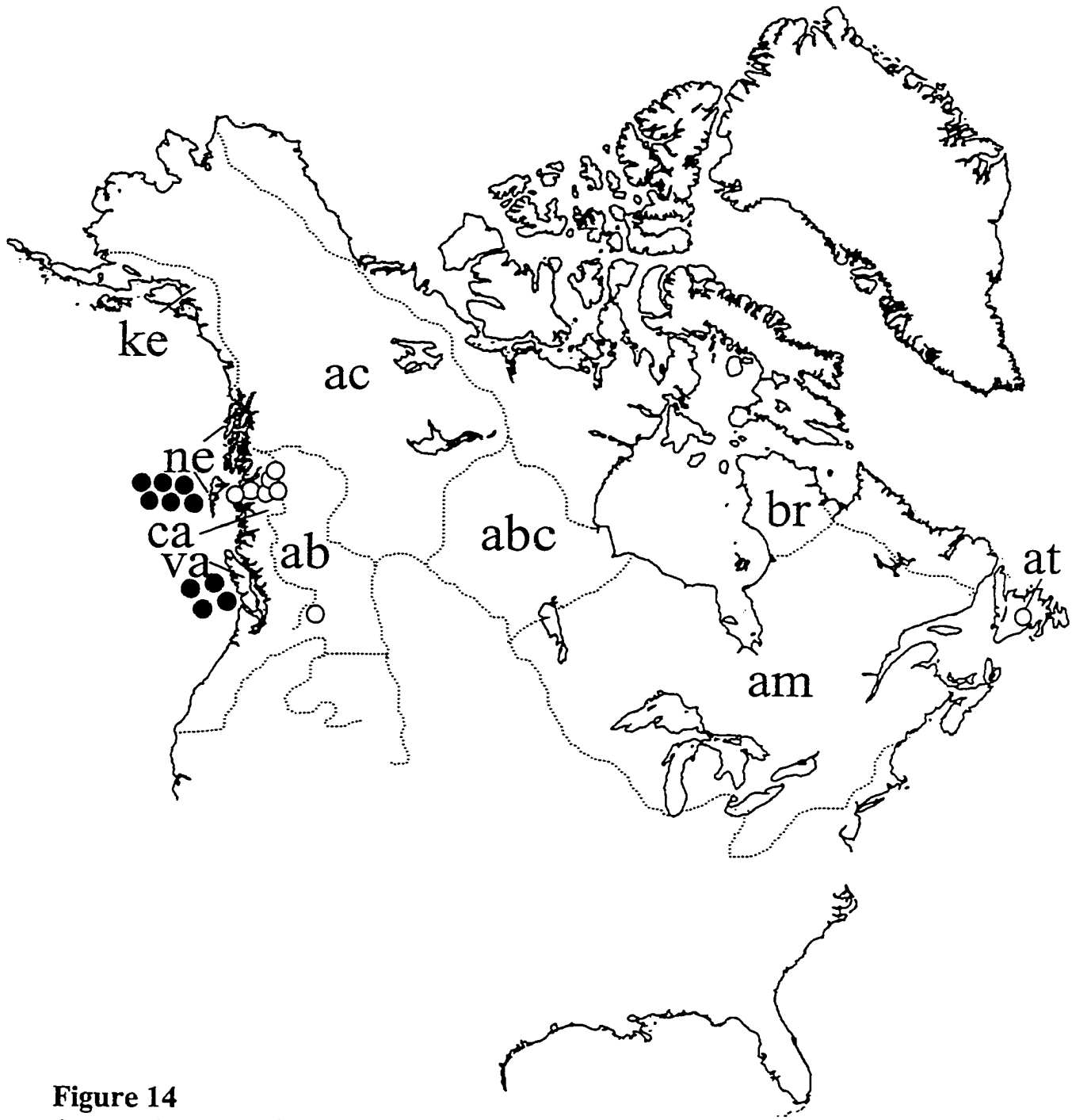



**Figure 14**

Geographic distribution of coastal (●) (n=10) and continental (○) (n=8) mtDNA lineages in marrten. The coastal lineage was found in subspecies *nesophila* (ne) (n=6) and *vancouverensis* (va) (n=4). The continental lineage was found on both the west and east coast of Canada in the subspecies *caurina* (ca) (n=6), *abietinoides* (ab) (n=1) and *atrata* (at) (n=1).

synapomorphies which defined each lineage were unambiguous. A single third base synapomorphy defined the continental lineage (G→A transition at position 100); two third base synapomorphies defined the coastal lineage (C→T transition at position 98 and C→T transition at position 111). The continental synapomorphy occurred in the transmembrane region of the cytochrome b protein. The latter two substitutions occurred in the $Q_i$ region. Within the coastal lineage, marten from Vancouver Island were further defined by another synapomorphy (a C→T first base transition at position 90) located in a transmembrane amino acid. The Vancouver Island lineage was also supported by bootstrap analysis.

*Maximum Likelihood*

A strict consensus and 50% majority rule tree were constructed from 100 trees of equal likelihood uncovered by PAUP*. The ln likelihood was -566.79. Figure 12 shows a maximum likelihood tree which not only reflects the topology of these consensus trees, but also shows branch lengths from one of 100 trees uncovered by a heuristic search. Branch lengths of $10^{-9}$ or less were collapsed to 0. Bootstrap analysis showed marginal support for the coastal lineage with a bootstrap of 54, as well as the Vancouver Island lineage and the Prince Rupert lineage which had bootstraps of 62 and 66 respectively. Removal of the Newfoundland marten and subsequent bootstrapping resulted in support for the continental lineage (57%).

*Distance*

Pairwise distances were calculated for marten using Kimura's two parameter

model (Table 6). The maximum distance observed within *Martes americana* was between the Newfoundland marten and Vancouver Island marten 3 (3.2%). Between most marten (16 pairs) no differences were observed. Figure 13 shows the neighbour-joining tree resulting from this corrected distance matrix. Branch lengths represent transformed distances; all negative branch lengths were collapsed to zero. The overall topology of the neighbour-joining tree was similar to trees generated by maximum parsimony. Marten were placed in one of two lineages, coastal and continental. However, only the coastal lineage was supported by a bootstrap of 59. No bootstrap support was found for the continental lineage. However, this lack of support was probably strongly influenced by the inclusion of the Newfoundland marten in this lineage. Given the current isolation between Newfoundland and BC marten, it is not surprising that the average genetic distance between them is quite high (2.1% or 4 autapomorphies). Removal of the Newfoundland marten and subsequent bootstrap analysis did result in marginal support for the continental lineage (50%). As in maximum parsimony, the Vancouver Island marten formed a lineage within the coastal lineage which was also marginally supported by bootstrapping.

Nonsynonymous variation ($K_a$) was negligible between all marten except for those from Vancouver Island and Newfoundland. Each of these marten were identified by the occurrence of one and two nonsynonymous substitutions respectively. $K_a$ between all continental BC marten was 0. $K_a$ within the continental marten including the marten from Newfoundland was 0.003. $K_a$ was 0 between Haida Gwaii marten. However, between Haida Gwaii and Vancouver Island marten, $K_a$ was 0.006. There were no synonymous differences between coastal marten ($K_s$=0). Within the continental BC

**Table 6 Kimura 2-parameter distance matrix for *Martes americana*.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Prince Rupert 1 | - | | | | | | |
| 2 | Prince Rupert 2 | 0.00000 | - | | | | | |
| 3 | Prince Rupert 3 | 0.00326 | 0.00326 | - | | | | |
| 4 | Smithers 1 | 0.00400 | 0.00400 | 0.00000 | - | | | |
| 5 | Smithers 2 | 0.01094 | 0.01094 | 0.00726 | 0.00795 | - | | |
| 6 | Smithers 3 | 0.00371 | 0.00371 | 0.00000 | 0.00000 | 0.00754 | - | |
| 7 | Kamloops | 0.00485 | 0.00485 | 0.00000 | 0.00000 | 0.00999 | 0.00000 | - |
| 8 | QCI 1 | 0.01839 | 0.01839 | 0.01475 | 0.01583 | 0.02233 | 0.01521 | 0.01864 |
| 9 | QCI 2 | 0.01473 | 0.01473 | 0.01114 | 0.01198 | 0.01874 | 0.01130 | 0.01439 |
| 10 | QCI 3 | 0.02299 | 0.02299 | 0.01923 | 0.01984 | 0.02668 | 0.01919 | 0.02407 |
| 11 | QCI 4 | 0.01468 | 0.01468 | 0.01109 | 0.01195 | 0.01854 | 0.01135 | 0.01426 |
| 12 | QCI 5 | 0.01639 | 0.01639 | 0.01210 | 0.01257 | 0.01222 | 0.01232 | 0.01401 |
| 13 | QCI 6 | 0.01539 | 0.01539 | 0.01162 | 0.01218 | 0.01926 | 0.01159 | 0.01490 |
| 14 | VI 1 | 0.01151 | 0.01151 | 0.00757 | 0.00800 | 0.01545 | 0.00792 | 0.01014 |
| 15 | VI 2 | 0.01975 | 0.01975 | 0.01586 | 0.01630 | 0.02337 | 0.01568 | 0.02062 |
| 16 | VI 3 | 0.01980 | 0.01980 | 0.01591 | 0.01626 | 0.02360 | 0.01594 | 0.01993 |
| 17 | VI 4 | 0.02004 | 0.02004 | 0.01609 | 0.01639 | 0.02370 | 0.01576 | 0.02070 |
| 18 | Newfoundland | 0.02021 | 0.02021 | 0.01678 | 0.01915 | 0.01820 | 0.01837 | 0.02351 |
| 19 | *Martes melampus* | 0.02886 | 0.02886 | 0.02519 | 0.03062 | 0.03785 | 0.03016 | 0.03527 |

**Table 6 Kimura 2-parameter distance matrix for *Martes americana* cont.**

| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|
| 8 QCI 1 | - | | | | | | |
| 9 QCI 2 | 0.00372 | - | | | | | |
| 10 QCI 3 | 0.01130 | 0.00765 | - | | | | |
| 11 QCI 4 | 0.00365 | 0.00000 | 0.00752 | - | | | |
| 12 QCI 5 | 0.00798 | 0.00409 | 0.01206 | 0.00398 | - | | |
| 13 QCI 6 | 0.00381 | 0.00000 | 0.00781 | 0.00000 | 0.00421 | - | |
| 14 VI 1 | 0.00797 | 0.00405 | 0.01191 | 0.00404 | 0.00879 | 0.00402 | - |
| 15 VI 2 | 0.00772 | 0.00390 | 0.01170 | 0.00389 | 0.00850 | 0.00387 | 0.00000 |
| 16 VI 3 | 0.00764 | 0.00396 | 0.01165 | 0.00389 | 0.00806 | 0.00409 | 0.00000 |
| 17 VI 4 | 0.00814 | 0.00395 | 0.01206 | 0.00394 | 0.00866 | 0.00392 | 0.00000 |
| 18 Newfoundland | 0.02971 | 0.02535 | 0.03481 | 0.02574 | 0.02043 | 0.02614 | 0.02276 |
| 19 *Martes melampus* | 0.03314 | 0.03275 | 0.03851 | 0.03313 | 0.03765 | 0.03300 | 0.02996 |

| 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|
| 15 VI 2 | - | | | |
| 16 VI 3 | 0.00000 | - | | |
| 17 VI 4 | 0.00000 | 0.00000 | - | |
| 18 Newfoundland | 0.03062 | 0.03166 | 0.03101 | - |
| 19 *Martes melampus* | 0.03826 | 0.04012 | 0.03868 | 0.04364 | - |

marten, $K_s$ was 0.007; when the Newfoundland marten was considered, $K_s$ was 0.010. The maximum distance was between Newfoundland and Vancouver Island marten with a $K_a$ of about 0.016. Synonymous variation ($K_s$) was highest between the members of the continental and coastal lineages, ranging from 0.05 to 0.06. $K_s$ within the coastal lineage ranged from 0 to 0.027.

The average sequence divergence between coastal and continental lineages was 1.8%. Average sequence divergence within lineages was low. For coastal and continental lineages, average distances were 0.8% and 1 % respectively. Average distance between BC marten of the continental lineage was about 0.6%. All marten from Vancouver Island were identical for the cytochrome b region examined.

Using the standard evolutionary rate of vertebrate mtDNA of 2%/million years for an average sequence divergence of 1.8%, the coastal and continental lineages appear to have diverged about 900,000 years ago. Using a divergence rate of 10% per million years for synonymous changes (Brown et al. 1982; Irwin et al. 1991) for a $K_s$ of 0.060, results in a similar putative separation time of about 600,000 years for coastal and continental lineages. Considering that *M. americana* first appears in the fossil record during the late Pleistocene and that marten are believed to have occupied North America for only 65,000-122,000 years, these molecular based divergences may be overestimates. Overestimation may be due to inappropriate use of molecular rates or inflated divergence values caused by small sequence length and limited number of substitutions. Because of limited variation, no attempt was made to estimate intralineage divergence.

*Relative Rate Test*

Relative rate test indicated no significant rate increase along continental and coastal lineages. The distance from *Martes melampus* to the continental lineage was 0.32±0.0017 (s= 0.0048); distance from *Martes melampus* to the coastal lineage was 0.036±0.0011 (s= 0.0036). These data suggest that the differences between the two lineages are not artifacts of a rate increase.

## Discussion

The Haida Gwaii marten (*nesophila*) is characterized by its exceptionally large size, robust rostrum and heavy dentition, as well as very pale pelage (Hagmeier 1955; Hagmeier 1961; Foster 1965). However, evolution of these characteristics and the characteristics defining the marten subspecies in general might be caused by postglacial selection across ecological gradients and/or rapid evolution due to population fragmentation. Whether *nesophila's* suite of distinct morphological traits originated postglacially or preglacially may be revealed using mtDNA sequence comparisons; significant genetic differentiation would indicate that this subspecies diverged from its conspecifics prior to the Fraser glaciation (27,500 years BP) and little genetic differentiation would imply that it diverged after glacial retreat (12,000 years BP).

The following discussion has two major limitations. First, it is based on extrapolations from a single locus gene tree. Second, low levels of sequence divergence and small number of synapomorphies permit only limited confidence that the topology of the gene tree is correct. However, confidence in the results is gained from the observation that substitutions defining the coastal and continental marten are

unambiguous (see Results-*Parsimony*) and that the marten tree is largely congruent with the trees obtained for black bear. Tree congruency across independent taxa is considered a reasonable indicator that gene topologies are an accurate reflection of species/subspecies trees (Avise 1994). Noting these limitations, the implications of these results to morphology and coastal refugia are discussed.

## Implications for Morphology

### Subspecies groups *caurina* and *americana*

Grinnell and Dixon (1926) were the first to suspect that there were two types of marten in North America, *caurina* from the west coast and *americana* from the rest of the continent. Recent mtDNA evidence (Carr and Hicks 1997) suggests that these two morphotypes are genetically distinct, a finding consistent with the hypothesis that *caurina* and *americana* are the result of two separate waves of dispersal into North America. Based on a 401 bp target of cytochrome b, Carr and Hicks (1997) found two mtDNA lineages apparently congruent with the *caurina* and *americana* morphotypes. These two lineages may be congruent to the continental and coastal lineages identified here. If so, then the two major marten lineages previously described by Carr and Hicks (1997) as *americana* and *caurina* are actually not congruent with the *americana* and *caurina* morphs as they surmised. In this study, marten from coastal British Columbia, morphologically described as *caurina*, had the same haplotype as marten from Newfoundland which is within the range of *americana*. Similar cytochrome b sequences in these morphotypes suggests that they differentiated in postglacial times, contradicting previous speculations that the physical features characterizing these two types of marten

evolved through isolation in different lateWisconsin refugia. The individual from Newfoundland (*americana*) shared a single synapomorphy with BC's mainland *caurina*. It is important to point out that this synapomorphy may be the result of convergence in which case the placement of the Newfoundland marten in the continental group would be erroneous. This can only be confirmed by more sequencing and sampling.

The presence of both coastal and continental lineages in the morphotype *caurina* may also be due to postglacial hybridizations. Such integration between morphotypes has been documented in northern Idaho and Montana (Wright 1953) as well as southwestern British Columbia (Hagmeier 1961). Although the $F_1$ hybrids may exhibit features intermediate with the two morphotypes (Wright 1953), successive generations may conform to the local ecotype which would be identified on the coast of BC as *caurina*. If the presence of the continental lineage within *caurina* is due to introgression then the coastal lineage should also be found in *caurina* populations on the mainland and should presumably be dominant. However, all six individuals of the subspecies *caurina* (three from Smithers and three from Prince Rupert) examined in this study possessed the continental haplotype. However, the higher frequency of continental haplotypes occurring on the coast of northwestern BC may be due to lineage sorting and/or sampling biases.

Occurrence of both coastal and continental lineages within the *caurina* morph may also be due to separation of mainland *caurina* (Prince Rupert and Smithers) from coastal *caurina* (Haida Gwaii and Vancouver Island) during the last glaciation. The coastal and continental marten lineages differ by an average sequence divergence of 1.8%, suggesting that they may have diverged prior to the Wisconsin. The two mtDNA

lineages may be the result of thousands of years of isolation in different refugia and the morphological similarity between coastal and mainland *caurina* may be the result of postglacial convergence. Whether the occurrence of both lineages in *caurina* populations on the mainland are the result of postglacial hybridization or its derivation from an *americana* ancestor and subsequent morphological change, the morphological differences between *caurina* and *americana* are likely a response to contemporary ecological conditions rather than retention of relictual features. In light of this information, taxonomic revisions suggesting that Vancouver Island marten (*vancouverensis*) is synonymous with *caurina* should be viewed with caution until further data are available.

**Morphological characteristics of *nesophila***

The Haida Gwaii marten is the most morphologically distinct and most isolated subspecies of all North American marten (Hagmeier 1961; Foster 1965; Giannico and Nagorsen 1989). Reasons for this strong divergence has been attributed to long isolation since pre-Wisconsin times (Hagmeier 1955; Foster 1965) as well as to rapid differentiation in response to Haida Gwaii's unique selective regime. Every divergent characteristic, including large body size, marked sexual dimorphism, heavy skull, jaws and teeth, can be accounted for as some functional response to an insular habitat (see Foster 1965). However, Foster (1965) found the pale pelage of *nesophila* puzzling. Like *nesophila*, *vancouverensis* has a pale colored pelage. Both *nesophila* and *vancouverensis* occupy the coastal western hemlock biogeoclimatic zone, an area of mild winters, cool summers and high annual precipitation. As pelage color varies clinally in marten according to Gloger's rule; marten in areas of high precipitation tend to have darker

pelage presumably for better camouflage. As *vancouverensis* shares the same genetic lineage as *nesophila*, pale pelage may be an ancestral condition. However, given the absence of competitors/predators from Haida Gwaii, Vancouver Island and the Alexander Archipelago like red fox (*Vulpes vulpes*), coyote (*Canis latrans*), lynx (*Lynx canadensis*), bobcat (*Lynx rufus*), and the fisher (*Martes pennanti*), there may have not have been a pressing need for more cryptic coloration. Furthermore, the absence of competitors may have increased diurnal activity and pale pelage may have been an adaptation to this change in foraging behaviour.

Based on the overall skull resemblance, Hagmeier (1961) considered that *nesophila* also occurred north of Haida Gwaii on the Alexander Archipelago although this was not supported in a more recent multivariate analyses of cranial and dental features (Giannico and Nagorsen 1989). Stone and Cook (pers. comm.) who have undertaken an analysis of Alaskan marten found that marten from the Alexander Archipelago have a divergent mtDNA lineage apparently synonymous with the coastal lineage of marten found on Haida Gwaii and Vancouver Island. Despite genetic similarities with *vancouverensis*, *nesophila* exhibits a greater degree of morphological divergence. As such, the features characterizing *nesophila* are likely to be reflections of its unique contemporary insular habitat rather than of its biogeographical history.

Although the coastal and continental marten lineages apparently diverged prior to the last glaciation, the subspecies that make up each of these lineages probably originated in post-glacial times.

**Implications for Refugia**

During the Fraser glaciation, marten were found in refugia both north (Youngman 1993) and south (Kurtén and Anderson 1980) of the Cordilleran ice sheet. If all marten in North America were derived from these two source populations, then two lineages would be expected, one localized in northern regions and the other found over the rest of the continent. Given that sequence data for northern marten were not obtained, one mtDNA lineage was expected which would include the subspecies *abietinoides*, *vancouverensis* and *nesophila*.

Two marten lineages were identified using partial sequences of cytochrome b. One lineage (continental) was found on the BC mainland and Newfoundland; the other lineage (coastal) was found on Haida Gwaii and Vancouver Island. Sequence divergence between the coastal and continental lineages suggests that they diverged prior to the Wisconsin glaciation. Though the number of synapomorphies identifying the continental and coastal marten lineages is low, the identification of the same marten lineage on the Atlantic and Pacific coast and a different lineage on Haida Gwaii and Vancouver Island make this distribution biologically significant. This is especially true considering that *atrata*, which also possesses the continental lineage identified in British Columbian marten, is currently isolated on Newfoundland and is believed to be derived from a refugium on the northeastern coast of North America (Buskirk 1994).

Other investigations have also revealed a divergent marten lineage in the Alexander Archipelago (Stone 1997, pers. comm.), in Montana (Cook 1998, pers comm.), in south-eastern Wyoming and in northern Idaho (Carr and Hicks 1997). If this divergent lineage is congruent to the 'coastal' lineage discovered here, then there are

several explanations which could account for its occurrence in continental areas.

Given the recent appearance of marten in North America, the coastal and continental lineages may actually be ancestral polymorphisms which scattered randomly during postglacial recolonization. Although this would account for the presence of the coastal lineage in areas like Montana, Wyoming and northern Idaho, it does not explain the apparent concentration of this lineage on islands of the Pacific Northwest

The current distribution of these lineages may be due to dispersal from southern refugia. If the divergent lineage which I have termed coastal actually originated south of the Cordilleran ice sheet, then the presence of this lineage on Haida Gwaii, Vancouver Island, and the Alexander Archipelago must be due to northward dispersal and eastward dispersal into Montana, Wyoming and Idaho.

While it is not impossible that marten arrived on Haida Gwaii postglacially, McCabe and Cowan (1945) suggested that the absence of marten on the more accessible islands east of this archipelago was evidence against such a colonization route. However, their absence could also be accounted for by their failure to establish stable populations on these small islands and the inability to uncover sub-fossil evidence of these early postglacial marten populations. Of more importance is the difficulty of early postglacial migration up the coast due to rising sea levels and unpredictable events such as advances and retreats at glacial margins. At the southern edge of the Cordilleran Ice Sheet, the enormous lakes which had formed by about 15,000 years BP also impeded early postglacial movement up the coast and into the interior (Pielou 1992). Furthermore, newly deglaciated areas were probably unsuitable for marten as these areas were likely to have lacked sufficient overhead cover, restricting significant northward dispersal during

the early stages of glacial retreat. Furthermore, by the time Vancouver Island was probably inhabited by marten (after 11,500 years BP), rising sea levels had severely restricted access to Haida Gwaii and the Alexander Archipelago making it extremely difficult for marten to have colonized these islands via water (Vancouver Island to Haida Gwaii to the Alexander Archipelago).

The occurrence of two marten lineages in the Pacific Northwest, one on Haida Gwaii, Vancouver Island and one found over the majority of the continent, may be better accounted for by the suggestion that the coastal lineage was derived from a source area in the vicinity of British Columbia's offshore islands. This is not impossible given that there is physical evidence of terrestrial, ice free areas in Hecate Strait adjacent to Haida Gwaii during the late Wisconsin (see Barrie and Conway for review in press). The black bear phylogeographic pattern described in the previous chapter which implied that this species may have recolonized North America from coastal and southern continental refugia suggests that the largely congruent marten phylogeography reported here may have also been similarly influenced .

The presence of three outliers in Montana, Idaho and Wyoming, is not entirely unexpected given the thousands of years that this lineage has had to disperse. However, the distribution frequency of the coastal lineage in North America needs to be documented through further sampling. Such data might provide important clues as to the source area.

Although it is difficult to determine whether this coastal lineage was derived from a southern or coastal source, multiple lines of evidence including its congruency with the black bear data, the existence of ice free areas in Hecate Strait, and the high frequency of

the coastal lineage on Haida Gwaii and Vancouver Island, suggest that postglacial recolonization of marten from coastal refugia is not unlikely (Fig 15).

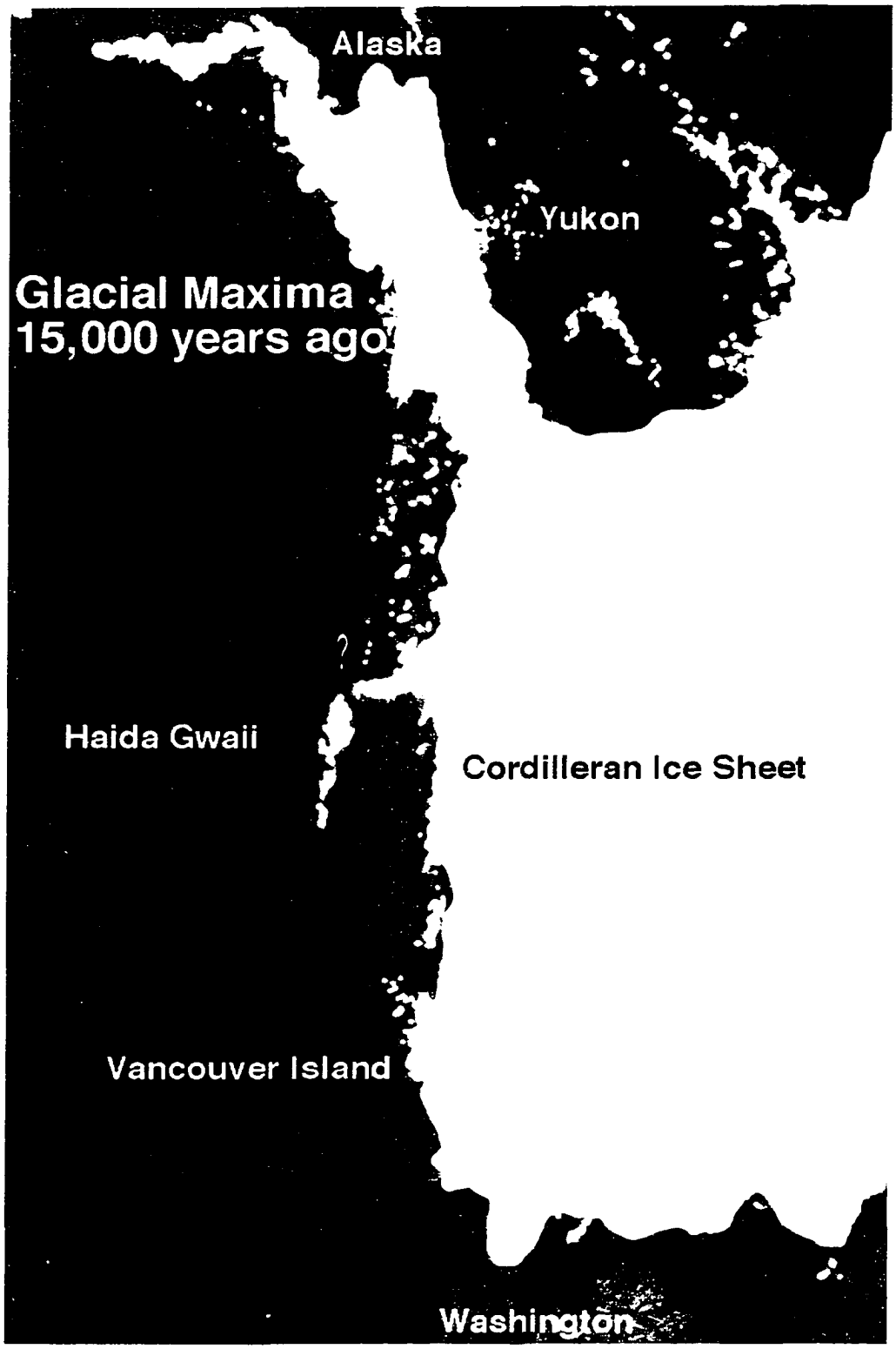

Alaska
Yukon
Glacial Maxima
15,000 years ago
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washington

fig 15

**Figure 15 Putative postglacial dispersal route of marten from the Hecate Refugium.**

MtDNA analyses suggests that marten on Haida Gwaii and Vancouver Island were derived from the same source population which may have existed on the continental shelf during the late Wisconsin. Marten from this coastal source population likely migrated to other parts of the coast. Stone and Cook (pers. Comm.) did find a divergent lineage north of Haida Gwaii which may be equivalent to the lineage found on Haida Gwaii and Vancouver Island. However, given the uncertainty, northern dispersal is indicated by a question mark. It is reasonable to assume that this coastal lineage has dispersed further inland and should appear with decreasing frequency in locales further from the Pacific Northwest.

# Chapter Four

## Short-tailed weasel (*Mustela erminea*)

### Introduction

The short-tailed weasel has a holarctic distribution. Weasels are found anywhere small mammals and birds reside such as rock slides, deciduous forests, margins of coniferous forests, sea-beach debris, and banks of rivers and streams (Cowan and Guiguet 1956; Banfield 1974). Like the black bear, short-tailed weasels are extremely opportunistic with a diverse diet consisting of small rodents, birds, insectivores, lagomorphs, insects, and large bodied mammals (King 1989a). They are strictly carnivorous and will consume almost anything that they can kill.

Short-tailed weasels exhibit extreme variability in body size and sexual dimorphism (Ralls and Harvey 1985; Powell and King 1997). Their body size probably evolved during the Pliocene as an adaptation for hunting small rodents on grassland and later became useful for hunting prey under snow during the glacial periods of the

Pleistocene (King 1989b). An unfortunate consequence of their small body size is their sensitivity to thermal stress and high metabolic rate. In order to supply their energy requirements, weasels need to consume more than a quarter of their body weight every day and as such typically require large home ranges (King 1989a). Short-tailed weasels need to forage constantly and usually do so in periods of 10-45 minutes interspersed with long resting periods lasting up to five hours. This foraging behaviour limits their ability to disperse long distances and weasels must often cache their food in order to survive food shortages (King 1989a).

**Evolution of *Mustela***

The ancestors of the weasels were probably marten-like forest carnivores which lived during the Miocene. With the coming of the Pliocene, the climate began to cool and savannas gradually replaced forests. These open grasslands soon became populated by early rodents and weasels underwent a size reduction in order to hunt these rodents in their runways and burrows (King 1989a). The ancestor of the short-tailed weasel, *M. plioerminea*, first appeared in Eurasia approximately four million years BP during the Pliocene (Kurtén 1968). Only one transitional form, *M. paleoerminea*, is known to have existed 700,000 years BP, eventually giving rise to *M. erminea* 200,000 years later. *M. erminea* reached North America during the late Kansan to early Illinoian glacial period (King 1989a), crossing the Bering land bridge into Alaska (Kurtén and Anderson 1980) and spreading extensively throughout North America. During the last glacial advance, the short-tailed weasel retreated both north and south of the Cordilleran ice sheet (Kurtén and Anderson 1980; Anderson 1980). Macpherson (1965) hypothesized that *M. erminea*

from Alaska, Yukon, and western NWT were likely to have been derived from eastern Beringia and *M. erminea* from southern Yukon, British Columbia, and southern NWT were likely to have originated south of the ice sheet.

Since the end of the Wisconsin glacial advance, the short-tailed weasel has undergone enormous morphological differentiation and has, according to Hall (1981) differentiated into twenty North American subspecies. Figure 16 shows the distribution of these subspecies. A brief description of those relevant to this study are given in Appendix II.

## The Haida Gwaii Weasel (*Mustela erminea haidarum*)

The short-tailed weasel exhibits enormous variation in body size, pelage color, length and color of tail and degree of sexual dimorphism. These characteristics have been used to identify subspecies but the variability of these characters has led to considerable confusion regarding the taxonomy of not only short-tailed weasels but the entire genus *Mustela* (King 1989a).

Of all short-tailed weasels, *M. e. haidarum* of Haida Gwaii is the most morphologically divergent and was originally described by Preble (1898) as a distinct species, *Putorius haidarum*. Although Hall (1945a) later reduced *P. haidarum* to a subspecies of *M. erminea*, given the numerous and significantly distinct morphological features characterizing *haidarum*, Hall considered this subspecies to have the greatest claim to full species status than any other subspecies of *M. erminea*. *M. e. haidarum* is distinguished by the great extent of white coloring on its underparts and extension of this color to the underside of the tail, which on the upperside is black (Cowan and Guiguet

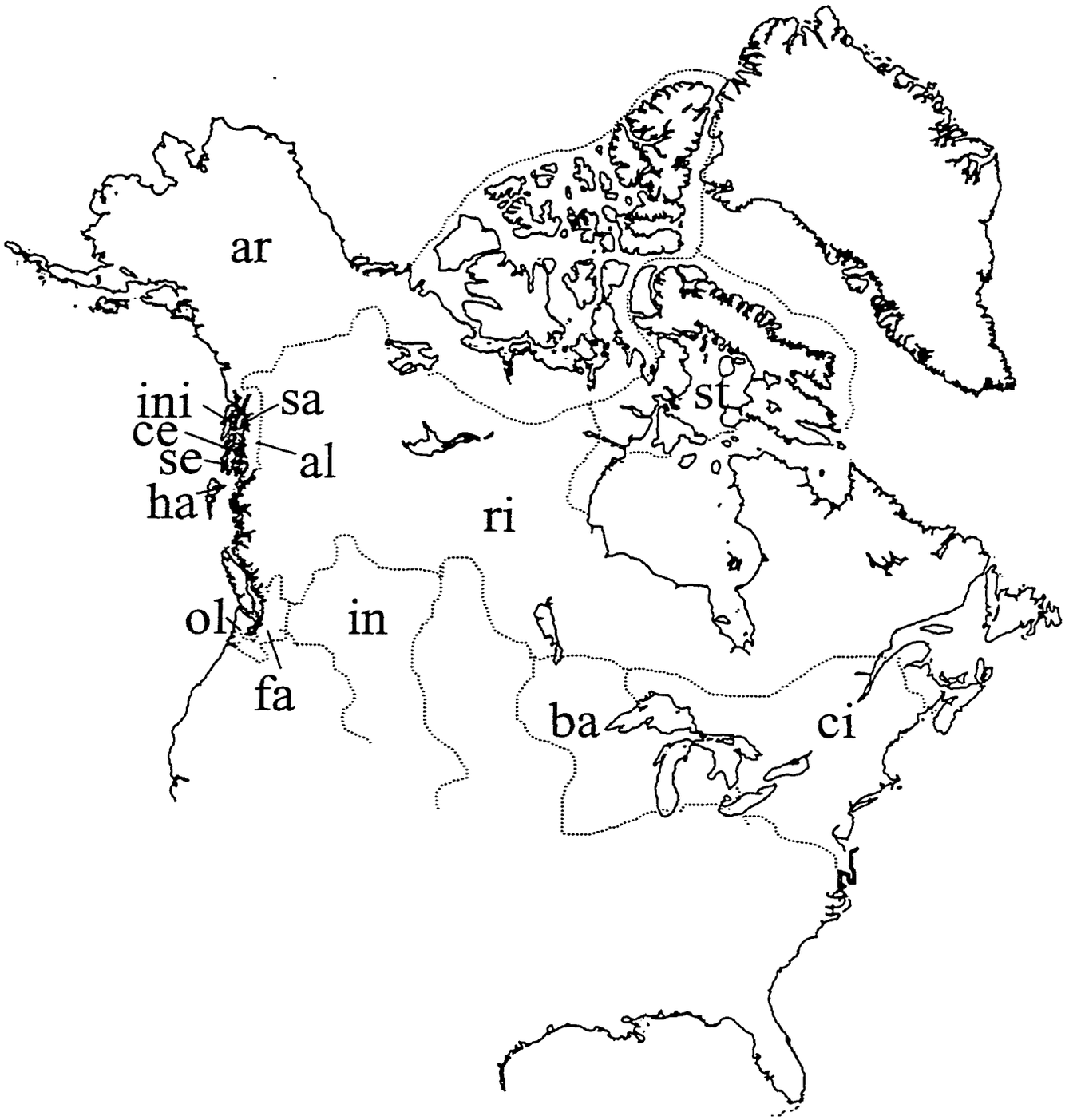



**Figure 16 Subspecies distribution map of *Mustela erminea*.**
Subspecies abbreviations: ha - *haidarum*, an - *anguinae*, fa - *fallenda*, in - *invicta*, ri - *richardsonii*, ar - *arctica*, ba - *bangsi*, ci - *cicognanii*, ini - *initis*, se - *seclusa*, ce - *celenda*, sa - *salva*, al - *alascensis*, ol - *olympica*, st - *streatori*, gu - *gulosa*, mu - *muricus*, sem - *semplei*, pol - *polaris*, ka - *kadiacensis*. Adapted from Hall 1981.

1956). Pelage color is noticeably lighter than the pelage color of surrounding insular species (Foster 1965). The skull is relatively flat, short and stocky, which is most obvious in its broad rostrum and interorbital regions (Osgood 1901). Degree of sexual dimorphism is minimal in *haidarum* (Hall, 1951; Foster 1965).

Short-tailed weasels are well adapted to periglacial environments and would have done quite well in glacial refugia. Given the ability of weasels to survive in harsh, cold environments and the degree of morphological divergence characterizing *haidarum*, both Hall (1951) and Foster (1965) considered that *M. e. haidarum* was likely to be a glacial relict which remained on Haida Gwaii during the Wisconsin, isolated from refugial populations north and south of the Cordilleran ice sheet.

To examine the relictual status of *M. e. haidarum*, its relationship with proximal conspecifics was established using mtDNA sequences of cytochrome b. Given the persistence of short-tailed weasels in both the Alaska/Yukon refugium and southern Washington, it is expected that two groups corresponding to each of these refugia currently exist. The depth of divergence between these groups would be expected to reflect a pre-Fraser separation of about 27,000 years. However, if *haidarum* is a glacial relict as suggested by Foster (1965) which was isolated from other refugial populations on the mainland, then *haidarum* should be genetically unique and three mtDNA groups instead of two would be expected.

## Materials and Methods

### Samples

A total of 31 individuals were examined in this study. These included subspecies *haidarum* (n=5), *anguinae* (n=7), *richardsonii* (n=8) *invicta* (n=4), *fallenda* (n=3), *arctica* (n=2), *bangsi* (n=1), and *cicognannii* (n=1). DNA from short-tailed weasels was obtained from fresh muscle and preserved skin. Preserved skins were obtained from the University of British Columbia's Cowan Vertebrate Museum. Small clippings or scrapings were taken from the ear lobes, corner of the lips and/or underside so as not to destroy the overall integrity of the mounts. I attempted to sample those areas that were also less likely to have been extensively handled.

As a precaution against exogenous and cross contamination, fresh gloves and scalpel blades were used for every sample taken. Each sample was stored individually in a sterile eppendorf tube and subsequently stored at room temperature. Sample details are given in Table 7.

### DNA Isolation

DNA was extracted from both muscle and preserved skin using the CTAB method (see Chapter 2). However, longer incubation time in CTAB was required (usually four to six hours) and at slightly higher temperatures (65-70°C). As a precaution against contamination with contemporary samples, all reagents were specifically reserved for ancient DNA work.

**Table 7**
**Subspecies, geographical location and sample sizes for short-tailed weasel.**

| Subspecies | Location | Sample number* | Sample size | Sample type | Source |
|---|---|---|---|---|---|
| *haidarum* | Tlell, Haida Gwaii | 8772 | 1 | museum skin | author |
| *haidarum* | Haida Gwaii | M39 | 1 | museum skin | author |
| *haidarum* | Haida Gwaii | M40 | 1 | museum skin | author |
| *haidarum* | Haida Gwaii | M42 | 1 | museum skin | author |
| *haidarum* | QCI city, Haida Gwaii | n/a | 1 | frozen muscle | author |
| *anguinae* | Vancouver Island (VI) | n/a | 4 | frozen muscle | author |
| *anguinae* | VI | 2736 | 1 | museum skin | author |
| *anguinae* | VI | 2371 | 1 | museum skin | author |
| *anguinae* | Port Hardy, VI | 4843 | 1 | museum skin | author |
| *richardsonii* | Gavin Lake, B.C. | 16894 | 1 | museum skin | author |
| *richardsonii* | Surf Inlet, B.C. | 3206 | 1 | museum skin | author |
| *richardsonii* | Hemp Creek, B.C. | 3209 | 1 | museum skin | author |
| *richardsonii* | Stum Lake, B.C. | 3326 | 1 | museum skin | author |
| *richardsonii* | Huntington | 4853 | 1 | museum skin | author |
| *richardsonii* | Saskatoon | 4833 | 1 | museum skin | author |
| *richardsonii* | Meyer's Pass | 7472 | 1 | museum skin | author |
| *richardsonii* | interior B.C. | n/a | 1 | frozen muscle | author |
| *richardsonii* | Britannia Beach, B.C. | 8780 | 1 | museum skin | author |
| *invicta* | Cameron Lake, B.C. | 3533 | 1 | museum skin | author |
| *invicta* | Hope, B.C. | 3535 | 1 | museum skin | author |
| *invicta* | Wentworth Lake, B.C. | 4835 | 1 | museum skin | author |
| *invicta* | Creighton Valley | 4836 | 1 | museum skin | author |
| *fallenda* | Point Grey, B.C. | 4852 | 1 | museum skin | author |
| *fallenda* | Sea Island, B.C. | 1976 | 1 | museum skin | author |
| *arctica* | Yukon | 10848 | 1 | museum skin | author |
| *arctica* | NWT | 3364 | 1 | museum skin | author |
| *bangsi* | Delta, Man. | 1948 | 1 | museum skin | author |
| *cicognanni* | Guelph, Ont. | 4854 | 1 | museum skin | author |
| *orientalis* | Hokkaido, Japan | n/a | 1 | a.p. muscle [1] | Masuda et al. 1992 |

*All sample numbers except M39, M40, M42, correspond to catalogue numbers in the Cowan Vertebrate Museum.
[1] a.p. stands for alcohol preserved

**Amplification**

Amplification using primers H15149 and L14841 was done using the same conditions described earlier for the black bear and marten. For contemporary tissues, 35 cycles were done, but for ancient samples, 40 cycles were required to maximize yields. For 14 samples, primers H15149 and L14841 would not give a product. There were several possibilities for this problem. The first was that the DNA extraction failed. Samples were retaken. However, this time they were taken from different areas on the skin/mount in order to avoid areas where samples had been taken previously. However, the second extraction also failed to give a PCR product. From previous extractions, it was known that with CTAB, the ancient DNA extraction success rate was at least 70%, so it seemed reasonable that the problem was either due to amplification or the degraded state of the DNA. The first possibility I considered was the change in house Taq from Stock E to stock G. G was found by others to be inefficient for amplifying genomic DNA. However, attempts to amplify using commercial Taq in combination with Taq extender buffers did not help. I observed that PCR reactions were resulting in smears, which when run upon high resolution gels (2% Nusieve) were actually multiple bands, all of which were smaller than 300 bp. It seemed likely that the DNA extracted from the preserved weasel skins were highly degraded and the multiple bands possibly the result of jumping PCR. Sequencing confirmed this.

New weasel specific primers were designed (L15009 GGC TGA ATC ATC CGA TAC ATA CAC GCA AAC, L15129 (L274) GGC ATT ATC TTA TTA TTC GCA GTT ATA GC, H15009 GTT TGC GTG TAT GTA TCG GGA TGA TTCAGC C, H15348 GAT GAA TGG TAG GAT RAA GTG GAA AGC, H15464 CTT TGA TGG TAT AGT

AGG GGT GAA ATG GG, H15539 TAG TTG TCT GGG TCT CCT TRT AGG TCG GG, H15678 GGC TAG GAT TAG GAT GGA GAR GAT TAG GGC). Primers were named relative to Kocher et al.'s (1987) universal primer L14841. Various combinations of these primers with the original H15149 and L14841 primers were used to produce products that ranged from 148 bp to 673 bp (which were done by combining two separate PCR products) in total length (Fig 17). PCR was done using in house Taq. PCR conditions were as follows: 40 cycles of 94°C for 1 minute, 55-60°C for 1 minute and 72°C for 1 minute followed by a 72°C extension. This was preceded by a 2 minute denaturation hold for 2 minutes and followed by 7 minutes of extension. Products were obtained from most of the DNA samples that had not successfully amplified before.

Because of the sensitivity of PCR and the poor quality of DNA extracted from preserved hides, precautions against contamination during the preparation of amplification reactions were taken. Reactions were set up in a flow hood apart from the main area where other laboratory procedures were done. The flow hood was wiped down with dilute bleach and then UV irradiated for at least 30 minutes. A mask was worn at all times and gloves were frequently changed. I did not do a hot start for ancient samples to minimize the risk of contamination.

**Purification of PCR Products**

PCR products were purified using either commercial columns alone (for manual sequencing) or purified using Nusieve and columns (for automated sequencing (as described in Chapter 2).

