

# Glacial refugia and the phylogeography of Steller's sea lion (*Eumatopias jubatus*) in the North Pacific

A. HARLIN-COGNATO,\* J. W. BICKHAM,† T. R. LOUGHLIN‡ & R. L. HONEYCUTT†

\*Department of Biology, Texas A&M University, TX, USA

†Department of Wildlife and Fisheries Sciences, Texas A & M University, TX, USA

‡National Marine Mammal Laboratory, National Marine Fisheries Service, Seattle, WA, USA

## Keywords:

*Eumatopias jubatus*;  
glacial vicariance;  
phylogeography;  
population structure;  
Steller's sea lion.

## Abstract

Mitochondrial DNA sequence data were used to examine the phylogeographic history of Steller's sea lions (*Eumatopias jubatus*) in relation to the presence of Plio-Pleistocene insular refugia. Cytochrome *b* and control region sequences from 336 Steller's sea lions reveal phylogenetic lineages associated with continental refugia south of the ice sheets in North America and Eurasia. Phylogenetic analysis suggests the genetic structure of *E. jubatus* is the result of Pleistocene glacial geology, which caused the elimination and subsequent reappearance of suitable rookery habitat during glacial and interglacial periods. The cyclic nature of geological change produced a series of independent population expansions, contractions and isolations that had analogous results on Steller's sea lions and other marine and terrestrial species. Our data show evidence of four glacial refugia in which populations of Steller's sea lions diverged. These events occurred from approximately 60 000 to 180 000 years BP and thus preceded the last glacial maximum.