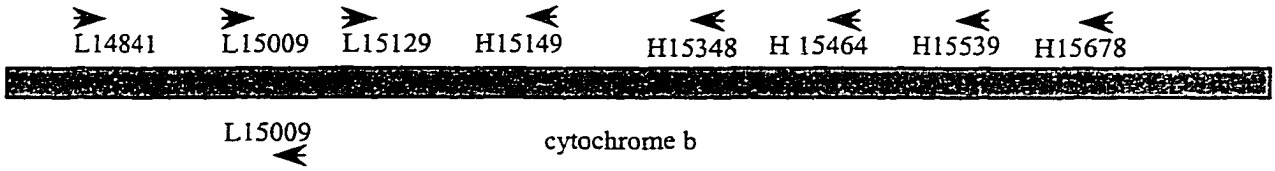



**Figure 17**
Schematic representation of primer positions and PCR strategy for *M. erminea*

**Cloning**

PCR products were ligated and transformed using the Invitrogen Cloning Kit. Clones were purified as described in Chapter 2 and sequenced using the ABI automated sequencer.

**Automated Sequencing**

Clones were sequenced according to the ABI suggested protocol. See Chapter 2 for details.

**Manual Sequencing**

PCR products were sequenced using both [$\alpha$-$^{32}$P] dATP end labeling and[$\alpha$- $^{35}$S] dATP direct incorporation. As with the black bear [$\alpha$-$^{35}$S] dATP was the preferred method of manual sequencing. See Chapter 2 for details.

**Phylogenetic Analyses**

Sequence consensus from ABI and sequence alignments for Short-tailed weasel were generated using Lasergene Navigator (DNASTAR, Madison, WI). A minimum of three sequences were used to generate the sequence consensus. Three types of analyses were used: maximum parsimony (Eck and Dayoff 1966; Fitch 1977), neighbour-joining (Saitou and Nei 1987), and maximum likelihood (Cavelli-Sforza and Edwards 1967). All tests were performed using PAUP * (Swofford in press).

Pairwise distance was calculated for all taxa using a two-parameter (Kimura 1980) model in PAUP * 4.0 (Swofford, in press). Because of the range of sequences

used in this data set, the longest sequence from each subspecies group was used as a representative in the following phylogenetic analyses. Selectively removing taxa was necessary because shorter sequences were often invariant, unnecessarily increasing computational load and decreasing the amount of usable data. Trees produced by removing taxa were compared to trees using all taxa.

*Parsimony*

Maximum parsimony analysis of equally weighted nucleotide sites and unordered character state transformations was used to generate trees based upon a branch and bound search in PAUP * 4.0 (Swofford, in press). Initial tree(s) were constructed using random stepwise addition and branch swapping implemented through tree bisection and reconnection (TBR) and steepest descent. A consensus of all trees was calculated and presented in the following results. Data was resampled using 2000 heuristic bootstrap replicates.

Rooting the trees was a significant problem as appropriate outgroups with sufficient sequence overlap were not available. Because of this, trees with selectively removed taxa were rooted using longer marten sequences. Based on a Kimura's two parameter model, marten are about 15% divergent from short-tailed weasels. Because saturation of nucleotide positions are believed to increase significantly past a 15% divergence (Moritz et al. 1987), the actual divergence may be significantly greater than what is observed. Such large divergences between the ingroup and outgroup can result in long branch attraction (see Maddison et al. 1992). Therefore, trees were rooted by connecting the outgroup after the ingroup phylogeny had already been established as

suggested by Lundberg (1972) and Nixon and Carpenter (1993). For phylogenetic trees constructed using all taxa, the least weasel (*Mustela nivalis*) was used as the outgroup. Although this was the most appropriate outgroup, short sequences (~300 bp) limited its use in the previous analysis.

*Maximum likelihood*

Maximum likelihood (Cavalli-Sforza and Edwards 1967) analyses was done using PAUP* 4.0 (Swofford, in press). Empirical base frequencies (A=0.28876, C=0.27388, G=0.13938, T=0.29797) and $T_i/T_s$ ratios 2 and 4 were used as well as a ratio estimated by maximum likelihood. Starting branch lengths were obtained using Rogers-Swofford approximation method and the Hasegawa - Kishino - Yano (HKY85) model (Hasegawa et al. 1985). Trees were generated using 100 heuristic search replicates. Trees were statistically evaluated using 2000 heuristic bootstrap replicates. Trees with selectively removed taxa were rooted using marten as described above. Trees constructed using all taxa were rooted using *M. nivalis*.

*Distance Analyses*

Distance trees were generated using neighbour-joining cluster analysis (Saitou and Nei 1987) on PAUP * 4.0 (Swofford, in press). A distance tree was produced using the same outgroup used in parsimony analysis. A two parameter distance matrix constructed in PAUP* was used. Data was resampled using 2000 heuristic bootstrap replicates. The tree with selectively removed taxa was rooted using marten as described above. *M. nivalis* was used for the tree constructed using all taxa. $K_s$ and $K_a$ were

calculated using MEGA 1.02 (Kumar et al. 1993).

*Minimum Spanning Tree (MST)*

Minimum spanning trees are alternative ways of looking at phylogenetic relationships. Although minimum spanning trees are constructed from genetic distances, they differ from conventional distance methods like neighbour-joining and UPGMA by allowing extant taxa to assume ancestral positions. Minimum spanning trees were constructed using uncorrected *p* distances in NTSYS PC 2.0 (Exeter Software). The use of uncorrected vs corrected distances is not of concern here due to low divergences between taxa.

*Relative Rate Test*

Based on trees generated from neighbour-joining, maximum parsimony and maximum likelihood, relative rate (Sarich and Wilson 1973) was calculated between the southern, coastal and Beringian lineages using the least weasel (*M. nivalis*) as an outgroup. The relative rate test was based upon uncorrected *p* values.

## Results

A total of 30 individuals were successfully amplified and sequenced (Table 8). Of these, 21 were from ancient DNA samples. Although a sequence was obtained from Haida Gwaii M42, it was omitted from subsequent analyses due to the presence of large gaps and large numbers of ambiguous sites which could not be resolved despite

## Table 8 Short-tailed weasel character matrix.

The following table shows The variable sites in reference to the sequence shown below. Position numbers are shown above the table, nucleotides are shown below position number.

? indicates missing data.

### VI 2

```
1                                                  45                                                  90
GAAACTTCGGCTCCCTCCTCGGAATCTGCCTAATTATTCAGATTCTTACAGGTTTATTTTTAGCCATGCATTATACATCAGACACAGCCA
91                                                 135                                                 180
CAGCCTTTTCATCAGTCACCCACATCTGCCGAGACGTCAACTACGGCTGAATCATCCGATACATACACGCAAACGGAGCTTCCATATTCT
181                                                225                                                 270
TTATCTGCCTATTCCTACACGTAGGACGAGGATTATATTACGGTTCTTATATATTCTCCGAAACATGAAACATTGGCATTATCTTATTAT
271                                                315                                                 360
TCGCAGTTATAGCAACTGCATTCATAGGTTACGTTTTACCATGAGGACAAATATCATTTTGAGGTGCAACCGTAATTACTAACTTACTAT
361                                                405                                                 450
CTGCTATCCCATACATTGGAACTAACCTTGTAGAATGAATTTGAGGCGGATTTTCAGTAGATAAAGCCACCCTAACACGATTCTTCGCCT
451                                                495                                                 540
TCCATTTTATCCTACCATTCATCATTTCAGCACTAGCAGCAGTCCACCTACTATTCCTCCACGAAACAGGATCCAACAACCCNTCCGGAA
541                                                585                                                 630
TCCCATCCGACTCTGACAAAATCCCATTCCACCCTTACTATACCATCAAAGACATCCTAGGCGCCCTATTCCTTATTCTAACACTAATAC
631                                               673
TACTAGTACTATTCTCAGACCTACAAGGAGACCCAGACAACTA
```

**Position Number**

| Taxon | 1111111111111111122222222222222233333333333444444455555566<br>1133667777888899011234444578889901222333556678800022233667023668223337 15<br>8472578014901373843939138980125346214825704568474562350636823335602134648 |
|---|---|
| Gavin Lake | ??????????????????????GACGACCTCCTCAACTTTTTATTATATTTTATTTTTTTTTCTAAGANCCTT? |
| Surf Inlet | CCCATTGATTCAGCGGTAYCGGACGRCTTTCTCARYWTTTTAYTAYATTTTATTCCTTTTTCTARGACCTTT? |
| Hemp Creek | ??????????????????????????????????????????TAYATTTTATTCCTTTTTCTARGACNTTT? |
| Stum Lake | CTCATTGATTCAGCGGTACCGGACGACCTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Saskatoon | ????????????????????????????CTCCYCMACTTTTTATTATGTTTT?????????????????????? |
| Meyers Passage | ????????????????????????????CTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Britannia Beach | CYCATWGAYTYAGCGGTAYCRRACGACYYYCTYARYTTTTTRYYAYAYTKYATTC?????????????????? |
| Interior BC | ?????TGTATCNNCGGTACCGGNCGACCTCCTCAACTTTTTATTATATTTTATTCCTTTTTCTAACNCCT??? |
| Cameron Lake | ????????????????????????????CTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Hope | ??????????????????????GACGACCTCCTCAACTTTTTATTATATTTTATTTTTTTTCTAAGACCCTT? |
| Wentworth Lake | ????????????????????????????CTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Creighton Valley | ??????????????????????????????????????????????ATTTTATTTTTTTTCYAAGACCCTT? |
| Sea Island | ????????????????????????????CTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Point Grey | ????????????????????????????CTCCTCAACTTTTTATTGTATTTTNNNTTTTTTCNAAGACCCTTN |
| Huntington | ????????????????????????????CTCCTCAACTTTTTATTGTATTTT?????????????????????? |
| Delta, Man. | ????????????????????????????CTCCTCAACTTTTTATTATATTTT?????????????????????? |
| Guelph, Ont. | ????????????????????????????CTCCTCAATTTTTTATTATATTTTATTTTTKTTTCTAAGACCCTT? |
| VI 1 | YCCATTGATTCAGCGGTWCCGGACGACTTTCTCAACYTTTTATTATATTTT?????????????????????? |
| VI 2 | CCCATTGATTCAGCGGTACCGGACGACTTTCTCAACTTTTTATTATATTTTATTTTTTTTTCCAAGACCNTTC |
| VI 3 | ??????????????????????????????????????????????ATTTTATTTTTTTTTCCAAGACCNNA? |
| VI 4 | CCCATTGATTCAGCGGTACCGGACGACTTTCTCAACTTTTTATTATATTTTATTC?????????????????? |
| 2371 VI 5 | ????????????????????????????TTTCTCAACTTTTTATTATATTTT?????????????????????? |
| 2376 VI 6 | ????????????????????????????TTTCTCAACTTTTTATTATATTTT?????????????????????? |
| Port Hardy,VI 7 | CCCATTGATTCAGCGGTACCGGACGACTTTCTCAACTTTTTATTATATTTT?????????????????????? |
| Haida Gwaii M39 | ?????TCATCCAGCGGTACCGGACGACTTTCTCAATTNTNTATTATATTTTATTTTCCCCCTCGAGATCC??? |
| Haida Gwaii M40 | ?????TGATCCACCNNTACCGGACGACTTTCTCAATTTTNTATTATATT?????????????????????? |
| QCI City | ??????TGATCCAGCGGTACCGGACGACTTTCTCAATTTCTTATTATATTTTATTTTCCCCCTCGAGATCC??? |
| Tlell | ????????????????????????????TTTCTCAATTTCTTATTATATTTT?????????????????????? |
| Yukon | CCCATTGATCCAGCGGCACCGGACGACTTTCTCACCDTTTCATTACATTTTNNNTTTTTTTCNAAGACCCTT? |
| NWT | CCCATTGATCCAGCGGCACCGGACGACTTTCTCACCTTTTCATTACATKTT?????????????????????? |
| Japan | CCCACTGATCCAGCGGCACCGGACGACTTCCTCACCTTTTCATTACA?????????????????????????? |
| *Mustela nivalis* | CCTTTTGACTCAGTGGTACTGGATAATTTTTTCACTTTTTTACTACA?????????????????????????? |

numerous attempts. Due to DNA degradation, several of the samples could not be amplified beyond 148 bp. The 5' end of the cytochrome b gene seemed to be particularly susceptible to DNA damage given the much lower rate of amplification success. Therefore a range of PCR products were obtained variable in both length and position along the entire 673 bp section of the gene.

Contamination was also a significant problem. Although some of this contamination did come from within the lab, a significant proportion of the contaminants were not from organisms that I had personally handled. Most common contaminants were from avian species and pufferfish.

Sequences from ancient DNA were considered bonafide sequences if they were novel or if the same sequences were obtained after multiple independent extraction, amplification and sequencing attempts.

**Phylogenetic Analyses**

Phylogenetic trees from maximum parsimony, maximum likelihood, and neighbour-joining revealed the existence of four mtDNA short-tailed weasel lineages (Fig 18a, b, 19a, b, 20a, b). The Haida Gwaii lineage was restricted to weasels found only on that archipelago, the VI lineage was composed of weasels from Vancouver Island and some areas along coastal BC, the Beringia lineage was found in the Yukon, NWT and Japan, whereas the continental lineage was dispersed throughout BC, and extended as far east as Manitoba and Ontario (Fig 21).

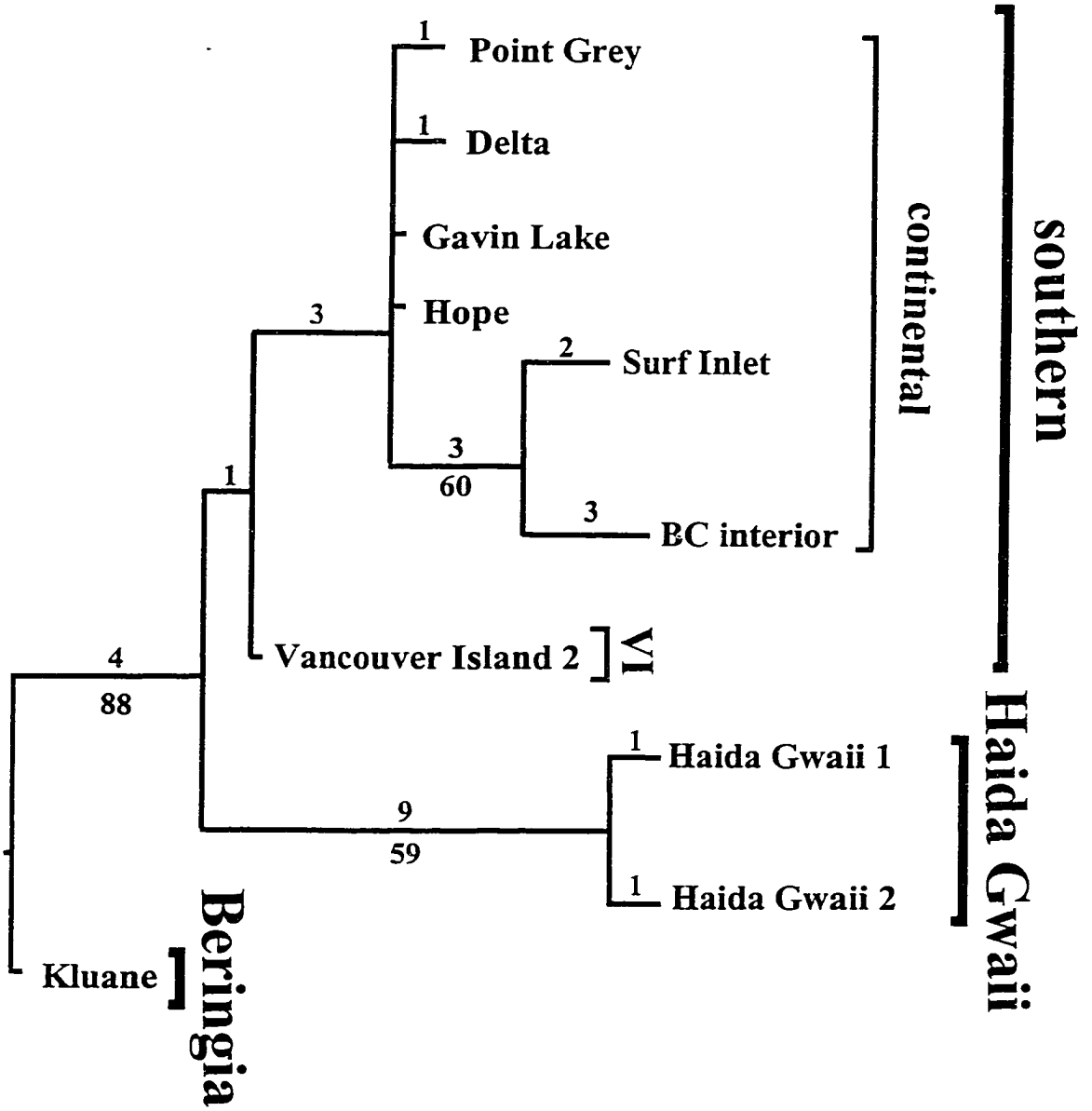



**Figure 18a Maximum parsimony tree for short-tailed weasel.**
This 50% majority rule tree was constructed using only selected individuals from each subspecies with sequence lengths of more than 600 bp. Topology of this tree is similar to the maximum parsimony tree constructed using all data shown in Fig 19b. Three major lineages are identified: Haida Gwaii, Beringia, southern. Within the southern lineage weasels were further structured into VI and continental lineages. Branch lengths are shown above branches and bootstrap values are shown below. The tree was rooted using *Martes americana*.

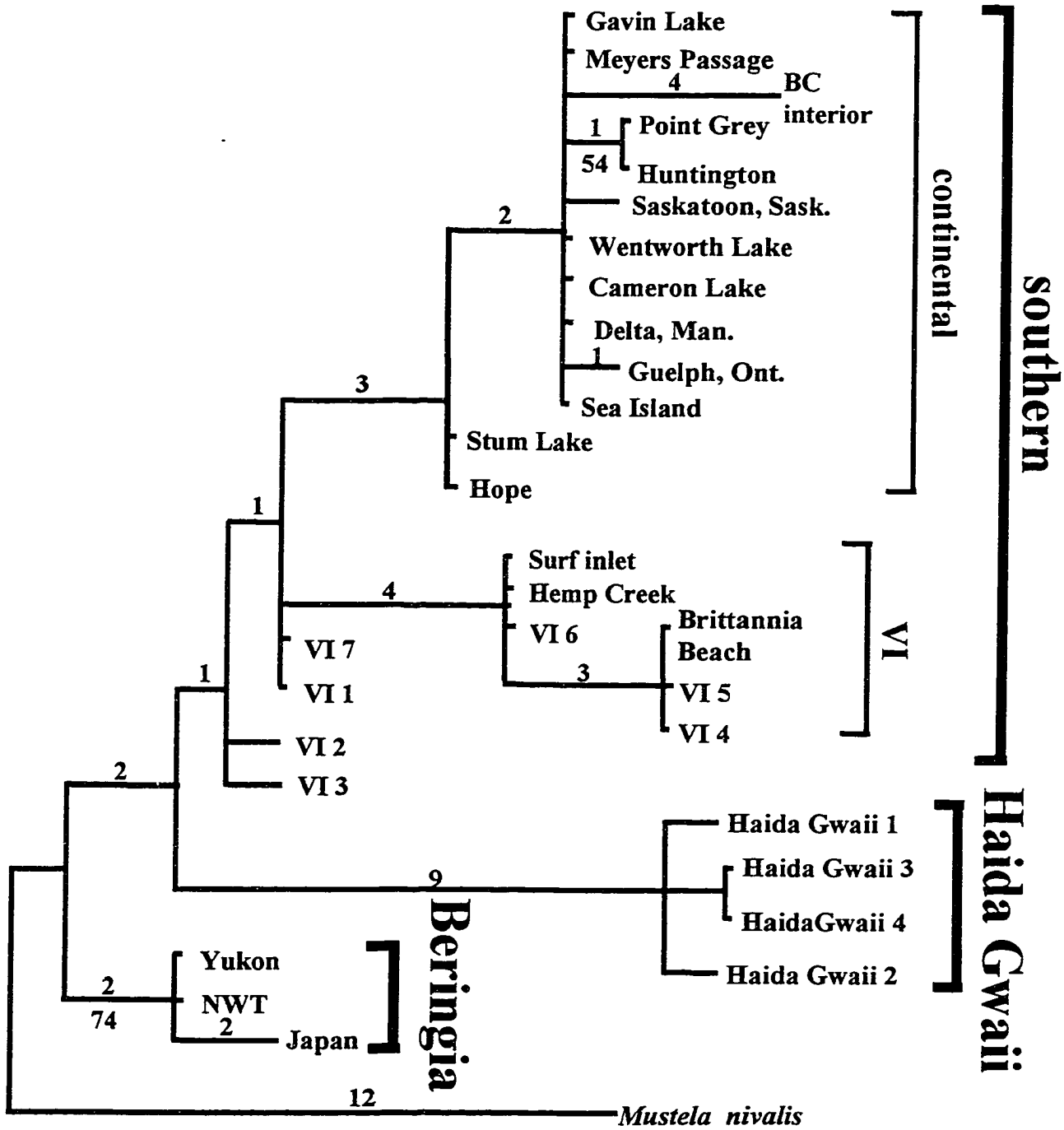



**Figure 18b Maximum parsimony for short-tailed weasel.**
Three major lineages of weasels are apparent from this 50% majority ruletree: Haida Gwaii, Beringia, and southern. Weasels excluded from the Haida Gwaii and Beringia lineages are collectively referred to as the southern group. Branch lengths are shown above branches, bootstraps are shown below. This tree was rooted using *Mustela nivalis*.

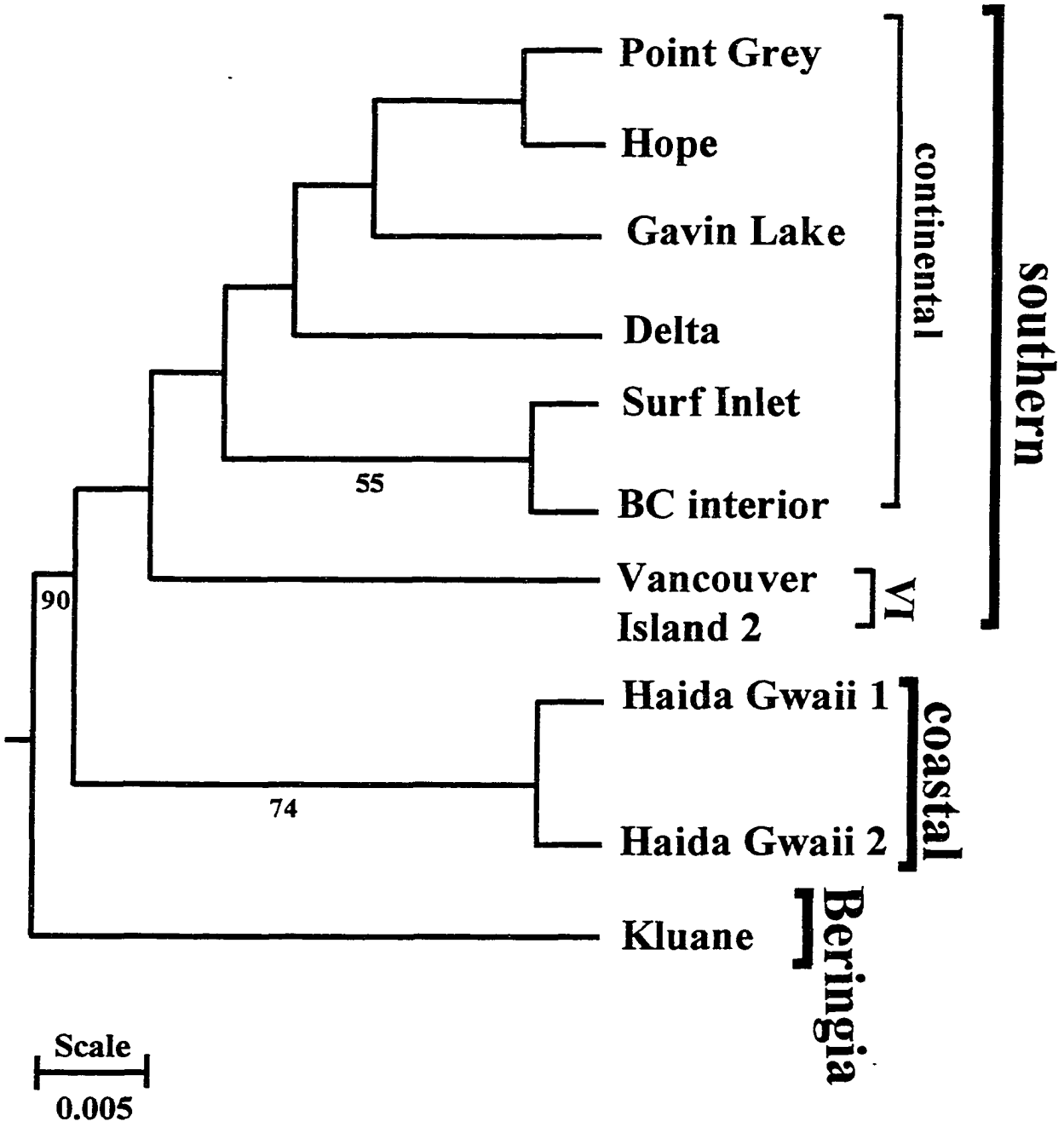



**Figure 19a Maximum likelihood tree for short-tailed weasels.**
This maximum likelihood tree was constructed (-ln likelihood 1644.56) using only selected taxa with sequence lengths of more than 600 bp. As with maximum parsimony, three weasel lineages are apparent: Haida Gwaii, Beringia, and southern. Of these, only the Haida Gwaii lineage was supported by bootstrapping (numbers below branches).

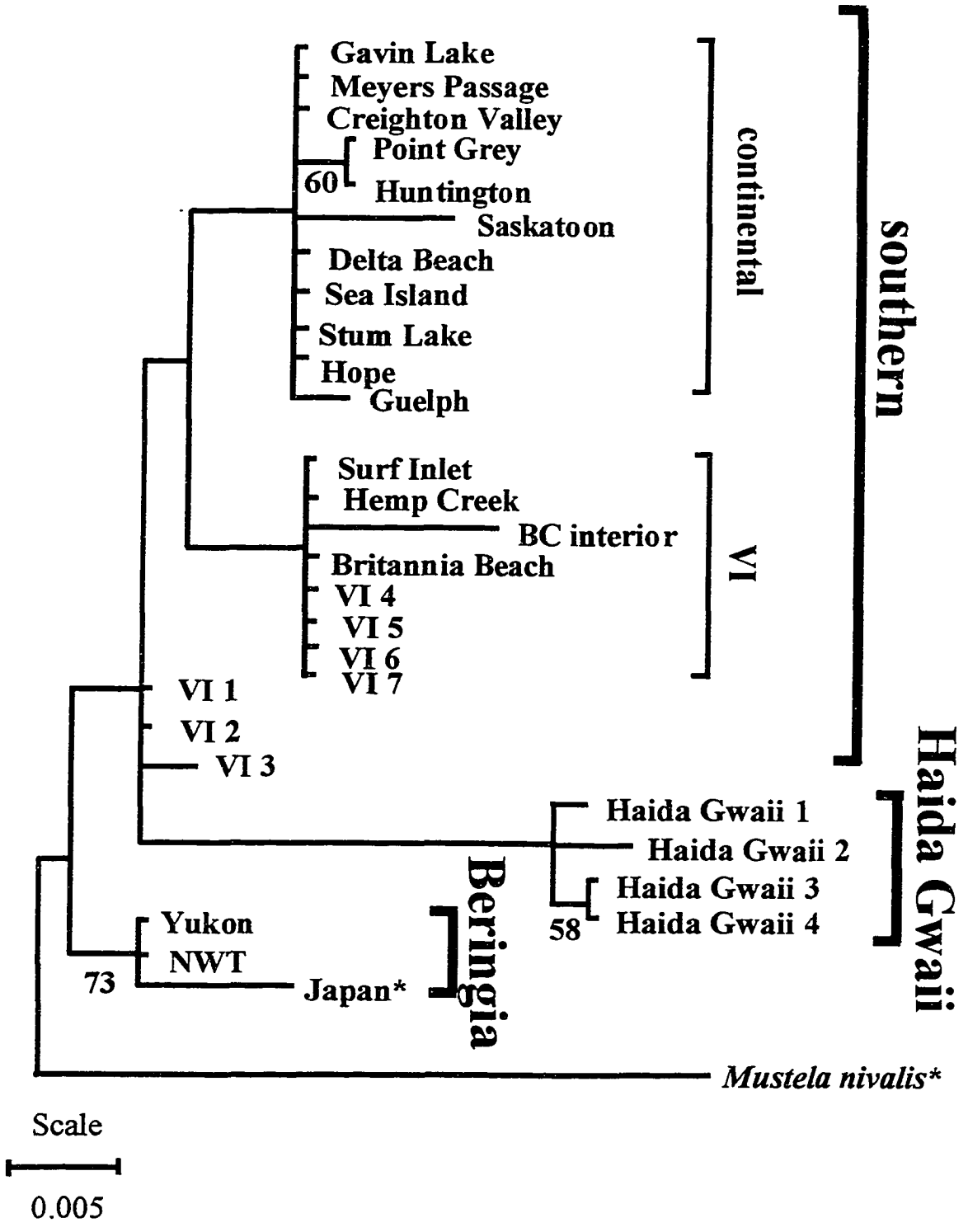



**Figure 19b Maximum likelihood tree for short-tailed weasel**
Topology of this maximum likelihood tree (ln likelihood 1190.09) is based on the complete data set. Branch lengths were introduced from one of 100 trees obtained from a heuristic search and are shown above the branch. Bootstrap values from 2000 heuristic replicates are shown below branches. As with maximum parsimony, maximum likelihood suggests that there are three major groups of weasels in North America: 1) a continental group 2) Beringian group and a 3) southern group. The weasels excluded from the Beringian and Haida Gwaii lineages are collectively referred to as the southern lineage. The * marks those sequences taken from GenBank.

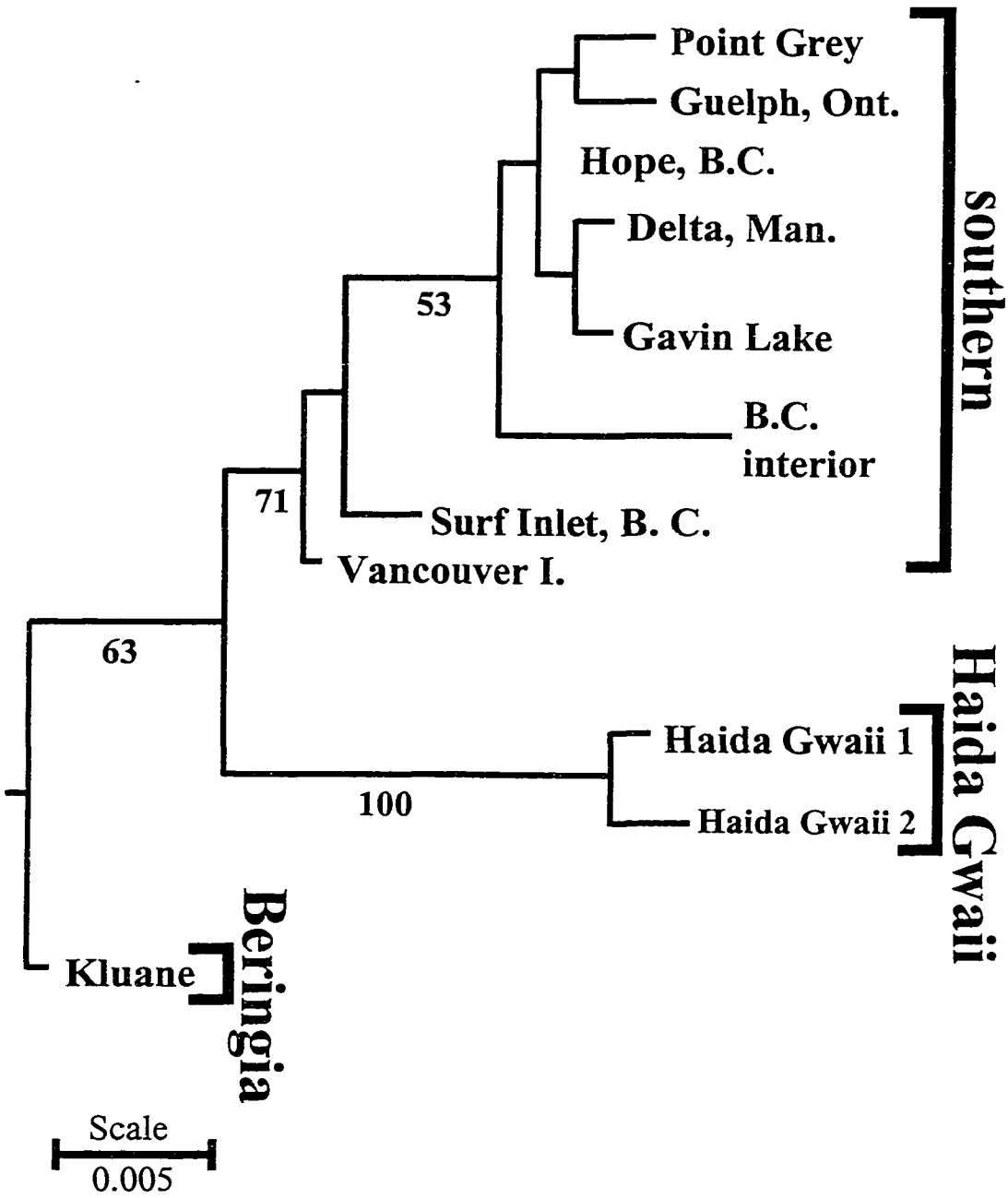



**Figure 20a Neighbour-joining tree for short-tailed weasel.** This neighbour-joining tree was constructed using only selected taxa with sequence lengths greater than 600 bp. As with maximum parsimony, Haida Gwaii, Beringia, and southern lineages were identified and supported by bootstrapping (values shown below branches). Within the southern lineage, a continental lineage was found but a VI lineage was not detected.

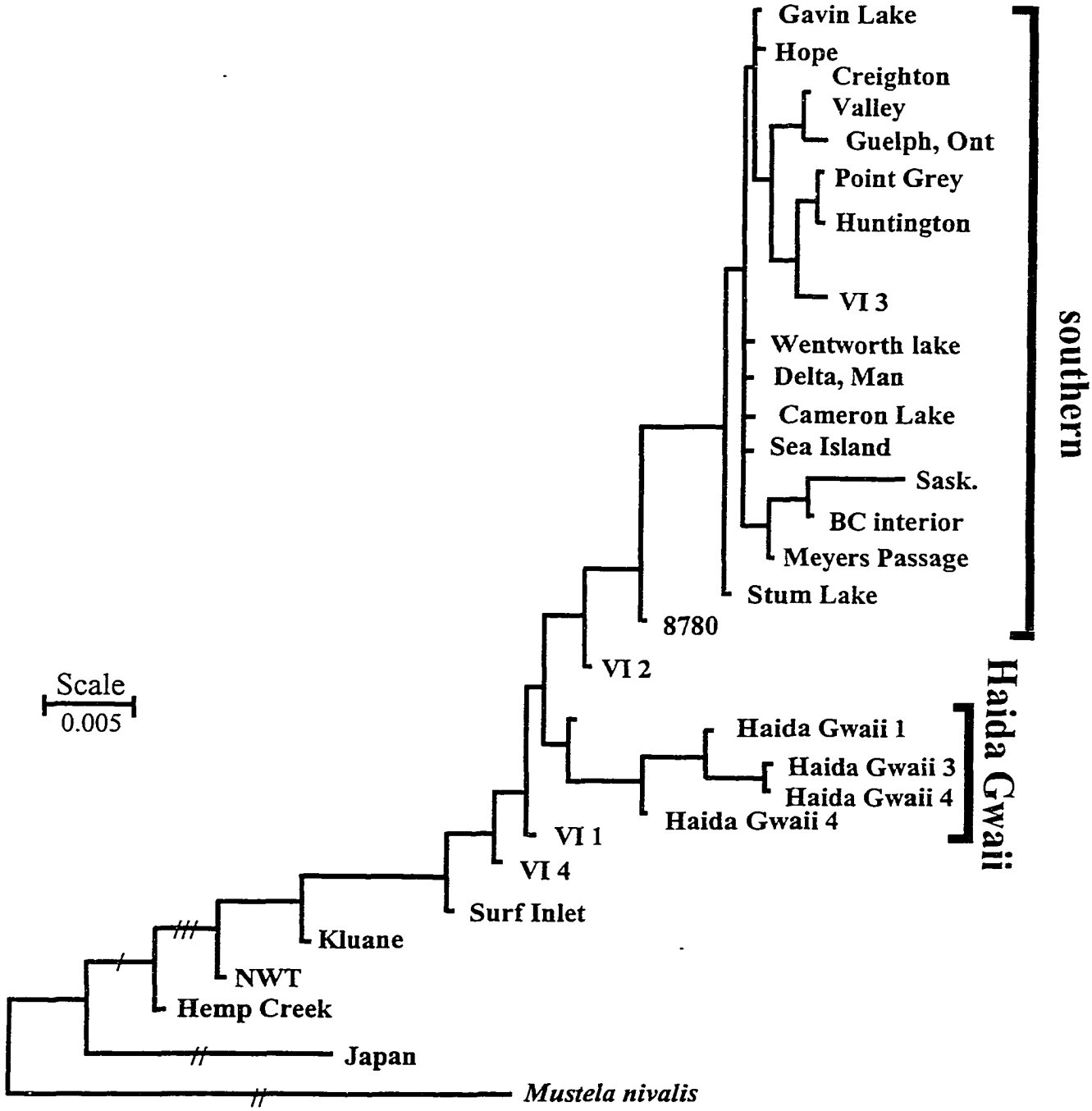



**Figure 20b Neigbour-joining tree of short-tailed weasel with all taxa.**
Both Haida Gwaii and southern lineages are evident from this tree. Although weasels from Kluane, NWT, and Japan were excluded from these lineages, they did not form a Beringian lineage. Vancouver Island weasels and weasels from Hemp Creek and Surf Inlet were scattered throughout the tree. Hatch marks indicate those branches which are not to scale.

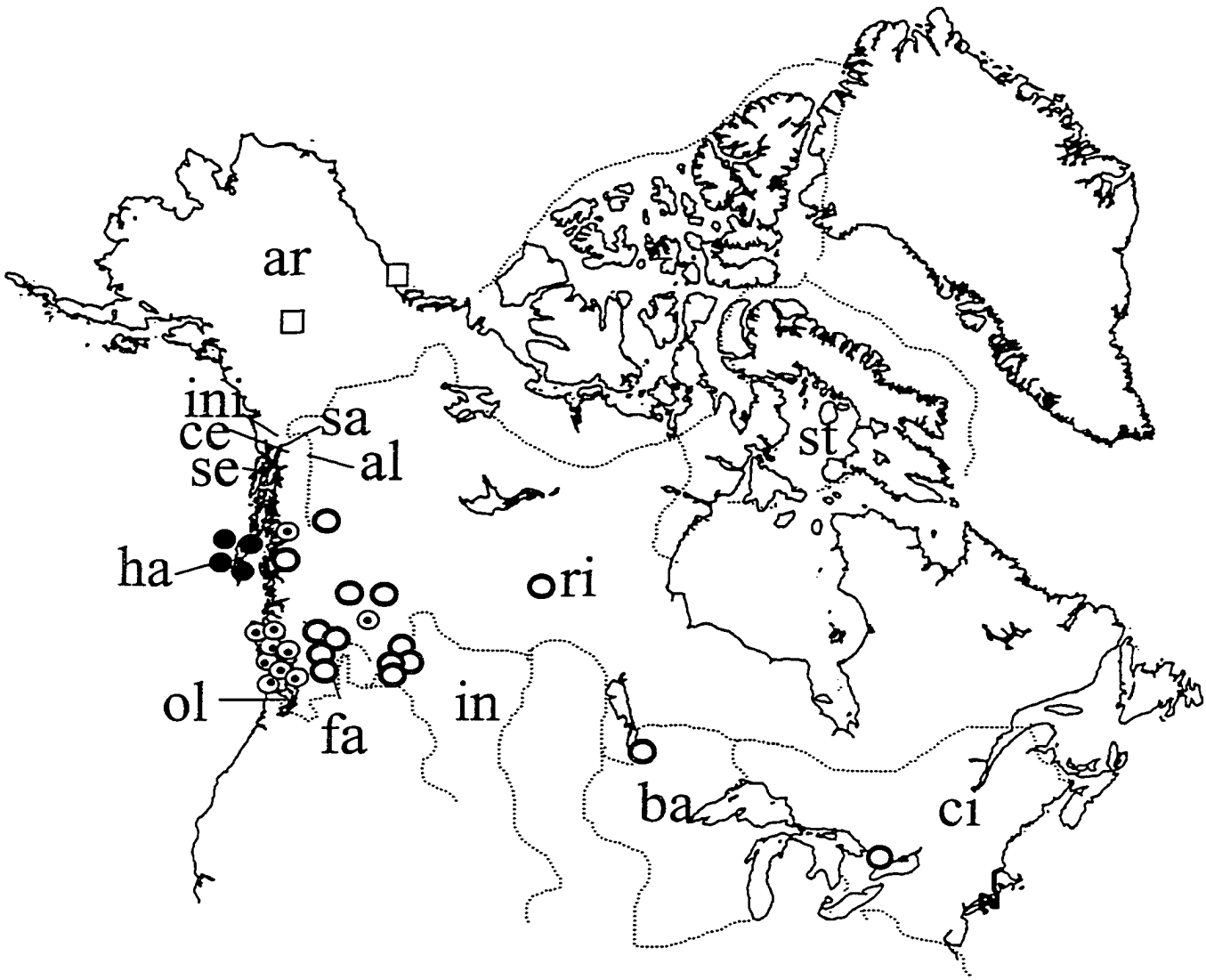



**Figure 21**

Geographic distribution of Haida Gwaii (●) (n=4), southern (○) (n=15) and Beringian (□) (n=2) lineages in short-tailed weasel as identified by parsimony and maximum likelihood. Within the southern lineage, short-tailed weasels from Vancouver Island, Surf Inlet and Hemp Creek were grouped together (VI lineage), sharing synapomorphies with both southern and Haida Gwaii weasels. These weasels are denoted by ◎ (n=9). This figure illustrates the phylogeographic structure in short-tailed weasels as the four lineages are restricted to particular regions. Exact locales for all samples are given except for the following: Vancouver Island 1, 2, 3, 4, 5; Haida Gwaii 1, 2; BC interior 1. I have not placed the latter samples (BC interior) on the map because the exact locale from which it was collected was not known. As such it is not known whether the sample lies within *richardsonii* or *invicta* subspecies boundaries.

*Maximum Parsimony*

Out of a total 673 possible characters, there were 27 parsimony informative sites. Eighty trees with a length of 55, consistency index (CI) of 0.8364 and retention index (RI) of 0.8163 were generated. Although the tree was originally generated using selected taxa (Fig 18 a), trees using all taxa were subsequently constructed (Fig 18 b). Consensus trees from these two methods were identical in their underlying topology. Figure 18 a and b illustrates the topology from 50% majority rule tree and strict consensus for all taxa and shows that four major weasel lineages exist in northwestern North America: Haida Gwaii, VI, continental and Beringia. Branch lengths were also shown to indicate the number of substitutions unique to each lineage.

Weasels which were excluded from Haida Gwaii and Beringian lineages were collectively referred to as the southern lineage as four individuals from Vancouver Island were not included in either continental or VI lineages. The latter two lineages were named to describe the geographical location from which the majority of the weasels were from. Mainland subspecies *richardsonii*, *fallenda*, *invicta*, *bangsi* and *cicognanii* were not well defined, with the exception of *richardsonii* samples from Surf Inlet, Hemp Creek and Britannia Beach. These weasels showed a strong affinity to some weasels from Vancouver Island (*anguinae*). Weasels from Vancouver Island did not comprise a single lineage but did appear to have affinities to both Haida Gwaii weasels (at positions 170, 182, 463) and southern weasels (at positions 74, 263, 265, 271, 301, 423, 433, 465, 531). Short-tailed weasels from Japan (*orientalis*), the Yukon (*arctica*) and the North West Territories (*arctica*) exhibited genetic affinity and were identified by 2 synapomorphies (position 98 and 237) and four additional sites at positions 212, 170, 221, and 278. The

subspecies *arctica* and *orientalis* differed by a single T to C autapomorphy in *orientalis* (position 182). Weasels from Haida Gwaii formed their own distinct group supported by a bootstrap of 50. Although support is weak, the Haida Gwaii weasel lineage was unambiguously defined from all other lineages at 17 sites. At nine of these sites (221, 363, 366, 378, 402, 423, 433, 465, and 531) the nucleotides were unique to the Haida Gwaii weasels.