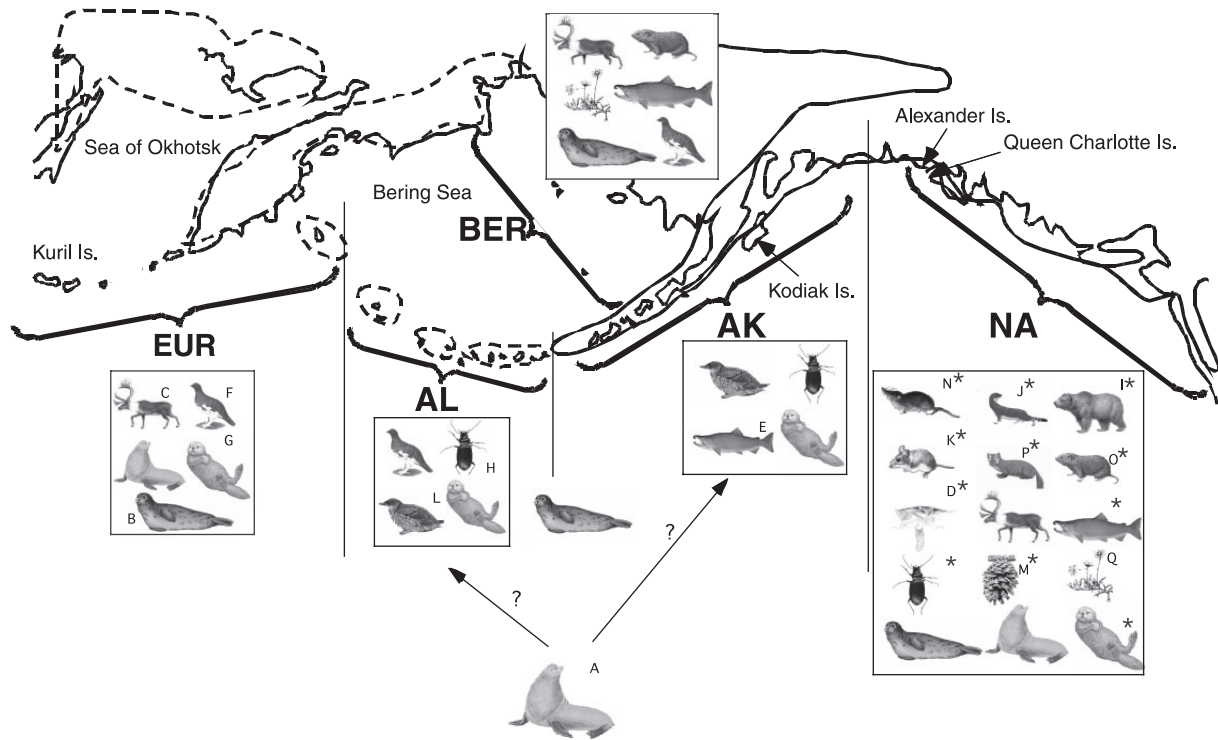
## Introduction

Several empirical studies have documented the impact of glacial cycles on phylogeographic patterns of terrestrial species of plants and animals with a Beringian distribution (Holder *et al.*, 1999,2000; Abbot *et al.*, 2000; Clarke *et al.*, 2001; Barnes *et al.*, 2002; Flagstad & Roed, 2003). The chronology and geomorphology of glaciation in the North Pacific has been well-documented, and there is a growing body of evidence indicating large ice-free regions in Beringia (BER) and south of the ice sheets in Eurasia (EUR) and North America (NA) that served as refugia during the Wisconsin glaciation 65 000–18 000 years ago (Fig. 1) (Pielou, 1991; Hewitt, 2000). Several species of terrestrial plants and animals confined to these regions reflect patterns of phylogeographic structure that are temporally concordant with these geological events (Holder *et al.*, 1999; Abbot *et al.*, 2000; Barnes *et al.*, 2002; Fedorov & Stenseth, 2002; Fleming &

Cook, 2002; Zink *et al.*, 2002; Flagstad & Roed, 2003; Hundertmark *et al.*, 2003). Support for ice-free regions during the last glacial maximum (LGM) also comes from patterns of endemism seen in organisms from coastal islands of British Columbia, Southeastern Alaska and the Gulf of Alaska (Clague, 1989; Heusser, 1989; Cook *et al.*, 2001; Lacourse *et al.*, 2003). For instance, terrestrial species occupying the Alexander Archipelago, Kodiak Islands and the Queen Charlotte Islands (Fig. 1) are considered genetically divergent from mainland relatives as a consequence of insular isolation (Cowan, 1989; Heusser, 1989; Byun *et al.*, 1997; Holder *et al.*, 1999,2000; Clarke *et al.*, 2001; Barnes *et al.*, 2002; Fedorov & Stenseth, 2002; Fleming & Cook, 2002; Brunhoff *et al.*, 2003; Fedorov *et al.*, 2003). As with these terrestrial species, marine mammals and birds, including the sea otter *Enhydra lutris* (Cronin *et al.*, 1996), harbour seal *Phoca vitulina* (Stanley *et al.*, 1996; Gorbics & Bodkin, 2001; Westlake & O'Corry-Crowe, 2002), marbled murrelet *Brachyramphus marmoratus* (Congdon *et al.*, 2000) and rock ptarmigan *Lagopus mutus* (Holder *et al.*, 1999,2000) show similar patterns of divergence. Many of these taxa display female breeding site fidelity, and mitochondrial (mtDNA) gene sequences support genetic

Correspondence: J. W. Bickham, Department of Wildlife and Fisheries Sciences, Texas A & M University, College Station, Texas 77843-2258, USA.

Tel.: +1 979 845 5777; fax: +1 979 845 4096  
e-mail: j-bickham@tamu.edu

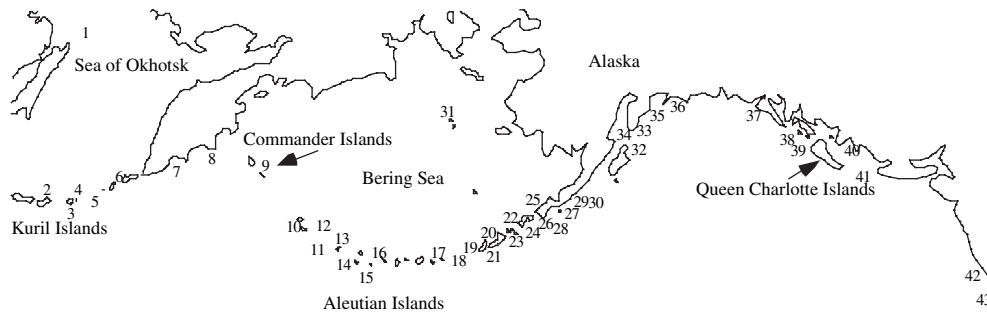


**Fig. 1** Association of putative refugia and genetic structure of North Pacific biota. This figure summarizes previous studies of genetic structure of species occupying regions that were subjected to glaciation during the Plio-Pleistocene. Congruence in the distribution of genetic diversity for a wide variety of plants and animals suggests glacial vicariance shaped the history of these species in a similar fashion. Vertical lines roughly correspond to statistically significant breaks in genetic diversity, each associated with a glacial refugium: EUR, Eurasia; AL, Aleutian Islands; AK, Gulf of Alaska and mainland Alaska; NA, Continental North America; BER, Beringia. Asterisks indicate mainland North American taxa which have forms endemic to the Queen Charlotte and Alexander Islands. The solid curve outlines the general limits of the Cordilleran ice sheet during the last glacial maximum (LGM) 18 000–20 000 years ago (Mann & Peteet, 1994). Boundaries of the EUR ice, which remain disputed (Mann & Hamilton, 1995; Rutter, 1995; Grosswald & Hughes, 2002), are represented by hashed curves. Taxa identification: A, *Eumetopias jubatus* (Baker *et al.*, 2005; Bickham *et al.*, 1998); B, *Phoca vitulina* (Westlake & O’Corry-Crowe, 2002); C, *Rangifer tarandus* (Flagstad & Roed, 2003); D, *Glaucomys* sp. (Kavanaugh, 1989; Abbot *et al.*, 2000); E, *Oncorhynchus kisutch* (Smith *et al.*, 2001); F, *Lagopus mutus* (Holder *et al.*, 1999); G, *Enhydra lutris* (Cronin *et al.*, 1996; Gorbics & Bodkin, 2001); H, *Nebria* sp. (Kavanaugh, 1989; Clarke *et al.*, 2001); I, *Ursus americanus* (Byun *et al.*, 1997; Barnes *et al.*, 2002); J, *Mustela erminea* (Fleming & Cook, 2002); K, *Peromyscus* sp. (Cowan, 1989); L, *Brachyramphus marmoratus* (Congdon *et al.*, 2000); M, *Pinus contorta* (Lacourse *et al.*, 2003); N, *Sorex* sp. (Cook *et al.*, 2001); O, *Microtus* sp. (Cook *et al.*, 2001; Brunhoff *et al.*, 2003); P, *Martes* sp. (Cook *et al.*, 2001); Q, *Dryas integrifolia* (Tremblay & Schoen, 1999).

differentiation between rookeries within regions associated with putative insular isolates (Fig. 1). For example, there are genetic breaks in haplotype diversity that define one or more genetic lineages within the Aleutian Islands (AL), the Gulf of Alaska (AK) and coastal continental NA, which contain one or more of the putative insular refugia that remained ice-free at the LGM (Fig. 1).

Steller’s sea lion (*Eumetopias jubatus*) is a highly vagile marine mammal with rookeries distributed along rocky continental coasts and near-shore islands from Northern California, through the Gulf of AK, along the AL Archipelago, to the Kamchatka Peninsula, Kuril Islands and the Sea of Okhotsk in Asia (Fig. 2). The species has been studied intensively since the mid-1970s, and as a result, genetic material from more than 2000 individuals has been collected from every rookery within the species’ range, and the breeding biology and dispersal behaviour of this species

has been well-documented (Raum-Suryan *et al.*, 2002). Previous studies of mtDNA haplotype diversity (Bickham *et al.*, 1996, 1998; Baker *et al.*, 2005) defined three genetic stocks of Steller’s sea lion – ‘eastern’ (California to Southeast AK), ‘western’ (Prince William Sound to the Commander Islands) and ‘Asian’ (Kamchatka Peninsula, Kuril Islands and Sea of Okhotsk) – with a geographic distribution of genetic diversity generally similar to that of the harbour seal, sea otter, rock ptarmigan, and marbled murrelet (Fig. 1). Combined, the results from mitochondrial (Bickham *et al.*, 1996, 1998; Baker *et al.*, 2005) and nuclear loci (Trujillo *et al.*, 2004) support behavioural observations that female Steller’s sea lions return to within 500 km of their natal rookeries to reproduce throughout their lifetimes, and that gene flow among regions is predominately mitigated through the long-distance dispersal of males (Raum-Suryan *et al.*, 2002).



**Fig. 2** Map of Steller's sea lion rookeries represented in this study. Sample sizes are in parentheses. 1, Iony Island (27); 2, Chernyya Brat'ya (13); 3, Srednego Island (15); 4, Raikoke Island (14); 5, Lovushki Island (19); 6, Antsiferova Island (13); 7, Zheleznaya Bay (9); 8, Kozlova Cape (11); 9, Medny Island (23); 10, Agatu Island (5); 11, Buldir (4); 12, Attu Island (4); 13, Kiska Island (13); 14, Amchitka Island (2); 15, Ulak Island (6); 16, Gramp Rocks (2); 17, Seguam Island (5); 18, Yunaska Island (3); 19, Adugak Island (4); 20, Bogosloff Island (2); 21, Ogchul Island (2); 22, Akun Island (2); 23, Akutan Island (24); 24, Ugamak Island (13); 25, Amak Island (1); 26, Clubbing Rocks (5); 27, Atkins Island (8); 28, Pinnacle Island (13); 29, Chowiet Island (2); 30 Chirikof Island (2); 31, Walrus Island (2); 32, Marmot Island (6); 33, Chiswell Island (2); 34, Sugarloaf Island (4); 35, Fish Island (9); 36, Seal Rocks (8); 37, White Sisters (6); 38, Hazy Islands (18); 39, Forester Island (5); 40, North Danger Rocks (3); 41, Triangle Island (5); 42, Rogue Reef (1); 43, St. George Reef (1).