*Maximum Likelihood*

One tree was generated from maximum likelihood and was similar in topology to trees produced from maximum parsimony (Fig 19 a and b). Differences between the two trees lie principally on the position of terminal taxa and branch lengths. There were only minimal differences in trees generated using $T_i/T_s$ ratios of 2 or 4. The tree presented in Figure 19 a and b illustrates the topology using a $T_i/T_s$ ratio of 2 and also shows the branch lengths. The tree shown in Figure 19 b shows all taxa and is consistent with the overall tree topology when only selected taxa (Fig 19 a) were used. As in parsimony analysis, four lineages were identified: continental, VI, Haida Gwaii and Beringia. Weasels not included in the latter two lineages were collectively referred to as the southern weasels. All lineages were marginally supported by bootstrapping.

*Distance*

As with maximum parsimony, distance analysis using a two parameter distance matrix (Table 9) identified a southern mainland group which included subspecies *richardsonii*, *fallenda*, *invicta*, *bangsi* and *cicognanii*, a Haida Gwaii lineage which

**Table 9 Kimura 2-parameter distance matrix for *Mustela erminea*.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Gavin Lake | - | | | | | | |
| 2 | Surf Inlet | 0.01009 | - | | | | | |
| 3 | Hemp Creek | 0.00769 | 0.00000 | - | | | | |
| 4 | Stum Lake | 0.00000 | 0.00985 | 0.00000 | - | | | |
| 5 | Saskatoon | 0.00762 | 0.01956 | 0.01980 | 0.00703 | - | | |
| 6 | Meyers Passage | 0.00000 | 0.01158 | 0.00000 | 0.00000 | 0.00705 | - | |
| 7 | Brittannia Beach | 0.00568 | 0.00000 | 0.00000 | 0.00000 | 0.00736 | 0.00000 | - |
| 8 | BC Interior | 0.00961 | 0.00972 | 0.00306 | 0.00736 | 0.00746 | 0.00000 | 0.00515 |
| 9 | Cameron Lake | 0.00000 | 0.01158 | 0.00000 | 0.00000 | 0.00705 | 0.00000 | 0.00000 |
| 10 | Hope | 0.00000 | 0.01006 | 0.00765 | 0.00000 | 0.00760 | 0.00000 | 0.00567 |
| 11 | Wentworth | 0.00000 | 0.01158 | 0.00000 | 0.00000 | 0.00705 | 0.00000 | 0.00000 |
| 12 | Brittania Beach | 0.00000 | 0.00821 | 0.00830 | 0.00000 | 0.03744 | 0.00000 | 0.02478 |
| 13 | Sea Island | 0.00000 | 0.01158 | 0.00000 | 0.00000 | 0.00705 | 0.00000 | 0.00000 |
| 14 | Point Grey | 0.00219 | 0.01285 | 0.01063 | 0.00611 | 0.01421 | 0.00604 | 0.01185 |
| 15 | Huntington | 0.00748 | 0.01917 | 0.02048 | 0.00694 | 0.01476 | 0.00705 | 0.00725 |
| 16 | Delta, Manitoba | 0.00000 | 0.01158 | 0.00000 | 0.00000 | 0.00705 | 0.00000 | 0.00000 |
| 17 | Guelph, Ontario | 0.00208 | 0.01056 | 0.00781 | 0.00667 | 0.01348 | 0.00572 | 0.00684 |
| 18 | VI 1 | 0.01280 | 0.00000 | 0.00000 | 0.00986 | 0.01942 | 0.01202 | 0.00000 |
| 19 | VI 2 | 0.00585 | 0.00465 | 0.00762 | 0.00944 | 0.01852 | 0.01062 | 0.00304 |
| 20 | VI 3 | 0.00572 | 0.01116 | 0.01124 | 0.00000 | 0.03789 | 0.00000 | 0.02456 |
| 21 | VI 4 | 0.03735 | 0.00962 | 0.03866 | 0.00949 | 0.01893 | 0.01151 | 0.00000 |
| 22 | 2371 VI | 0.01822 | 0.00000 | 0.00000 | 0.01739 | 0.02221 | 0.01405 | 0.00000 |
| 23 | 2736 VI | 0.01723 | 0.00000 | 0.00000 | 0.01650 | 0.02111 | 0.01361 | 0.00000 |
| 24 | Port Hardy, VI | 0.01267 | 0.00000 | 0.00000 | 0.00971 | 0.01910 | 0.01167 | 0.00000 |
| 25 | M39 Haida Gwaii | 0.02756 | 0.02831 | 0.04162 | 0.01842 | 0.02428 | 0.01624 | 0.01174 |
| 26 | M40 Haida Gwaii | 0.05421 | 0.10828 | 0.00000 | 0.13714 | 0.06829 | 0.06675 | 0.11630 |
| 27 | M42 Haida Gwaii | 0.02035 | 0.00725 | 0.00000 | 0.01956 | 0.02699 | 0.01882 | 0.00844 |
| 28 | QCI City | 0.02980 | 0.02843 | 0.04159 | 0.01847 | 0.03365 | 0.02545 | 0.01179 |
| 29 | Tlell,Haida Gwaii | 0.03210 | 0.00571 | 0.00000 | 0.03122 | 0.03538 | 0.02759 | 0.00644 |
| 30 | Kluane, Yukon | 0.01009 | 0.01113 | 0.00767 | 0.02708 | 0.04671 | 0.03834 | 0.01572 |

**Table 9 Kimura 2-parameter distance matrix for *Mustela erminea* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 31 | Aklavik, NWT | 0.03091 | 0.01107 | 0.00000 | 0.02624 | 0.04535 | 0.03747 | 0.01331 |
| 32 | Hokkaido, Japan | 0.02680 | 0.02891 | 0.03206 | 0.02744 | 0.04056 | 0.03220 | 0.02231 |
| 33 | *Mustela nivalis* | 0.06984 | 0.03264 | 0.00000 | 0.05518 | 0.07347 | 0.06302 | 0.02850 |

| | | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|
| 8 | BC Interior | - | | | | | | |
| 9 | Cameron Lake | 0.00000 | - | | | | | |
| 10 | Hope | 0.00955 | 0.00000 | - | | | | |
| 11 | Wentworth Lake | 0.00000 | 0.00000 | 0.00000 | - | | | |
| 12 | Brittania Beach | 0.01443 | 0.00000 | 0.00000 | 0.00000 | - | | |
| 13 | Sea Island | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | - | |
| 14 | Point Grey | 0.01275 | 0.00604 | 0.00219 | 0.00604 | 0.00000 | 0.00604 | - |
| 15 | Huntington | 0.00732 | 0.00705 | 0.00746 | 0.00705 | 0.00000 | 0.00705 | 0.00000 |
| 16 | Delta, Manitoba | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00604 |
| 17 | Guelph, Ontario | 0.01297 | 0.00572 | 0.00208 | 0.00572 | 0.00000 | 0.00572 | 0.00438 |
| 18 | VI 1 | 0.01536 | 0.01202 | 0.01286 | 0.01202 | 0.00000 | 0.01202 | 0.02221 |
| 19 | VI 2 | 0.01745 | 0.01062 | 0.00584 | 0.01062 | 0.00000 | 0.01062 | 0.00597 |
| 20 | VI 3 | 0.01829 | 0.00000 | 0.00570 | 0.00000 | 0.00278 | 0.00000 | 0.00287 |
| 21 | VI 4 | 0.02794 | 0.01151 | 0.03743 | 0.01151 | 0.09712 | 0.01151 | 0.05025 |
| 22 | 2371 VI | 0.01821 | 0.01405 | 0.01831 | 0.01405 | 0.00000 | 0.01405 | 0.02535 |
| 23 | 2736 VI | 0.01722 | 0.01361 | 0.01732 | 0.01361 | 0.00000 | 0.01361 | 0.02418 |
| 24 | Port Hardy, VI | 0.01519 | 0.01167 | 0.01273 | 0.01167 | 0.00000 | 0.01167 | 0.02207 |
| 25 | M39 Haida Gwaii | 0.04013 | 0.01624 | 0.03004 | 0.01624 | 0.02987 | 0.01624 | 0.03210 |
| 26 | M40 Haida Gwaii | 0.06116 | 0.06675 | 0.05440 | 0.06675 | *0.30275 | 0.06675 | 0.08080 |
| 27 | M42 Haida Gwaii | 0.02383 | 0.01882 | 0.02045 | 0.01882 | 0.00000 | 0.01882 | 0.03249 |
| 28 | QCI City | 0.03992 | 0.02545 | 0.03228 | 0.02545 | 0.02987 | 0.02545 | 0.03454 |
| 29 | Tlell,Haida Gwaii | 0.03204 | 0.02759 | 0.03223 | 0.02759 | 0.00000 | 0.02759 | 0.03927 |

**Table 9 Kimura 2-parameter distance matrix for *Mustela erminea* cont.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 30 Kluane, Yukon | 0.02625 | 0.03834 | 0.01004 | 0.03834 | 0.00000 | 0.03834 | 0.01221 |
| 31 Aklavik, NWT | 0.03406 | 0.03747 | 0.03094 | 0.03747 | 0.00000 | 0.03747 | 0.04443 |
| 32 Hokkaido, Japan | 0.04489 | 0.03220 | 0.02872 | 0.03220 | 0.02580 | 0.03220 | 0.02923 |
| 33 *Mustela nivalis* | 0.05757 | 0.06302 | 0.07003 | 0.06302 | 0.00000 | 0.06302 | 0.07695 |

| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|
| 15 Huntington | - | | | | | | |
| 16 Delta, Manitoba | 0.00705 | - | | | | | |
| 17 Guelph, Ontario | 0.01331 | 0.00572 | - | | | | |
| 18 VI 1 | 0.01908 | 0.01202 | 0.02309 | - | | | |
| 19 VI 2 | 0.01816 | 0.01062 | 0.00833 | 0.00000 | - | | |
| 20 VI 3 | 0.00000 | 0.00000 | 0.00592 | 0.00000 | 0.00278 | - | |
| 21 VI 4 | 0.01861 | 0.01151 | 0.05092 | 0.00000 | 0.01552 | 0.09725 | - |
| 22 2371 VI | 0.02194 | 0.01405 | 0.02499 | 0.00000 | 0.00538 | 0.00000 | 0.00000 |
| 23 2736 VI | 0.02104 | 0.01361 | 0.02361 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| 24 Port Hardy, VI | 0.01878 | 0.01167 | 0.02278 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| 25 M39 Haida Gwaii | 0.02382 | 0.01624 | 0.02842 | 0.01152 | 0.02154 | 0.02972 | 0.02806 |
| 26 M40 Haida Gwaii | 0.06645 | 0.06675 | 0.06488 | 0.11548 | 0.11266 | *0.30275 | 0.11447 |
| 27 M42 Haida Gwaii | 0.02651 | 0.01882 | 0.01703 | 0.01176 | 0.01138 | 0.00000 | 0.01189 |
| 28 QCI City | 0.03319 | 0.02545 | 0.03089 | 0.01153 | 0.02157 | 0.02972 | 0.02806 |
| 29 Tlell,Haida Gwaii | 0.03515 | 0.02759 | 0.02357 | 0.01475 | 0.01485 | 0.00000 | 0.01410 |
| 30 Kluane, Yukon | 0.04631 | 0.03834 | 0.01287 | 0.01747 | 0.00761 | 0.00277 | 0.03212 |
| 31 Aklavik, NWT | 0.04500 | 0.03747 | 0.04526 | 0.01640 | 0.01533 | 0.00000 | 0.01607 |
| 32 Hokkaido, Japan | 0.04026 | 0.03220 | 0.03197 | 0.02465 | 0.02430 | 0.02851 | 0.03872 |
| 33 *Mustela nivalis* | 0.07251 | 0.06302 | 0.05787 | 0.04414 | 0.04564 | 0.00000 | 0.04245 |

**Table 9 Kimura 2-parameter distance matrix for *Mustela erminea* cont.**

| | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|
| 22 2371 VI | - | | | | | | |
| 23 2736 VI | 0.00000 | - | | | | | |
| 24 Port Hardy, VI | 0.00000 | 0.00000 | - | | | | |
| 25 M39 Haida Gwaii | 0.00563 | 0.00534 | 0.01144 | - | | | |
| 26 M40 Haida Gwaii | 0.04342 | 0.04359 | 0.11464 | 0.05323 | - | | |
| 27 M42 Haida Gwaii | 0.00621 | 0.00619 | 0.01172 | 0.00794 | 0.05185 | - | |
| 28 QCI City | 0.01538 | 0.01452 | 0.01143 | 0.00383 | 0.05235 | 0.00788 | - |
| 29 Tlell Haida Gwaii | 0.01436 | 0.01361 | 0.01453 | 0.00535 | 0.04378 | 0.00621 | 0.00000 |
| 30 Kluane, Yukon | 0.02854 | 0.02686 | 0.01728 | 0.02821 | 0.11907 | 0.02440 | 0.02827 |
| 31 Aklavik, NWT | 0.02673 | 0.02524 | 0.01624 | 0.02181 | 0.12045 | 0.02346 | 0.02186 |
| 32 Hokkaido, Japan | 0.04274 | 0.03585 | 0.02437 | 0.01613 | 0.11849 | 0.02918 | 0.01610 |
| 33 *Mustela nivalis* | 0.04646 | 0.04647 | 0.04364 | 0.04843 | 0.15137 | 0.04762 | 0.04846 |

| | 29 | 30 | 31 | 32 | 33 |
|---|---|---|---|---|---|
| 29 Tlell,Haida Gwaii | - | | | | |
| 30 Kluane, Yukon | 0.04194 | - | | | |
| 31 Aklavik, NWT | 0.04011 | 0.00000 | - | | |
| 32 Hokkaido, Japan | 0.05096 | 0.01687 | 0.00687 | - | |
| 33 *Mustela nivalis* | 0.04647 | 0.04936 | 0.04721 | 0.05727 | - |

included only the subspecies *haidarum* and a Beringian lineage which included the subspecies *arctica* (Fig 20 a and b). Based on the neighbour-joining tree with selectively removed taxa (Fig 20 a), synonymous ($K_s$) and nonsynonymous ($K_a$) distances were calculated between and within the three identified lineages. This was done in order to remove those taxa with small sequence lengths which spanned non-variant regions. $K_s$ within the southern and Haida Gwaii lineage was 0.027 and 0 respectively. Based on $K_s$, the Haida Gwaii lineage was the most distinct with a distance of 0.096 from the southern lineage and 0.084 from the Beringian lineage. $K_s$ between southern and Beringian lineages was 0.053. The number of synonymous substitutions were relatively low and as such provided no phylogeographic information. $K_a$ within the southern and Haida Gwaii lineages was 0.0003 and 0.008 respectively. Between southern and Haida Gwaii lineages, $K_a$ was 0.0019. $K_a$ between southern and Beringian lineages was 0.0039. $K_a$ between Haida Gwaii and Beringian lineages was 0.008.

Based on the two parameter distance matrix shown in Table 9, the average genetic divergence within the southern lineage was 1%. Weasels from Haida Gwaii were also identified as a distinct lineage with an average sequence divergence of 2.5% from the southern lineage and an intralineage divergence of 0.8%. As in the maximum parsimony tree, the weasel from the Yukon was also excluded from either the southern or Haida Gwaii lineages and differed by an average sequence divergence of 2.2 % and 2.4% respectively. Application of either a standard molecular clock of 2% per million years or 10% per million years for silent substitutions suggests that these lineages separated from one another more than a million years ago. This number is likely to be an overestimate

given that the short-tailed weasels are believed to have originated and entered North America only about 500,000 years BP.

*Minimum Spanning Tree*

The minimum spanning tree was overlaid on a map of the Pacific Northwest. The number of substitutions separating these individuals are indicated on the tree (Fig 22). Based on the mtDNA gene cytochrome b and the knowledge that the ancestor to the short-tailed weasels originated in Eurasia, it appears that the ancestral mtDNA haplotypes in British Columbia exist on the coast. This is not what one would expect if Haida Gwaii had been recolonized from the mainland. Instead, the minimum spanning tree suggests that the maternal lineage of short-tailed weasels entered North America through Beringia and colonized North America via the coast.

*Relative Rate Test*

Using *M. nivalis* as the outgroup, the southern group showed a divergence of 0.053±0.006 (s= 0.018). This was higher than the divergence calculated for the coastal lineage which was 0.043±0.005 (s= 0.015) and for either the subspecies *orientalis* (0.047) or for *arctica* (0.039). Rates of evolution along the ermine lineages are not significantly different when the lower rate limit of the continental lineage (0.047) and the upper limit of the coastal lineage (0.048) are considered. Rates are also not appreciably different from evolutionary rates of either *orientalis* and *arctica*.

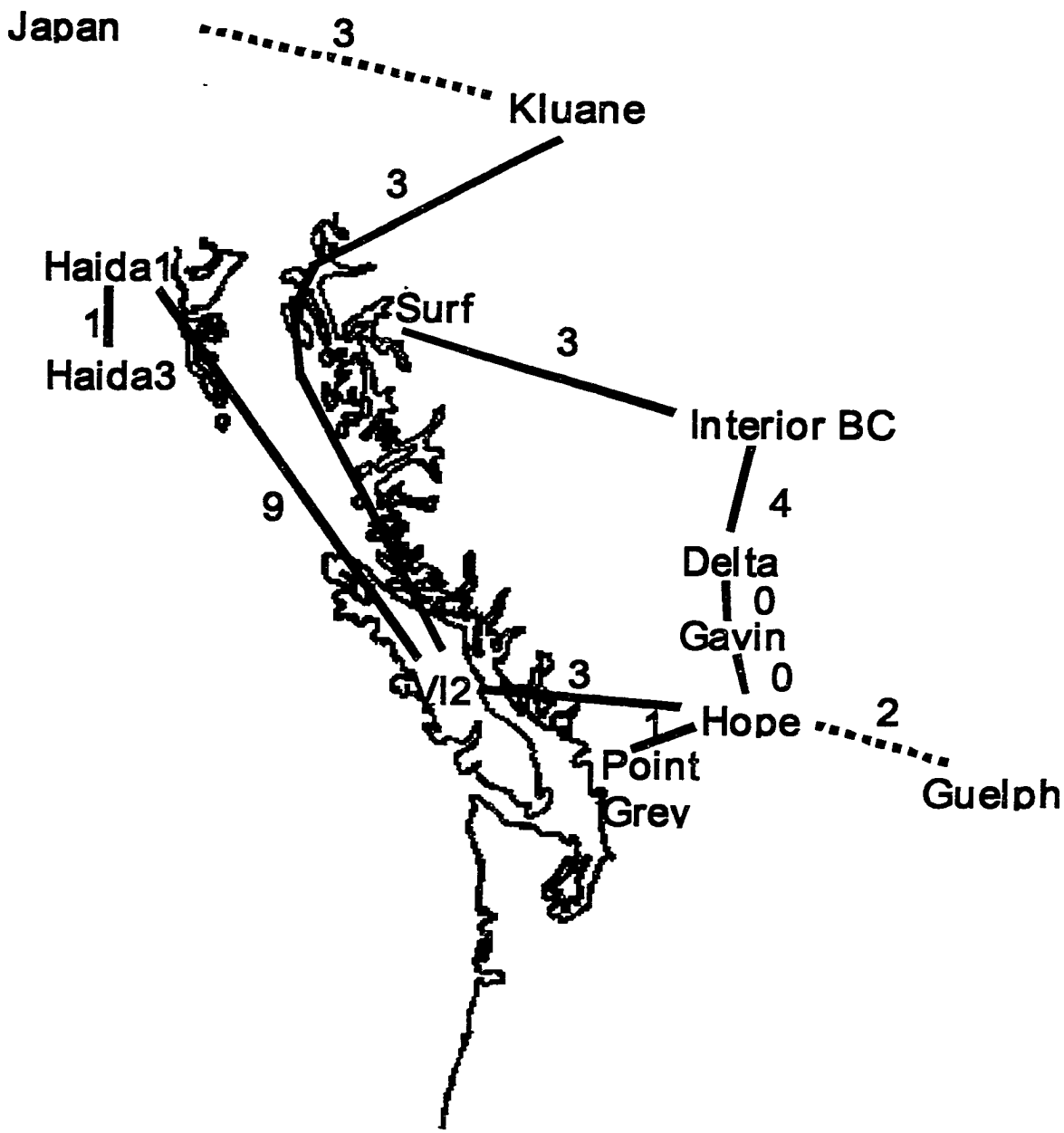



**Figure 22**

By overlaying the minimum spanning network onto a map and orienting it based on fact that short-tailed weasels originated and migrated from Asia into North America, it appears that short-tailed weasels might have actually colonized North America via the coast. The network suggests that coastal populations may have eventually given rise to short-tailed weasel populations on the mainland.

## Discussion

All methods of analyses converged upon a similar gene tree topology which suggested that the Haida Gwaii short-tailed weasels are the most genetically distinct subspecies of ermine. Although bootstrap values were low, maximum parsimony, neighbour-joining and maximum likelihood all suggest that mainland subspecies *richardsonii*, *invicta*, *fallenda*, *bangsi* and *cicognanii* are genetically indistinguishable and that *anguinae* from Vancouver Island has affinities to both continental and Haida Gwaii lineages. An examination of the sequences clearly show that all lineages can be unambiguously identified based on specific nucleotide substitutions (see Results - *Maximum Parsimony*)

Though in the following discussion I have used the gene tree to make certain statements regarding subspecies relationships, the reader is cautioned that the results provided by partial sequences of cytochrome b is a reflection of a maternal gene lineage of short-tailed weasels and as such, is not necessarily congruent with the species tree. Noting these limitations, I will discuss the implications that this data has on morphology and the issue of coastal refugia.

### Implications for Morphology

Close genetic affinity of mainland subspecies *richardsonii*, *invicta*, *fallenda*, *bangsi*, and *cicognanii* is not surprising given the nature of their differences. In most cases, morphological distinctions are clinal (Hall 1923; Hall 1945a, b; Cowan and Guiget 1956; Banfield 1974; Hall 1981) and are likely to have an ecological rather than historical basis. Subspecies distinctions generally consist of size and pelage color differences

which are known to vary according to habitat and latitude.

Although short-tailed weasels from Vancouver Island have genetic affinities to both mainland and Haida Gwaii weasels, morphologically there is no indication that *anguinae* is an intermediate form. Most of its characteristics are typically insular. It exhibits reduced sexual dimorphism and a dark colored pelage, the latter of which might be a functional response to high precipitation in coastal regions. Like *fallenda*, which is located immediately east on the mainland, *anguinae* has a narrow ventral white band. However, there are no indications of a particularly close genetic affinity between these two adjacent subspecies. In fact, *anguinae* seems to be closely related to a coastal group of *richardsonii* with which it shares no obvious morphological affinity.

Morphological distinction of *haidarum* is likely due in part to its insular habitat and geographical isolation. Although its pale colored pelage did seem curious to Foster (1965) given the high levels of precipitation in this archipelago and the relatively dark coloring of weasels on islands north and south of Haida Gwaii, this particular feature is also common in the Haida Gwaii marten and as such may be due to selective pressure or relaxation of selective pressure particular to this insular habitat. The extent of *haidarum's* white ventral band is in accordance with northern subspecies *arctica*, *polaris*, *kadiacensis* and *semplei* (Foster 1965).

Skull size in short-tailed weasels varies clinally and appears to be a function of habitat. However, an analysis of skull shape by Eger (1990) suggested that short-tailed weasels could be categorized into three major groups: northern, southern and coastal. These three groups are congruent with the findings in this study. As such, skull shape in short-tailed weasels may be a relictual feature.

**Implications for Refugia**

Short-tailed weasels are known to have existed both north and south of the Wisconsin ice sheets (Kurtén and Anderson 1980). Subspecies of *M. erminea* in western Canada including *haidarum* from Haida Gwaii are presumed to be derived from southern source populations largely because northern areas remained ice locked long after southern areas had access to coastal and continental regions. As such, two short-tailed weasel lineages were expected to exist in North America, representing each of these different refugial populations: a southern lineage comprised of subspecies *haidarum*, *anguinae*, *richardsonii*, *fallenda*, *invicta*, *bangsi*, and *cicognanii* and a Beringian lineage consisting of *arctica*.

Phylogenetic analyses using parsimony, neighbour-joining and maximum likelihood did identify a southern and Beringian lineage. However, these analyses also showed that *haidarum* did not belong to either of these lineages and presumably was not derived from either of these source populations (Fig 21).

The average genetic distance between southern and Beringian weasels is 2.2%. These two groups of weasels were largely isolated south and north of the Cordilleran ice sheet. Although the stochastic nature of the molecular clock prevents exact divergence time estimates, extrapolation from the genetic distance between Beringian and southern weasels suggests that *haidarum*, which is divergent by 2.5% and 2.4% respectively may have also diverged from southern and Beringian weasel populations prior to the beginning of the Wisconsin.

Although the large degree of divergence observed between *haidarum* and

Beringian/southern weasels could be due to founder effects and/or bottlenecks, such an event is often accompanied by an apparent increase in the rate of evolution. This is not to mean that such events inflate the mutation rate ($\mu$). Rather, large divergences are caused by the founding of populations by rare and divergent haplotypes. However, based on the relative rate test, no significant difference in the rates of evolution was detected.

Ermine populations in northern North America are currently believed to have first become established during the Illinioan glaciation 500,000 years BP (King 1989). During this initial colonization, some ermines remained in Beringia, while others probably dispersed southward either through the continent when Illinoian glaciers retreated and/or via a series of coastal refugia. If these refugia were substantial enough to maintain populations of weasels, they may have eventually found their way down to Haida Gwaii, Vancouver Island and coastal British Columbia and eventually established populations there. The minimum spanning tree (Fig 22) supports such a scenario. Because extant taxa can assume ancestral positions in a minimum spanning tree, a possible migration route can be seen when the tree is overlaid on a map as in Fig 22 and geographically oriented based on the knowledge that short-tailed weasels entered North America over Beringia. Based on this logic, the short-tailed weasels may have entered North America via a coastal route, accounting for the intermediate position held by some coastal and Vancouver Island weasels. Although the intermediate position of Vancouver Island weasels and some coastal weasels might be due to current gene flow and hybridization, given the formidable barriers that currently exist, it is unlikely. As such, the phylogeographic pattern observed in short-tailed weasels may actually be a reflection of a structure established through its initial entry into North America during the Illinoian

glaciation via the coast. The original distribution of these lineages remained more or less intact through the Wisconsin as weasels on Haida Gwaii and perhaps the coast were separated from weasels north and south of the Cordilleran ice sheet by persisting in coastal refugia. In other words, continuity of a Beringian, southern and Haida Gwaii lineage throughout the Wisconsin would have been possible if at least three separate refugia existed which maintained these groups. Maintenance of phylogeographic structure through two glacial advances is not unusual and has also been documented in the European meadow grasshopper (*Chorthippus*) (see Hewitt 1996 for a review).

The degree of genetic divergence that *M. e. haidarum* exhibits is unlikely to have been achieved within the last 12,000 years when colonization from known southern and Beringian source areas became possible. Therefore, is reasonable to suggest that *haidarum* derived its genetic divergence *in situ*, isolated from other ermine populations for at least the duration of the Wisconsin in a coastal refugium in close proximity to Haida Gwaii (Fig 23).

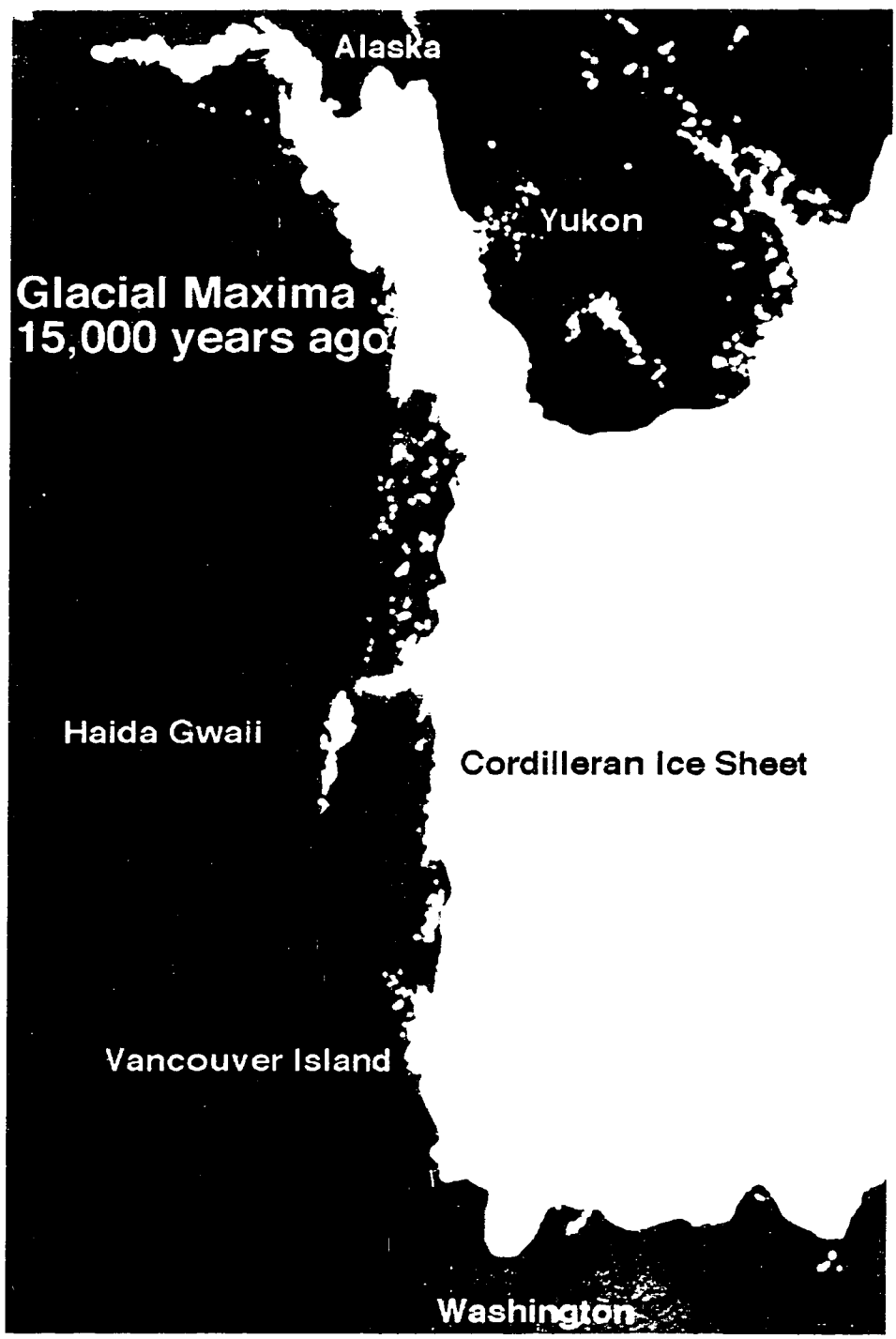
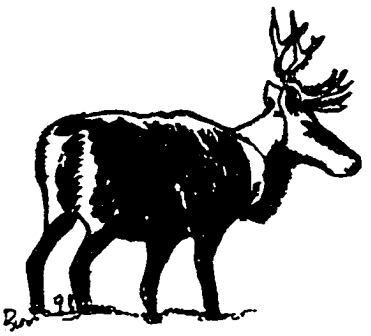

Alaska
Yukon
Glacial Maxima
15,000 years ago
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washington

fig 23

**Figure 23 Putative postglacial dispersal route of short-tailed weasel from the Hecate Refugium.**

Short-tailed weasels may have dispersed to Haida Gwaii from a coastal refugial source population which existed on the now submerged continental shelf. This may account for the unique mtDNA lineage characteristic of this population.

# Chapter Five



# Caribou (*Rangifer tarandus*)

## Introduction

The taxon *Rangifer tarandus* is comprised of at least nine (Banfield 1961) subspecies distributed across the Holarctic. They are found in a diversity of habitats such as tundra, northern boreal forests and alpine habitats (Cowan and Guiguet 1956; Nagorsen 1990) between which they migrate according to season and their reproductive cycle. In northern regions, caribou are almost always in constant motion while feeding. As such, herds tend to feed extensively rather than intensively covering areas of about 2 million km $^2$ in northern Canada (Kelsall 1968).

Although caribou consume a variety of vegetation depending upon locale and season, one of the most important types of food are terrestrial and arboreal lichens (Kelsall 1968; Cowan and Guiguet 1956). Although quantitative analysis of caribou diet has been impeded by technical limitations, direct observations imply that lichens are of

great importance for the persistence of caribou in extreme arctic climates (Kelsall 1968; Thomas et al. 1996).

During winter months when snow depths are minimal, caribou will often restrict their grazing to exposed vegetation. However, in greater snow depths, caribou will dig feeding craters and expose clumps of widely spaced vegetation with amazing accuracy. Caribou have been known to dig these craters in more than two feet of snow suggesting that food is being located by smell (Kelsall 1968). Despite speculations that caribou use their antlers to dig these feeding craters, observation of feeding animals and examination of antlers for wear has failed to substantiate these claims (Kelsall 1968). Instead, caribou dig feeding craters using their broad, deeply clefted hooves, which are not only well adapted for digging but for migrating over boggy ground and large expanses of ice and snow (Kurtén and Anderson 1980)

Caribou are capable of long migrations. During these migration periods which can occupy three to four months of the year, caribou migrate between various ranges 800-1200 km apart (Kelsall 1968). They are capable of moving quickly on land (as fast as 7 km/hour) and have been observed swimming across large bodies of water such as Aberdeen Lake and against tidal currents such as those found in Bathurst Inlet (Seton 1927; Kelsall 1968). Direct observations have shown that caribou are capable of swimming 6.5 km/hour and up to 12 km/hour over short distances (Seton 1927; Banfield 1954). They can move considerable distances by swimming, although they have not been observed swimming more than 6 km at a time (Kelsall 1968).

Migratory caribou herds in northern North America undertake two purposeful migrations a year. The first takes place between April 15 to June 15 when caribou move

from winter ranges to calving grounds. The second migration takes place during the late summer as caribou move from the barren tundra towards winter feeding grounds. The more sedentary caribou of the south also migrate during the spring but instead of congregating like northern caribou, they tend to disperse. In fact, dispersion is greatest at this time of year (Bergerud et al. 1990).

## Evolution of *Rangifer*

Although much about the evolutionary history of caribou is unknown, it is generally accepted that caribou originated in Eurasia (Osborn 1910) or Beringia (Kurtén and Anderson 1980) and reached North America at least 500,000 years ago (Kurtén and Anderson 1980). During the late Wisconsin, caribou were restricted to three refugia, Alaska/Yukon, Peary Land, and south of the continental glaciers, coinciding with the three major groups of North American caribou, mainland tundra caribou (Barren Ground), Arctic insular caribou and forest caribou (Woodland), respectively.

## The Subspecies

Substantial geographical variation in body size, antler morphology, pelage color and secondary sexual markings has led to considerable confusion in caribou taxonomy. In 1961, Banfield reduced the previously identified 15 North American subspecies (Hall and Kelson 1959) to six . These six subspecies are subdivided into two types: the barren ground caribou and the woodland caribou.

**Barren Ground Caribou**

Barren ground caribou occur in the northern part of the North American range. They include northern subspecies *R. t. granti*, *R. t. pearyi*, and *R. t. grönlandicus*, and *R. t. eogroenlandicus* (extinct), although recent revisions based upon secondary sexual characteristics have led to suggestions that the name, barren ground, should be restricted to those caribou in northwestern North America (Geist 1991). Barren ground caribou are distinguished by their relatively small to moderate body size, relatively short legs, and broad hooves. The skull is comparatively short to moderate in size with broad, flattened nasal arches (Banfield 1961).

Antlers of barren ground caribou are highly variable but can generally be described as long, well developed and lightly colored. Beams are cylindrical and the brow tines are typically more palmate. Both sexes usually possess antlers. Barren ground caribou are highly gregarious and often undergo extensive seasonal migrations, typically moving to taiga during winter months.

**Woodland caribou**

Woodland caribou occupy the southern portion of the North American range. Two subspecies included in this group are *R. t. caribou* and *R. t. dawsoni* (extinct). Banfield (1961) and Geist (1991) considered the mountain caribou of British Columbia, Alberta and Idaho to be synonymous with woodland caribou. Distinguishing characteristics of woodland caribou include a relatively large body size with rather elongate skull. Pelage is thinner and generally darker than the barren ground caribou, and the extent of white fur on the belly, legs and rump is more restricted (Banfield 1961).

Woodland caribou also possess antlers with variable morphology. However, they can be characterized as short, heavy and weakly developed compared to barren ground caribou. Beams are flattened and tines are generally more palmate. Although both sexes can possess antlers, approximately 30-40% of females are naturally antlerless (Banfield 1961).

Woodland caribou are moderately gregarious and are often found in small herds of usually no more than nine individuals. They undergo local migrations but these typically involve altitudinal rather than long distance movement (Bass 1995, Cowan and Guiguet 1956).

**Dawson caribou (*Rangifer tarandus dawsoni*)**

The three subspecies of caribou that are of greater concern in this study *are R. t. granti*, *R. t. caribou*, and *R. t. dawsoni*. Figure 24 shows the distribution of these subspecies. A brief description is given in Appendix II.

*R. t. dawsoni* was the rarest and most restricted form of caribou. It was first reported by Dawson in 1890 and formally described for science by Seton-Thompson in 1900. That same year, Osgood made his expedition to Haida Gwaii and based upon his own failure to uncover conclusive evidence, he suggested that the type specimen from which Seton-Thompson described *dawsoni* was a hoax. Additional evidence from the Haida and a shed antler found in 1906, later confirmed the existence of the Haida Gwaii caribou. In 1908 a small herd of Dawson caribou consisting of two bulls, a cow and a calf was seen. The three adults were shot and the skins and skulls were sent to the Royal British Columbia Museum in Victoria. The smaller male was mounted. These

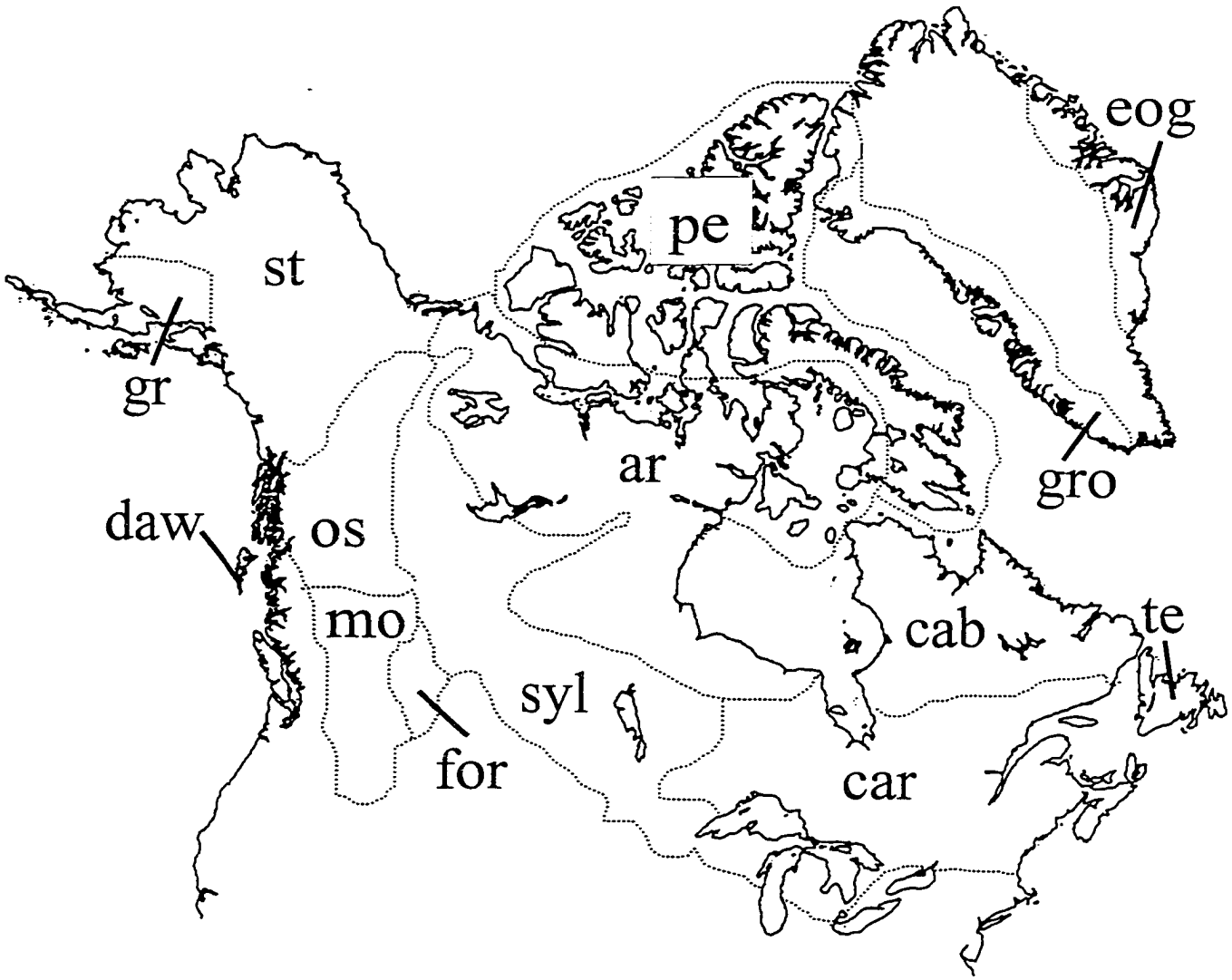



**Figure 24 Subspecies distribution map of *Rangifer tarandus*.**
Subspecies abbreviations: daw - *dawsoni*, gr - *granti*, st- *stonei*, os - *osborni*, mo - *montanus*, fo - *fortidens*, syl - *sylvestris*, car - *caribou*, cab - *caboti*, ar - *arcticus*, pea - *peayri*, gro - grönlandicus, eog - *eogroenlandicus*, te - *terraenovae*. Adapted from Hall 1981. In a recent reevaluation of caribou taxonomy, Geist (1991) suggested that the subspecies *R. t. caribou* should include all mainland caribou. Therefore, the subspecies designated by Hall as *osborni*, *montanus*, and *fortidens* are collectively referred to in this thesis as the subspecies *caribou*.

individuals were the last Dawson caribou ever seen on the islands and it is believed that they became extinct shortly thereafter during the 1920's (Cowan and Guiguet 1956) or 1930's (Banfield 1961) (Fig 25 a and b).

Not much is known about Dawson caribou. They were probably never abundant and the Haida, who generally restricted their activities to the coast, did not have much knowledge regarding the caribou which inhabited the dense coastal forests (Banfield 1961). What little is known about their morphology is based on the type skull and the three individuals shot in 1908.

*R. t. dawsoni* was small statured. Their antlers were poorly developed and remarkably irregular while females were typically antlerless (Cowan and Guiguet 1956). They are described as having a pale coloured pelage with no distinguishing black, dark brown or white markings (Cowan and Guiguet 1956).

Banfield (1961) and Foster (1965) considered *dawsoni's* diagnostic morphological characteristics typical of insular populations. In addition to the highly plastic nature of caribou morphology, the absence of suitable caribou habitat on Haida Gwaii during the last glacial maximum led Foster (1965) to believe that *dawsoni* was an early occupant of Haida Gwaii, which actually evolved its particular characteristics during postglacial periods.

The six subspecies of caribou in the western Hemisphere are allopatric and exhibit intergration along the margins of their geographical ranges (Banfield 1961). Although these subspecies probably evolved postglacially *in situ*, Banfield (1961) surmised that the two groups of caribou, barren ground and woodland, evolved in different Wisconsin glacial refugia and that the anomalies in their distribution pattern could be explained by

FIG 25a

FIG 25b

**Figure 25a and b**

These Dawson Caribou were the last ever reported seen on Haida Gwaii. They were shot in 1908 by Haida hunters. Photo courtesy of the BC Archives.

their migration through corridors which became accessible sometime after deglaciation. If it is true that Dawson caribou was recently derived from southern refugia along with *R. t. caribou*, then there should not be any significant genetic differences between them. Furthermore, *R. t. caribou* and Dawson caribou should show similar levels of divergence from barren ground caribou.

## Materials and Methods

### Samples

DNA from four *R. t. caribou* (northern BC) and two *R. t. granti* (Ray Mountain Herd) was obtained from muscle and blood tissue and antler velvet. DNA from *R. t. dawsoni* was obtained from the three adults shot in 1908. Small pieces of hide from two individuals (RBCM 1487 and RBCM 1486) and small scrapings from the ear, belly, and foot of the mount (RBCM 1484) were collected and stored as described in Chapter Four. Samples were also taken from the type specimen (RCBM 1483). Small scrapings of desiccated flesh were taken off the skull and base of the antlers and subsequently stored at room temperature.