The congruent distribution of genetic variation among terrestrial-breeding marine taxa supports the presence of insular refugia during the LGM. However, the extent to which these insular refugia shaped the phylogeographic history of terrestrial-breeding marine taxa remains inconclusive (Holder *et al.*, 1999). To investigate this, we present three testable predictions of the patterns of mtDNA population structure expected from species isolated among insular refugia during the Plio-Pleistocene. First, the effects of glacial vicariance should be detectable in the analyses of maternal lineages, especially in species with female philopatry. Second, geographic regions that demonstrate significant genetic structure are expected to be associated with putative glacial refugia. Third, the rate of coalescence events within maternal lineages is predicted to be synchronized with the chronology of geological events.

To test these hypotheses we studied the mitochondrial DNA control region and complete cytochrome *b* gene from 338 Steller's sea lions, distributed among 43 rookeries. Control region sequences were previously reported in a much larger study of population genetics designed to investigate stock structure of this species (Baker *et al.*, 2005). Based upon haplotype frequencies, they concluded that there are three stocks of Steller's sea lions: an eastern stock includes rookeries from California to southeastern AK, a western stock includes rookeries from Prince William Sound to the Commander Islands, and an Asian stock includes rookeries on the Kamchatka Peninsula, Kuril Islands and Sea of Okhotsk. Their study did not employ a phylogenetic analysis of the sequences or any specific tests to relate population subdivision to geological history. In this study we report new sequence data for the cytochrome *b* gene from a subset of the animals used by Baker *et al.* (2005), and we perform analyses to investigate the

relationships among geographic distributions of haplotypes, the phylogenetic history of the haplotypes, and the geological history of the North Pacific region. Our results provide support for the presence of glacial refugia proposed by the congruent distribution of multiple taxa with similar distributions, dispersal capabilities, and dependence on suitable terrestrial breeding habitat. Specifically, we present evidence that geographic variation of the Steller's sea lion is consistent with one or more insular refugia in the Pacific Northwest, Gulf of AK, AL Islands and Russia, and that following glacier recession, rookeries were re-colonized in a linear pattern, resulting in contemporary isolation-by-distance (IBD) between historical refugia. Collectively, this study provides one of the most thorough tests of insular refugia hypotheses, and it illustrates the response of a terrestrially breeding marine mammal to habitat perturbations resulting from glacial cycles.

## Materials and methods

### Sampling

Tissues were selected from an archive of flipper punches from pups taken at their natal rookeries (Fig. 2) collected from 1994 to 2003. Because Steller's sea lions are not known to twin, it is assumed that all samples are from unrelated individuals. Emphasis was placed on obtaining representation of previously recognized genetic stocks (Bickham *et al.*, 1996) and from regions containing putative insular refugia (Fig. 2), as well as representatives of all 80 control region haplotypes known from pups taken at rookeries at the time we conducted this work. All samples had previously been sequenced for a 238 bp segment of the control region (Bickham *et al.*, 1996, 1998; Trujillo *et al.*, 2004; Baker *et al.*, 2005).

### Data collection

Total genomic DNA was isolated using either a standard phenol–chloroform protocol (Maniatis *et al.*, 1982) or a Qiagen DNeasy kit (Qiagen, Valencia, CA, USA). The complete cytochrome *b* (*cyt b*) gene and 238 bp of the 5' end of the mitochondrial control region (Bickham *et al.*, 1996) were amplified with the polymerase chain reaction (PCR). External primer sets included: (1) *cyt b* – LGL 765F, 5'-GAAAAACCAAYCGTTGTWATTCAACT-3' and LGL 766R, 5'-GTTAATTACAATYTYAGCTTTGGG-3'; (2) control region – LGL 283F, 5'-TACTACTGGTCTTGTAACC-3', LGL 1115R, 5'-ATGACCCTGAAGAA-RGAACCAG-3'. Methods for analysis of the control region were reported previously (Bickham *et al.*, 1996). For cytochrome *b*, approximately 1–2  $\mu$ L of DNA template were included in 50  $\mu$ L PCR reactions containing the following: 5  $\mu$ L each 10X Amplitaq PCR buffer (Perkin Elmer, Boston, MA, USA), 25 mM MgCl<sub>2</sub> and 10 mM deoxynucleotidetriphosphates (dNTP's, 2.5 mM each), 1  $\mu$ L each of 10 mg mL<sup>-1</sup> bovine serum albumin (BSA), 10  $\mu$ M of each primer, and 5 units (U) of Amplitaq (Perkin Elmer) DNA polymerase. Reactions were performed in an ABI 2700 (Applied Biosystems, Foster City, CA, USA) thermocycler with an initial denaturation at 95 °C for 2 min, followed by 25 cycles of 95 °C (45 s), 50 °C (40 s), 70 °C (2 min 30 s), and a final extension at 72 °C for 10 min.

Amplicons were electrophoresed in 1.5% agarose-TBE (tris, boric acid, EDTA) and visualized under UV light. Prior to sequencing, excess oligonucleotides and dNTP's were removed with a QIAquick PCR purification kit (Qiagen, Valencia). Cleaned PCR products were sequenced with ABI (Applied Biosystems) BigDye sequencing chemistry and an ABI 377 automated sequencer. All amplicons were sequenced in both directions, and two internal primers (560, 5'-GCAACCCTAACACGATTCTTCG-3'; 610, 5'-CCAGTTTCGTGTAGGAATAATAGG-3') designed for Steller's sea lion were used to sequence cytochrome *b* to obtain complete overlap in both directions.

Sequences were aligned with default parameters in Clustal X (Thompson *et al.*, 1994), and published sequences of *E. jubatus* (GenBank accession X82311), *C. ursinus* (AF380888, AF384390, AF380890, AF384389), *P. vitulina* (L39204, AF522864, X82306, AF522865), and *Zalophus californianus* (X82310, L37031), the sister-taxon to Steller's sea lion (Wynen *et al.*, 2001), as guides. The alignment of *cyt b* was unambiguous and contained no insertions or deletions. Subsequent to the Clustal X alignment of the control region, visual alignment in MACCLADE VERSION 4.0 (Maddison & Maddison, 1992) was used to make minor adjustments. Because the mitochondrial genome is inherited as a single genetic locus without recombination, the cytochrome *b* and control region sequences were concatenated to produce a fragment of 1378 base

pairs. This alignment is available from authors upon request. All subsequent statistical analyses were performed on this combined data set. The sequences for 28 cytochrome *b* haplotypes were submitted to GenBank under accession numbers DQ144995–DQ145022. All control region sequences were previously submitted to GenBank by Baker *et al.* (2005).

### Diversity indices, model of evolution, and rate of substitution

DNASP VERSION 3.99.5 (Rozas & Rozas, 1999) was used to estimate haplotype diversity (Nei, 1987), nucleotide diversity ( $\pi$ ) (Nei, 1987), theta, and number of segregating sites. Standard error of these measurements was determined from a null distribution generated from 10 000 random permutations of the data keeping sample size constant. The average number of pair-wise differences was estimated with the program MEGA VERSION 2.1 (Kumar *et al.*, 1993, 2001) with gamma-corrected Tamura–Nei (Tamura & Nei, 1993) distances. A 95% confidence interval for the pair-wise distances was calculated from 100 bootstrap replications.

MODELTEST VERSION 3.06 (Posada & Crandall, 1998) was used to test 54 nested hypotheses of nucleotide substitution. The Tamura–Nei model (Tamura & Nei, 1993) with a correction for rate variation among sites (Yang, 1996) and a proportion of invariant sites [ $I = 0.9176$ ; (Waddell *et al.*, 1996)] was selected as the model that best fit the substitution pattern for both genes, and was used in all analyses that employed a model of evolution.

A maximum likelihood analysis of edited *E. jubatus* ( $n = 2$ , this study) sequences, aligned with GenBank sequences from *C. ursinus* ( $n = 3$ ) and *Z. californianus* ( $n = 2$ ), was performed in PAUP\* VERSION 4.0B10 (Swofford, 1999) with model parameters selected via ModelTest Version 3.06 (Posada & Crandall, 1998) and rooted with *P. vitulina* as an outgroup. The resultant phylogeny (not shown) was congruent with a previous analysis (Wynen *et al.*, 2001), with *E. jubatus* and *Z. californianus* as sister taxa and *C. ursinus* at the base of the *E. jubatus* and *Z. californianus* clade. This phylogeny with maximum likelihood optimized branch-lengths was subsequently used in RHINO VERSION 1.2 (Rambaut, 2003) to estimate the divergence time and substitution rate of the *E. jubatus* lineage. Two calibration points, the divergence of *E. jubatus* and *Z. californianus* at 2.0 ybp and *C. ursinus* and the family Otariidae at 6.0 ybp (Repenning, 1976), were used to estimate substitution rates. The rate of evolution obtained with RHINO was compared to that derived with the formula  $r = K/2T$  (Li & Graur, 1991), where  $K$  is the number of pair-wise differences between Steller's sea lion and *Z. californianus*, and  $T$  is the date of divergence in millions of years (ybp). The date of divergence of 2.0 ybp (Repenning, 1976) from the fossil record was used as a calibration point in this analysis.