Samples from two caribou metapodials from aracheological excavations (Blue Jackets Creek and Honna River - see Fig 26) and a sample from a putative caribou antler found on Haida Gwaii carbon dated (accelerator mass spectrometry) at 40,000 years BP were also used. The caribou antler was sampled in a fume hood, which had been washed down with dilute bleach and lined with bench paper. Drill bits were soaked in dilute HCL, rinsed with 70% ethanol and thoroughly dried. The antler was drilled directly through the center. Bone powder was collected in sterile eppendorf tubes and stored at -

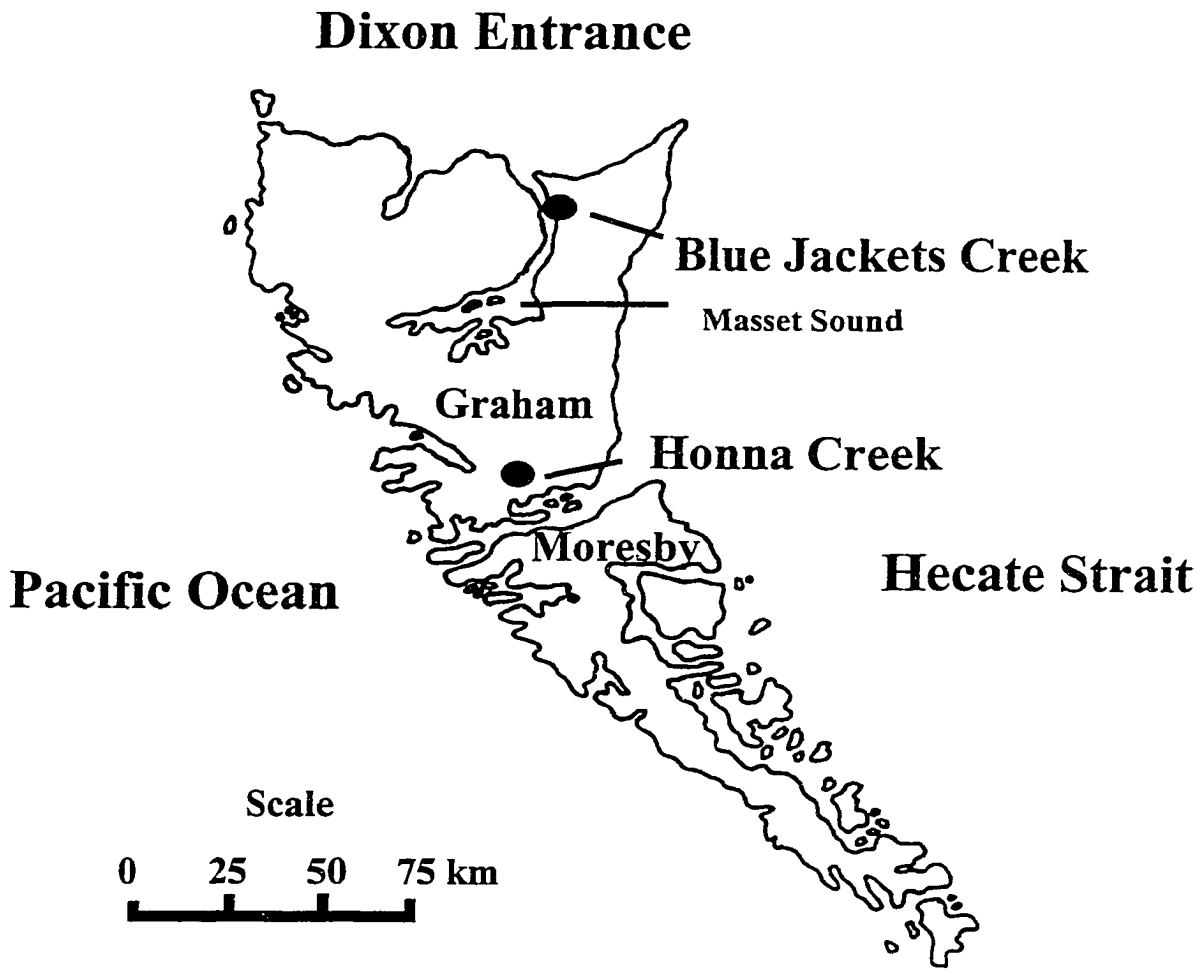



**Figure 26 Location of Blue Jackets Creek and Honna River archaeological sites.**

Blue Jackets Creek is located approximately 2.4 km south of Masset on the eastern shore of Masset Sound. Radiocarbon estimates suggest that the date of this midden is between 4300 to 2000 years BP. In addition to the worked caribou metapodials which were used in this study, remains of 28 human individuals, numerous cultural artifacts, and shells have been found here. A diversity of faunal remains also suggests the presence of caribou, black bear, dogs, ravens, loons, seals, sea otters, salmon and Pacific halibut during this time (Severs 1973).

Honna River is located approximately 6.4 km west of Queen Charlotte City. Relatively little is known about this site except that a small assemblage of cultural artifacts such as microblades and unworked flakes has been found. Unfortunately, this site has been badly damaged by current logging operations (British Columbia Site Inventory Form).

70°C

Initial sampling from the long bones was done by S. Crockford of the Department of Anthropology. I later resampled these same long bones using the following protocol. A flow hood in the Department of Anthropology was wiped down with dilute bleach. Drill bits and bone were soaked separately in dilute bleach, the former for 3 minutes and the latter for 1 minute. Bleach was necessary to remove contaminant DNA. Drill bits were thoroughly dried. Bone and the drill bits were UV irradiated in the flow hood for one hour. The bone was secured using a retort stand and clamp and surrounded by a clean plexiglass shield. Bone was drilled at an upward angle so a stream of bone powder could be collected in a sterile 15 ml falcon tube. Efforts were taken to minimize damage and maintain the integrity of the bone's diagnostic features. To minimize cross contamination, only one bone was sampled within a 24 hour period. Samples details are given in Table 10.

**DNA Isolation**

*Muscle/ Blood/Preserved Skin*

DNA was extracted from blood using DTAB/CTAB reported earlier. DNA was extracted from muscle, desiccated muscle, and preserved skin using the CTAB protocol described in Chapter 2, using the same precautions for ancient DNA.

*Bones*

DNA from bones were extracted using a protocol originally developed for DNA

**Table 10**
**Subspecies, geographical location and sample sizes for caribou**

| Subspecies | Location | Sample number | Sample size | Sample type | Source |
|---|---|---|---|---|---|
| *caribou* | northern BC[1] | n/a | 4 | muscle/antler | author |
| *granti* | Alaska [2] | 104342 | 1 | blood | author |
| *granti* | Alaska | 104334 | 1 | blood | author |
| *dawsoni* | Graham Island (GI) [3] | 1486 | 1 | preserved skin | author |
| *dawsoni* | GI | 1487 | 1 | preserved skin | author |
| *dawsoni* | Virago Sound (GI) | 1483 | 1 | dried muscle | author |
| *dawsoni*? | Blue Jackets Creek (GI) [4] | Flua-004 | 1 | long bone | author |
| *dawsoni*? | Blue Jackets Creek (GI) | Flua-004 | 1 | long bone | author |
| *dawsoni*? | Honna River (GI) [5] | Flua-15 | 1 | long bone | author |

[1] Four samples of *R. t. caribou* were from the following places in northern BC: Tucho Lake,
Prophet River, and Johiah Lake. Exact locale for the fourth individual was not given.
[2] Both samples of *R. t. granti* were from the Ray Mountain herd in central Alaska.
[3] Sample of *R. t. dawsoni* were obtained from the Royal British Columbia Museum in Victoria.
[4] The Blue Jackets Creek midden is located on the eastern shore of Masset Sound approximately 2.4 km south of Masset. Samples were provided by P. Severs.
[5] The Honna River Bridge site is found about 6.4km south of Queen Charlotte City on the eastern bank of the Honna River. Sample was provided by P. Severs.

extraction from preserved skin (Thomas et al. 1990). Approximately 1-1.5 grams of bone powder was immersed in 15 ml of 10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 2 mM EDTA, 1% SDS, 10 mg/ml dithiothreitol (DTT) and 0.5 mg/ml of proteinase K. This mixture was subsequently incubated at 37°C for 40 hours with constant gentle agitation. The resulting solution was then extracted twice with an equal volume of phenol and once with choloroform:isoamyl (24:1). The extract was subsequently concentrated and desalted using a Centricon 30 microconcentrator and stored at -20°C.

**Amplification**

Amplification using primers H15149 and L14841 was done using the same conditions described in previous chapters. Although products were retrieved using these primers, amplification was not efficient. New primers H15183 (YCC TCA RAA TGA TAT TTG TCC TCA) and L14850 (CCA TCA AAY ATC TCR TCA TGA AA), were designed using aligned cervid sequences from GenBank. These primers were used with the following PCR conditions: 40 cycles of 94°C for 2 minutes, 55°C for 1 minute, 72°C for 1 minute. These cycles followed an initial 2 minute hold at 94°C and followed by a 7 minute extension at 72°C.

Additional primers (L15058 GCT TAT TTA TAC ATG TAG GAC GAG GCC, H15058 CCG GAG CAG GAT GTA CAT ATT TAT TCG, H15144 GGA CAT ATC CTA CAA ATG CTG TAG CTA TTA, L14968 TCT CCT CTG TTA CTC ACA TCT GCT GAG ACG TC) for ancient DNA were designed so a range of PCR products spanning 50 to 400 bp could be amplified. For convenience, the same PCR conditions were used for all of the caribou primers so samples could be run with multiple primers

simultaneously. Primer positions are illustrated in Figure 27.

All amplifications with ancient DNA were done using the precautions described in Chapter Four.

**Purification of PCR Products**

PCR products were purified using either commercial columns alone (for manual sequencing), purified using Nusieve and columns or low melting agarose (Eclipse) and columns (for manual sequencing). See Chapter 2 for full description.

**Cloning**

PCR products were ligated and transformed using the Invitrogen Cloning Kit. Clones were purified as described earlier in Chapter 2 and sequenced using the ABI automated sequencer.

**Automated Sequencing**

Clones were sequenced according to the ABI suggested protocol. See Chapter 2 for more details.

**Manual Sequencing**

PCR products were sequenced using [$\alpha$-$^{35}$S] dATP direct incorporation. See Chapter 2 for more details.

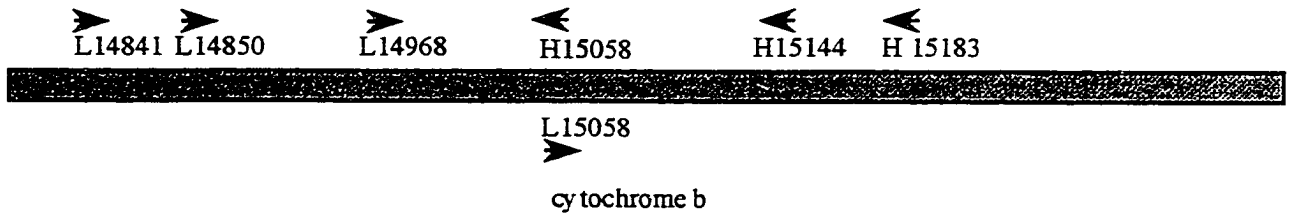



**Figure 27**
Schematic representation of primer positions and PCR sstrategy for Rangifer tarandus.

**Phylogenetic Analyses**

Sequence consensus from ABI and sequence alignments for caribou were generated using Lasergene Navigator (DNASTAR, Madison, WI). A minimum of three sequences were used to generate the sequence consensus. These consensus sequences were subsequently analyzed using maximum parsimony (Eck and Dayoff 1966; Fitch 1977), maximum likelihood (Cavelli-Sforza and Edwards 1967) and neighbour-joining (Saitou and Nei 1987). All tests were performed using PAUP * (Swofford in press).

Pairwise distances using Kimura's two parameter model in PAUP* 4.0 (Swofford, in press) indicated that the most appropriate outgroup for *Rangifer* was the muntjac (*Muntiacus muntjak*) and the Chinese water deer (*Hydropotes inermis*). However large genetic divergence of the outgroups compared to the genetic distance within the ingroup suggested that long branch attraction might be a problem. Therefore, in all of the following analyses, outgroups were added to constrained ingroup topologies.

Appropriateness of muntjac and the Chinese water deer as outgroups was subsequently evaluated using maximum likelihood ratios on PHYLIP 3.5c (Felsenstein 1989). A maximum likelihood tree was generated on PHYLIP, using a $T_i/T_v$ ratio of 2, and empirical base frequencies. The outgroups were then moved to 7 other positions within the tree to determine whether the placement of the outgroup in the original maximum likelihood tree was significantly better than all other placements. This was done for each outgroup alone and together.

*Maximum Parsimony*

Maximum parsimony trees were generated using a branch and bound search of equally weighted characters in PAUP * 4.0 (Swofford, in press). Initial tree was obtained by random stepwise addition and branch swapping implemented using TBR and steepest descent. Data was resampled using 2000 heuristic bootstraps.

*Maximum Likelihood*

Maximum likelihood analysis was done using PAUP* 4.0 (Swofford, in press). Empirical base frequencies (A=0.30, C= 0.23, G=0.15, and T=0.32)and $T_i/T_v$ ratios 2 and 4 were used. $T_i/T_v$ ratios estimated by maximum likelihood was also used. Starting branch lengths were obtained by Rogers-Swofford approximation method. Trees were generated using the HKY85 model (Hasegawa et al. 1985) and branches swapped using TBR and steepest descent. Trees were evaluated using 2000 heuristic bootstrap replicates.

*Distance*

$K_s$ and $K_a$ was determined using MEGA 1.02 (Kumar et al. 1993). Pairwise distance were also calculated using two parameter models in PAUP* (Swofford, in press). The two parameter distance matrix was used to calculate average sequence divergence and to generate a neighbour-joining tree (Saitou and Nei 1987). Because long distances tend to compound systematic error, no outgroup was used in this analysis because sequence data for closely related taxa was unavailable. Data was resampled using 2000 heuristic bootstrap replicates.

## Results

A total of nine individuals were sequenced, three of which were Dawson caribou (RBCM 1483, 1486, and 1487). Fragment lengths of 313 bp to183 bp were obtained. Sequences are presented in Table 11. Attempts were made to amplify fragments of cytochrome b from antler and long bone samples. However, amplification products were only obtained with the antler. Unfortunately, yield from antler DNA was extremely low and as such, purification attempts always resulted in total loss of product.

The appropriateness of the outgroups, muntjac and Chinese water deer, was evaluated using maximum likelihood ratios. Eight maximum likelihood trees which differed only in the position of its outgroup were examined in order to evaluate the suitability of the muntjac and water deer as outgroups. In almost every case random placement of the outgroup yielded trees that were not significantly worse than any other. These results suggests that rooting the tree with these outgroups is neither relevant nor meaningful. As such, the trees produced by maximum parsimony, neighbour-joining and maximum likelihood were rooted at the midpoint.

### Phylogenetic Analysis

The information provided by this mtDNA gene target was minimal. Limited number of synapomorphies and low genetic distances did not allow for significant resolution. Although parsimony, maximum likelihood and neighbour-joining analyses generated trees with similar topology (Fig 28, 29, 30), most nodes were not well

## Table 11 Caribou character matrix.

The following table shows the variable sites in reference to the sequence shown below. Position numbers are shown above the table, nucleotides are shown below position number.

? indicates missing data.

### Dawson caribou 1487

```
1                                        40                                      80
AATTTTGGCTCTCTACTAGGAATCTGCTTAATTCTACAAATCCTTACCGGTCTATTTCTAGCAATACATTATACATCCGA
81                                       120                                     160
TACAATAACAGCATTCTCCTCTGTTACTCACATCTGTCGAGACGTCAATTATGGCTGAATAATCCGATACATACATGCCA
161                                      200                                     240
ACGGAGCATCAATATTTTTCATCTGCTTATTTATACATGTAGGACGAGGCCTAYACTACGGATCATACACCTTCCTAGAA
241                                      280                             311
ACATGAAATATTGGAGTGATCCTCTTATTTACAGTAATAGCTACAGCATTTGTAGGATATGTCCTACCATG
```

**Position Number**

| Taxon | 111111111111111111112222222222222222222222233333<br>112234555778800111233445556788899011223333456666677789900000<br>548696703614234703980113258132595706817036961036791284390125 8 |
|---|---|
| Alaska 1 | TTCCCCTCTATTATTTTCTCATACCATCACCTAATTCCACCCAYTGCCTATTATTATATCA |
| Alaska 2 | TTCCCCTTTATTATTTTCTCATACCATCACCTAATTCCACCCATTGCTTATTATTATATCA |
| Johiah Lake | TTCCCCTCTATTATTTTCTCATACTATCACCTAATTCTACCTATTGCCTATTATTTATGCA |
| Prophet River | ?????CTCTATTAYTTTCTCATACTATCACCTAATTCTACCCATTGCCTATTATTATATCA |
| Tucho Lake | ??????????TTATTTTCTCNTNCTATCACCTAATTCTACCTATTGCCTCTTATTATATCA |
| northern BC | ?????CTCTATTTTTTTCTCATACCATCACCTAATTCCACCCATTGCCTATTATTATATCA |
| Dawson 1487 | TTCCCCTCTATTATTTTCTCATAACATCACCTAATYCCACCCATTGCCTATTATTATATCA |
| Dawson 1483 | ????????????????TTTCTCATACTATCACCTAATTCCACCCGTTGCCTAATATTATATCA |
| Dawson 1486 | ??????????????????????ATACTATCACCTAATTCCACCCGTTGCCTATTATTATATCA |
| Reindeer | TTCCCGTCTATTATTTTCTCATACCATCACCTAATTCCATCCATTGCCTATTATTATATCA |
| Muntjac | CCCTCCCACACTATGTCTCCACATTACATTTCACCTCTACCTATTATCCATTTCCATATTA |
| Chinese water deer | TCTTTTTACCCCACTCTTCTACATCGTAATCTCACTTTTCTCATCATCCATCACCGTATCT |

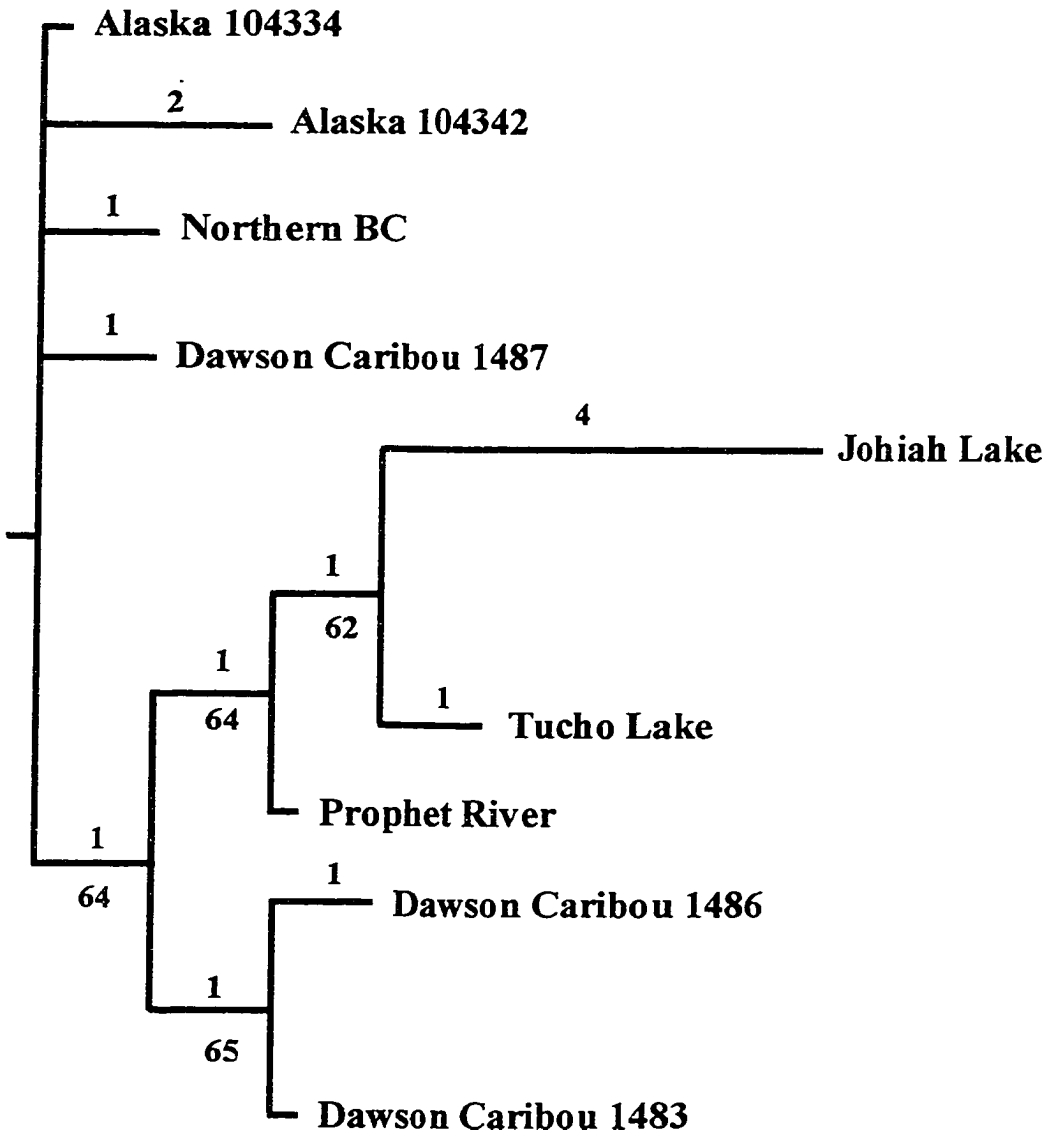



**Figure 28 Maximum parsimony tree for caribou.**
The woodland caribou are distinguished from barren ground caribou based on a single substitution. Dawson caribou (1486 and 1483) were further identified by another substitution. However, Dawson caribou 1487, was excluded from this woodland group along with one individual from northern BC. Branch lengths are shown above branches, bootstrap values are shown below.

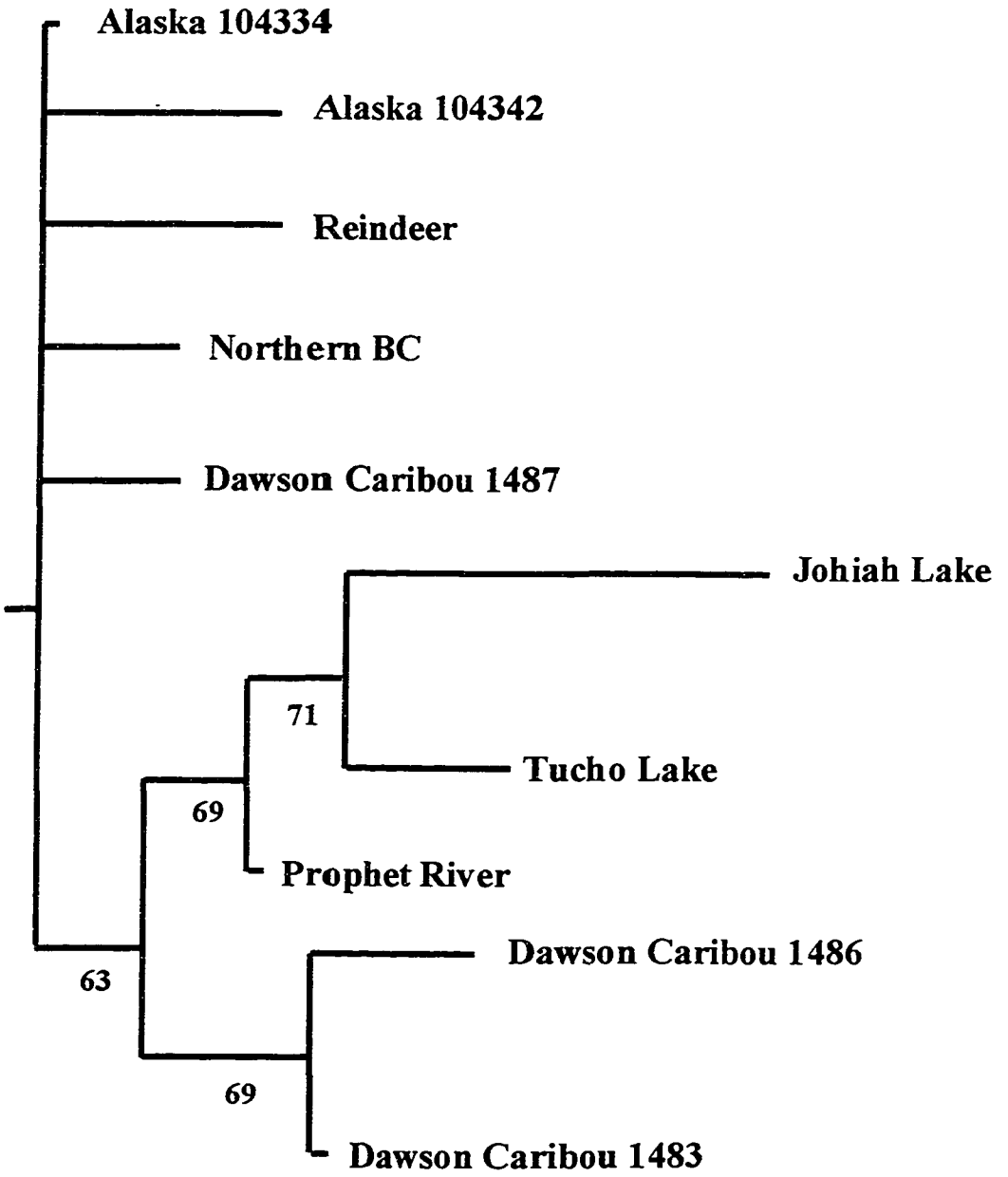



**Figure 29 Maximum likelihood tree for caribou.**
The tree generated from maximum likelihood was identical in topology to the tree generated from maximum parsimony. Branch lengths reflect maximum likelihood values. Bootstrap values are shown below branches

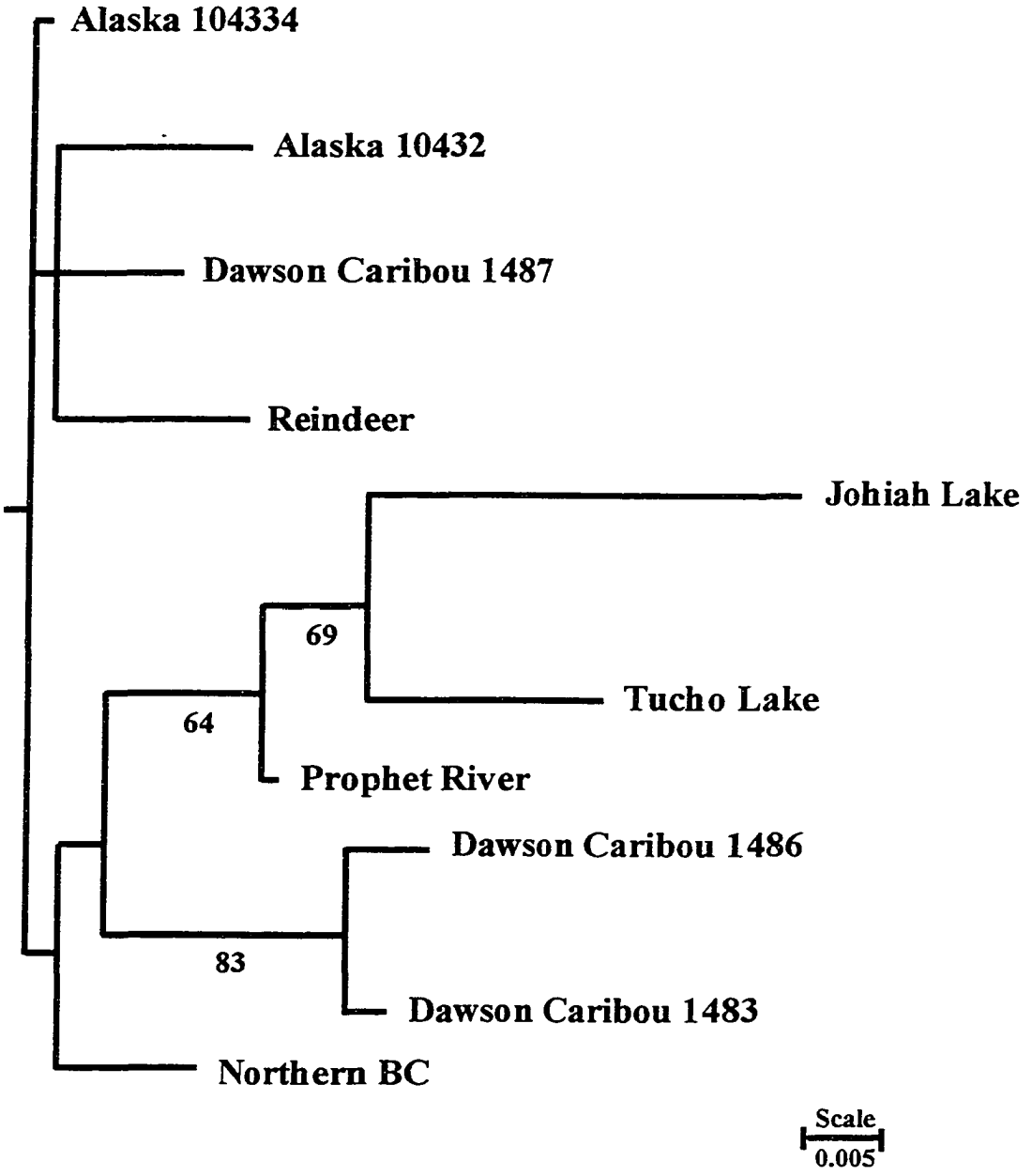



**Figure 30 Neighbour-joining tree for caribou.**
The neighbour-joining tree was almost identical to the maximum parsimony and maximum likelihood trees. The only difference was the inclusion of the individual from northern BC in the woodland group. However, this node was not supported by bootstrap analysis. As with parsimony and likelihood, Dawson caribou 1486 and 1583 were included in the woodland group; Dawson caribou 1487 was excluded. Branch lengths reflect genetic distance. Bootstrap values are shown below branches. The neighbour-joining tree was generated based on genetic distances generated by Kimura's two parameter model.

supported by bootstrapping. Nonetheless, I have reported the results from these analyses in the following sections.

Out of the nine individuals that were sequenced, nine haplotypes were identified. Despite the high number of haplotypes, degree of differentiation between haplotypes was relatively low. Woodland caribou and barren ground caribou were not recognized as distinct mtDNA assemblages although some geographic separation is evident. Distribution of haplotypes are shown in Figure 31.

*Maximum Parsimony*

Out of a total of 313 characters, there were 4 parsimony informative sites. Due to the paucity of informative characters, the resolving power of these data were very limited. A single tree from a branch and bound search was found that had a tree length of 16, CI of 1.00 and RI of 1.00. The tree is shown in Figure 28. Most of the woodland caribou were grouped together by a single synapomorphy at position 152. Within this woodland lineage, caribou from Johiah Lake, Tucho Lake and Prophet River, and Dawson caribou 1486 and 1483 were further defined by a single synapomorphy at position 221. Dawson caribou 1487, Alaska 104334, Alaska 104342, and the individual from northern BC were excluded from this lineage and differed from one another by one to three substitutions.

A total of 10 substitutions were found. These occurred in the transmembrane:outer ($Q_o$):inner($Q_i$) segments in the ratio 6:1:3. Six of the substitutions were transitions, 67% were C/T and 33% were A/G. Of the four transversions observed,

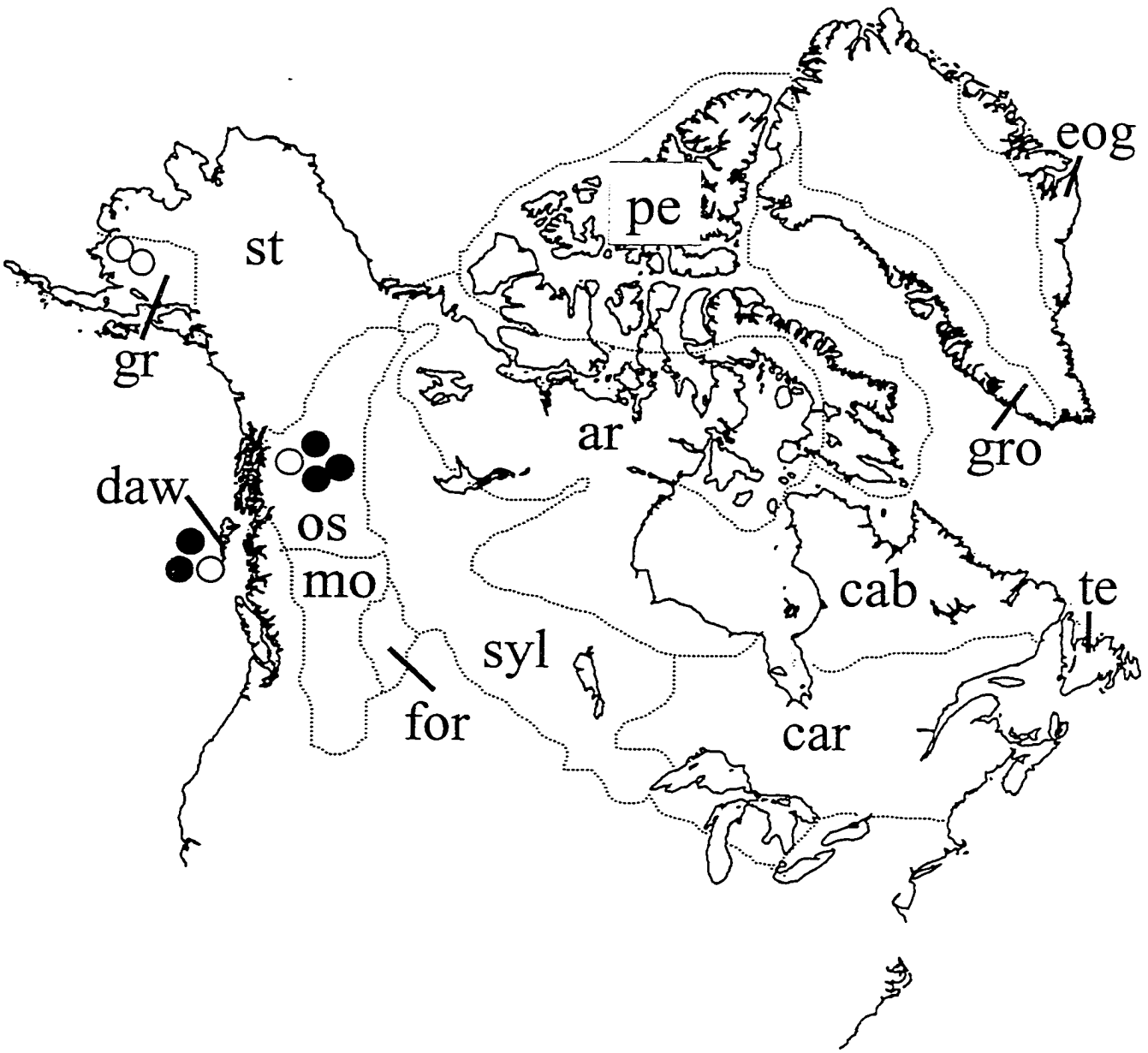



**Figure 31.**
Geographic distribution of mtDNA groups ( ○ and ● ) (n=4 and n=5 respectively) in caribou based on relationships shown in parsimony and maximum likelihood. Genetic differentiation between groups was small. The important point of this figure is to show that Dawson caribou is not genetically unique and is composed of haplotypes found in both *caribou* and *granti*..

two occurred in amino acid residues known to be hypervariable (Irwin et al. 1991).

*Maximum Likelihood*

The topology of maximum likelihood trees were similar to the branch and bound tree produced by maximum parsimony (Fig 29). The -ln likelihood is 536.54.

*Distance*

Pairwise distances using Kimura's two parameter model were calculated. The minimum genetic distance was 0.0033 between Dawson caribou 1487 and Alaska 104334 (which is equivalent to a single substitution) and maximum distance was 0.0386 between Dawson caribou 1486 and Johiah Lake (which is equivalent to seven substitutions). Details are given in Table 12.

The tree constructed from neighbour-joining cluster analysis grouped most woodland caribou together (Johiah Lake, Tucho Lake, Prophet River, Dawson caribou 1486, Dawson caribou 1483, and northern BC). Another lineage was identified which included Alaska 104342, Dawson caribou 1487 and reindeer. Alaska 104334 was excluded from both of these lineages (Fig 30). Neither the barren ground or woodland caribou were supported by bootstraps. However, there was reasonable support for the *caribou* (64) and *dawsoni* (83) lineages

There were no synonymous substitutions between Dawson caribou 1483 and 1486 ($K_s$=0). There was however one nonsynonymous change at position 122 ($K_a$=0.007). $K_s$ and $K_a$ between Dawson caribou 1483 and 1486 and Dawson caribou 1487 was 0.013 and 0.007 respectively. $K_a$ within the *caribou* lineage was 0.014; not

**Table 12 Kimura 2-parameter distance matrix for *Rangifer tarandus*.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 Alaska 104334 | - | | | | | | | | | |
| 2 Alaska 104342 | 0.0066 | - | | | | | | | | |
| 3 Johiah Lake | 0.0233 | 0.0300 | - | | | | | | | |
| 4 Prophet River | 0.0073 | 0.0150 | 0.0186 | - | | | | | | |
| 5 Tucho Lake | 0.0168 | 0.0209 | 0.0206 | 0.0084 | - | | | | | |
| 6 northern BC | 0.0035 | 0.0111 | 0.0299 | 0.0109 | 0.0210 | - | | | | |
| 7 Dawsons 1487 | 0.0033 | 0.0099 | 0.0265 | 0.0107 | 0.0208 | 0.0072 | - | | | |
| 8 Dawsons 1486 | 0.0145 | 0.0193 | 0.0384 | 0.0141 | 0.0240 | 0.0145 | 0.0194 | - | | |
| 9 Dawsons 1483 | 0.0114 | 0.0172 | 0.0386 | 0.0108 | 0.0228 | 0.0115 | 0.0167 | 0.0051 | - | |
| 10 *Rangifer tarandus* | 0.0066 | 0.0131 | 0.0296 | 0.0145 | 0.0207 | 0.0110 | 0.0097 | 0.0192 | 0.0171 | - |

surprisingly, $K_s$ was higher at 0.028. Between the Dawson caribou and *caribou* lineage, $K_s$ was 0.065. $K_a$ was 0.012. Within the woodland lineage (Dawson caribou and *caribou*), $K_s$ was 0.045. $K_a$ was considerably lower at 0.01. Within the barren ground lineage $K_s$ was 0.0134. However, $K_a$ was considerably higher at 0.059. Between the woodland and barren ground lineages, $K_s$ was 0.052 and $K_a$ was 0.013.

Average distance within the woodland lineage was 0.020 whereas intralineage distance within the barren ground lineage was 0.040. These distances are comparable to interlineage distances of 0.018 between the woodland and barren ground lineages and between *dawsoni* and *caribou* lineages. Because of the low number of variable sites average distance was initially used to estimate divergence time. Using the average genetic distance of 1.8 % between woodland and barren ground lineages and the standard vertebrate molecular clock, the two lineages diverged approximately one million years ago. Intralineage divergence occurred one million to two million years ago. Based on paleontological data, which indicates that caribou entered North America approximately 500,000 years ago, the molecular divergence dates may overestimate the actual subspecies divergence. A more reasonable divergence estimate is obtained using Brown et al's (1982) rate for synonymous substitution of 10% per million years. $K_s$ between woodland and barren ground is 5.2%, suggesting that these groups separated shortly after arriving in North America. The Dawson caribou lineage is estimated to have diverged from the *caribou* lineage about the same time. Using this 10% rate, intralineage divergence may have occurred sometime within the Holocene.

Although these latter divergence estimates are consistent with the fossil evidence, the small number of changes and small sequence lengths limits confidence in these

results. A single random substitution can significantly elevate genetic divergence and as such elevate divergence estimates. However, it is clear from the data collected in this study that none of the caribou groups, including Dawson caribou, were significantly different from one another.

## Discussion

Foster (1965) referred to Dawson caribou as the most intriguing of all native mammals on Haida Gwaii because of its very distinctive morphology and recent extinction. Due to the lack of suitable caribou habitats on Haida Gwaii during the late Wisconsin, Foster surmised that Dawson caribou was a postglacial immigrant derived from the same source population as *R. t. caribou*. Based on partial sequences from the mitochondrial gene cytochrome b, Dawson caribou was not recognized as a unique mtDNA assemblage and as such, supports Foster's hypothesis that Dawson caribou is not a glacial relict. Furthermore, the lack of significant genetic differentiation between *dawsoni*, *caribou* and *granti* suggest that all of subspecies may have diverged relatively recently.

Although these statements are tenuously based on extrapolations from a single locus gene tree, the lack of genetic differentiation between caribou subspecies has also been supported by other studies. The inability to unambiguously identify barren ground caribou and woodland caribou as distinct mtDNA assemblages using a small single locus is not surprising given that RFLP analysis (Cronin 1992) has also failed to resolve *caribou* and *granti* as distinct mtDNA groups. While Strobeck (1994) did find some genetic differentiation between woodland and barren ground caribou based on

preliminary investigations using RFLP analysis and D-loop sequence comparisons, the apparent lack of phylogenetic structure in caribou populations and the high number of haplotypes found in this study is consistent with what has been found by other workers (Purdue 1995 pers. comm.). Although I believed that the best approach to resolving the phylogenetic relationship between caribou was through a broad analysis of multiple loci using a method like RFLP, this was not done because of technical limitations. Obtaining a region long enough for RFLP analysis was not possible given the ancient state of Dawson caribou DNA. As the aim of this study was not to establish a caribou phylogeny but to provide insight into the relative relationship of Dawson caribou with conspecifics, I did not consider it useful to compare extended sequences of *caribou* and *granti* when such comparisons were not possible with *dawsoni*.

**Implications for Morphology**

Many of Dawson caribou's divergent characteristics such as small stature and reduced antlers are typical of insular ungulates (Huxley 1932; Foster 1964; Case 1978). This is clearly illustrated by the recent decrease in body size and allometric reduction in antler size of black tailed deer which were introduced to Haida Gwaii in the 1920's. These morphological changes to body and antler size has been attributed to a variety of factors including lack of predators and poor nutrition ( Foster 1965; DeBlase and Martin 1981) while antler reduction has further been interpreted as an adaptation to improve mobility through dense forest (Lister 1993).

A 66 % reduction in antler beam length in moose (*A. alces*) from its mid-Pleistocene ancestor *A. latifrons* appears to have coincided with a shift from an open,

steppe-like habitat (Sher 1974) to coniferous forest (Lister 1993). Similarly, the reduction in antler size observed in Dawson caribou may be an adaptive response to a shift into coastal temperate rainforest. While large antlers would not be an encumbrance in open habitats such as tundra where caribou typically live, they could have impeded mobility within the forest.

The metapodials used in this study that were recovered at Blue Jackets Creek and Honna River are significantly larger than is typical for Dawson caribou. In fact, the bones found at this site are similar in size to barren ground caribou and may represent individuals present on the islands prior to the dwarfing process (Severs 1973). Radiocarbon dates taken at this midden suggest that the bones are approximately 4200 years old (British Columbia Archaeological Site Inventory Form) and that the change in body size occurred sometime thereafter. However, considering that the bones showed evidence of human workmanship, these bones may have also been brought to Haida Gwaii from the adjacent mainland postglacially. Such rapid reduction in body size is not unusual in insular ungulates. Similar reduction have been observed in the Sitka deer recently introduced to Haida Gwaii. Caribou on Arctic islands are similarly stunted presumably due to poor nutrition.

The pale colored pelage characteristic of Dawson caribou is unusual given the high levels of precipitation of their coastal forest habitat. However, this feature might be an artifact of preservation since photographs of the individuals shot in 1908 do not appear to have the mousy color considered particular to this subspecies (Fig 25 a, b) (Banfield 1961; Nagorsen 1997 pers. comm.). Lack of genetic differentiation of Dawson caribou is consistent with the supposition that it is a postglacial immigrant to Haida Gwaii and its

highly distinctive form a consequence of rapid *in situ* evolution.

**Implications for Refugia**

Dawson (1890) regarded the absence of other native cervids on Haida Gwaii as evidence that Dawson caribou arrived on the islands during the early stages of deglaciation. Woodland caribou (represented by *dawsoni* and *caribou*) are believed to have evolved south of the Cordilleran Ice Sheet, separated from barren ground caribou (represented by *granti*) which were isolated in northern refugia. There is some evidence for separation between these two groups as the average genetic distance between *granti* and the woodland lineage was 0.018 and comparable to the average distance between *granti* and *tarandus* (0.02) which have been separated since the end of the last glaciation (Roed and Whitten 1986).