### Prediction 1: genetic structure of female lineages

Phylogenetic relationships among mtDNA haplotypes within the *E. jubatus* lineage were estimated following the statistical parsimony procedure of Templeton *et al.* (1992) with the program TCS VERSION 1.1.3 (Clement *et al.*, 2000). The haplotype network obtained from TCS was used as the framework for sequential nesting of haplotypes into clades (Templeton, 1998). Reticulations, or equally parsimonious connections within the network, were resolved with two procedures. First, alternative connections between haplotypes were broken following a series of rules based on coalescence theory (Crandall & Templeton, 1993). Second, synapomorphies in the form of nonsynonymous substitutions in the *cyt b* gene, a conservative class of substitution, was used to resolve all cases in which assignment to a nested series was ambiguous ('stranded clades') (Templeton *et al.*, 1987; Templeton & Sing, 1993). The program GEODIS VERSION 2 (Posada *et al.*, 2000) was used to test the null hypothesis of random geographic distribution of genetic variation. This program performs two analyses. The first is an  $r \times c$  contingency test of geographic locality and frequency of haplotypes. The second is the estimation of haplotype dispersion within and among nested clades (Templeton, 1998). In both cases, the significance of results was derived from comparison to a null distribution generated from 10000 randomizations of the data matrices.

Templeton's nested clade procedure (Templeton *et al.*, 1987, 1992; Templeton, 1998) was used to define genetic boundaries of populations by simultaneously incorporating evolutionary history and contemporary geographic distribution and frequency of haplotypes. With this method, it is not required that the number or geographic boundaries of populations be defined *a priori*, but rather genetically distinct populations are defined via statistical tests for association of evolutionary lineages and their geographic distribution. Statistical significance is determined with a permutation procedure that increases the power to detect population structure in species whose genetic variation otherwise does not display a definitive geographic pattern (Templeton, 1998, 2004). Because the relative age of clades increases with nesting level, the most contemporary population boundaries were determined from the distribution of 1-step clades that had a significant geographic association in the NCA. The geographic distribution of haplotypes within clades at deeper nested levels (> 1-step) was used to characterize the temporal shift in the geographic distribution of population boundaries over time. A hierarchical AMOVA (Excoffier *et al.*, 1992) was performed with ARLEQUIN VERSION 2.00 (Schneider *et al.*, 1997) to measure the strength of the genetic structure among populations with boundaries defined with NCA, and to quantify the partitioning of variation within rookeries, within populations of rookeries, and among populations. Significance

values of  $\phi$  statistics (Weir & Cockerham, 1984) were calculated by comparison to a null distribution derived from 10 000 random permutations of the data.

### Prediction 2: association of glacial refugia and population boundaries

The program IBD VERSION 1.5 (Bohonak, 2002) was used to perform a Mantel test that assessed significance of the correlation between geographic and genetic distances. The slope of the regression plus 95% confidence intervals was estimated with reduced major axis regression (RMA), which is considered a more appropriate estimator of slope in IBD analyses than ordinary least squares regression (Hellberg, 1994).

A partial regression was performed with IBD to test for significant association of genetic divergence and putative insular refugia while controlling for the effect of geographic distance. A third variable matrix was created from pair-wise comparisons of rookeries that were or were not potentially separated by inhospitable (ice covered) rookeries at the LGM. For example, a '1' was assigned to individuals from rookeries that were not putatively separated by ice sheets at the LGM, and a '0' was given to comparisons among rookeries that were potentially isolated or extirpated via glacial vicariance. In this manner, we tested for an effect of ice sheets on genetic divergence among rookeries. Furthermore, population boundaries derived from NCA were compared to the geographic location of putative refugia (Fig. 1).

### Prediction 3: dates of population divergence and glacier chronology

The stochastic nature of lineage sorting within species makes estimation of intraspecific divergence with a single locus problematic. In an attempt to circumvent this potential problem, times of divergence among populations of Steller's sea lion were calculated with two methods. First, the time to most recent common ancestor (TMRCA) of clades was estimated with the program BEAST VERSION 1.0.3 (Drummond *et al.*, 2002; Drummond & Rambaut, 2003a) that uses a Bayesian approach with a MCMC search strategy to calculate from an aligned sequence matrix, a model of evolution, and a rate of nucleotide substitution, the posterior probability density distribution of divergence dates of clades. One important feature of BEAST is the ability to analyze concatenated DNA sequences from multiple data partitions that have different models of evolution. Therefore, the concatenated cytochrome *b* and control region partitions were assigned independent models of evolution derived from MODELTEST. Three independent MCMC chains were run for 1 000 000 steps each to optimize the scale factors to an acceptance rate probability of 0.25 for each parameter (Drummond *et al.*, 2002). Each initial MCMC chain was run with scale factors adjusted as