Lack of genetic differentiation between subspecies *caribou*, *granti* and *dawsoni* suggest that the latter is not a glacial relict and was actually postglacially derived from southern populations, northern populations, or combinations thereof. From the cytochrome b sequence data, Dawson caribou appears to be comprised of haplotypes bearing close affinity with both barren ground and woodland caribou suggesting that it may be paraphyletic. The affinity of *dawsoni* haplotypes to both woodland and barren ground caribou may be due to gene flow which occurred during the glacial period, shortly after glaciers receded and/or to recent introgression. Given that populations of Dawson caribou have probably been isolated since sea levels rose about 10,000 years BP, recent introgression is not likely. However, persistence of woodland and barren ground haplotypes in Dawson caribou may be the result of ancestral polymorphism which are

still present in Dawson caribou due to its recent origin. Another explanation may be that Dawson caribou was postglacially derived from both northern (barren ground) and southern (woodland) populations rather than a single refugial source population.

Based on high levels of mtDNA variation, large caribou populations are believed to have existed in eastern Beringia during the late Wisconsin (Cronin 1992). These populations probably consisted of both *granti* and *tarandus* which were separated approximately 11,000 years BP with the submergence of the Bering land bridge (Elias et al. 1996). Although *R. t. tarandus* was recently introduced into Alaska during the late 1800's, there has been limited gene flow between these subspecies (Cronin et al. 1995). Average genetic distance between *tarandus* and *granti* (0.02) is comparable to the average genetic distance between *dawsoni* and *caribou* (0.016), *caribou* and *granti* (0.018), and *dawsoni* and *granti* (0.016), suggesting that these subspecies may have been in contact and interbreeding up until the end of the Wisconsin.

Lack of significant genetic differentiation between *caribou*, *granti* and *dawsoni* may be caused by recent origin of these three subspecies and/or a history of recent colonization following the retreat of Wisconsin glaciers and gene flow. Early postglacial movement up the coast might have been possible for caribou given their ability to migrate over boggy ground and ice, swim short distances and subsist on sparse tundra vegetation. Recovery of a skull believed to be that of a caribou in 1915 from a glacier near the mouth of the Skeena River (Banfield 1961) suggests that caribou were present on the coast during early postglacial times. A partial metacarpal retrieved from the Prince of Wales Island (see Table 16 for more details) suggest that caribou had reached offshore islands by about 10, 000 years BP (Heaton et al. 1996) and caribou subfossils at Blue Jackets

Creek suggest that they had reached Haida Gwaii no later than 4000 years BP.

Movement of caribou from coastal BC to Haida Gwaii could have taken place over ice or Hecate Strait when it was above sea level. Despite its swimming ability, it is unlikely that caribou colonized Haida Gwaii by swimming across the Strait. As mentioned previously, lengthwise currents prevent direct access and swimming would have probably taken too long for many animals to survive. Osgood's frequent observations of dead deer on the shores of Haida Gwaii exemplifies this difficulty (1901). Although caribou are known to migrate over ice, empirical evidence suggests that long crossings where islands or an opposing shore is not visible at the outset are rarely made except during purposeful migrations to calving grounds or winter ranges (Kelsall 1968). Evidence from Cape Ball shows that Haida Gwaii was deglaciated 16,000 years BP and parts of the Hecate Strait are known to have been terrestrial during the glacial maxima (Warner et al. 1982). The assemblage of plant remains in these cores implies that this area was surrounded by patches of herbaceous vegetation akin to the treeless tundra-like regions that barren ground caribou inhabit today. Given their ability to migrate over snow and ice and persist in periglacial environments, it may be that caribou, possibly ancestors to *dawsoni*, used this area as feeding or calving grounds (Fig 32). As the Cordilleran ice sheet retreated and readvanced, locations of refugial areas may have frequently changed, promoting population panmixis along the coast as a diversity of herds congregated in these areas. Furthermore, caribou feeding behaviour probably encouraged migrations all over the coast as herds moved from one refugium to another in search of food. Perhaps the caribou in Beringia and caribou south of the Cordilleran ice sheet were largely isolated but occasionally met and bred in coastal refugia, ultimately

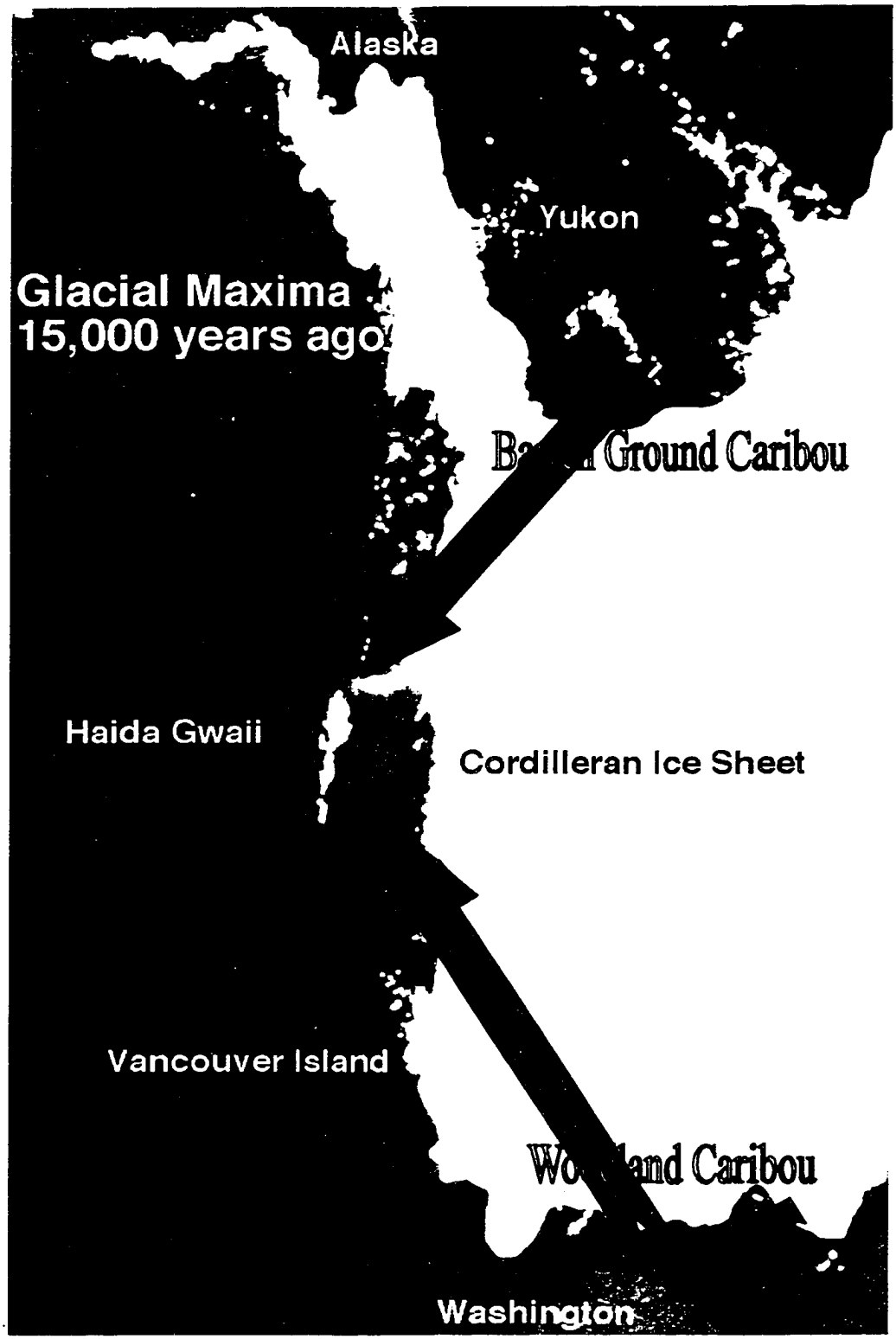
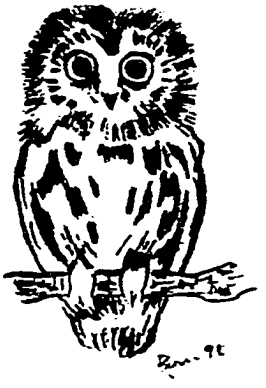

Alaska
Yukon
Glacial Maxima
15,000 years ago
Ground Caribou
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washington

fig 32

**Figure 32 Putative Late Wisconsin dispersal routes of caribou.**
Haida Gwaii may have been recolonized by caribou source populations found both north and south of the Cordilleran ice sheet. Given the ability of caribou to travel over snow, ice and water, dispersal probably began during the early stages of deglaciation (Fraser Glaciation) and possibly intermittently throughout the late Wisconsin.

giving rise to Dawson caribou near the end of the Wisconsin. Although Dawson caribou is not likely to be a glacial relict and offers no conclusive evidence of coastal refugia in the Pacific Northwest, it is a classic example of the morphological differentiation that can occur on island habitats over a short period of time.

# Chapter Six



## Saw-whet Owl (*Aegolius acadicus*)

### Introduction

The Saw-whet Owl is typically found in moist coniferous and deciduous forests. They are found all across Canada in any woodland habitat from Haida Gwaii to Newfoundland and are known to breed from southeastern Alaska to Mexico with the exception of southeastern states. During the winter, the range of the Saw-whet Owl shifts slightly south but as this species is a short distance migrant (Terres 1980) its winter range is generally congruent with their breeding range.

The Saw-whet Owl is amongst the smallest of all owls in North America with adult males having an average height of about 170-190 mm. They are nocturnal and hunt a variety of small mammals such as squirrels, bats, and shrews, small songbirds, and most commonly insects (Terres 1980).

Owls are known to have existed in North America since at least the Paleocene (Feduccia 1996). Fossils of owls are relatively rare (Feduccia 1996) and as such provide

little information regarding the distribution of various owl species during the Wisconsin glaciation. Although there is no information regarding the post-glacial source population for the Saw-whet Owl, considering its high dependence on trees for nesting sites, it is unlikely to have survived in the tundra regions north of the Cordilleran ice sheet and was more likely to have persisted and recolonized northern North America from southern refugia.

**The Haida Gwaii Saw-whet Owl (*Aegolius acadicus brooksi*)**

There are two subspecies of Saw-whet Owl found in Canada, *A. a. acadicus* and *A. a. brooksi* (Fig 33). The Haida Gwaii Saw-whet Owl (*A. a. brooksi*) was first officially described by Fleming in 1916 as a dark, slightly larger race and was later regarded as a distinct insular species (Brooks and Swarth 1925). These two features which characterize *brooksi* are typical for insular avifauna (Murphy 1938; Foster 1965) and as such are likely to have evolved *in situ* as a response to local selective pressure. However, of the three other endemic subspecies of birds found on Haida Gwaii, the non-migratory and solitary behaviour of the Saw-whet Owl limits its potential success as a colonizer and makes it one of the birds more likely to have survived in a glacial refugium.

If the Haida Gwaii Owl is a relict of the last glacial advance on the coast, then these owls should be genetically distinct and some phylogeographic structure should be observed. If the Haida Gwaii Owl is a post-glacial migrant which arrived to the archipelago from mainland refugia, then these owls should not show significant genetic differentiation from adjacent conspecifics.

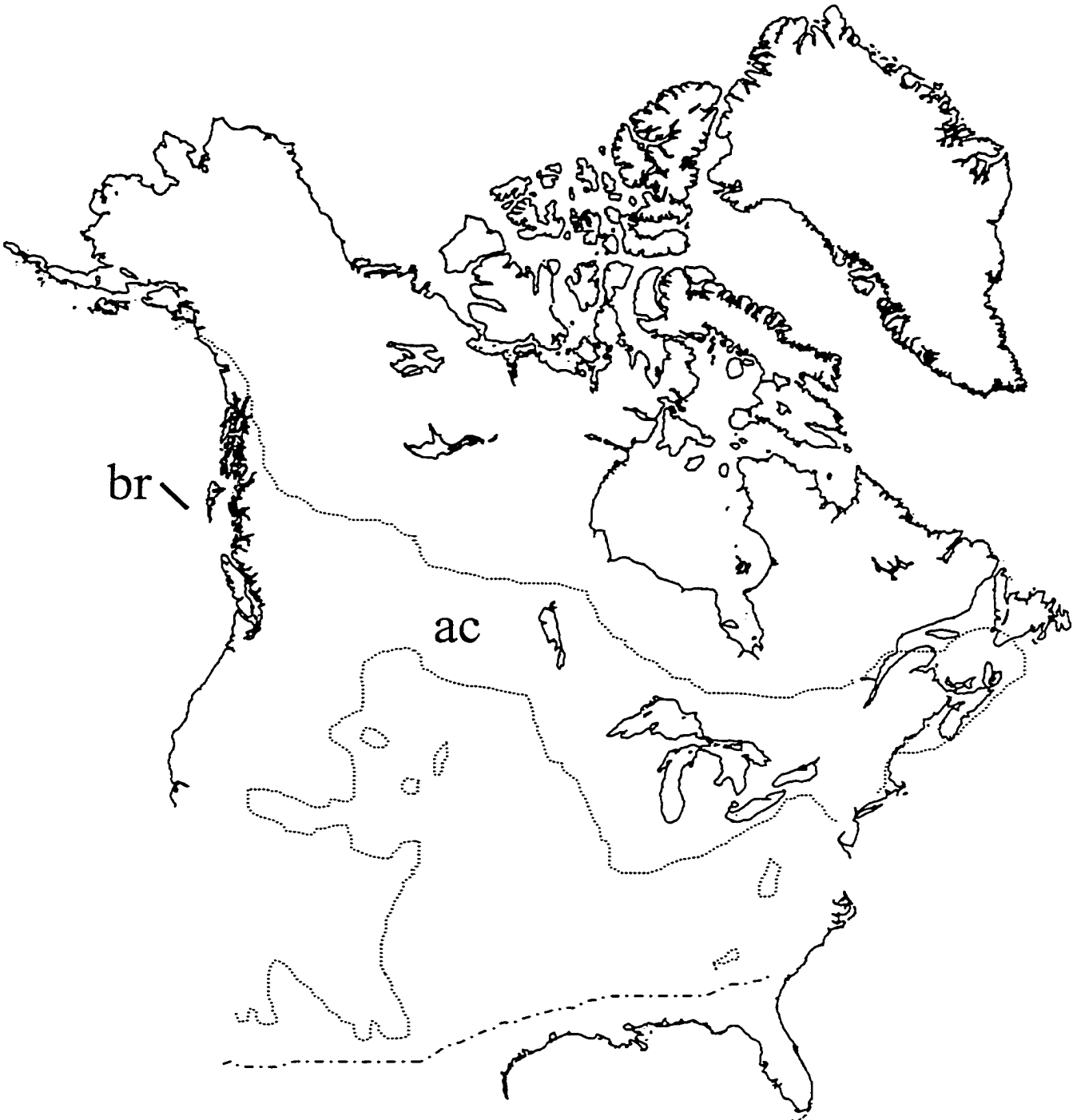



**Figure 33 Subspecies distribution map of *Aegolius acadicus*.**
Subspecies abbreviations: br - *brooksi* and ac - *acadicus*. Adapted from Johnsgard (1988). The map shows the breeding range of the two subspecies of Saw-whet Owl in North America. Differences between the two subspecies have already been outlined in Chapter 6.

-·-·-·- southern limit of winter migrants
····· subspecies boundary lines

## Materials and Methods

### Samples

A total of nine individuals were used and included the two subspecies *brooksi* (n=2) and *acadicus* (n=6). For *acadicus*, six individuals were from various locales around BC, and 1 was from Manitoba. DNA from Saw-whet Owls was primarily obtained from preserved skin. Sample details are given in Table 13.

### DNA Isolation

DNA was isolated from muscle and preserved tissue as described earlier.

### Amplification

Primers were made based on the most conserved regions within a nightjar alignment generated using sequences from GenBank and Megalign. Nightjars were chosen as they are considered to be a close relative of the owl (Sibley and Alhquist 1990). The following primers were designed: H705 GCT AGG GTT GTT AGT GGG AGG AGT AT, H589 CCY GAT TCG TGG AGG AAG GTG AGG TG, H648 TRT GAA YCC TAG TGG GAT TTT GTC, H616 ATG CCT AGR GGG TTG TTA G, H685 YAG TAT GAA YCC TAG GAT GTC, L127 GTA ACA CAA ATC CTA ACC GG.

### Purification of PCR Products

PCR products were purified using commercial columns or purified using Nusieve and columns. These methods were described previously.

**Table 13**
**Subspecies, geographical location and sample sizes for Saw-whet Owl.**

| Subspecies | Location | Sample number* | Sample size | Source |
|---|---|---|---|---|
| *acadicus* | Kamloops, BC | 14113 | 1 | author |
| *acadicus* | Yew Lake, BC | 14417 | 1 | author |
| *acadicus* | Vancouver, BC | 14734 | 1 | author |
| *acadicus* | Sechelt Lake, BC | 17410 | 1 | author |
| *acadicus* | Hope, BC | 516 | 1 | author |
| *acadicus* | Oak Lake, Man | 13016 | 1 | author |
| *brooksi* | Haida Gwaii | 910 | 1 | author |
| *brooksi* | Haida Gwaii | 919 | 1 | author |

*These samples are from the Royal British Columbia Museum. All of the samples were from preserved skin.

**Cloning**

PCR products were ligated and transformed using the Invitrogen Cloning Kit See Chapter 2 for details.

**Automated Sequencing**

Clones were sequenced according to the ABI suggested protocol.

**Phylogenetic Analyses**

Consensus sequences were generated using a minimum of three clones using Lasergene Navigator (DNASTAR, Madison, Wisconsin). The resulting consensus sequences were aligned using Megalign. Sequence consensus from ABI and sequence alignments for marten were generated using Lasergene Navigator (DNASTAR, Madison, WI). A minimum of three sequences were used to generate the sequence consensus. Three types of analyses were used on the resulting consensus sequences: maximum parsimony (Eck and Dayoff 1966; Fitch 1977), neighbour-joining (Saitou and Nei 1987), and maximum likelihood (Cavelli-Sforza and Edwards 1967). All tests were performed using PAUP * (Swofford in press).

*Maximum Parsimony*

Trees were generated by adding randomly adding taxa. Heuristic searches were conducted using tree bisection and reconnection (TBR) and with the steepest descent option in effect. Nodes were statistically evaluated using 2000 heuristic bootstrap replicates.

*Maximum Likelihood*

Maximum likelihood tests were conducted using PAUP* (Swofford, in press). Trees were generated through heuristic searches, TBR, and steepest descent. Maximum likelihood tests were based on the HKY85 (Hasegawa et al. 1985) model using empirical base frequencies of A=0.30, C=0.32, G=0.15, and T=0.23. $T_i/T_v$ ratios of 2 and 4 were used. Starting branch lengths were approximated using Rogers-Swofford approximation method. Trees were generated based on a heuristic search, TBR branch swapping and steepest descent. Trees were evaluated using 2000 heuristic bootstrap replicates.

*Distance Analyses*

Pairwise distance was calculated for all taxa using Kimura's two parameter (Kimura 1980) model in PAUP * 4.0 (Swofford, in press). The resulting distance matrix model was subsequently used to determine average sequence divergence and to construct a neighbour-joining tree (Saitou and Nei 1987) in PAUP* 4.0. Nodes were evaluated using 2000 bootstrap replicates. Synonymous ($K_s$) and nonsynonymous variation ($K_a$) were also calculated using MEGA 1.02 (Kumar et al. 1993).

## Results

Fragment lengths of 241 bp were obtained for all individuals except for Haida Gwaii 1 in which only 218 bp was amplified. There was very little difference in sequence between most individuals (Table 14).

## Table 14 Saw-whet Owl character matrix.

The following table show the variable sites in reference to the sequence shown below. Position numbers are shown above the table, nucleotides are shown below position number.

? indicates missing data.

**Yew Lake**

```
1                                                40                                                80
CTACTAGCCATACACTACACCGCTGACTCAACTCTAGCCTTCACATCAGTCTCACACACATGCCGATATGTCCAATACGG
81                                               120                                               160
CTGACTAATCTGCAAGCTACACGCAAACGGAGCATCCATATTCTTCATCTGCATCTATCTACACATCGGACAGGGCCTAT
161                                              200                                               240
ACTACTGCTCATACCTCTACAAAGAAACCTGAAACACAGGAGTCATACTCCTACTGAGCCTRATAGCAACCGCCTTYGTA
241
G
```

| Position Number | |
|---|---|
| | 222222222 |
| | 5023333333 |
| **Taxon** | 31021235789 |
| Yew Lake | ACGRCGCTYGT |
| Kamloops | GCGACGCTCGT |
| Vancouver | GCGRCCGCTYY |
| Sechelt | AMGRCGCTYGT |
| Hope | RMGRCGCTTGT |
| Haida Gwaii 1 | RCG????????? |
| Haida Gwaii 2 | GCGGYGCTCGT |
| Oak Lake, Man. | GCKRCGCTCGT |

Three substitutions were found at positions 3, 222, and 237. All three were third base transitions, including two A/G and one C/T change. Two (one A/G and one C/T) occurred within the transmembrane region of the cytochrome b protein and one (A/G) was found on the outer surface adjacent to the $Q_o$ redox centre. Unfortunately, in all 3 positions where these substitutions occurred, considerable difficulty in sequencing was experienced. Despite numerous attempts to sequence through these regions, ambiguous bases consistently appeared. This may be due to DNA degradation, a typical feature of preserved samples (Pääbo 1989; Pääbo et al. 1989; Higuchi 1992). Therefore, these substitutions may actually be artifacts and as such the distances could be an overestimate. Given the ambiguity in the sequence data, I estimate that there are at least three haplotypes within the individuals examined.

Pairwise distances using Kimura's two parameter model was used to estimate average genetic distance (Table 15). The maximum distance observed between pairs was 0.0043 (a single substitution). Maximum synonymous variation ($K_s$) was 0.0172. There were no nonsynonymous ($K_a$) substitutions. Because of the low level of genetic divergence within cytochrome b, all methods of phylogenetic analyses indicated no significant phylogeographic structure within the Saw-whet Owls. The two subspecies *brooksi* and *acadicus* were not distinguishable using this region of cytochrome b. However, as this is not a Saw-whet phylogeny, the lack of resolution within this gene target is informative as it implies that the Haida Gwaii Saw-whet Owl is not genetically distinct from other owls on the mainland at this locus. Results from parsimony, neighbour-joining and maximum likelihood analyses are shown in Figs. 34, 35, and 36 respectively. The distribution of haplotypes are shown in Figure 37.

**Table 15 Kimura 2-parameter distance matrix for *Aegolius acadicus*.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 Haida Gwaii 1 | - | | | | | | | |
| 2 Haida Gwaii 2 | 0.0000 | - | | | | | | |
| 3 Hope | 0.0000 | 0.0042 | - | | | | | |
| 4 Kamloops | 0.0000 | 0.0043 | 0.0000 | - | | | | |
| 5 Sechelt | 0.0000 | 0.0043 | 0.0000 | 0.0000 | - | | | |
| 6 Yew Lake | 0.0000 | 0.0000 | 0.0042 | 0.0042 | 0.0042 | - | | |
| 7 Vancouver | 0.0000 | 0.0000 | 0.0000 | 0.0042 | 0.0042 | 0.0000 | - | |
| 8 Oak Lake, Man. | 0.0000 | 0.0000 | 0.0042 | 0.0042 | 0.0042 | 0.0000 | 0.0000 | - |

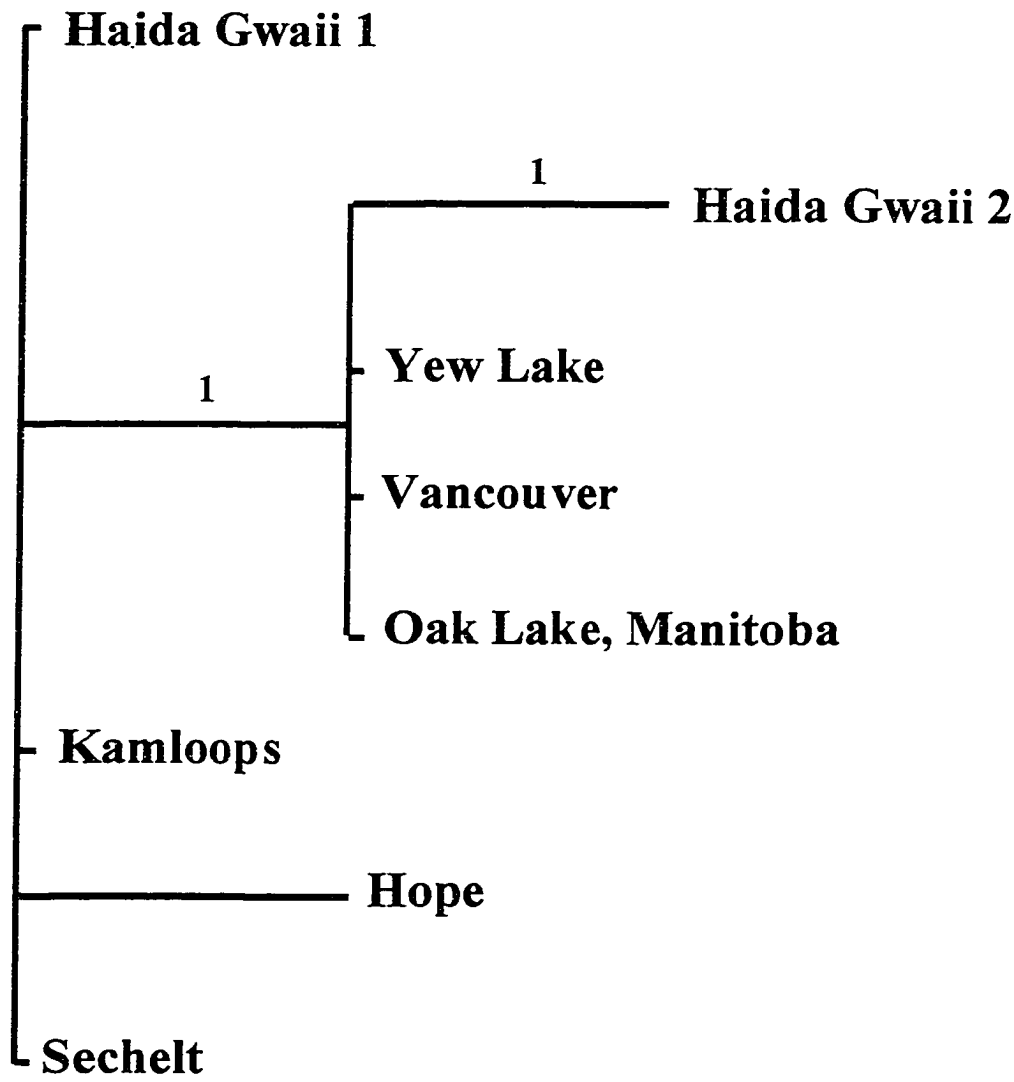



**Figure 34 Maximum parsimony for Saw-whet Owl.**
This maximum parsimony tree is an example of one of 100 equally parsimonious trees uncovered by PAUP*. Numbers above branches indicate branch lenghts. Due to the low numbers of substitutions, bootstrap analysis failed to support the tree topology.

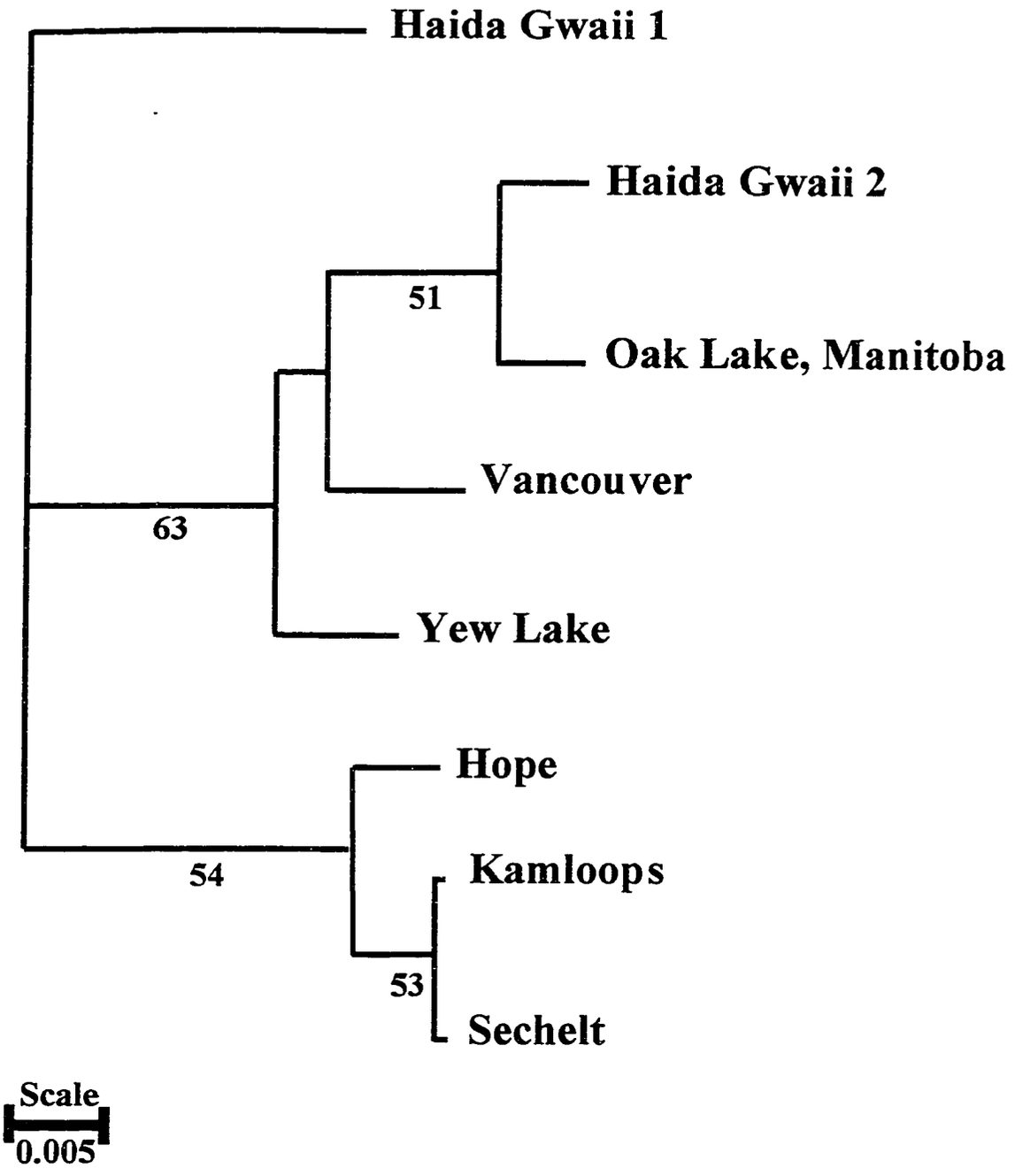



**Figure 35 Neighbour-joining tree for Saw-whet Owl**
There is no indication from the neighbour-joining tree that the Haida Gwaii owls are genetically distinct from Saw-whet Owls on the mainland. Branch lengths reflect Genetic distance. Bootstrap values (based on 2000 replicates) are shown below branches.

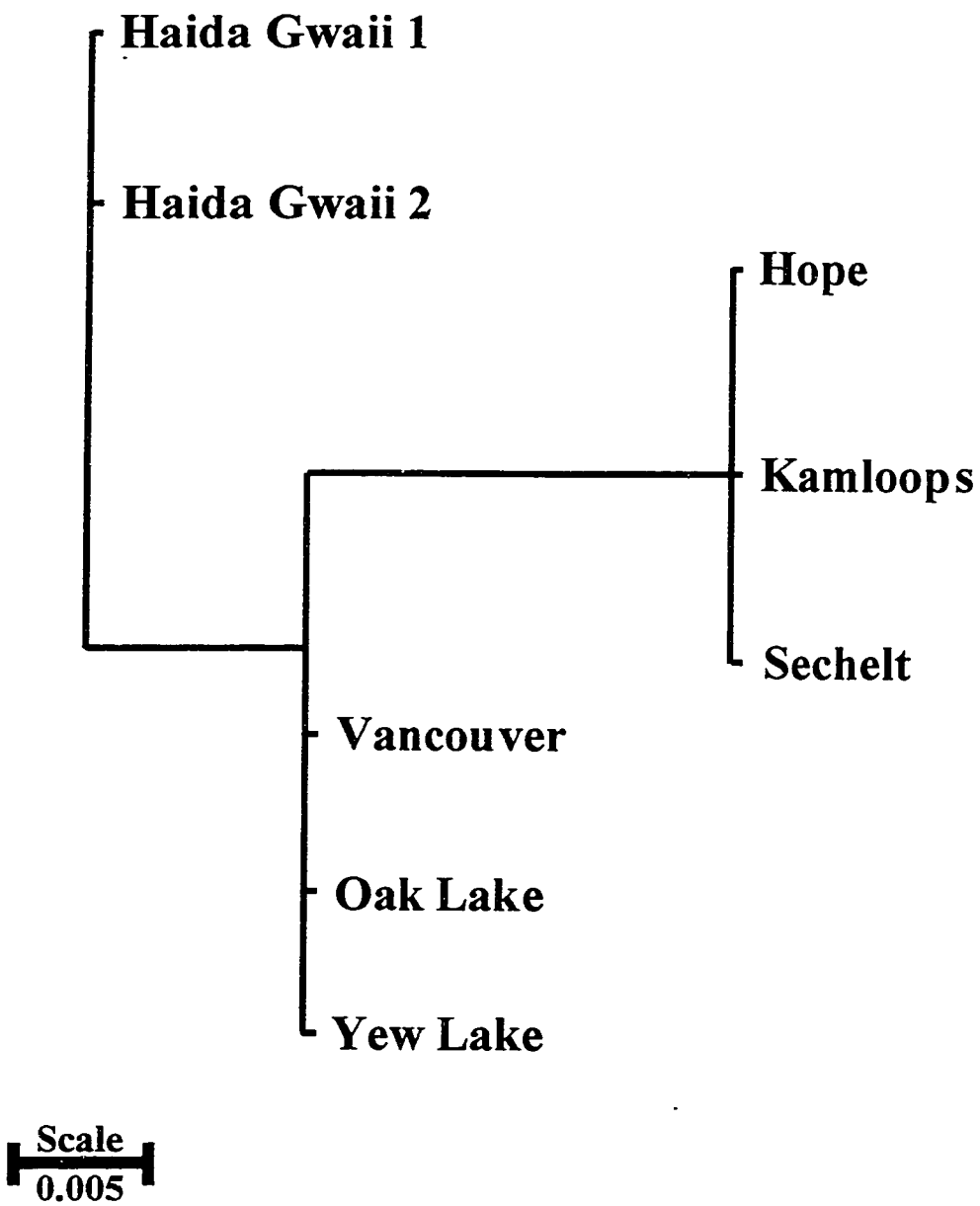



**Figure 36 Maximum likelihood for Saw-whet Owl.**
One maximum likelihood tree with a -ln likelihood ratio of 1677.99 was uncovered. Although the Haida Gwaii Saw-whet Owl is excluded from other owls in this analysis, there was no support for this topology from 2000 bootstrap replicates. Branch lengths reflect maximum likelihood values.

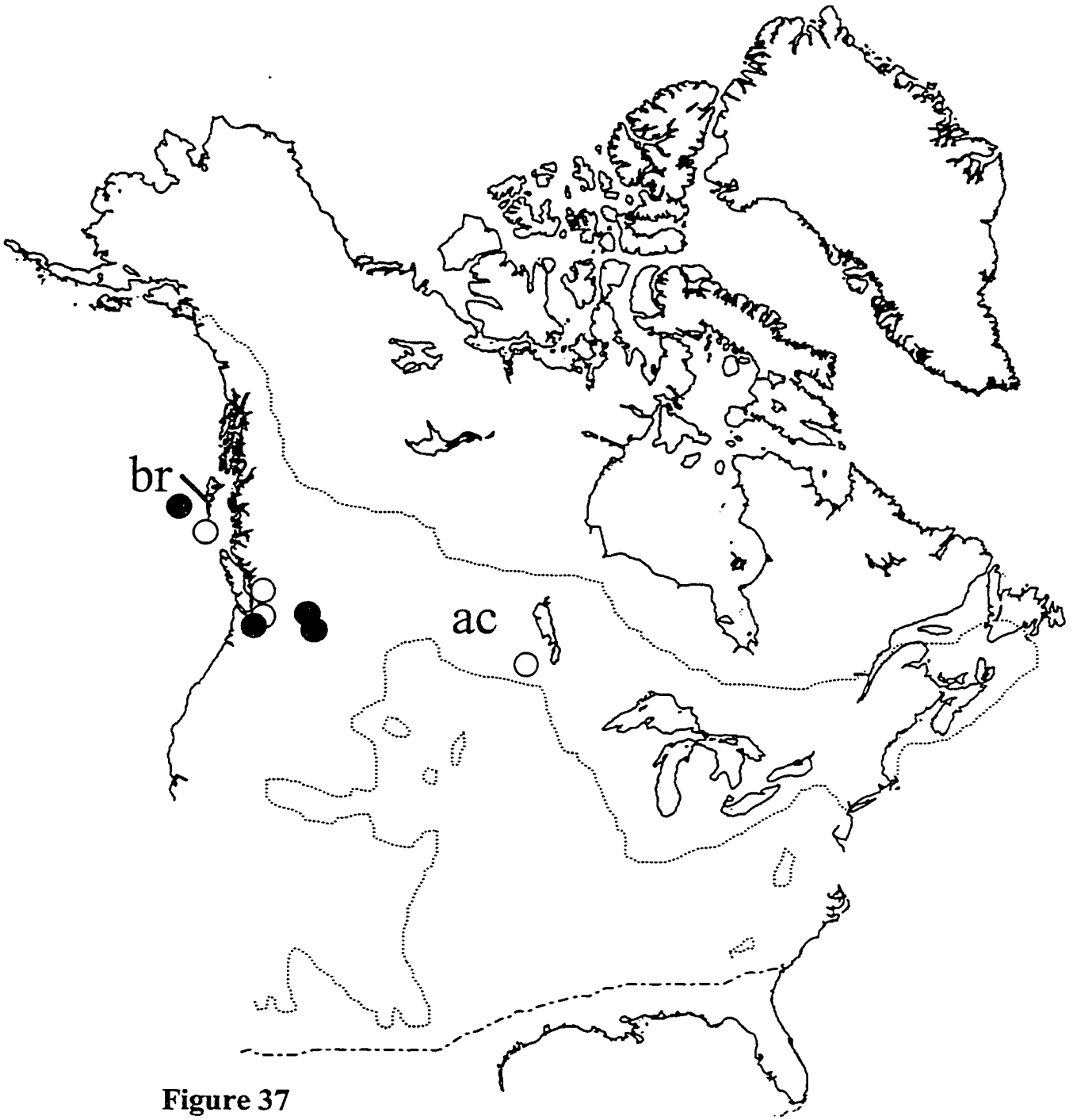



**Figure 37**

Geographic distribution of the two mtDNA groups (● (n=4) and ○ (n=4)) uncovered by phylogenetic analyses. These two groups were only weakly differentiated. This figure demonstrates the lack of phylogeographic structure in the Saw-whet Owl using this portion of cytochrome b and the lack of genetic differentiation between *brooksi* and *acadicus*.

## Discussion

North temperate birds generally exhibit low levels of genetic diversity (Barrowclough 1983), a likely consequence of high levels of contemporary gene flow or recent origins. As such, there are relatively few examples of intraspecific mtDNA variation within birds (Zink 1991). However, population substructuring has been observed in some bird species such as the Rufous-sided Towhee (*Pipilo erythrophthalmus*), Common Yellowthroat (*Geothlypis trichas*) (Ball and Avise 1992) and the Rock Ptarmigan (*Lagopus mutus*) (Holder pers. comm.).

In general, highly vagile species such as the red-winged black bird (*Agelaius phoniceus*), exhibit little mtDNA differentiation (Ball et al. 1988) whereas relatively sedentary species such as the seaside sparrow (*Ammodramus maritimus*) (Avise and Nelson 1989) exhibit significant phylogeographic structure. Given the relatively sedentary lifestyle of owls, the apparent low levels of genetic differentiation within this species ranging from Haida Gwaii to central Canada may seem unusual. However, of all owls in North America, the Saw-whet Owl is one of the most migratory, exhibiting fairly extensive eastern/western movements (Johnsgard 1988). The lack of phylogeographic structure using this small portion of the cytochrome b gene may be due to substantial movements of this species making it unamenable to this type of analysis. It can also imply that the two subspecies of Saw-whet Owl, *acadicus* and *brooksi*, are recently derived and probably post-glacial in origin.

There are numerous examples in the literature of morphological variation in avifauna in the absence of phylogeographic structure (for examples see Zink and Dittmann 1993; Ball and Avise 1992). The generally lower levels of genetic

differentiation of avian conspecifics compared to other vertebrates (Avise and Aquadro 1982) suggests recent establishment of populations and relatively rapid phenotypic response to environmental factors (Zink 1991). The apparent lack of genetic differentiation of the Haida Gwaii Saw-whet Owl suggest that it is not a glacial relict. This owl probably arrived on the archipelago sometime after glaciers retreated and subsequently differentiated during post-glacial times in response to its insular habitat (Fig 38).

The short sequences of cytochrome b used to determine lack of phylogeographic structure (218 -241 bp) and the small sample size needs to be confirmed by more sequencing, preferably of faster evolving targets like the D-loop or internal transcribed spacer regions. However, even if further investigation of this species corroborates preliminary findings of no phylogeographic structure within the Saw-whet Owl, it would not eliminate the possibility that other avian endemics on Haida Gwaii are glacial relicts.

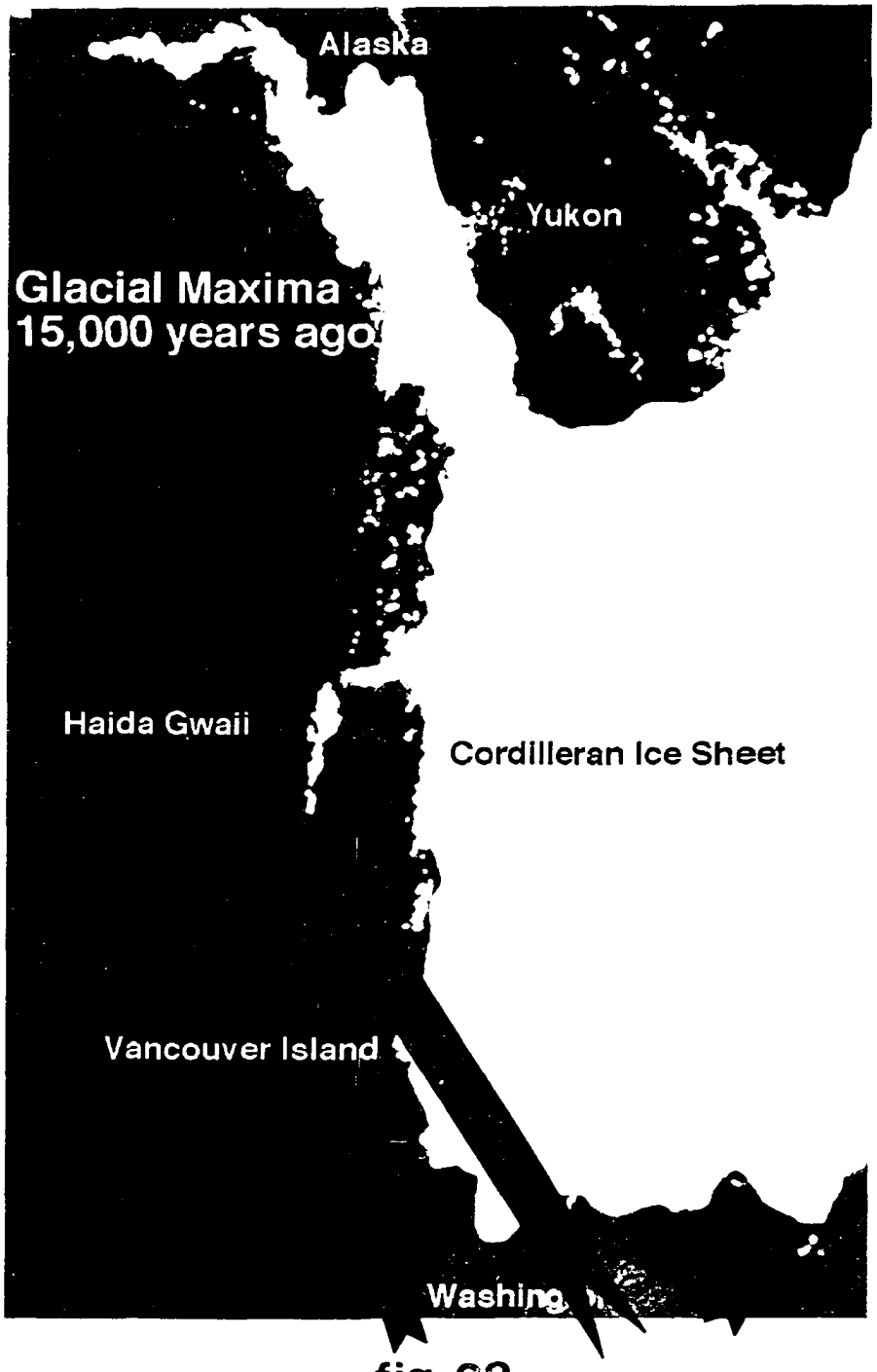

Alaska
Yukon
Glacial Maxima
15,000 years ago
Haida Gwaii
Cordilleran Ice Sheet
Vancouver Island
Washing

fig 38

**Figure 38 Putative postglacial dispersal route of the Saw-whet Owl.**
The Haida Gwaii Saw-whet Owl is probably a postglacial migrant from the mainland. Although it is not known if Saw-whet Owls persisted both north and south of the Cordilleran ice sheet during the late Wisconsin, given its high dependence on trees, it is likely that they largely resided in southern refugia. As such, I have indicated only northward dispersal.