suggested by the operator analysis (Drummond & Rambaut, 2003a) until the scale factors and the length of chains were adequate to produce an effective sample size (ESS) (Drummond *et al.*, 2002) greater than 100 for all estimated parameters. The final BEAST analysis consisted of two independent MCMC chains of 2 500 000 steps each with optimized search settings. TRACER VERSION 1.0.1 (Drummond & Rambaut, 2003b) was used to examine the burn-in period, the degree of mixing, the shape of the probability density distribution, and 95% confidence intervals for estimated divergence dates. A final examination of the two runs combined suggested that the two chains sampled from similar probability density distributions, and that mixing during the search was adequate. The quality of the MCMC search was also supported by high (> 100) ESS values. Second, the TMRCA of clades was estimated by calculating the average number of pair-wise differences between haplotypes within each clade, and multiplying this by the point estimate and 95% confidence intervals of the maximum-likelihood derived mutation rate of the concatenated data partitions (see previous section).

## Results

### Diversity indices, model of evolution, and rate of substitution

Comparisons of 336 individuals of *E. jubatus* yielded 64 variable sites and 107 haplotypes for the combined data. Haplotypes differed from each other by an average of 2.26 substitutions, and nucleotide diversity was relatively low at 0.18%. Of the 107 haplotypes, 93 were found only in a single individual, and haplotype diversity was 0.89 ( $\pm 0.01$ ).

The maximum likelihood estimation of the rate of substitution for *E. jubatus* was 1.9% per myr with a 95% confidence interval of 1.5–2.8%. Similarly, the method based on the average number of pair-wise differences produced a substitution rate that ranged between 1.5 and 1.9% per myr with an average of 1.7%, well within the 95% confidence interval of the maximum likelihood estimate.

The maximum likelihood analysis of divergence times proposed a separation of the *E. jubatus* and *Z. californianus* lineages approximately 2.25 myr ago, with a 95% confidence interval of 1.57–3.06. This divergence estimate was cross-validated with independent calibrations, i.e. the 2 myr *E. jubatus*/*Z. californianus* and the 6 myr sea lion/*C. ursinus* fossil dates fell within the 95% confidence intervals of divergence dates derived from the ML analysis of molecular data.

### Prediction 1: genetic structure of female lineages

The nested clade procedure produced a network with four nested levels (Fig. 3), each with clades that demon-