# Chapter Seven

# Discussion

Studies of patterns of geographic variation are the foundation upon which processes of population differentiation such as natural selection, isolation, gene flow, genetic drift and ultimately speciation have been identified (Gould and Johnston 1972). Inferences from these patterns have helped to reveal the role of vicariant events in substructuring populations and have allowed us to assess the relative impact that historical and ecological factors have had on the origin and maintenance of morphological and genetic diversity.

Patterns of such genetic variation were obtained in this study through a broad mtDNA phylogeographic survey of black bears, short-tailed weasels, marten, Saw-whet Owls and caribou. For the black bear and marten, two mtDNA lineages (continental and coastal) which apparently diverged prior to the late Wisconsin were identified. Inclusion of two or more subspecies in each of these lineages suggest that these subspecies originated postglacially from two source areas, one on the continent and the other on the coast. Three mtDNA lineages were identified in the short-tailed weasel, corresponding to known refugial areas north and south of the Cordilleran ice sheet, as well as a putative refugium on the coast. As with the black bear and marten, subspeciation in weasels appears to have predominately occurred after range expansion from refugia following glacial retreat. Both the Saw-whet Owl and caribou mtDNA analyses demonstrated no significant phylogeographic structure, suggesting that the subspecies, including the Haida Gwaii Saw-whet Owl and Dawson caribou originated within the last 10,000 years. Examination of these patterns from an ecological/morphological and historical

perspective provide insight into some of the evolutionary processes that have influenced species diversity in the Pacific Northwest. The following is a discussion of these perspectives.

**Morphology**

It is generally assumed that climatic changes and population fragmentation which occurred during the multiple Pleistocene glacial advances promoted taxonomic differentiation (Mengel 1964; Hewitt 1993; Hewitt 1996; Bernatchez and Dodson 1991; Brown and Chapman 1991; however, see Coope 1979; Klicka and Zink 1998). Limited gene flow between populations due to isolation in different refugia is considered to be a major impetus for morphological differentiation. As such, it is not unreasonable to expect that the subspecies for each of the five species examined here originated during the Pleistocene.

However, for most of the subspecies examined in this study, morphological diversity appears to have occurred during post-glacial times and the uniformity of the changes observed in the endemic subspecies of black bear, marten, short-tailed weasel, caribou and Saw-whet Owl on Haida Gwaii suggest that strong ecological influences were paramount in their differentiation. Such regularity of morphological changes like modifications in body size, increased robustness of skull and teeth, and differentiation in the colour of fur and feathers are probably an adaptive response to insular habitats (Foster 1965), which Endler (1982) suggested would not only account for endemism and species diversity, but concordant patterns of morphological divergence.

One of the changes typically observed in insular fauna is an increase or decrease

in body size (Foster 1964; Case 1978, Sondaar 1994; Lawlor 1986). Such changes are known to occur in a diversity of taxa and may be governed by resource limitation (Case 1978), predator release (Sondaar 1994), competitor release (McNab 1971), and combinations thereof (Lomlino 1985). The universality of this modification in a variety of island habitats suggest that in most cases, changes in body size are not caused by historical factors but rather by prevailing ecological conditions.

Incredible morphological variation can occur in insular faunas, classically exemplified by the assemblage of divergent sticklebacks on the northeastern corner of Graham Island ( see Moodie and Reimchen 1973, 1976 a, b). Absence of competitors and predators in some of these lakes has apparently allowed for the evolution of novel adaptations resulting in as much morphological variation in Haida Gwaii sticklebacks as seen throughout its entire circumboreal distribution. The significance of biotic impoverishment in allowing for new phenotypes, is clearly illustrated by the spine reduction which occurs in sticklebacks of Boulton Lake (Reimchen 1994).

Spine reduction in sticklebacks is an unusual morphological characteristic and is known to occur only a small number of locales including Texada Island and the Outer Hebrides, U.K.. The common environmental correlate of spine reduction appears to be the absence of predatory fish (Bell 1976; Moodie and Reimchen 1976b). In Boulton Lake, the lack of salmonids appear to play a significant, albeit an indirect role in the evolution of this phenotype (Reimchen 1980). Absence of salmonid predation may allow for greater densities of macroinvertebrates, including odonate nymphs (*Aeshna*). These nymphs, which prey upon sticklebacks, do not appear to be affected by the presence of dorsal or pelvic spines. Furthermore, the presence of spines may actually serve as a

disadvantage for the stickleback since it may increase frictional contact reducing opportunities for escape (Reimchen 1980).

In many cases, the morphological changes that occur in insular populations are closely associated with feeding mechanisms. This is exemplified by the diversity of beak sizes and shapes in the Hawaiian honeycreepers and finches of the Galapagos Islands. The endemic carnivores of Haida Gwaii are typically noted for their broad heavy skulls and robust molars. It is known that cheek teeth (pre-molars and molars) which are involved in food mastication, show the greatest diversity in form correlated with variations in diet (DeBlase and Marten 1981). Larger, more robust skulls, increase the surface area for muscle attachment and permitted greater power for mastication. As Foster (1965) surmised, these adaptations likely evolved in response to the addition of marine invertebrates to their diet.

Processes of evolutionary change generally require some degree of isolation whether it be through geographical separation or ecological differences. Such isolation is often best achieved on islands and consequently, island forms typically possess peculiar morphological and sometimes odd behavioural traits such as changes in body size (e.g. the Komodo Dragon), loss of dispersal ability (e.g. carabid beetles) and loss of defensive adaptations (e.g. spine reduction in sticklebacks of Boulton Lake). Intense selective pressure, long isolation, founder effects and bottlenecks are all presumed, with variable effectiveness, to accelerate evolutionary changes. In some cases, such changes can occur within extremely short periods of time (e.g. Chernoff 1982; Sondaar 1994). As such, the major morphological characteristics that distinguish the Haida Gwaii endemics from their conspecifics can be entirely explained by changes that can take place relatively rapidly in

insular habitats and does not require explanations of extended isolation.

If endemism on Haida Gwaii is caused by a rapid response to alternative selective gradients which were established post-glacially, then the high incidence of endemism on Haida Gwaii would say little about historical factors such as population fragmentation and isolation caused by the onset of glaciers. Given the low genetic divergence found in this study between subspecies within mtDNA lineages and morphological characteristics which are correlated with habitat, morphological endemism in the Haida Gwaii black bear, marten, weasel, caribou and Saw-whet Owl are probably examples of post-glacial evolution. This implies that local adaptation to different ecological conditions has been of greater importance to the formation of these subspecies than vicariance events that occurred during the Pleistocene glacial advances.

**Implications for Speciation**

Allopatric speciation is currently believed to be the most common form of speciation in nature. As isolation in different refugia allowed for both genetic and morphological divergence, population fragmentation during the multiple Pleistocene glacial advances provided enormous opportunities for such speciation. In cases where sufficient differentiation was not achieved in order to complete speciation, postglacial range expansion from these refugia allowed divergent populations to become sympatric along hybrid zones or secondary zones of contact.

For example, two subspecies of the European meadow grasshopper (*Chorthippus parallelus*) form a hybrid zone within the Pyrenees Mountains (Butlin and Hewitt 1985a). The hybrid zone between *C. p. paralellus* and *C. p. erythropus* is believed to have

become established about 9000 years BP and exhibits what Hewitt (1983) describes as the 'classical attributes of postglacial contact'. There are numerous examples of such hybrid zones, most of which are believed to be a result of secondary contact (Barton and Hewitt 1985; see Hewitt 1983 for a review)

However, it has become apparent in this study that significant intralineage subspeciation may occur during or after postglacial range expansion. For example, the continental mtDNA lineage of short-tailed weasels apparently diverged postglacially into at least 5 different subspecies: *cicognanii*, *bangsi*, *fallenda*, *invicta* and *richardsonii*. The coastal lineage of black bear diverged into at least two mainland subspecies, *altifrontalis* and *kermodei*, and the continental lineage of marten may have diverged into at least three, *atrata*, *abietinoides*, and *caurina*. The assemblage of subspecies comprising each of these mtDNA lineages apparently diverged after expansion from refugia and in response to environmental conditions in new habitats. If such differentiation did occur along an environmental gradient in a more or less geographically continuous population, then the hybrid zones between these various subspecies may be primary, not secondary zones of contact. If this is true, then subspeciation within these species may be examples of the initial stages of parapatric speciation. However, it cannot be assumed that these postglacial populations were continuous, especially when considering the fluctuating stability of ecosystems caused by the retreating ice. It may be that populations did differentiate allopatrically while temporarily isolated by new physical barriers or extinction of intermediate populations.

Although there is no evidence of genetic differentiation of subspecies using cytochrome b, further investigation of these subspecies and their hybrid zones using other

molecular markers like RFLPs and microsatellites may provide insight into how genetically distinct these subspecies are. Furthermore, it would be worth examining interactions at subspecies boundaries to assess the amount of gene flow and the nature of the hybrids. In the case of *kermodei* and *cinnamomum*, two adjacent black bear subspecies, there may be very little gene flow. Despite the close proximity of these taxa, they are characterized by very different mtDNA lineages; *kermodei* is coastal while *cinnamomum* is continental. Further sampling using nuclear, mtDNA and morphological markers within this zone of contact would be desirable to determine whether a hybrid zone actually exists.

**Relevance of Subspecies as a Taxonomic Unit**

A subspecies is classically defined as "an aggregate of local populations of a species inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species" (Mayr 1963). As a taxonomic unit, the term subspecies can be both ambiguous and arbitrary as any number of morphological and molecular characters can be used to define it (Wilson and Brown 1953).

The lack of genetic differentiation between subspecies, akin to what was found in this study, has been used to question the evolutionary significance of current subspecies designations (Ball and Avise 1992). While it may be reasonable to apply subspecies names for major, concordant molecular subdivisions as Ball and Avise (1992) suggest, the absence of such subdivision as a criterion with which to revoke subspecies status is not. Just as we cannot define a species based solely on genetic differentiation, we cannot base the definition of a subspecies on this criterion either. It is the nature of the

differences which is important. For example, several species of leafroller moths (Family Tortricidae) are reproductively segregated by relatively minor changes in the chemistry of female pheremones, apparently equating to mutations within a single gene (Wilson 1992). If the lack of overall genetic variation cannot be used to dismiss these leafrollers as a species, then it does not logically follow that lack of genetic variation can be used to deem certain subspecies as irrelevant. In the case of the black bear, marten, short-tailed weasel, caribou and Saw-whet Owl, the lack of genetic variation between subspecies, especially within a single locus, cytochrome b, cannot be used to invalidate these designations. They are still morphologically distinct and it is reasonable to recognize them as such.

Many have argued that the inherent non discrete nature of subspecies is justification for removing them completely as part of the biotic classification system (Frost et al. 1992; Wiley 1992; Wilson and Brown 1953). However, systematics and classification serve two functions: to reflect phylogeny and biodiversity (Smith et al. 1997; Simpson 1961). Although there are difficulties in incorporating subspecies in phylogenetic analyses (see Introduction pg. 18), ignoring subspecies is a misrepresentation of diversity and as such a misrepresentation of nature. Subspecific nomenclature is a method of documenting major patterns of variation (Smith et al. 1997) and some of the best examples of evolutionary processes such as adaptation and allopatric speciation have come from studies founded on such geographical diversity (Mayr and Ashlock 1991). While the subspecies is not an independent evolutionary unit as suggested by Mayr's definition, it is a product of evolution and should be categorized, recognized and studied from that perspective.

Therefore, with regards to the subspecies examined in this study, the lack of genetic differentiation within one locus, cytochrome b, cannot be used to make assumptions regarding its taxonomic status and should not overshadow the morphological differences for which they were originally named.

**Rates of Morphological Evolution**

It has always been of interest to quantify the rate at which organisms evolve (Gingerich 1983, 1993a, 1993b; Haldane 1949; Simpson 1944). Rapid morphological change such as the kind documented in the sticklebacks of Haida Gwaii (Moodie and Reimchen 1973, 1976b) or the cichlid fish of Lake Victoria (Meyer et al. 1990) may imply processes such as intense selective pressure, isolation, and in the case of non-adaptive features, founder events/and or bottlenecks. In an attempt to understand how glacial retreats and advances during the Pleistocene affected evolutionary rates, Kurtén (1959) used morphological measurements and estimated that the rate of mammalian evolution had increased 300 fold from the Tertiary to the Quaternary. He concluded that this apparent increase was either due to drastic climatic changes during the Pleistocene and Holocene or an artifact of the time interval over which morphological changes were measured.

The latter of these suspicions was later confirmed by Gingerich (1983) when he demonstrated that rates of evolution, when measured in darwins, are inversely proportional to the interval length over which they are measured. The Tertiary fossils which Kurtén had employed in his study were separated by a much longer period of time than the Quaternary fossils and as such, appeared to evolve at slower rates. The effect

that time interval has on quantification of evolutionary rates is partly due to averaging of fluctuating evolutionary rates (Gingerich 1993a, b). Therefore, quantification of these changes occurring over millions of years cannot be used to examine these rate fluctuations in detail. Unfortunately, it is often these particular fluctuations occurring at intermediate time scales which are of interest. Although the fossil record can be used to study changes occurring over long periods of time, and laboratory/field experiments can be used to examine changes occurring over short periods of time, there is a lack of information regarding changes which occur at intermediate time intervals, say in the order of hundreds or thousands of years (Gingerich 1993a).

The molecular data obtained in this study offer an excellent opportunity to examine rates of change at such time scales and to look at these changes which occurred during a period of enormous environmental upheaval. Because the subspecies within mtDNA lineages in black bears, marten, short-tailed weasels, caribou and Saw-whet Owls, are characterized by low average genetic divergences (0.1%-1% ), the implication is that these subspecies originated sometime during the Holocene (within the last 10,000 years). By having a molecular based estimate of the divergence time, evolutionary rates of change can then be calculated using Haldane's equation:

$$\text{Rate (darwins)} = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

where $x_2$ represents a measurement at time 2 ($t_2$) and $x_1$ represents a measurement at time 1 ($t_1$).

Although there can be considerable error when estimating divergence times based on a stochastic molecular clock, time estimates using the fossil record may be no better.

The appearance of a fossil provides the minimum divergence time and does not necessarily provide information regarding its taxonomic status. Recognition of morphologically different forms are often assumed in paleontology to represent speciation events; subspecies are not recognized (Groves 1992). By using extant taxa, where divergences can be estimated based on genetic information, a large number of different morphological characters can be collected and knowledge of their biology such as generation time (see Gingerich 1993a and 1993b for a discussion on the importance of generation time) can be incorporated. Utilizing substantial museum collections and calculating evolutionary rates of the subspecies examined in this study, may provide some insight into the evolutionary processes which have caused the diversification within these species and as such bring a greater understanding to the origin of biodiversity during the Holocene.

**Alternative Explanations for a Coastal Refugium**

The presence of divergent intraspecific lineages in the absence of current physical and/or reproductive barriers typically imply historical substructuring of existing populations (Rising and Avise 1993; Avise 1994). Identification of geographical genetic variation and concordance of such structure across unrelated taxa is generally assumed to be the result of vicariant events which similarly fragmented populations along congruent geographical positions.

Sequence comparisons of the mitochondrial gene cytochrome b revealed divergent lineages with significant geographical structure in black bears, marten and short-tailed weasels. The locality of the mtDNA phylogeographic split within these

carnivores is largely congruent and consistent with there being a refugium along the exposed portions of the Hecate Strait during the last glacial maximum.

Despite the congruency of phylogenetic trees obtained for black bears, marten and short-tailed weasels, interpretations based on gene trees, especially those constructed from one locus like mtDNA, can be problematic as they do not necessarily reflect species trees. This is especially true when small target sequences with low sequence divergence are used. The difference between organismal and gene trees may be significant in our study since phylogenetic patterns based on mtDNA are potentially biased due to maternal inheritance and male-biased dispersal patterns in mammals (Rodgers 1987). However, these same characteristics may make mtDNA more informative than nuclear markers. For certain investigations, mtDNA is more likely to retain ancient phylogeographic patterns because of the slower dispersal of haplotypes. As such, mtDNA may be a better indicator of where particular lineages have originated. Congruent localization of divergent haplotypes around Haida Gwaii, Vancouver Island and northern coast of British Columbia, suggest that these coastal haplotypes may have originated in that region and that the division in mtDNA trees presents a true representation of biogeographical history.

Despite the advantages of using mtDNA in intraspecific phylogeographic studies, possibility of nuclear integration of mtDNA sequences has recently raised questions that clusters on phylogenetic trees may be an artifact of comparisons between mtDNA and nuclear counterparts (Zhang and Hewitt, 1996). The divergent lineages identified in black bear, marten, and short-tailed weasels are not likely to be an artifact of nuclear integration for the following reasons: accidental amplification of a nuclear version of

cytochrome b would result in 1) in a random distribution of the two lineages and not a geographical cluster and 2) inconsistent PCR with regards to numbers and types of amplification products within an individual. Our PCR was consistent in both respects. Furthermore, phylogeographic studies based on nuclear versions of mtDNA sequences are expected to result in unrealistic divergences. Although the magnitude of divergence differed between black bears, marten and short-tailed weasels, this may be due to evolutionary rate heterogeneity across different taxa or isolations which occurred during different Pleistocene glacial or interglacial periods. Black bears are believed to have first entered North America during the Pliocene (Thenius 1990). Short-tailed weasels are believed to have crossed over into North America about 500,000 years BP (King 1989) while marten did not arrive until about 65,000-122,000 years BP (Anderson 1994). Relative differences in tree depth agree with estimated times of entry into North America based on paleontological data.

Pre-Wisconsin divergence between coastal and continental lineages in black bear and marten and between short-tailed weasel lineages was estimated using a standard mammalian mtDNA evolutionary rate of 2% per million years. In the case of black bears, an additional clock calibrated with the fossil record was used and resulted in similar divergence estimates. However, the molecular clock is highly stochastic and its reliability is dependent upon a variety of factors which could led to substantial errors in divergence estimates. One such factor is the possibility of founder effects and/or bottlenecks which might occur during colonization. The divergent mtDNA lineages observed in black bear, marten and weasel might have originated within the last 10,000 years if a rare and divergent haplotype became established by a founder effect and

subsequent prolonged bottlenecks during recolonization. MtDNA is particularly susceptible to such effects due to its smaller effective population size relative to nuclear genes. In order for the observed changes to have occurred within the last 10,000 years, a significant rate increase along the divergent lineage would have probably occurred. Relative rate tests indicate no such increase took place within mtDNA lineages in black bears, marten, or short-tailed weasels. In addition, because the majority of nucleotide substitutions were silent, the substitution rate varies according to the overall mutation rate of the population and is independent of its effective size (Ohta 1992).

**Evidence of Long Biotic Continuity on the Coast**

For all three species, black bear, marten, and short-tailed weasel, the phylogeographic split appears to have occurred sometime during the mid-Pleistocene. Although divergence estimates based on molecular clocks are subject to considerable error, these estimates are congruent to estimates of other taxa in the Pacific Northwest with similar phylogeographic structure (Talbot and Shields 1997; Cook et al. 1998 pers. comm.; see Additional Evidence for a Hecate Refugium). This apparent mid-Pleistocene split suggests that the phylogeographic structure of some of these taxa may have actually been established during the penultimate glaciation (the Illinoian) when many of these taxa first entered North America and was maintained throughout the Wisconsin through habitat continuity on the coast. Given the possibility that a coastal refugium existed during the last glaciation and the likelihood that similar processes of glacial growth and sea level changes occurred during the Illinoian, it is not unreasonable to presume that the continental shelf served as a refugium through at least two different glacial advances and

that this coastal refugium may have facilitated the entry of some taxa into North America. Growing evidence that divergence of northwestern forms occurred sometime during the mid to late Pleistocene corresponding approximately with the last two glacial advances, suggests that for some taxa, divergence occurred during the Illinoian. These divergent populations may have been maintained during the interglacial on Haida Gwaii and perhaps other parts of the Pacific Northwest when the continental shelf was submerged and later during the Wisconsin, may have migrated back to the shelf as the mainland and islands were covered in ice.

Evidence of long habitat continuity is also found in the assemblage of organisms on Haida Gwaii. Haida Gwaii's possession of four genera of endemic mammalian carnivores and only one type of rodent (Cowan 1989) is unusual given the fact that carnivores are usually underrepresented in island biota due to low vagility, low carrying capacities, and high extinction rates (Alcover and McMinn 1994). Such disharmony (overabundance of one group and paucity of another) as exemplified by Haida Gwaii's biota, is typically the result of isolation and age. For example, Madagascar is located approximately 500 km off the coast of Africa and has probably been in existence since the breakup of Gondwanaland. As such, Madagascar exhibits radical disharmony. On the other hand, Sri Lanka, located approximately 100 km off the shore of India, was connected to this continent as recently as 12,000 years BP. Sri Lanka, known as a land bridge island, shows no great disharmony in its species assemblage (Quammen 1996).

Haida Gwaii is located about 80 km off the coast of British Columbia and like Sri Lanka is also considered a landbridge island (Lawlor 1986). Core data indicate that parts of the Hecate Strait located between Haida Gwaii and the mainland were terrestrial

during the height of the Wisconsin and like Sri Lanka, Haida Gwaii may have been connected to the continent. As Haida Gwaii was covered by ice until about 16,000 years BP (Warner et al. 1982), continuity of terrestrial areas along the Hecate Strait throughout the Wisconsin and then on Haida Gwaii during the interglacial, may have contributed to the particular features of this archipelago's biota that make it seem older and more isolated than is possible given its relative proximity to the mainland and glacial history.

**Interpreting the Congruent Phylogeographic Patterns**

A central tenet of biogeography is that vicariant events such as habitat fragmentation during glacial advances result in congruent distributions across diverse taxa (Croziat 1962). However, dispersal can also result in congruent patterns such as those produced during the invasion of Central America from North America over the Isthmus of Panama (Savage 1982). As such, the patterns observed in this study may be caused by incremental dispersal from mainland refugia north and south of the Cordilleran Ice Sheet.

At the height of the last glaciation, the continental shelf between Haida Gwaii and the mainland possessed both terrestrial and freshwater habitats (Josenhans et al. 1993). This uncovered shelf may have been part of the refugium from which Haida Gwaii, Vancouver Island, and the north western coast of North America were postglacially colonized. Occurrence of a glacial refugium in this area would account for the distribution of divergent molecular lineages in black bear, marten and the short-tailed weasel by temporarily connecting and allowing access to Haida Gwaii, Vancouver Island and coastal British Columbia.

There is little doubt that the Hecate refugium was probably a part of a series of coastal refugia which extended further up and down the coast and presumably were inhabited by various refugial populations. However, it is unlikely that the divergent lineages observed in the black bear, marten and short-tailed weasel in the Pacific Northwest are derived by incremental northward migration from southern refugia.

Dispersal from southern refugia probably began during the early stages of deglaciation. However, massive recessional lakes, floods and marine transgression all severely impeded northward migration (see Introduction - *Deglaciation*). On the coast, eustatic and isostatic changes resulted in large fluctuations in sea level resulting in massive flooding of the Puget Lowland (Easterbrook 1992). For the first few thousand years (13,000 BP and 9000 BP) following deglaciation, sea levels rose along the Coast Mountains and eastern shores of Haida Gwaii respectively. These changes were exacerbated by the tilting of tectonic plates largely due to isostatic depression, which effectively increased sea levels on the coastal mainland while decreasing sea levels further west. Dispersal to remote archipelagoes like Haida Gwaii is extremely difficult, even during the rather benign environmental conditions of the present. During deglaciation, dispersal would have been much more challenging and was probably limited to more vagile organisms. As such, the entire assemblage of carnivores, (which are typically poor colonizers ) (Carlquist 1974) on Haida Gwaii is unlikely to have originated due to post-glacial dispersal alone.

The dramatic changes which were occurring along the coast and margins of retreating ice severely impeded northward dispersal from the south. However, dispersal from the Alaska/Yukon refugium north of the Cordilleran ice sheet was probably even

more limited because surrounding ice kept it ice locked for a much longer than southern refugia. Approximately 10,000 - 13,000 years BP, marine transgression of up to 230 m occurred in south-eastern inland coast of Alaska (Mann and Hamilton 1995) further hindering dispersal to Haida Gwaii. Significant southward animal migration probably did not start occurring until a coastal corridor from this northern refugium finally opened approximately 9000 years BP (Youngman 1975). However by this time, Haida Gwaii was even more isolated than at present by higher sea levels (Fladmark 1974). The concentration of highly divergent mtDNA lineages on Haida Gwaii, Vancouver Island and in some cases the coastal mainland, suggest that these regions were recolonized by the same, nearby source area which existed somewhere around the Hecate Strait. Although it is likely that this refugium was at least transiently connected to other coastal refugia during the Fraser glaciation, given its close location to Haida Gwaii, Vancouver Island and coastal mainland, it is the most likely source area for these three regions.

Recolonization of the Pacific Northwest by black bear, marten, and short-tailed weasel was probably significantly influenced by dispersal from the Hecate refugium. Therefore, to discount the existence of this refugium and its influence on recolonization ignores sonar profile data which indicate freshwater and terrestrial habitats on the shelf (Josenhans et al. 1993), ignores the existence of congruent phylogeographic patterns of three carnivore species, ignores the presence of these divergent lineages on Haida Gwaii and ignores the extreme difficulty of dispersal to this archipelago in present and postglacial periods.

**Additional Evidence for a Hecate Refugium From Other Taxa**

Numerous nunataks and open areas along the Pacific Northwestern coast must have been transiently available as the margins of the Cordilleran ice sheet continuously retreated and readvanced during the Wisconsin. However, these coastal refugia during the Fraser glaciation were probably marginal ecosystems and were presumed to have been relatively insignificant source areas. The persistence of top carnivores such as black bears, marten, and short-tailed weasels on the continental shelf during the last glacial maximum changes the perception of coastal refugia from unproductive nunataks to ones which were inhabited by an assemblage of organisms. If the Hecate Refugium was a major source area for postglacial recolonization and the phylogeographic pattern observed in these carnivores is the result of isolation and subsequent dispersal from this refugium, then the current distributions of other taxa of the Pacific Northwest should have also been influenced. As such, the phylogeographic patterns exhibited by these taxa should be congruent to the patterns found in black bears, marten and short-tailed weasels.

The following section reviews the evidence from a number of different and largely independent taxa which show phylogeographies consistent with the hypothesis that a refugium existed on the currently submerged shelf between Haida Gwaii and the mainland. Although each study on its own may not offer compelling evidence for a coastal refugium, collectively, these data along with molecular data presented from this study, strongly imply that a major phylogeographic break exists in the Pacific Northwest and that this break may be related to the persistence of coastal source populations during the Wisconsin.

**Plants**

*Tellima*

*Tellima grandiflora* is a diploid perennial herb found from the Alaskan peninsula to central California. Restriction site analysis of chloroplast DNA (cpDNA) using 20 endonucleases on over 51 populations revealed two divergent cpDNA lineages. The northern lineage, which extends from the Kenai Peninsula to Oregon and the southern lineage which is generally restricted to California. Outliers of this southern lineage occur on Prince of Wales Island, Alaska about 1200 km north of its major southern range and on the Olympic Peninsula, Washington. The occurrence of this lineage in these two regions is suggestive as they are thought to have been Wisconsin glacial refugia (Fig 39). In addition, the southern lineage was found in two populations just north of Seattle, Washington, as well as one population from Vancouver British Columbia. Although the disjunct distribution of the southern lineage may be due to long distance dispersal, the fact that the chloroplast genome is maternally inherited and seed dispersal is limited in *T. grandiflora* makes this alternative hypothesis unlikely (Soltis et al. 1991).

*Senecio* (*Packera*) complex

The members of the new genus *Packera* have until recently been referred to as members of the *Senecio* complex. Recent RFLP chloroplast (cp) DNA analysis of *Packera* species and specifically of *P. pseudaurea* has revealed a phylogeographic split which largely separates those populations (Yates et al. 1998) and species (Bain 1997 pers comm.; Bain and Jansen 1996) located west of the Rocky Mountains and those found

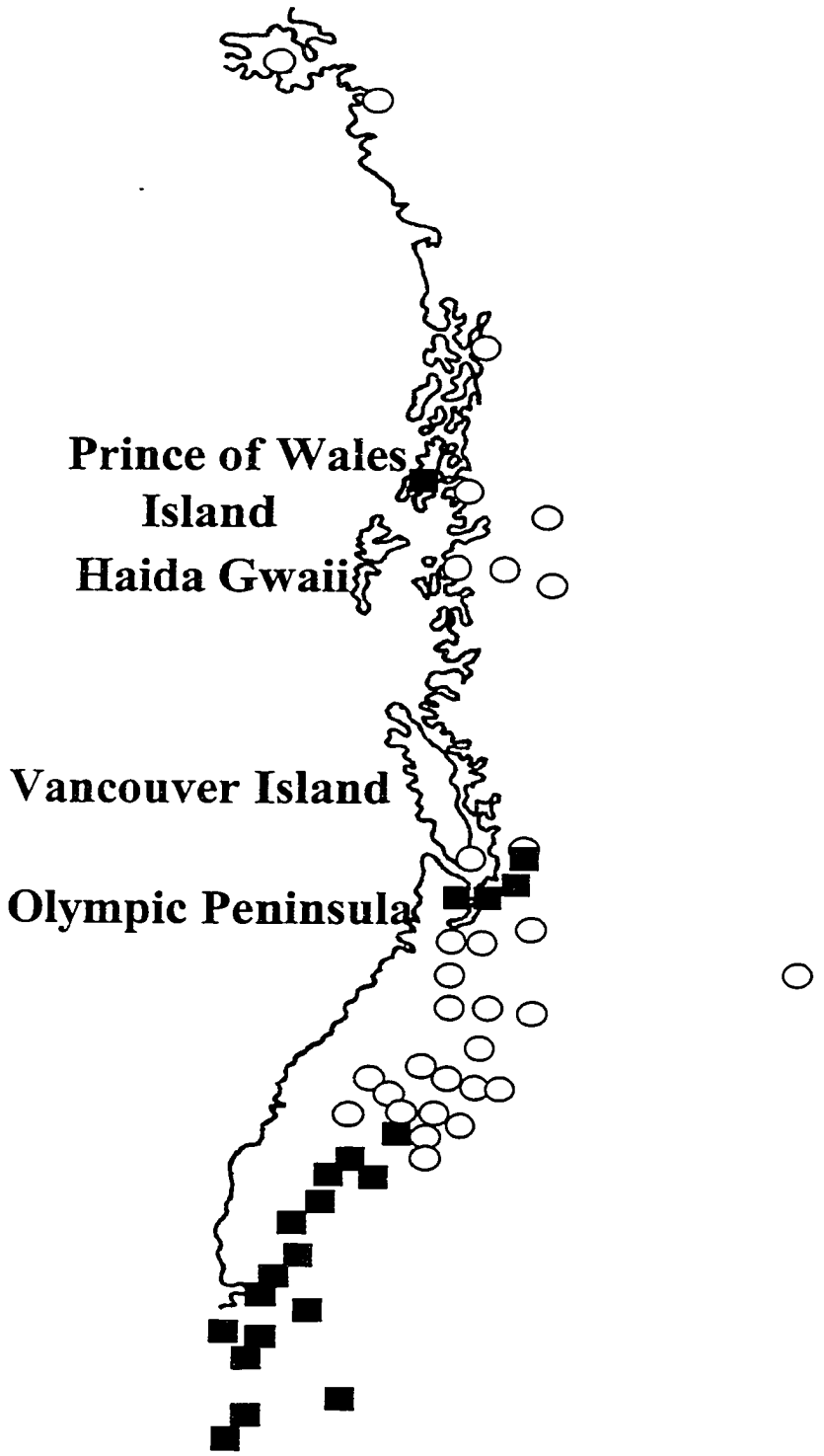



**Figure 39**
Distribution map of cpDNA variation in *Tellima grandiflora*. The southern lineage is indicated by black squares; the northern lineage is indicated by open circles. Notice the disjunct locations of this southern lineage on the Prince of Wales, Alaska and the Olympic Peninsula (adapted from Soltis et al. 1991).

over the rest of the species range (Fig 40). This division within the genus *Packera* is congruent with the coastal/continental split found in black bears. Such a division in *Packera* is consistent with dispersal from the coastal and continental refugia and subsequent differentiation into species during postglacial periods.

**Fish**

*Sticklebacks*

As discussed in the Introduction (see MtDNA: Useful Features for Examining Biogeographical History), genetic work on the sticklebacks, beginning with RFLP analysis by Gach and Reimchen (1987), was the first indication of genetically divergent forms on Haida Gwaii. This work, which was followed up by O'Reilly et al. in 1993 and Orti et al. in 1994, indicated that a divergent mtDNA haplotype existed on Haida Gwaii (Rouge Lake), and a few locales in Alaska which belonged to a mtDNA lineage commonly found in Japan (Fig 41). Orti et al (1994) suggested that the Japanese lineage was widely distributed prior to the Wisconsin and was extirpated in all but a few refugial areas along the northwestern coast of North America. This view was altered by a more recent study by Deagle et al. (1996), who found that the Japanese haplotype was concentrated in locales on the northeastern tip of Haida Gwaii as well as two locations on the western coast. The Japanese and Pacific Northwest stickleback lineages were also both found in the mid-Pacific suggesting ongoing gene flow. Although this accounts for the occurrence of the Japanese lineage on Haida Gwaii and various areas around the coast, questions still remain regarding its apparent restriction to particular locales in the

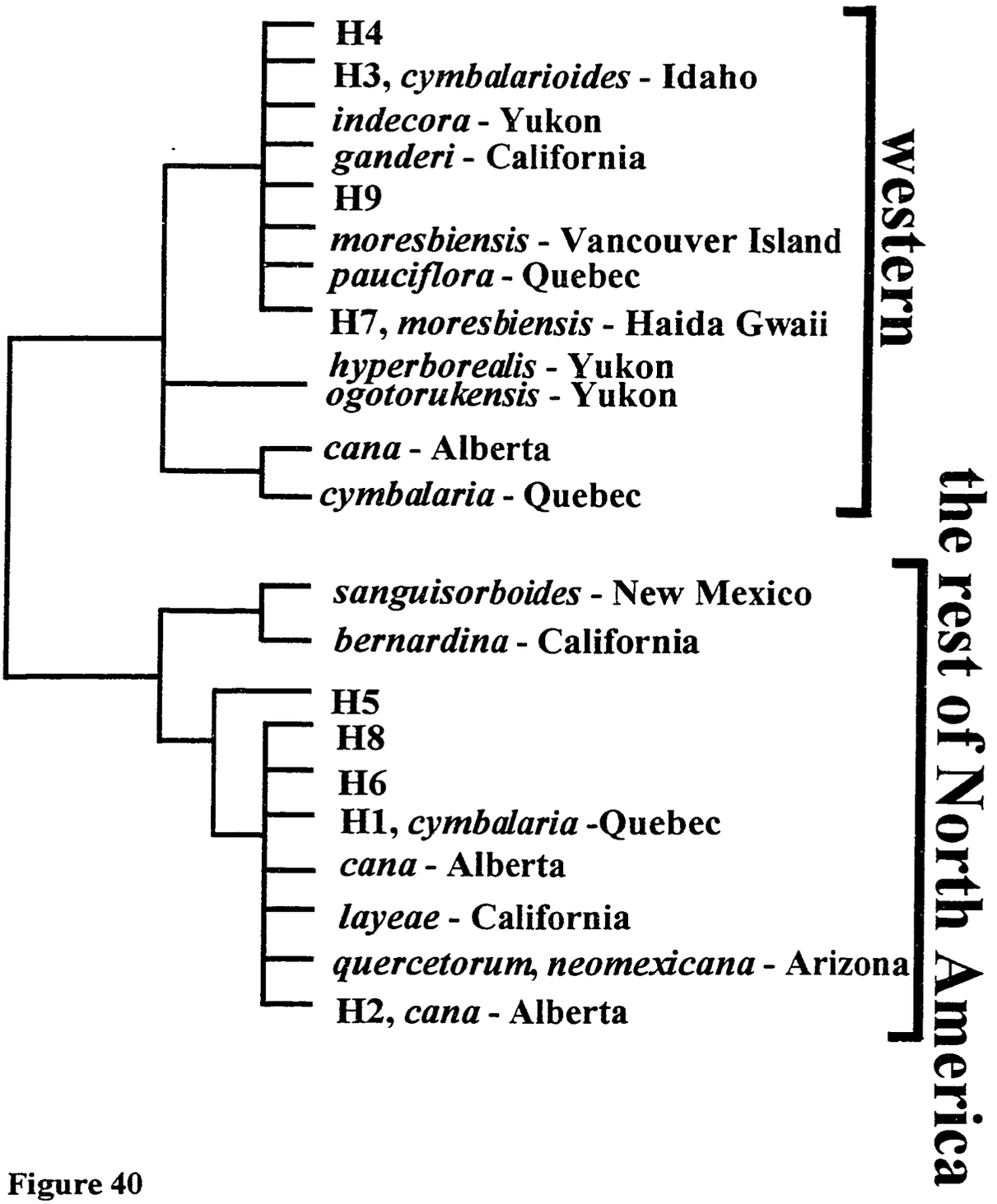



**Figure 40**

This strict consensus tree was generated using four restriction enzymes: *Dra* I, *Ava* I, *Ava* II, and *Hae* III on *Packera pseudaurea*. The nine haplotypes discovered by this assay were separated into two well defined lineages: one widely distributed over most of North America and the other largely localized west of the Rocky Mountains. This separation of western populations is mirrored by other species of the *Packera* genus (adapted from Yates et al. 1998).

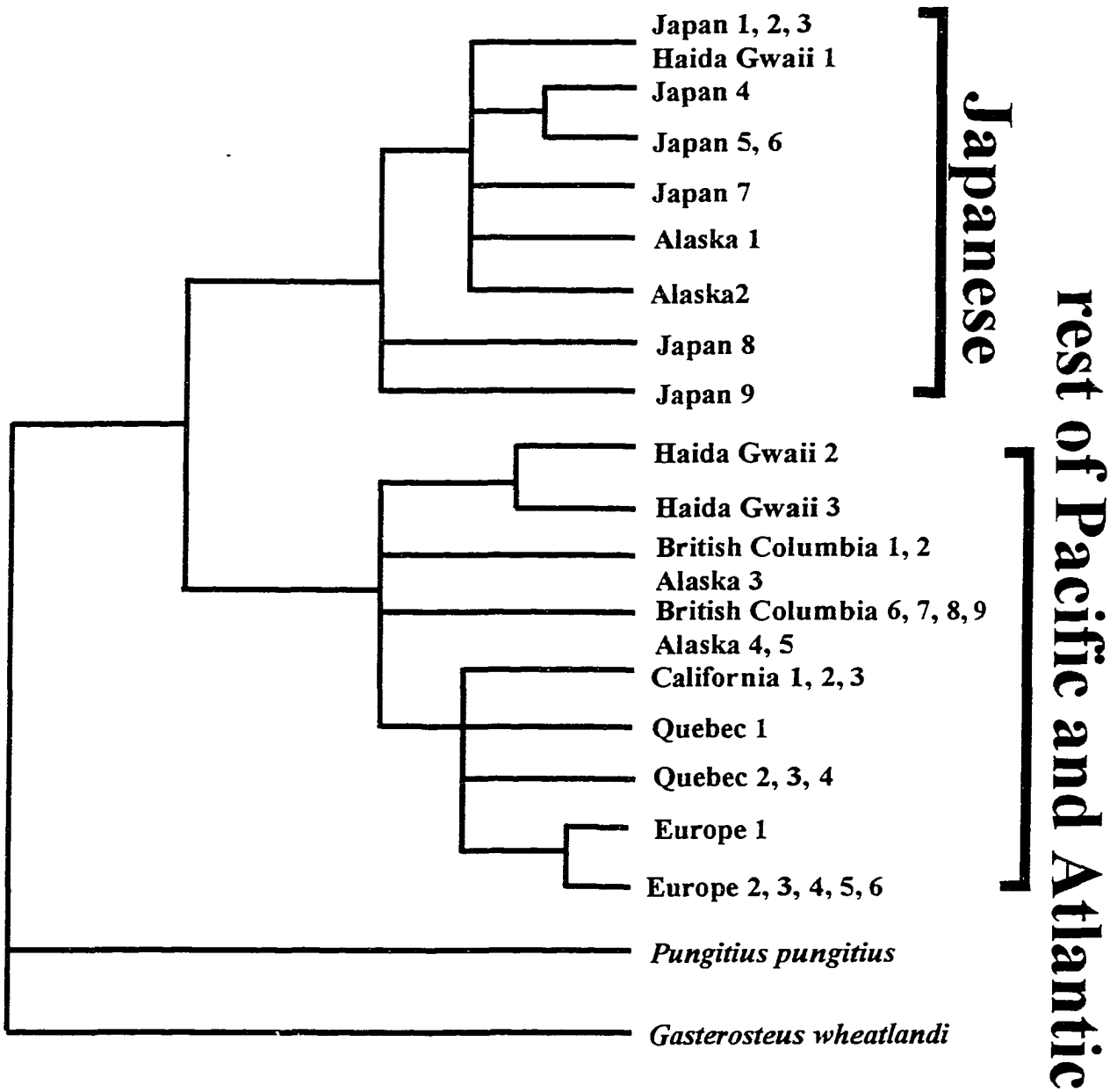



**Figure 41**

This 50% majority rule tree, based on 831 bp of cytochrome b and assays using a diagnostic restriction enzyme *Bst* XI, demonstrates the existence of divergent lineages of sticklebacks. The first lineage occurs in both the Pacific and Atlantic Oceans. The other lineage, referred here as the Japanese lineage, is restricted to Japan and specific locales on Haida Gwaii and Alaska. The Japanese lineage is assumed to have been more widely distributed prior to the Wisconsin and largely extirpated during the glacial period. The presistence of this lineage in the Pacific Northwest may reflect the persistence freshwater habitats during the last glaciation (adapted from Orti et al. 1994).

Pacific Northwest and its original divergence during the early Pleistocene despite the great potential intermixing of oceanic populations.

*Sockeye Salmon*

Allozyme variation at highly structured loci from 83 sockeye salmon (*Oncorhynchus nerka*) spawning sites revealed significant geographical structuring (Fig 42). Three lineages were identified and localized to 1) southern rivers (the Fraser and Columbia), 2) northern rivers (Taku, Stikine, Nass, and upper Skeena), and the 3) coastal mainland (most coastal mainland sites, most lower Skeena River sites and one site from the lower Nass River). This phylogeographic structure corroborates the view that current sockeye populations are derived from Beringia and a Columbian refugium. Furthermore, the existence of the coastal mainland lineage suggests that this was derived from coastal refugia in British Columbia (Wood et al. 1994).

**Birds**

*Rufous-Sided Towhee*

The Rufous-Sided Towhee(*Pipilo erythrophthlmus*) ranges from southern British Columbia south to Baja California and across north America to the east Gulf coast (Terres 1980). Using 16 restriction enzymes, Ball and Avise (1992) identified five different mtDNA haplotypes and a clear pattern of phylogeographic structure which separated eastern (represented by the subspecies *rileyi* from Georgia and South Carolina) and western (represented by *curtatus* from Washington) populations (Fig 43). Based on a

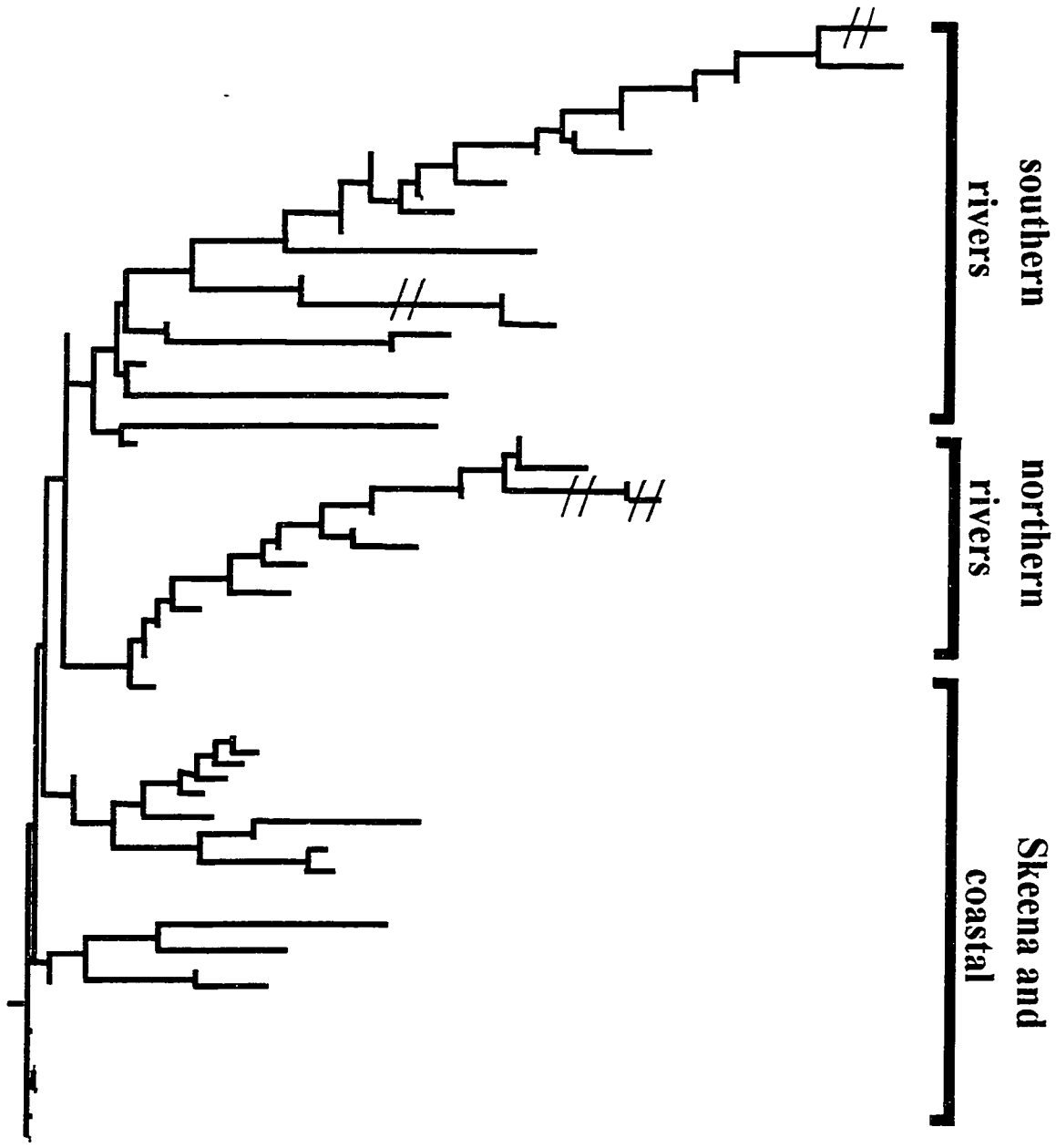



**Figure 42**
This neighbour-joining tree reveals the existence of three sockeye salmon lineages: southern, northern, and Skeena/coastal. Wood et al. (1994) attributed these lineages to postglacial derivation from three refugia in the Pacific Northwest: Beringia, Columbia, and coastal. Due to the complexity of the tree, exact locales were not included in this figure but the distribution of the lineages can be seen in Figure 47. Hatch marks indicate those branches which are not to scale.

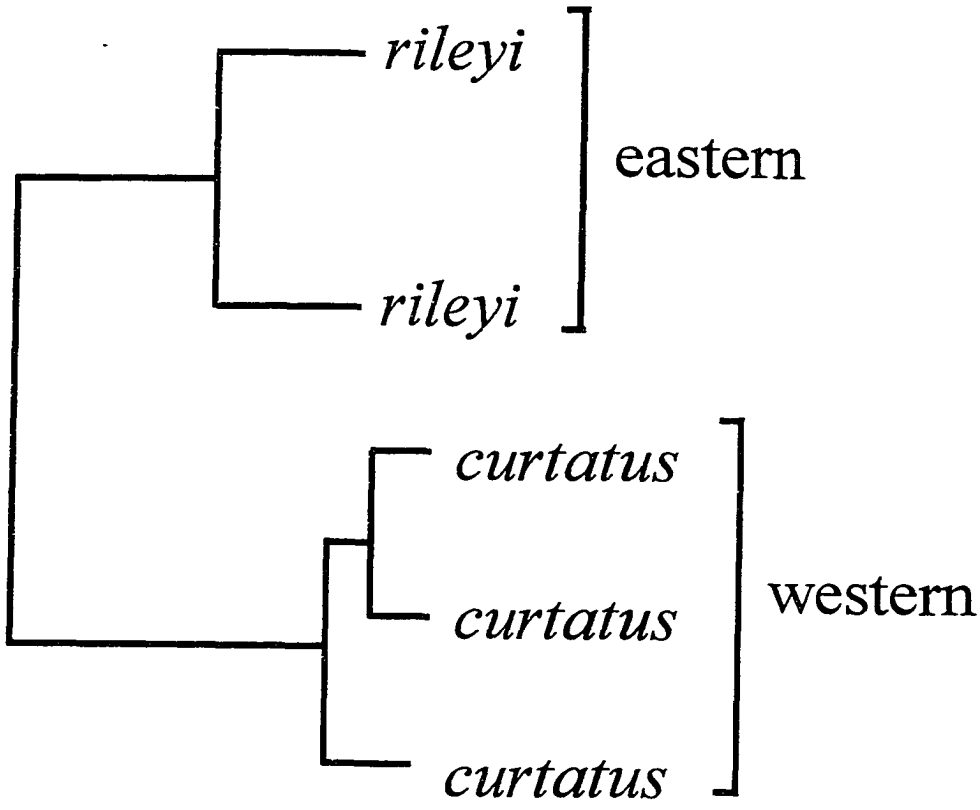



**Figure 43**
UPGMA dendrogram showing phylogenetic differentiation between eastern and western populations of the Rufous-sided Towhee. The differences between lineages is approximately 0.8% (adapted from Ball and Avise 1992).

2% divergence/million years ago, the phylogeographic split in this species originated approximately 400,000 years ago.

*Common Yellowthroat*

The Common Yellowthroat ( *Geothylpis trichas*) ranges over most of North America, from southeastern Alaska, across Canada to Newfoundland and south to the Gulf coast and Baja, California (Terres 1980). Using 19 restriction enzymes, 10 phylogeographically structured haplotypes were identified which separated locales found in eastern (represented by the subspecies *typhicola* from Georgia and South Carolina) and midwestern (represented by *brachidactylus* from Michigan and Minnesota) locales from those populations found in the west (represented by *arizela* from Washington) (Ball and Avise 1993) (Fig 44). The sequence divergence of the western lineage from all other Common Yellowthroats, was 1.2%. Using a rate of 2% divergence/million years, this separation originated about 600,000 years ago.

**Mammals**

*Deer Mice*

Based on chromosomal, allozyme, and mtDNA analysis, Hogan et al. (1993) determined that two distinct groups of deer mice (*Peromyscus*) exist in the Pacific Northwest. One group included deer mice found on Vancouver Island and the surrounding Gulf Islands (*P. m. austerus*), whereas the other group included deer mice from southern Alaska to northern Washington (Fig 45). This included Haida Gwaii's *P.*

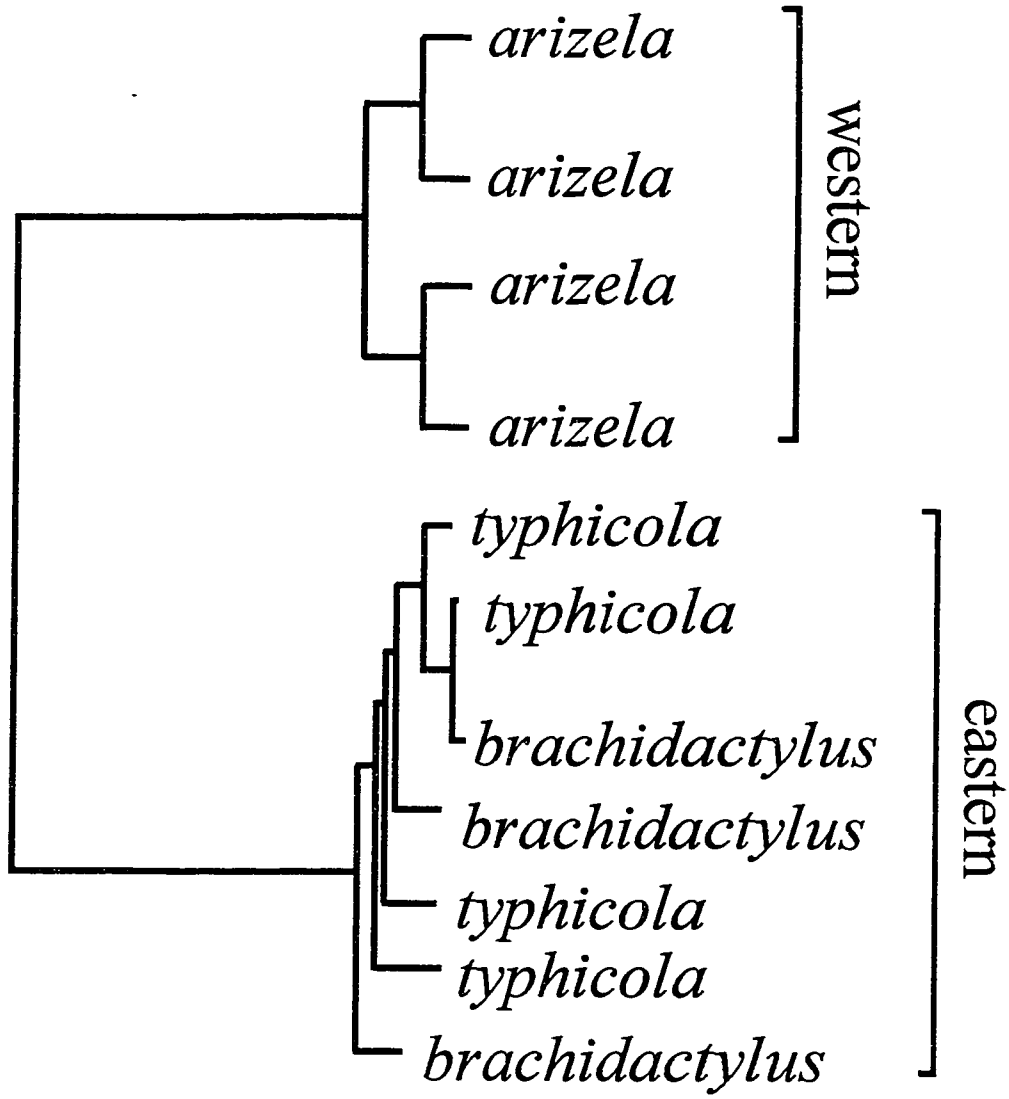



**Figure 44**
UPGMA dendrogram showing the phylogeographic differentiation between western and eastern/midwestern Common Yellowthroat populations. The sequence divergence between these two lineages is about 1.2% (adapted from Ball and Avise 1992).

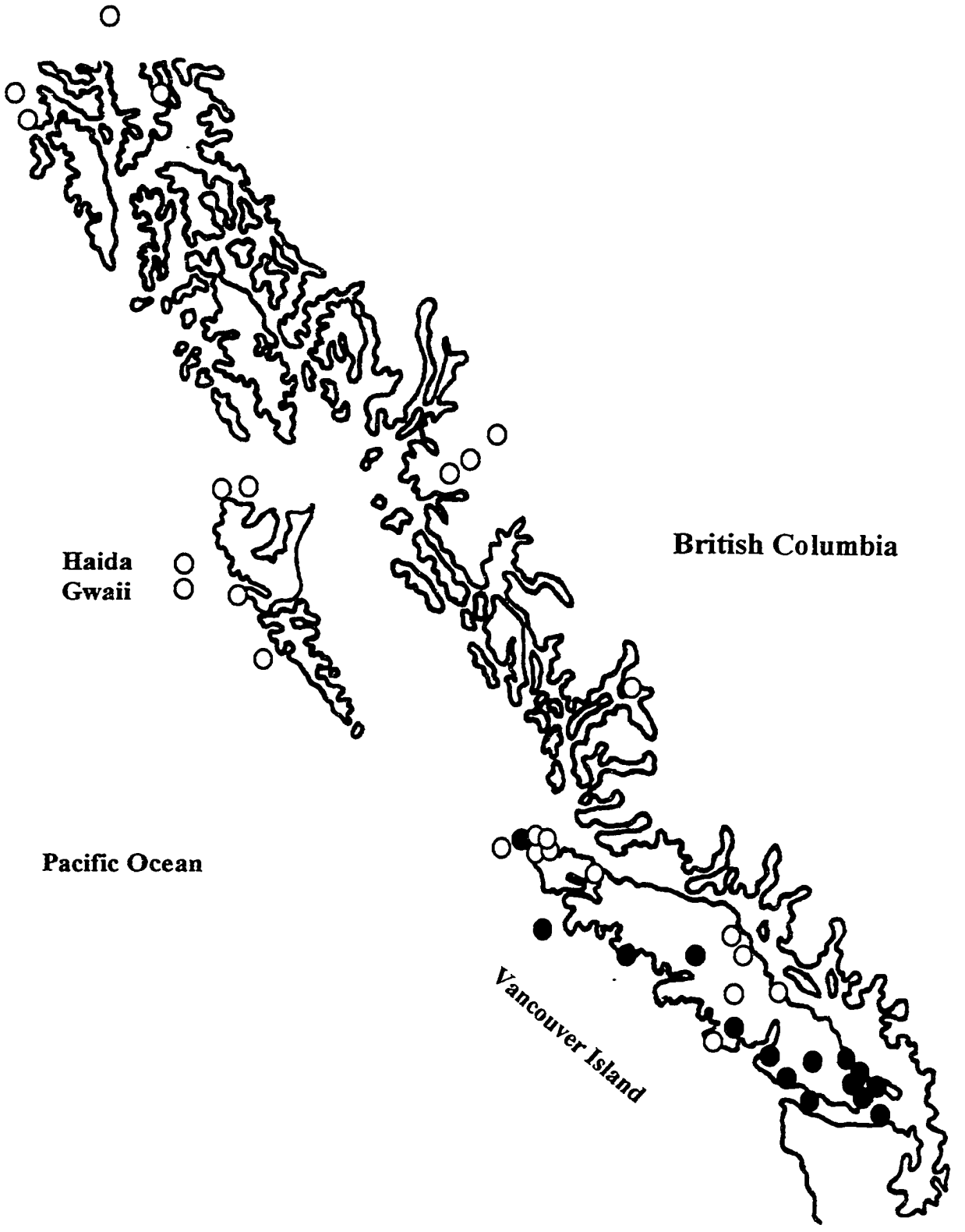



**Figure 45**
Two genetically distinct groups of deer mice have been found in the Pacific Northwest. Their distribution is indicated by the ● and ○ symbols. Adapted from Hogan et al. 1993.

*sitkensis*, *P. prevostensis*, and *P. m. keeni*, confirming Foster's (1965) hypothesis that the former was not a glacial relict (see Introduction - *Land Mammals*). The existence of two genetically distinct group of deer mice around the coast suggest that Vancouver Island and Gulf Islands were recolonized by the one source population, while Haida Gwaii, the coast and parts of southeastern Alaska were colonized by another. Although further sampling needs to be done before any strong statements can be made about this data in reference to coastal refugia, the phylogeographic pattern is largely congruent with the pattern found for black bear, marten and short-tailed weasel.

*Homo sapiens*

Native Americans can largely be divided into three main groups: 1) the Eskimo-Aleuts of the Arctic coast 2) the Na-Dene which include populations from the northwest coast, western subarctic interior and southwestern US and 3) the Amerind group which encompass the remainder of native Americans (Greenberg et al. 1986). These three groups, which were identified based on linguistic divergence (Greenberg 1987), dental morphology (Turner 1987) and genetic and immunoglobin data (Suarez et al. 1985; Szathmay 1985; Williams et al. 1985) were proposed by Greenberg et al. (1986) to be the result of three distinct migrations from Eurasia starting with the Amerind at 14,000 years BP, the Na-Dene at 10,000 years BP and the Eskimo-Aleuts at 4500 years BP (Dumond 1977). An alternative view of this tripartite division, based primarily on linguistic distribution and diversity, was proposed by Rogers (1985) and Rogers et al. (1991). They suggested that the three groups were actually the result of isolation in refugia north

(Eskimo-Aleuts), south (Amerind) and west (Na-Dene) of the Cordilleran Ice Sheet. Although there is evidence of congruency between gene and language trees (Penny et al. 1993), a recent study failed to uncover significant genetic divergence between Na-Dene and Amerind (Ward et al. 1993), despite their great linguistic differences.

The hypothesis that humans may have migrated down the coast via a series of coastal refugia (Fladmark 1979; Luternauer et al. 1989) has been gaining support (see Easton 1992; Barrie and Conway 1998; Heaton et al. 1996) due to physical evidence that the continental shelf was exposed and free of ice. The molecular data on the black bear, marten and short-tailed weasel offer support that the continental shelf was indeed habitable. If humans did persist on the coast during the late Wisconsin then they were most likely limited to resource rich coastal regions and rivers which supported salmon runs. As such, many of these prehistoric sites (pre-12,000 years BP) are likely to be submerged up to 100 m below present sea levels. Recently, human artifacts, carbon dated to be at least 10,200 years BP, were found in cores 100 metres at the bottom of the Hecate Strait (Josenhans et al. 1997). If the coast was amenable for human migration, then colonization of the Americas may have been much more rapid than previously thought, as traveling along coastal waters was much faster than traveling on land (Crockford 1998 pers. comm.). Recently, a site in Monte Verde Chile was confirmed to be about 33,000 years old indicating that human occupation occurred much earlier than the previous dates of 14,000 years BP. Verification of such a site also suggests that much older sites exist to the north (see Adovasio and Pedler 1997 for review).

The absence of archaeological sites and fossils deposited during the critical time when the Cordilleran Ice Sheet built up to a maximum (27,500 to 16,000 years BP) is

typical for this region (Heusser 1989). However, as the coastal refugium was likely to have been on the continental shelf, marine transgression following deglaciation probably submerged most paleontological evidence of refugial populations on the coast, resulting in this fossil hiatus. Continued exploration of the continental shelf may further uncover some of these missing sites and fossils.

*Brown Bears*

The Alexander Archipelago is located approximately 55 km northeast of Haida Gwaii, and like the rest of the region, its flora and fauna are believed to have been established postglacially (Klein 1965). However, since 1987 remains from a variety of vertebrates have been discovered in its extensive network of limestone caves on the following islands: Prince of Wales, Dall and Heceta. (Heaton et al. 1996) (Table 16). One of the oldest subfossils found was that of a small black bear tibia carbon dated at 41,600±1500 years BP from 'On Your Knees Cave' on the Prince of Wales Island. A large femur from a brown bear was also discovered in this cave (35,365±800 years), suggesting that these two large mammals may have coexisted in this region just prior to the beginning of the Fraser glaciation. Further discoveries of brown bear and black bear remains from El Capitan Cave radiocarbon dated at 12,295±120 years BP and 11,565±115 years BP respectively suggests that these mammals were also present during early postglacial periods.

Continuity of brown bears throughout the Wisconsin in southeastern Alaska is also supported by a genetically distinct group of brown bears (*Ursus arctos*) recently identified on Admiralty, Baranof, and Chichagof Islands (ABC Islands) (Talbot and

**Table 16 List, locale, and radiocarbon dates of faunal remains discovered in the Alexander Archipelago (Heaton et al. 1996)**

| Cave Name | Location | $^{14}C$ age (yr BP) | Description |
|---|---|---|---|
| **El Capitan** | Prince of Wales | 12,295±120 | brown bear |
| | | 9760±75 | brown bear |
| | | 11,565±115 | black bear (cranium) |
| | | 11,540±110 | black bear (skull) |
| | | 10745±75 | black bear (skeleton) |
| | | 6415±130 | black bear (cranium) |
| | | 6810±65 | fish bone |
| | | 8535±70 | fish bone |
| | | 3290±60 | river otter |
| | | 5770±130 | fish bone |
| **Bumper** | Prince of Wales | 11,640±80 | brown bear (skeleton) |
| | | 11,225±110 | brown bear (humerus) |
| | | 10,970±85 | brown bear (molar) |
| | | 7205±65 | brown bear (jaw) |
| | | 10,515±90 | caribou (metacarpal) |
| **Blowing in the wind** | Prince of Wales | 9995±95 | brown bear (skeleton) |
| **Kushtaka** | Prince of Wales | 8725±70 | black bear (femur) |
| | | 8630±60 | black bear (rib) |
| | | 2820±60 | spear point (artifact) |
| **Enigma** | Dall | 11,175±120 | black bear (skeleton) |
| **Nautilus** | Heceta Island | 8180±70 | Mule deer (humerus) |
| **On Your Knees** | Prince of Wales | 35,365±800 | brown bear (femur) |
| | | 41,600±1500 | black bear (tibia) |
| | | 17,565±160 | ringed seal (ulna) |
| **Devil's Canopy** | Prince of Wales | 44,500+ | marmot (incisor) |

Shields 1996) (Fig 46). Divergence time estimates based on mtDNA sequence data suggested that the ABC brown bears had been isolated for the last 500,000 to 700,000 years. A possible mid-Pleistocene divergence for these brown bears is similar to the divergence times estimated for the black bear, marten and short-tailed weasel.

Although conclusive evidence for the persistence of flora and fauna on the northwestern coast of North America in the form of a continual ice-free late Wisconsin - age fossil sequence is still lacking, the congruent phylogeographic patterns observed across a diversity of taxa is nonetheless compelling (Fig 47) and requires an explanation. Given the current state of knowledge, I consider the most reasonable interpretation to be that at least some of these genetically divergent forms dispersed from the Hecate Refugium to Haida Gwaii, the mainland, Vancouver Island and various other areas in the Pacific Northwest at the end of the last glaciation. The absence of these forms in any of these regions can be accounted for by their failure to establish a population, their failure to reach these areas before sea levels reached their present state or lineage sorting. Eventually their counterparts, which had been isolated north and south of the Cordilleran ice sheet, dispersed from their refugia and secondary postglacial contact was made between these relict populations.

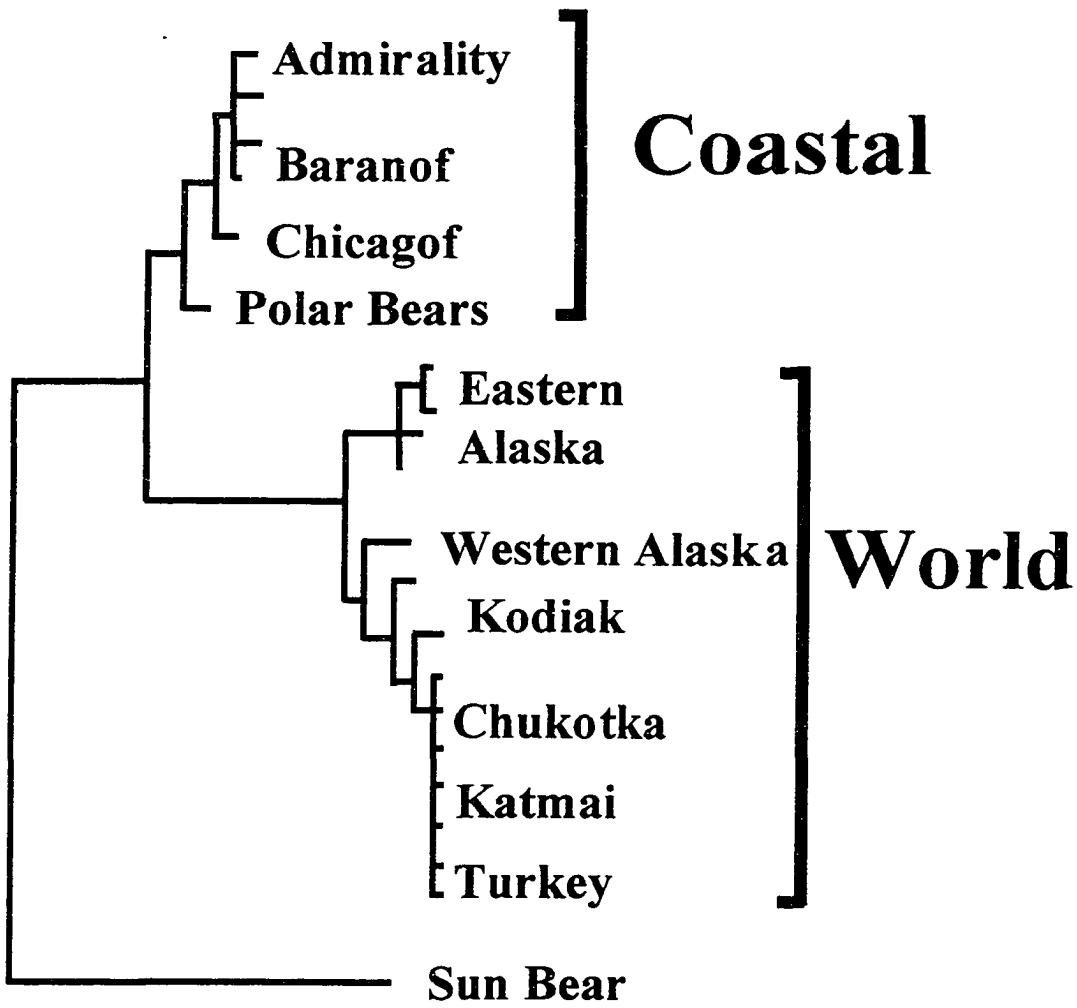
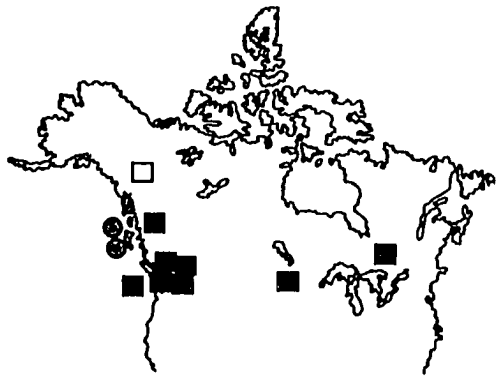
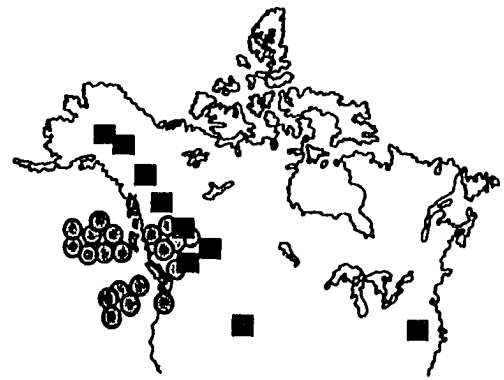


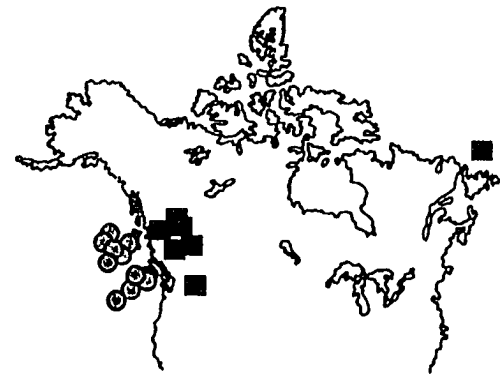

**Figure 46**
MtDNA sequence analysis (cytochrome b and D-loop) revealed the existence of two distinct lineages of brown bears, one composed exclusively of brown bears from the ABC Islands and polar bears. A minimum of 27 point mutations was estimated to reflect a divergence between these lineages of approximately 550,000 - 700,000 years. The ABC brown bears are assumed to be relicts which colonized these islands prior to the late Wisconsin glaciation (adapted from Heaton et al. 1996).



**Short-tailed weasel**



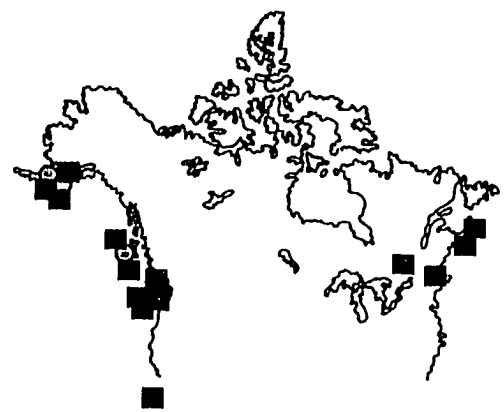
**Black bear**



**Marten**

**Sockeye Salmon**

**simplified from Wood et al. 1994**



**Stickleback**

**simplified fromOrti et al. 1996**

**Brown Bear**

**simplified from Talbot and Shields 1996 and Heaton et al. 1996**

**Figure 47**

**Habitat Suitability of the Hecate Refugium**

There is convincing but still controversial evidence that portions of the Hecate Strait were exposed and free of ice during the late Wisconsin. However, because of the lack of fossils, there is no definitive evidence of that this area supported a variety of animal and plant populations and as such served as a major centre of postglacial biotic dispersal. Based on molecular evidence, a diversity of organisms including mammals (black bears, brown bears, marten, short-tailed weasels, deer mice), fish (sticklebacks and salmon), angiosperms (*Tellima grandiflora, Packera pseudaurea*), birds (Rufous-Sided Towhee and Common Yellowthroat), may have persisted on the northwestern coast of North America during the most intense period of the late Wisconsin glaciation. However, to support the ecological requirements of these populations, particularly the large-bodied taxa, would require a large coastal refugium rather than a nunatak or small ephermal refuge as is currently assumed to have existed on the north Pacific coast of North America.

Marten, one of the species that may have survived in the Hecate refugium, requires minimal overhead cover. Although marten are known to live above the treeline (Streeter and Braun 1968) they generally require an abundance of complex physical structures near the ground (Buskirk 1994) to provide them with protection from predators, access to subnivean space where most of their winter prey are captured and protective thermal microenvironments (Buskirk 1994). These structures include coarse woody debris, rocks, and/or herbaceous plants (Buskirk 1994). Although the pollen assemblage from Cape Ball suggest a tundra like environment in adjacent regions about

16,000 years BP (Warner et al. 1982; Mathewes 1989), there is some indirect evidence suggesting that trees were present in coastal areas during the Wisconsin. Hebda (1982, 1995) and Hebda and Whitlock (1997) proposed that the rapid expansion of pine during the late glacial period suggests that this species survived in coastal refugia like the Brooks Peninsula and adjacent exposed continental shelves (Hebda 1997). Based on spruce pollen found at Cape Ball, Mathewes (1989) suggested that small number of stunted spruce trees might have existed along the coast. Presence of such trees may not have been necessary for marten but certainly would have provided a more diverse habitat.

Based on the mtDNA data, both black bears (Byun et al. 1997) and brown bears (Talbot and Shields 1996) may have persisted on the coast during the Wisconsin. On the coast, these two species only co-occur in areas which are least partially forested. Although it is unlikely that the Hecate refugium supported large forests, the possible coexistence of black bears and brown bears suggests that there may have been small stands of stunted trees which allowed these two species to inhabit the refugium simultaneously.

Black bears are highly opportunistic foragers; however, on the coast, they are highly dependent upon salmon. This dependence suggests that if black bears persisted in the Hecate refugium, there was likely to have been reasonable access to salmon. Allozyme studies by Wood et al. (1994) suggest that during the Fraser glaciation, sockeye may have had spawning grounds in coastal refugia around the area of the Hecate Strait. The possible occurrence of sockeye in the Hecate refugium requires that rivers were present and possibly freshwater nursery lakes. The presence of a freshwater lake in the Hecate refugium has been confirmed by core analysis (Josenhans et al. 1993).

Marten consume a variety of items. However, short-tailed weasels appear to be more limited and subsist primarily on small rodents. The possibility of deer mice persisting on the coast during the late Wisconsin makes the survival of this mustelid much more feasible.

It is not my intention to imply that the Hecate refugium was an ecological oasis. Dynamic changes in sea levels and glacier position probably altered the boundaries of this refugium, preventing long term stability of plant and animal populations. Open tundra was probably interspersed with small stands of shrubs and the animal populations that inhabited this area were probably forced to constantly shift their range in order to accommodate these environmental changes.

## Concluding Remarks

From this study and examination of current literature, it becomes apparent that a major phylogeographic split exists within a diversity of North America taxa which differentiates populations on the northwestern coast from the rest of the continent. In light of the emerging evidence such as congruent molecular phylogeographies (Byun et al. 1997; Talbot and Shields 1996), global plant disjunctions (Ogilvie 1989; Schofield 1989: Hebda 1997), stratigraphic data (Warner et al. 1982; Barrie et al. 1993; Josenhans et al.1995; Barrie and Conway, in press), and paleontological remains (Heaton et al. 1996) which have indirectly supported the existence of a refugium near Haida Gwaii on the continental shelf, I suggest that this phylogeographic division is largely a consequence of persistence and dispersal from this coastal source area.

If the phylogeographic distributions observed in at least some of these taxa have

been influenced by recolonization by coastal source populations, then two major predictions can be made from this hypothesis: 1) The divergent populations on the northwestern coast should become less common in more southern and eastern locales. This however would be highly dependent upon such things as dispersal rate and time of divergence and 2) Further investigation should reveal congruent phylogeographic divisions between coastal and continental populations in other taxa, especially those which are relatively sessile.

The data presented in this paper provide support for an ecologically productive biological refugium on the northwest coast of North America during the last glacial maximum. This refugium may have provided the source populations that contributed to modern populations along coastal British Columbia and its offshore archipelago, Haida Gwaii and Vancouver Island. As previous morphological analysis has not been able to identify the close biogeographic affinity of these regions, I conclude that in this case, morphological endemism does not accurately indicate refugia and that the morphological attributes that differentiate subspecies in the coastal region are likely to be postglacially derived. This study provides insight into the historical processes that have influenced the region's biota and as such may play a role in modifying prevailing concepts in the zoogeography of the Pacific Northwest.

## Literature Cited

Adovasio, J. M. and D. R. Pedler. 1997. Monte Verde and the antiquity of humankind in the Americas. Antiquity 71:573-580.

Allen, J. A. 1902. List of mammals collected in Alaska by the Andrew J. Stone Expedition of 1901. Bulletin of the American Museum of Natural History 14:213-218.

Anderson, E. 1994. Evolution, prehistoric distribution and systematics of *Martes*. In S. W. Buskirk, A. S. Harestad, and M. G. Rapheal, comps., (eds.), Marten, sables, and fishers: biology and conservation. Pp. 13-25. Ithaca, N.Y. Cornell University Press.

Alcover, J. A. and M. McMinn. 1994. Predators of vertebrates on islands. Bioscience 44(1): 12-18.

Avise, J. C. and C. F. Aquadro. 1982. A comparative summary of genetic distances in the vertebrates. Evolutionary Biology. 15: 151-185.

Avise, J. C. and W. S. Nelson. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. Science 243: 646-648.

Avise, J. C. , Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 449-522.

Avise, J. C. 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, New York.

Baccus, R., Ryman, N., Smith, M. H., Reuterwall, C., and D. Cameron. 1983. Genetic variability and differentiation of large grazing mammals. Journal of Mammalogy 64(1): 109-120.

Bain, J. F. and R. K. Jansen. 1996. Numerous chloroplast DNA polymorphisms are shared among different populations and species in the aureoid *Senecio* (*Packera*) complex. Canadian Journal of Botany 74: 1719-1728.

Ball, R. M. Jr. and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. The Auk 109(3): 626-636.

Ball, R. M., Jr., James, F. C., Freeman, S., Bermingham, E. and J. C. Avise. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proceedings of the National Academy of Science. U.S.A. 85: 1558-1562.

Banfield, A. W. F. 1954. Preliminary investigation of the barren-ground caribou. Canadian Wildlife Services, Wildlife Management Bulletin Series 1, No 10A and 10B.

Banfield, A. W. F. 1961. A Revision of the Reindeer and Caribou, Genus *Rangifer*. National Museum of Canada, Bulletin No. 177. Biological Series No. 66.

Banfield, A. W. F. 1974. The Mammals of Canada. National Museum of Natural Sciences. University of Toronto Press. Toronto, Ont. Pp. 304 - 323.

Bangs, O. 1897. Preliminary description of the Newfoundland marten American Naturalist 31: 161-167.

Barrie, J. V., Conway, K. W., Mathewes, R. W., Josenhans, H. W. and M. J. Johns. 1993. Submerged late Quaternary terrestrial deposits and paleoenvironments of northern Hecate Strait, British Columbia continental shelf, Canada. Quaternary International 20: 123-129.

Barrie, J. V. and K. W. Conway. 1998. Late Quaternary glaciation and postglacial stratigraphy of the northern Pacific margin of Canada. Submitted to Quaternary Research.

Barrowclough, G. F. 1983. Biochemical studies of microevolutionary processes. In A. H. Brush and C. A. Clark, Jr. (eds.), Perspectives In Ornithology. Pp. 223-261. Cambridge University Press, New York.

Barton, N. H. and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113-148.

Bass, R. 1995. The woodland caribou. Audubon 76: 76-115.

Bell, M. A. 1976. Evolution of phenotypic diversity in *Gasterosteus aculeatus* superspecies on the Pacific coast of North America. Systematic Zoology 25: 211-227.

Bergerud, A. T., Ferguson, R., and H. E. Butler. 1990. Spring migration and dispersion of woodland caribou at calving. Animal Behavior 39: 360-368.

Bernatchez, L. and J. J. Dodson. 1991. Phylogeographic structure in mtDNA of Lake Whitefish. Evolution 45(4): 1016-1035.

Bissonette, J. A. and S. S. Sherburne. 1983. Subnivean access: the prey connection. In I. D. Thompson (ed.), International Union of Game Biologists, XXI Congress. Vol. 1: 225-228.

Blaise, B., Clague, J. J., and R. W. Mathewes. 1990. Time of maximum glaciation, west coast of Canada. Quaternary Research 34: 282-295.

Blanchard, M. M., Taillon-Miller, P., Nowotny, P., and V. Nowotny. 1993. PCR buffer optimization with uniform temperature regimen to facilitate automation. PCR Methods and Applications 2: 234-240.

Bradshaw, R. H. W. and O. Zackrisson. 1990. A two thousand year history of a northern Swedish boreal forest stand. Journal of Vegetation Science 1: 519-528.

Brooks, A. and H. S. Swarth. 1925. A distributional list of the birds of British Columbia. Museum of Vertebrate Zoology of the University of California No. 423

Brown, B. L. and R. W. Chapman. 1991. Gene flow and mitochondrial DNA variation in the killifish, *Fundalus heteroclitus*. Evolution 45(5): 1147-1161.

Brown, W. M., George, M. J., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences. USA 76: 1967-1971.

Brown, W. M. 1985. The mitochondrial genome of animals. In R. J. MacIntyre (ed.) Molecular Evolutionary Genetics. Pp. 95-130. Plenum Press, New York.

Brown, W. M., Prager, E. M., Wong, A., and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Evolution 18: 225-239.

Butlin, R. K. and G. M. Hewitt. 1985a. A hybrid zone *between Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* (Orthoptera: Acrididae): morphological and electrophoretic characters. Biological Journal of the Linnean Society 26: 287-299.

Buskirk, S. W. 1994. An introduction to the genus *Martes*. In S. W. Buskirk, A. S. Harestad, and M. G. Rapheal, comps., (eds.), Marten, Sables, and Fishers: Biology and Conservation. Pp. 1-10. Ithaca, N.Y. Cornell University Press.

Byun, S. A., Koop, B. F. and T. E. Reimchen. 1997. North American black bear mtDNA phylogeography: Implications for morphology and the Haida Gwaii glacial refugium controversy. Evolution 51(5): 1647-1653.

Calder, J. A. and R. L. Taylor. 1968. Flora of the Queen Charlotte Islands, Part 1. Systematics of the vascular plants. Research Branch, Canada Department of Agriculture Monograph 4(1). Queen's Printer, Ottawa.

Carlquist, S. 1974. Island Biology. Columbia University Press. New York, U.S.A.

Carr, S. M. and Hicks, S. A. 1997. Are there two species of pine marten in North America? Genetic and evolutionary relationships within *Martes*. In G. Proulx, R. Goddard, and H. Bryant (eds.). *Martes*: Taxonomy, Ecology, Techniques and Management. Pp. 1528-2002. Provincial Museum of Alberta, Edmonton.

Case, T. J. 1978. A general explanation for insular body size: trends in terrestrial vertebrates. Ecology 59(1): 1-18.

Case, T. J. and M. L. Cody. 1987. Testing theories of island biogeography. American Scientist 75: 402-411.

Cavalli-Sforza, L. L. and A. W. F, Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32: 550-570.

Churcher, C. S. , Parmalee, P. W., Bell, G. L., and J. P. Lamb. 1989. Caribou from the late Pleistocene of northwestern Alabama. Canadian Journal of Zoology 67: 1210-1216.

Chernoff, B. 1982. Character variation among populations and the analysis of biogeography. American Zoology 22: 425-439.

Clague, J. J. 1989. Quaternary Geology of the Queen Charlotte Islands. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 65-74. Queen Charlotte Island Museum Press, Second Beach.

Clague, J. J. 1975. Late Quaternary sediments and geomorphic history of the southern Rocky Mountain Trench, British Columbia. Canadian Journal of Earth Sciences 12: 595-605.

Clark, T. E. Molecular Reevaluation of the *Nebria gregaria* Infra group and the Implications for the Existence of an Ice Age Refugium on the Queen Charlotte Islands. MSc. Thesis. University of Victoria, Victoria, B.C..

Clarke, J. S., Fastie, C., Hurtt, G., Jackson, S. T., Johnson, C., King, G. A., Lewis, M., Lynch, J., Pacala, S., Prentice, C., Schupp, E. W., Webb, T. III, and P. Wyckoff. 1998. Reids' paradox of rapid plant migration. Bioscience 48(1): 13- 24.

Coope G. R. 1979. Late Cenozoic Coleoptera: evolution, biogeography, and ecology. Annual Review Ecology and Systematics 10: 249-267.

Cowan, I. McT. and C. J. Guiget. 1956. The Mammals of British Columbia. British Columbia Provincial Museum. Handbook No. 11, Victoria.

Cowan, I McT. 1989. Birds and mammals on the Queen Charlotte Islands. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 175-186. Queen Charlotte Island Museum Press, Second Beach.

Cowan, I. McT. 1935. A distributional study of the *Peromyscus sitkensis* group of white-footed mice. University of California Publications in Zoology 40(13): 429-438.

Cox, C. B. and P. D. Moore. 1985. Biogeography: An Ecology and Evolutionary Approach. Blackwell Scientific Publications.

Cronin, M. A., Armstrup, S. C., Garner, G. W., and E. R. Vyse. 1991. Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). Canadian Journal of Zoology 69: 2985-2992.

Cronin, M. A. 1992. Intraspecific variation in mitochondrial DNA of North American cervids. Journal of Mammalogy 73(1): 70-82.

Cronin, M. A., Renecker, L., Pierson, B. J., and J. C. Patton. 1995. Genetic variation in domestic reindeer and wild caribou in Alaska. Animal Genetics 26: 427-434.

Crooks, K. R. and D. Van Vuren. 1995. Resource utilization by two insular endemic mammalian carnivores, the island fox and island spotted skunk. Oecologia 104: 301-307.

Croziat, L. 1962. Space, Time, Form: The Biological Synthesis. Published by the author, Caracas. (available from Wheldon and Wesley, Lytton Lodge, Codicote, Herts, UK.)

Davis, J. I. and K. C. Nixon. 1982. Populations, genetic variation, and the delimination of phylogenetic species. Systematic biology 1(4): 421-435.

Dawson, G. M. 1890. Later Physiographical Geology of the Rocky Region in Canada. Transactions of the Royal Society of Canada 8(4): 51.

Dawson, A. G. 1992. Ice Age Earth: Late Quaternary Geology and Climate. Routledge, New York.

DeBlase, A. F. and R. E. Martin. 1981. A Manual of Mammalogy with Keys to Families of the World. Wm. C. Brown, Dubuque, Iowa.

Deagle, B. E., Reimchen, T. E., and D. B. Levin. 1996. Origins of endemic stickleback from the Queen Charlotte Islands: mitochondrial and morphological evidence. Canadian Journal of Zoology 74: 1045-1056.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Doyle, J. J. 1997. Trees within trees: genes and species, molecules and morphology. Systematic Biology 46(3): 537-553.

Dumond, D. 1977. The Eskimo and Aleuts. Thames and Hudson. London.

Easton, N. A. 1992. Mal de mer above terra incognita, or, "What ails the coastal migration theory?". Arctic Anthropology 29(2): 28-42.

Easterbrook, D. J. 1992. Advance and retreat of Cordilleran ice sheets, Washington, U.S.A. Geographie Physique et Quaternaire 46(1): 51-68

Eck, R. V. and M. D. Dayoff. 1966. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, M. D.

Eger, J. L. 1990. Patterns of geographic variation in the skull of Nearctic ermine (*Mustela erminea*). Canadian Journal of Zoology 68: 1241-1249.

Elliot, D. G. 1903. A list of mammals collected by Edmund Heller in the San Pedro Martir and Hanson Languna mountains and the accompanying coast regions of Lower California with descriptions of apparently new species. Field Columbian Museum Publications 79(3) 199-232.

Endler, J. A. 1982. Problems in distinguishing historical from ecological factors in biogeography. American Zoology 22: 441-452.

Feduccia, A. 1996. Origins and Evolution of Birds. Vail-Ballou Press, Binghamton. New York. Pp. 291-296.

Felsenstein, J. 1989. PHYLIP-Phylogeny Inference Package (Version 3.2). Cladistics 5: 164-166.

Fitch, W. M. 1977. On the problem of discovering the most parsimonious tree. American Naturalist 111: 223-257.

Fladmark, K. R. 1970. Honna River Bridge Site (HCB). No. 6.

Fladmark, K. R. 1974. A Paleoecological Model for Northwest Coast Prehistory. Ph.D. Dissertation. Department of Archaeology, University of Calgary, Alberta.

Fladmark, K. R. 1979. Routes: Alternate migration corridors for early man in North America. American Antiquity 44: 55-69.

Fleming, J. H. 1916. The Saw-whet Owl of the Queen Charlotte Islands. Auk 33: 420-423.

Foster, J. B. 1964. Evolution of mammals on islands. Nature 202: 234-235.

Foster, J. B. 1965. The Evolution of the Mammals of the Queen Charlotte Islands, British Columbia. Province of British Columbia Department of Recreation and Conservation, Victoria.

Frost, D. R., Kluge, A. G., and D. M. Hillis. 1992. Species in contemporary herpetology: comments on phylogenetic inference and taxonomy. Herpetological Review 23(2) :46-54

Fulton, R. J. 1967. Deglaciation studies in Kamloops region ,an area of moderate relief, British Columbia. Geological Survey of Canada, Bulletin 154.

Fulton, R. J. 1969. Glacial lake history, southern Interior Plateau, British Columbia. Geological Survey of Canada, Paper 69-37.

Fulton, R. J. 1991. A conceptual model for growth and decay of the Cordilleran ice sheet. Geographie Physique et Quaternaire 45(3):281-286.

Gach, M. H. and T. E. Reimchen. 1987. Mitochondrial DNA patterns among endemic stickleback from the Queen Charlotte Islands: preliminary survey. Canadian Journal of Zoology 67: 1324-1328.

Geist, V. 1991. Taxonomy: On an objective definition of subspecies, taxa as legal entities, and its application to *Rangifer tarandus* Lin 1758. In C. E. Butler and S. P. Mahoney (eds.). Proceedings 4th North American Caribou Workshop. Pp. 1-36. St. John's, Newfoundland.

Giannico, G. R. 1986. Geographic and sexual variation of the American Pine Marten (*Martes americana*) in the Pacific Northwest, with special reference to the Queen Charlotte Islands. University of Victoria. MSc. thesis. 119 p.

Giannico, G. R. and D. W Nagorsen. 1989. Geographic and sexual variation in the skull of the Pacific coast marten (*Martes americana*). Canadian Journal of Zoology 67: 1386-1393.

Gilbert, B. K. and R. M. Lanner. 1995. Energy, diet selection and restoration of brown bear populations. In Proceedings of the 9th International Conference of Bear Research and Management. Pp. 231-240. Pateris: French Ministry of the Environment and the Natural History Museum of Grenoble.

Gingerich, P. D. 1983. Rates of evolution: effects of time and temporal scaling. Science 222: 159-161.

Gingerich, P. D. 1993a. Rates of evolution in Plio-Pleistocene mammals: six case studies. In R. A. Martin and A. D. Barnosky (eds.) Morphological Change in Quaternary Mammals of North America. Pp. 84-106. Cambridge University Press, New York.

Gingerich, P. D. 1993b. Quantification and comparison of evolutionary rates. American Journal of Science 293: 453-478.

Gould, S. J. and R. F. Johnston. 1972. Geographic variation. Annual Review of Ecology and Systematics 3: 457-498.

Gould, S. J. and N. Eldredge. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3: 115-151.

Gray, J. E. 1865. Revision of the genera and species of Mustelidae contained in the British Museum. Proceedings of the Zoological Society of London. Pp. 100-154.

Greenberg, J. H., Turner, C. G. II, and S. L. Zegura. 1986. The settlement of the America: a comparison of the linguistic, dental, and genetic evidence. Current Anthropology 27: 477-497.

Greenberg, J. H. 1987. Language in the America. Stanford University Press, Stanford.

Grinnell, J. and J. S. Dixon. 1926. Two new races of the pine marten from the Pacific coast of North America. University of California Publications in Zoology 21: 411-417.

Groves, C. P. 1992. How old are subspecies? A tiger's eye view of human evolution. Archaeology in Oceania 27(3): 153-160.

Gustincich, S., Carninci, P., and C. Schneider. 1992. A rapid method to extract high-quality genomic DNA from yeast. Technique (Philadelphia) 3(2): 76-77.

Haffer, J. 1969. Speciation in Amazonian forest birds. Science 165: 131-137.

Hagmeier, E. M. 1955. The genus *Martes* (Mustelidae) in North America: its distribution, variation, classification, phylogeny, and relationship to Old World forms. Ph. D. thesis. Dept. of Zoology, University of British Columbia, Vancouver, B.C.

Hagmeier, E. M. 1958. Inapplicability of the subspecies concept to North American marten. Systematic Zoology 7: 1-7.

Hagmeier, E. M. 1961. Variation and relationships in North American marten. Canadian Field-Naturalist 75: 122-138.

Haldane, J. B. S. 1949. Suggestions as to quantitative measurement of rates of evolution. Evolution 3: 51-56.

Hall, E. R. 1928. A new race of black bear from Vancouver Island, British Columbia, with remarks on other northwest coast forms of *Euarctos*. University of California Publications in Zoology 30: 231-242.

Hall, E. R. 1945a. Four new ermines from the Pacific Northwest. Journal of Mammalogy 26(1): 75-85.

Hall, E. R. 1945b. A revised classification of the American ermines with description of a new subspecies from the western Great Lakes region. Journal of Mammalogy 26(2): 175-182.

Hall, E. R. 1951. American Weasels. University of Kansas Publications, Museum of Natural History 4: 1-466.

Hall, E. R. 1981. The Mammals of North America. Vol.2. Second Edition. John Wiley and Sons, New York, USA.

Hall, E. R. and K. R. Kelson. 1959. The Mammals of North America. Ronald Press.

Hasegawa, M., Kishino, H. and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 21: 160-174.

Hare, F. K. and J. E. Hay. 1974. The climate of Canada and Alaska. In R. A. Bryson and F. K. Hare (eds.), Climates of North America. Elsvier, Amsterdam.

Hawley, V. D. and F. E. Newby. 1957. Marten home ranges and population fluctuations in Montana. Journal of Mammalogy 38: 174-184.

Heaton, T. H., Talbot, S. L. and Sheilds, G. F. 1996. An ice age refugium for large mammals in the Alexander Archipelago, southeastern Alaska. Quaternary Research 46: 186-192.

Hebda, R. J. 1982. Late-glacial and postglacial vegetation history at Bear Cove Bog, northeast Vancouver Island, British Columbia. Canadian Journal of Botany 61: 3172-3192.

Hebda, R. J. 1995. British Columbia vegetation and climate history with focus on 6KA BP. Geographie Physique et Quaternaire 49: 55-79.

Hebda, R. J. 1997. Late Quaternary paleoecology of Brooks Peninsula. Chapter 9. In J. Hebda and J. C. Haggerty (eds.), Brooks Peninsula: An Ice Age Refugium on Vancouver Island. Pp. 9.1-9.48. Occasional Papers No. 5. R. BC Parks, Ministry of Environment, Lands and Parks, Victoria, BC.

Hebda, R. J., and C. Whitlock. 1997. Environmental history of the coastal temperate rainforest or northwest North America. In P. K. Schoonmaker, B. von Hagen, and E. C. wolf (eds.), The Rain forests of Home: Profile of a North American Bioregion. Pp. 227-254. Island Press, Covelo, CA.

Hedges, B. S., Hass, C. A., and L. R. Maxson. 1992. Caribbean biogeography: molecular evidence for dispersal in West Indian terrestrial vertebrates. Proceedings of the National Academy of Sciences 89: 1909-1913.

Henault, M. and F. Renaud. 1993. Weight of marten (*Martes americana*) in forests of southwestern Quebec, Canada. In I. D. Thompson, (ed.), International Union of Game Biologists, XXI Congress. Vol. 1: 216-219.

Heusser, C. J. 1989. North Pacific coastal refugia. The Queen Charlotte Islands in perspective. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 91-106. Queen Charlotte Island Museum Press, Second Beach.

Hewitt, G. M. 1993. Postglacial distribution and species substructure: lessons from pollen, insects and hybrid zones. In D. R. Lees and D. Edwards (eds.), Evolutionary Patterns and Processes. Pp. 97-123. Academic Press Inc., San Diego, CA. U.S.A.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

Higuchi, R. 1992. Dr. Russ's problem corner. Ancient DNA Newsletter 1(1): 6-8.

Hicks, S. A. and S. M. Carr. 1992. Genetics analysis of a threatened subspecies, The Newfoundland Pine marten (*Martes americana atrata*), by means of the polymerase chain reaction. In Martin Willison, J.H., Bondrup-Nielsen, S., Drysdale, C., Herman, T.B., Munro, N.W.P., and Pollock, T.L.(eds.), Elsvier, New York. Science and the Management of Protected Areas.

Hicock, S. R. and J. E. Armstrong. 1985. Vashon Drift: definition of the formation in the Georgia Depression, southwest British Columbia. Canadian Journal of Earth Sciences 22: 748-757.

Hogan, K. M., Hedin, M. C., Koh, H. S., Davis, S. K., and I. F. Greenbaum. 1993. Systematic and taxonomic implications of karyotypic, electrophoretic, and mitochondrial-DNA variation in *Peromyscus* in the Pacific Northwest. Journal of Mammalogy 74(4): 819-831.

Huxley, J. 1932. Problems in Relative Growth. Methuen, London.

Irwin, D. M., Kocher, T. D. and A. C. Wilson. 1991. Evolution of the cytochrome b gene of mammals. Journal of Molecular Evolution 32: 128-144.

Janovec, J. P. and T. M. Barkley. 1996. *Sinosenecio newcombei* (Asteraceae: Senecioneae): A new combination for a North American plant in an Asiatic genus. Novon 6: 265-267.

Johnsgard, P.A. 1988. North American Owls. Biology and Natural History. Smithsonian Institution Press. Washington, USA.

Johnson, W. N. and T. F. Paragi. 1993. The relationship of wildfire to lynx and marten populations and habitat in interior Alaska. Galena, A.K: U.S. Depart. Of the Interior, Fish and Wildlife Service, Koyukyuk/Notwitna Refuge Complex; Annual Report for 1992.

Josenhans, H. W., Barrie, J. V., Conway, K. W., Patterson, T., Mathewes, and G. J. Woodsworth. 1993. Surficial geology of the Queen Charlotte Basin: evidence of submerged proglacial lakes at 170 m on the continental shelf of western Canada. Geological Survey of Canada, Current Research, Paper 93-1A, 119-127.

Josenhans, H. W., Fedje, D. W., Conway, K. W., and J. V. Barrie. 1995. Postglacial sea levels on the western Canadian continental shelf: evidence for rapid change, extensive subaerial exposure, and early human habitation. Marine Geology 125: 73-94.

Josenhans, H. W., Fedje, D., Pientiz, R., and J. Southon. 1997. Early humans and rapidly changing sea levels in the Queen Charlotte Islands-Hecate Strait, British Columbia, Canada. Science 277: 71-74.

Jukes, T. H. and C. R. Cantor. 1969. Evolution of protein molecules. Pp. 21-132. In H. N. Munro (ed.), Mammalian Protein Metabolism. Academic Press, New York.

Kavanaugh, D. H. 1992. Carabid beetles (Insecta: Coleoptera: Carabidae of the Queen Charlotte Islands, British Columbia. Memoirs of the California Academy of Sciences Number 16, 113.

Kelsall, J. P. 1968. The Caribou. Crown Copyrights. Ottawa, Canada.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 10: 111-120.

Kindberg, N. C. 1899. Note Sur Hypoterygium du Canada. Review of Bryology 26: 46-48.

King, C. M. 1989a. The advantages and disadvantages of small size to weasels, *Mustela* species. In J. L. Gittleman (ed.), Carnivore Behaviour, Ecology and Evolution, Pp. 302-334. Cornell University Press, Ithaca, New York, U.S.A.

King, C. 1989b. The Natural History of Weasels and Stoats. Cornell University Press. Ithaca, New York, U.S.A.

Klein, D. R. 1965. Postglacial distribution patterns of mammals in the southern coastal regions of Alaska. Arctic 18: 7-20.

Klein, N. and W. M. Brown. 1994. Intraspecific molecular phylogeny in the yellow warbler (*Dendroica petechis*), and implications for avian biogeography in the West Indies. Evolution 48(6): 1914-1932.

Klicka, J. and R. M. Zink. 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277: 1666-1669.

Kocher, T. D. Thomas, W. K., Myer, A., Edwards, S. V., Pääbo, S. Villablanca, F. X., and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Science 86: 6196-6200.

Kullman, L. 1996. Norway spruce present in the Scandes Mountains, Sweden, at 8000 BP: New light on Holocene tree spread. Global Ecology and Biogeography Letters 5: 94-101.

Kumar. S. Tamura, K., and M. Nei. 1993. MEGA: Molecular evolutionary genetics analysis. Pennsylvania State University, University Park, Pennsylvania.

Kurtén, B. 1959. Rates of evolution in fossil mammals, Cold Spring Harbor Symposia on Quantitative Biology 24: 204-215.

Kurtén, B. 1964. The evolution of the polar bear, *Ursus maritimus*. Acta Zoologica Fennica 108: 1-26.

Kurtén, B. 1968. Pleistocene Mammals of Europe. Wiedenfeld and Nicholson, London. Pp. 179.

Kurtén, B. and E. Anderson. 1980. Pleistocene Mammals of North America, Columbia University Press, New York.

Lawlor, T. E. 1986. Comparative biogeography of mammals on islands. Biological Journal of the Linnean Society 28: 99-125.

Li, W. H. and D. Graur. 1991. Fundamentals of Molecular Evolution. Sinauer Associates, Inc. Publishers, Sunderland.

Lindsey, C. C. 1989. Biotic Characteristics of the Queen Charlotte Islands. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 107-108. Queen Charlotte Island Museum Press, Second Beach.

Lister, A. M. 1993. Evolution of mammoths and moose: the Holarctic perspective. In R. A. Martin and A. D. Barnosky (eds.), Morphological Change in Quaternary Mammals of North America. Pp. 178-204. Cambridge University Press., Cambridge.

Lomlino, M. V. 1985. Body size of mammals on islands: the island rule reexamined. American Naturalist 125: 310-316.

Lundberg, J. G. 1972. Wagner networks and ancestors. Systematic Zoology 21: 398-413.

Luternauer, J. L., Clague, J. J., Conway, K. W., Barrie, J. V., Blaise, B., and R. W. Mathewes. 1989. Late Pleistocene terrestrial deposits on the continental shelf of western Canada: evidence for rapid sea level change at the end of the last glaciation. Geology 17: 357-360.

Maddison, W. P. 1997. Gene trees in species trees. Systematic Biology 46(3): 523-536.

MacPherson, A. 1965. The origin of diversity in mammals of the Canadian Arctic tundra. Systematic Zoology 14: 153-173.

Maddison, D. R., Ruvolo, M., and D. L. Swofford. 1992. Geographic origins of human mitochondrial DNA.: Phylogenetic evidence from control region sequences. Systematic Biology 41: 111-124.

Mann, D. H. and T. D. Hamilton. 1995. Late Pleistocene and Holocene paleoenvironemnts of the North Pacific Coast. Quaternary Science Reviews 14: 449-471.

Matthewes, J. V. Jr. 1974. Wisconsin environment of interior Alaska: Pollen and macrofossil analysis of a two metre core from the Isabella Basin (Fairbanks, Alaska). Canadian Journal Earth Science 11: 828-841.

Mathewes, R. W. 1989. Paleobotany of the Queen Charlotte Islands. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 75-90. Queen Charlotte Island Museum Press, Second Beach.

Matsch, C. L. 1976. North America and the Great Ice Age. McGraw-Hill Book Company, Toronto.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press. Cambridge , Massachusetts.

Mayr, E. and P. D. Ashlock. 1991. Principles of Systematic Zoology. Second Edition McGraw-Hill. New York.

McCabe, T. T. and I. McT. Cowan. 1945 . *Peromyscus maniculatus macrorhinus* and the problem of insularity. Transactions of the Royal Canadian Institute 25: 177-215.

McCall, R. A. 1997. Implications of recent geological investigations of the Mozambique Channel for the mammalian colonization of Madagascar. Proceedings of the Royal Society of London (B) 264(1382): 663-665.

McNab, B. K. 1971. On the ecological significance of Bergmann's rule. Ecology 52: 845-854.

Mengel, R. M. 1964. The probable history of species formation in some northern wood warblers (Parulidae). Living Bird 3: 9-43.

Merriam, C. H. 1890. Descriptions of twenty six new species of North American mammals. North American Fauna 4.

Meyer, A., Kocher, T. D., Basasibwaki, P., and A. C. Wilson. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347: 550-553.

Moodie, G. E. E. and T. E. Reimchen. 1973. Endemism and conservation of stickleback populations of the Queen Charlotte Islands. Canadian Field Naturalist 87: 173-175.

Moodie, G. E. E. and T. E. Reimchen. 1976a. Glacial refugia, endemism, and stickleback populations of the Queen Charlotte Islands, British Columbia. Canadian Field Naturalist 90: 471-474.

Moodie, G. E. E. and T. E. Reimchen. 1976b. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25: 49-61.

Moritz, L. Dowling, T. E., and W. M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics 18: 269-292.

Murphy, R. C. 1938. The need for insular exploration as illustrated by birds. Science 88: 533-539.

Myers, G. S. 1960. The endemic fish fauna of Lake Lanao, and the evolution of higher taxonomic categories. Evolution 14: 232-333.

Nagorsen, D. W. 1990. The mammals of British Columbia. Royal British Columbia Museum and the Wildlife Branch. Victoria.

Nagorsen, D. W., Keddie, G., and R. Hebda. 1995. Early Holocene black bears, *Ursus americanus*, from Vancouver Island. Canadian Field Naturalist 109: 11-18.

Nei, M. and T. Gojobori. 1986. Simple methods for estimating the number of synonymous substitutions and nonsynonymous nucleotide substitutions . Molecular Biology and Evolution 3: 418-426.

Nixon, K. C. and J. M. Carpenter 1993. On outgroups. Cladistics 9: 413-426.

Ogilvie, R. T. and H. L. Roemer. 1984. The rare plants of the Queen Charlotte Islands. BC Naturalist 22(2): 17-18.

Ogilvie, R. T. 1989. Disjunct vascular flora of northwestern Vancouver Island in relation to Queen Charlotte Islands' endemism and Pacific Coast refugia. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 127-130. Queen Charlotte Island Museum Press, Second Beach.

Ogilvie, R. T. 1997. Vascular plants and phytogeography of Brooks Peninsula. In R. J. Hebda and J. C. Haggarty (eds.), Brooks Peninsula: An Ice Age Refugium on Vancouver Island. Occasional Paper No. 5. Pp. 5.1-5.48. BC Parks, Ministry of Environment, Lands and Parks, Victoria, BC.

Ohta, T. 1992. The nearly neutral theory of molecular evolution. Annual Review of Ecological Systematics 23: 263-286.

Osborn, H. F. 1910. The Age of Mammals in Europe, Asia, and North America. Macmillian Co., New York.

Osgood, W. H. 1901. Natural history of the Queen Charlotte Islands, British Columbia. Natural History of the Cook Inlet region, Alaska. North American Fauna 21: 1-87.

O'Reilly, P., Reimchen, T. E., Beech, R., and C. Strobeck. 1993. Mitochondrial DNA in *Gasterosteus* and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution 47: 678-684.

Orti, G., Bell, M. A., Reimchen, T. E., and A. Meyer. 1994. Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. Evolution 48: 608-622.

Pääbo, S. 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proceedings of the National Academy of Sciences. USA 86: 1939-1943.

Pääbo, S., Higuchi, R., and A. C. Wilson, A. C. 1989. Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology. Journal of Biological Chemistry 264: 9709-9712.

Paetkau, D. and C. Strobeck. 1996. Mitochondrial DNA and the phylogeny of Newfoundland black bears. Canadian Journal of Zoology 74: 192-196.

Penny , D. Watson, E. E., and M. A. Steel. 1993. Trees from languages and genes are very similar. Systematic Biology 42(3): 382-383.

Pielou, E. C. 1992. After the Ice Age: The Return of Life to Glaciated North America. University of Chicago Press. Chicago.

Pitelka, L. F. and the Plant Migration Workshop Group. 1997. Plant Migration and Climate Change. American Scientist 85: 464-473.

Powell, R. A., Zimmerman, J. W., and D. E. Seaman 1997. Ecology and Behaviour of North American Black Bears: Home Ranges, Habitat and Social Organization. Chapman and Hall, Cornwall, UK.

Powell, R. A. and C. M. King. 1997. Variation in body size, sexual dimorphism and age-specific survival in stoats, *Mustela erminea* (Mammalia: Carnivora), with fluctuating food supplies. Biological Journal of the Linnean Society 62: 165-194.

Preble, E. A. 1898. Description of a new weasel from the Queen Charlotte Islands, British Columbia. Proceedings of the Biological Society of Washington 12: 169-170.

Purdue, J. R. and E. J. Reitz. 1993. Decrease in body size of white tailed deer (*Odocoileus virginianus*) during the late Holocene in South Carolina and Georgia. In R. A. Martin and A. D. Barnosky (eds.), Morphological Change in Quaternary Mammals of North America. Pp. 281-298. Cambridge University Press., Cambridge.

Quammen, D. 1996. The Song of the Dodo: Island Biogeography in an Age of Extinctions. Scribner. Toronto, Canada.

Ralls, K and P. H. Harvey. 1985. Geographic variation in size and sexual dimorphism of North American weasels. Biological Journal of the Linnean Society 25: 119-167.

Rampton, V. 1971. Late Quaternary vegetational and climatic history of the Snag-Klutlan area, south western Yukon territory, Canada Geological Society of American Bulletin 82: 959-978.

Reimchen, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 58: 1232-1244.

Reimchen, T. E. 1994. Predators and morphological evolution in threespine stickleback. In M. A. Bell and S. A. Foster (eds.), Evolutionary Biology of the Threespine Stickleback. Pp. 240-276. Oxford University Press, Oxford.

Rising, J. D. and J. C. Avise. 1993. An application of genealogical concordance principles to the taxonomy and evolutionary history of the Sharp-tailed Sparrow (*Ammodramus caudacutus*). Auk 110(4): 844-856.

Roed, K. H. and K. R. Whitten. 1986. Transferrin variation and evolution of Alaskan reindeer and caribou, *Rangifer tarandus*. *Rangifer* 1: 247-251.

Rodgers, L. L. 1987. Factors influencing dispersal in the black bear. Pp. 75-84. In B. D. Chepeko-Sade and Z. T. Halpin, eds. Mammalian Dispersal Patterns. University of Chicago Press. Chicago.

Rogers, R. A. 1985. Glacial geography and native North American languages. Quaternary Research 23: 130-137.

Rogers, R. A., Rogers, L. A., Hoffman, R. S., and L.D. Martin. 1991. Native American biological diversity and the biogeographic influence of Ice Age refugia. Journal of Biogeography 18: 623-630.

Rounds, R. C. 1987. Distribution and analysis of colourmorphs of the black bear (*Ursus americanus*). Journal of Biogeography 14: 521-538.

Ryder, J. M., Fulton, R. J., and J. J. Clague. 1991. The Cordilleran ice sheet and the glacial geomorphology of southern and central British Columbia. Geographie Physique et Quaternaire 45(3): 365-377.

Saitou, N. and M. Nei. 1987. The neighboring method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sanger, F. G., Air, M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., Slocombe, P. M., and M. Smith. 1977. Nucleotide sequence of the øX174 DNA. Nature 265: 687-695.

Sarich, V. M. and A. C. Wilson. 1973. Generation time and genomic evolution in primates. Science 179: 144-1147.

Saunders, I. R., Clague, J. J., and M. C. Roberts. 1987. Deglaciation of Chilliwack River valley, British Columbia. Canadian Journal of Earth Sciences 24: 915-923.

Savage, J. M. 1982. The enigma of the Central American herpetofauna: dispersals or vicariance? Annals Missouri Botanical Garden 69: 464-547.

Schofield, W. B. 1965. Correlations between the moss floras of Japan and British Columbia, Canada. Journal. Hattori Botanical Laboratory 28: 16-42.

Schofield, W. B. 1984. Bryogeography of the Pacific coast of North America. Journal. Hattori Botanical Laboratory 55: 35-43.

Schofield, W. B. 1989. Structure and affinities of the bryoflora of the Queen Charlotte Islands. In G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Pp. 109-120. Queen Charlotte Island Museum Press, Second Beach.

Servheen, C. 1990. The Status and Conservation of the Bears of the World. International Conference of Bear Research and Management Monograph Series No. 2.

Seton-Thompson, E. 1900. Preliminary descriptions of a new caribou from the Queen Charlotte Islands. Ottawa Naturalist 15: 257-261.

Seton, E. T. 1927. Lives of game animals. Vol. III. Doubleday Page, New York.

Severs, P. D. S. 1973. Report on continued archaeological investigations at Blue Jackets Creek, Flua 4, Queen Charlotte Islands (HCB). No. 14.

Sher, A. V. 1974. Pleistocene mammals and stratigraphy of the far northeast USSR and North America. International Geological Review 16: 1-206.

Shields, G. F. and T. D. Kocher. 1991. Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. Evolution 45: 218-221.

Sibley, C. G. and J. E. Ahlquist. 1990. Phylogeny and Classification of Birds. A Study in Molecular Evolution. Yale University Press. New Haven, U.S.A.

Simpson, G. G. 1944. Tempo and Mode in Evolution. Columbia University Press. New York.

Simpson, G. G. 1961. Principles of Animal Taxonomy. Columbia University Press, New York.

Smith, H. M. , Chiszar, D. and R. R. Montanucci. 1997. Subspecies and classification. Herpetological Review 28(1): 13-16.

Sober, E. 1988. The conceptual relationship of cladistic phylogenetics and vicariance biogeography. Systematic Zoology 37(3): 245-253.

Soltis, D. E., Mayer, M. S., Soltis, P. S., and M. Edgerton. 1991. Chloroplast DNA variation in *Tellima grandifora* (Saxifragacea). American Journal of Botany 78: 1376-1390.

Sondaar, P. Y. 1994. Paleoecology and evolutionary patterns in horses and island mammals. Historical Biology 8: 1-13.

Streeter, R. G. and C. E. Braun. 1968. Occurrence of pine marten, *Martes americana* (Carnivora: Mustelidae) in Colorado alpine areas. Southwestern Naturalist 13: 449 -451.

Strobeck, C. 1994. Molecular Genetic Research Final Report. Environment Canada Parks Service.

Suarez, B. K., Crouse, J., and D. O'Rourke. 1985. Genetic variation in North American populations: the geography of gene frequencies. American Journal of Physical Anthropology 67: 217-232.

Sutherland, P. 1972. Preliminary report on the archaeological investigation of the site at Blue Jackets Creek, Queen Charlotte Islands, FlUa 4 (HCB). No 23.

Sutherland Brown, A. and H. Nasmith 1962. The glaciation of the Queen Charlotte Islands. Canadian Field Naturalist 76: 209-219.

Swarth, H. S. 1911. Birds and mammals of the 1909 Alexander Alaska expedition. University of California Publications in Zoology 7(9): 9-172.

Swofford, D. L. 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1, Smithsonian Institution, Illinois Natural History Survey, Illinois.

Swofford, D. L. (in press). PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0. Sinauer Associates, Sunderland MA.

Szathmary, E. J. E. 1985. Peopling of North America: clues from genetic studies. pp. 79-104. In R. Kirk and E. Szathmary (eds.), Out of Asia: Peopling the Americas and the Pacific. The Journal of Pacific History, Canberra.

Taberlet, P. and J. Bouvet. 1994. Mitochondrial DNA polymorphism, phylogeography and conservation genetics of the brown bear *Ursus arctos* in Europe. Proceedings of the Royal Society of London B 255(1344): 195-200.

Talbot, S. L. and G. F. Shields. 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. Molecular Phylogenetics and Evolution 5: 567-575.

Taylor, R. L. 1989. Vascular plants of the Queen Charlotte Islands. pp. 121-125. In: G.G.E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Island Museum Press, Second Beach.

Terres, J. K. 1980. The Audubon Society Encyclopedia of North American Birds. Alfred A. Knopf Inc. Random House of Canada, Toronto.

Thenius, E. 1990. Carnivores: Phylogeny. In B. Grezimek, ed. Grezimek's Encyclopedia of Mammals, vol 3. Pp. 370-464. McGraw-Hill Publishing Company, New York.

Thomas, W. K., Pääbo, S., Villablanca, F. X., and A. C. Wilson. 1990. Spatial and temporal continuity of Kangaroo Rat populations shown by sequencing mitochondrial DNA from Museum Specimens. Journal of Molecular Evolution 31: 101-112.

Thomas, D. C. , Edmonds, E. J., and W. K. Brown. 1996. The diet of woodland caribou populations in west-central Alberta. In K. Brown, D. Cichowski, J. Edmonds, D. Seip, S. Stevenson, D. Thomas, and M. Wood (eds.), *Rangifer*: Proceedings of 6th North American Caribou Workshop, Special Issue No. 9. Pp. 337-342. Nordic Council for Reindeer Research, Tromso, Norway.

Turner, C. 1987. Telltale teeth. Natural History 96(1): 6-10.

Vrana, P. B., Milinkovitch, M. C., and J. R. Powell. 1994. Higher level relationships of the Arctoid carnivora based on sequence data and "total evidence". Molecular Phylogenetics and Evolution 3: 47-58.

Walsh, P. S., Metzger, D. A., and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10(4): 506-513.

Ward, R. H., Redd, A., Valencia, D., Frazier, B., and S. Pääbo. 1993. Genetic and linguistic differentiation in the America. Proceedings of the National Academy of Science USA 90: 106633-10667.

Warner, B. G., Mathewes, R., and J. J. Clague. 1982. Ice free conditions on the Queen Charlotte Islands, British Columbia, at the height of the Late Wisconsin glaciation. Science 218: 675-677.

Wiley, E. O. 1988. Parsimony analysis and vicariance biogeography. Systematic Zoology 37(3): 271-290.

Wiley, E. R. 1992. The evolutionary species concept reconsidered. In M. Ereshefsky (ed.), The Units of Evolution: Essays on the Nature of Species. Pp. 79-92. Massachusetts Institute of Technology. Cambridge.

Wilson, E. O. and W. L. Brown. 1953. The subspecies concept and its taxonomic application. Systematic Zoology 2(3): 97-111.

Wilson, A. C., Ochman, H., and Prager, E. M. 1987. Molecular time scale for evolution. Trends in Genetics 3(9): 241-247.

Wilson, E. O. 1992. The Diversity of Life. W.W. Norton and Company. New York.

Williams, R., Steinberg, A., Gershowitz, H., Bennett, P., Knowler, W., Pettitt, D., Butler, W., Baird, R., Dowda Rea, L., Burch, T., Morse, H., and C. Smith. 1985. GM allotypes in Native Americans: evidence for three distinct migrations across the Bering land bridge. American Journal of Physical Anthropology 66: 1-19.

Wolstenholme, D. R. 1992. Animal mitochondrial DNA: Structure and evolution. International Review of Cytology 141: 173-216.

Wood, C. C., Riddell, B. E., Rutherford, D. T., and R. E. Withler. 1994. Canadian Journal of Fisheries and Aquatic Science 51(Suppl. 1): 114-130.

Wooding, S. and R. Ward. 1997. Phylogeography and Pleistocene evolution in the North American black bear. Molecular biology and Evolution 14(11): 1096-1105.

Woods, K. D. and M. B. Davis. 1989. Paleoecology of range limits: Beech, in the upper peninsula of Michigan. Ecology 70: 681-696.

Wright, P. L. 1953. Intergradation between *Martes americana* and *M. caurina* in western Montana. Journal of Mammalogy 34: 74-86.

Yates, J. S., Golden, J. L., and J. F. Bain. 1998. A preliminary phytogeographical analysis of inter and intrapopulational cpDNA variation in *Packera pseudaurea* from southwestern Alberta and adjacent Montana. Submitted.

Youngman, P. M. 1975. Mammals of the Yukon Territory. National Museum of Canada, Publications in Zoology. 192 pages.

Youngman, P. M. 1993. The Pleistocene small carnivores of eastern Beringia. Canadian Field-Naturalist 107(2): 139-163.

Zhang, Y. P. and O. A. Ryder. 1995. Phylogenetic relationships of bears (the Ursidae) inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 3(4): 351-359.

Zhang, D. and G. M. Hewitt. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology and Evolution 11: 2478

Zielinski, W. J., Spencer, W. D., and R. H. Barrett. 1983. Relationship between food habits and activity patterns of pine marten. Journal of Mammalogy 64(3): 387-396.

Zink, R. M. 1991. The geography of mitochondrial DNA variation in sympatric sparrows. Evolution 45(2): 329-339.

Zink, R. M. and D. M. Dittmann. 1993. Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution 47: 717-729.

# Appendix I

## Summary of Haida Gwaii's Endemic Fauna

### Land Mammals

**Vespertilionidae**
Northwest Bat *(Myotis californicus caurinus)*
Keen Bat *(Myotis keeni keeni)*
Sooty Big-Footed Bat *(Myotis lucufugus alascensis)*
Silver Haired Bat *(Lasionycteris noctivagans)*

**Cricetidae**
Keen's Deer Mouse *(Peromyscus maniculatus keeni)*
Prevost Island Deer Mouse *(Peromyscus. sitkensis prevostensis)*

**Soricidae**
Queen Charlotte Dusky Shrew *(Sorex monticolus elassodon)*
Prevost Island Dusky Shrew *(Sorex monticolus prevostensis)*

**Ursidae**
Queen Charlotte Black Bear (*Ursus americanus carlottae)*

**Mustelidae**
River Otter *(Lutra canadensis periclyzomae)*
Queen Charlotte Marten (*Martes americana nesophilia*)
Haida short-tailed weasel (*Mustela erminea haidarum*)

**Cervidae**
Dawson caribou (*Rangifer tarandus dawsoni)*

### Endemic Birds

**Strigidae**
Saw-whet Owl (*Aegolius acadicus brooksi*)

**Picidae**
Hairy woodpecker (*Picoides villosus picoideus* )

**Corvidae**
Steller's jay (*Cyanocitta stelleri carlottae*)

**Fringillidae**
Pine grosbeak (*Pinicola enucleator carlottae*)

## Endemic Freshwater Fish

**Gasterosteidae**
Threespine Stickleback (*Gasterosteus aculeatus*)

## Endemic Invertebrates

**Pontogeniidae**
*Paramoera carlottensis*

**Carabidae**
*Nebria haida*
*Nebria charlottae*
*Nebria louiseae*

## Appendix II Descriptions of subspecies referred to in this study for black bear, marten, short-tailed weasel and caribou:

### Black bear

*Ursus americanus americanus*, otherwise known as the common black bear, is the most widely distributed subspecies. It occupies most of Canada and the US and was originally described from a type specimen from eastern North America in 1780. *U. a. americanus* has also been known as *U. a. sornborgeri* (Okak, Labrador), *U. arctos schwenki* (Union Co., Pennsylvania), *Euarctos randi* (Mount Sheldon, Canol Road, Mile 222, Yukon), and *Euarctos hunteri* (Prarie Creek, South Nahanni River) (Hall 1981;Youngman 1975).

*Ursus americanus cinnamomum* was first described in 1854. As its name suggests, *cinnamomum* is more brown than black even in its dark phase and up to 50% of the population has a cinnamon colored pelage (Cowan and Guiget 1956). Despite having a relatively large skull, its molar teeth are fairly narrow, and significantly smaller than the teeth of coastal bears like *carlottae*, *vancouveri* or *altifrontalis* (Cowan and Guiget 1956). *U .a. cinnamomum* is similar in both form and size to *americanus* and in fact *randi* and *hunteri* (now ssp. *americanus*) were once considered synonymous with *cinnamomum* (Cowan and Guiget 1956).

*Ursus americanus altifrontalis* is a coastal race ranging from Bella Coola to Oregon (Nagorsen 1990) and originally described by Elliot in 1903. The most distinguishing characteristic of *altifrontalis* is its high frontal region and wide molar teeth. Although *altifrontalis* does have a brown color phase, it tends to be uncommon and when it does occur, is limited to a dark chocolate brown. It is morphologically similar to *cinnamomum* but *cinnamomum* lacks the elevated forehead which is so characteristic of *altifrontalis*.

*Ursus americanus kermodei* was originally described by the occurrence of a white color morph in 1904 which is currently known to be caused by a single recessive allele. Although it was later discovered that only about 10% of the bears from Burke Inlet to the Nass River, were white, these bears did share some common cranial characteristics and

the name *kermodei* currently applies to both white and black color phases. Hall (1928) describes *kermodei* as having a small skull with a narrow and elongated rostrum. However, Cowan and Guiget (1956) expressed uncertainty concerning diagnostic cranial features for *kermodei* because of great individual variation.

*Ursus americanus vancouveri*, originally described by Hall in 1928, is restricted to Vancouver Island and larger adjacent islands. Like other island populations of black bear, no brown phase has been observed. Black bears on Vancouver Island are further characterized by their massive skulls and large bodies. However, its teeth tend to be less robust when compared to other coastal forms.

*Ursus americanus pugnax* was first described by Swarth (1911). This subspecies is found in the Alexander Archipelago and is distinguished by its depressed frontal shield, and short broad skull (Hall 1928; Foster 1965). Like *vancouveri, pugnax* lacks the brown color phase (Foster 1965). Hall (1928) considered the cranial features characterizing *pugnax* to be highly variable and thought that *pugnax* might even be separated into additional subspecies with further study.

**Marten**

*M. a. abietinoides* was originally described by Gray in 1865. It is found all over the forested regions of British Columbia north of the Omineca and Peace Rivers and east of the Coast Mountains (Cowan and Guiget 1956). It is recognized as a small, dark marten with pale-yellow throat patch.

*M. a. atrata* was described by Bangs in 1897 as a small, dark race restricted to insular Newfoundland and Anticosti Island. However, Hagmeier's (1961) subsequent examination revealed that Bangs mistook females for males and Hagmeier suggests that *atrata* is not significantly different from the adjacent marten subspecies, *M. a. brumalis*.

*M. a. caurina* was first characterized by Merriam (1890). It is a small marten with a pelage that varies considerably in color from brown to cinnamon. They are generally

paler than adjacent subspecies *abietinoides* and *actuosa*, and have deep orange rather than yellowish colored underparts.

*M. a. vancouverensis* was first described by Grinnell and Dixon (1926) as a small pale race restricted to Vancouver Island and possibly some of the larger Gulf Islands, However, the validity of *vancouverensis* is questionable as it is indistinguishable from *caurina* in many respects.

**Short-tailed weasel**

*M. e. richardsonii* is the most widespread of all short-tailed weasel subspecies, extending from western to eastern Canada. Originally characterized in 1893, it is distinguished by it relatively large size, white upper lip, and yellowish underparts. Sexual dimorphism is particularly pronounced in this subspecies (Hall 1981).

*M. e. invicta* resembles *richardsonii* in many respects. It is distinguished by its slightly smaller size and more whitish rather than yellowish underparts (Cowan and Guiget 1956). Hall (1945a) reported small cranial differences between these conspecifics which included *invicta* having a slightly lighter and narrower skull. The winter pelage of *invicta* is white with the exception of the tip of the tail which remains black (Hall 1945a).

*M. e. cicognanii* was first characterized by in 1893 and was considered by Hall (1945a) to be comparable to *invicta*. Considering that these species' ranges do not meet, Hall states that *cicognanii* and *invicta* may actually be independent southern derivatives of *richardsonii*. The differences between *invicta* and *cicognanii* are slight must be considered concurrently to be significant. The differences are as follows: the underparts in *invicta* are more extensive, the tail is longer by 4% relative to the head and body, the hind feet tend to be proportionately longer in *invicta* and there appears to be greater sexual dimorphism.

*M. e bangsi* was once considered synonymous with *cicognanii* despite cranial differences, more pronounced sexual dimorphism in *bangsi* and its larger size. It is

slightly smaller than *richardsonii* and was considered to be an intermediate of these two adjacent conspecifics. Differences between *bangsi* and *richardsonii* are more subtle. The light colored underparts of *bangsi* are more narrow than in *richardsonii* there are slight cranial differences (Hall 1945b).

*M. e. fallenda* was first identified by Hall (1945a). It differs from *richardsonii* in its smaller size, the darker color of its pelage and its extensive encroachment onto the light colored underparts. In contrast to *invicta* and *richardsonii*, its upper lip is brown rather than white (Hall 1945a; Cowan and Guiget 1956). However, *fallenda* does resemble *richardsonii* is several respects. There are similarities in some cranial features, the black color on the tip of the tail is longer and both subspecies demonstrate considerable sexual dimorphism and the black tipped portion of the tail is much (Hall 1945a).

*M. e arctica* was first recognized by in 1989 and largely distinguished from *richardsonii* by its larger, more robust skull. Cranial differentiation is most pronounced in the posterior section of the skull.

*M. e anguinae* was characterized by Hall (1932) and distinguished from mainland conspecifics *richardsonii* and *cicognanii* by its smaller size, narrow white ventral band, smaller skull and significantly different skull shape. The upper lips are usually white and sexual dimorphism is much reduced They usually remain brown all winter (Cowan and Guiget 1956; Banfield 1974).

**Caribou**

*R. t. granti* was originally described by Allen (1902) based upon great cranial differences from various forms of woodland caribou. However, these differences were later attributed to sampling artifacts as over half of the measurements were from sub-adults (Banfield 1961). Banfield (1961) retained the subspecies *granti* but considered that caribou populations in Alaska represented a broad area of integration between *groenlandicus* and *caribou*. The introduction of *R. t. tarandus* from Eurasia into Alaska

during the late 1800's, caused speculations that *granti* was still a pure stock due to hybridization with *tarandus*. However, using three allozyme loci DQA, K-casein, and D-loop, Cronin et al. (1995) concluded that gene flow between these subspecies has been limited. I refer to the northern subspecies (*granti* and *stonei*) defined by Hall (1981) collectively as barren ground caribou.

*R. t. caribou* is the archetype of the woodland caribou first described in 1788. It exhibits enormous variation over its wide geographic range and I will not reiterate its diagnostic features as these were summarized previously. Geist (1991) suggested that *R. t. caribou* should be restricted to mainland caribou including the mountain caribou of British Columbia, Alberta and Idaho and not include those caribou from Newfoundland. *R. t. caribou* are dependent upon old growth forests, and as such are no longer contiguous throughout its range. I will refer to southern subspecies *dawsoni*, *osborni*, *montanus*, *sylvestris*, *fortidens* and *caribou* described by Hall (1981) collectively as woodland caribou.

# Appendix III

## GenBank Accession Numbers for *Ursus americanus* sequences

| | |
|---|---|
| Haida Gwaii 1 | AF007936 |
| Haida Gwaii 2 | AF007917 |
| Haida Gwaii 3 | AF007935 |
| Haida Gwaii 4 | AF007918 |
| Haida Gwaii 5 | AF007937 |
| Haida Gwaii 6 | AF007919 |
| Haida Gwaii 7 | AF007920 |
| Haida Gwaii 8 | AF007922 |
| Haida Gwaii 9 | AF007921 |
| Haida Gwaii 10 | AF007915 |
| Haida Gwaii 11 | AF007916 |
| Vancouver Island 1 | AF007929 |
| Vancouver Island 2 | AF007930 |
| Vancouver Island 3 | AF007931 |
| Vancouver Island 4 | AF007932 |
| Vancouver Island 5 | AF007928 |
| Terrace 1 | AF007924 |
| Terrace 2 | AF007925 |
| Terrace 3 | AF007906 |
| Tweedsmuir 1 | AF007906 |
| Tweedsmuir 2 | AF007926 |
| Prince Rupert 1 | AF007912 |
| Prince Rupert 2 | AF007913 |
| Prince Rupert 3 | AF007914 |
| Khutzymateen | AF007908 |
| Lakelse | AF007909 |
| Moricetown | AF007910 |
| Williston Lake | AF007933 |
| Yukon | AF007934 |
| Alaska 1 | AF007907 |
| Olympic Peninsula | AF007911 |
| Jasper | AF007809 |