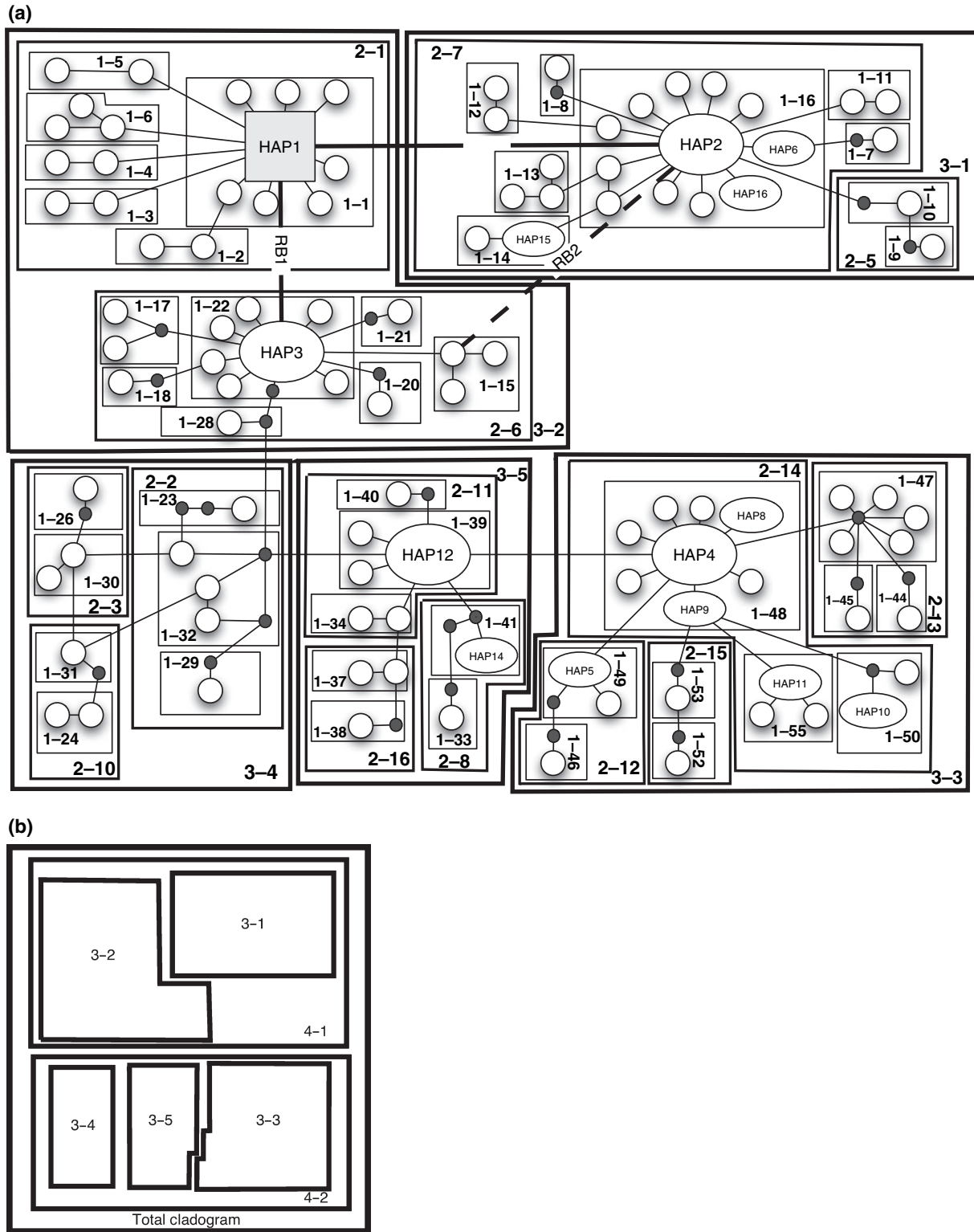
strated statistically significant geographic patterns (Table 1). For example, Clade 4-2 contained 100% of the haplotypes collected in NA rookeries (British Columbia to California, Fig. 1), and Clade 4-1 showed the opposite pattern containing the majority of haplotypes from EUR (west of the Commander Islands) (Fig. 4). Those rookeries in the central portion of the range, from the Gulf of AK (Fig. 1) through the AL Islands (Fig. 1), most often had haplotypes distributed equally between the two 4-step clades (Fig. 4). There is a similar pattern to the geographic distribution of haplotypes in 1-, 2-, and 3-step clades, but geographic resolution increases as the nested level decreases. For example, 2-step clades show association between EUR and NA, but also clearly demarcate a lineage (clade 2-6) geographically restricted to the AL and AK regions (Fig. 4). Clades at the first nested level defined four lineages, each associated with EUR, NA, AL and AK regions (Fig. 4). Boundaries of contemporary Steller's sea lion populations were defined from the geographic limits of these four regionally associated lineages. Measures of population structure provided additional statistical support for genetic subdivision of *E. jubatus* into these four populations (Table 2).

### Prediction 2: association of glacial refugia and population boundaries

The four populations identified with the nested clade procedure roughly corresponded to the location of the NA, EUR, AL and AK refugia (Fig. 4). The partial regression revealed statistical support for the association of genetic distances between rookeries and putative glacial refugia ( $r = 0.18$ ,  $P < 0.05$ ). Additionally, the Mantel and RMA analyses supported a significant positive correlation between genetic and geographic distance between rookeries ( $r = 0.36$ ,  $P < 0.001$ ).

### Prediction 3: dates of population divergence and glacier chronology

Estimates of TMRCA derived from Bayesian and maximum likelihood methods varied, so that no two estimates from different methods overlapped in their dates (Table 3). When the results of each method were examined independently, the confidence intervals tended to be large regardless of the method used, such that in most cases estimates of TMRCA among lineages were not significantly different from each other (Fig. 5). Dates derived by the Li & Graur (1991) method produced the smallest confidence intervals with the greatest degree of discrimination of inter-clade divergence (Table 3). These dates were generally 150 000–200 000 years younger than those of the Bayesian method, and the confidence intervals were more consistent (Table 3). Nevertheless, regardless of the method used to derive the dates, analyses supported a divergence of *E. jubatus* populations prior to the Wisconsin glaciation.



**Fig. 3** Nested cladogram. (a) Nested levels 0–3. Haplotype codes are as in Table 1. Ovals designate haplotypes with a frequency > 1 and are roughly proportional to frequency. Solid circles represent inferred intermediate haplotypes. Alpha-numeric designations refer to individual sea lions possessing unique haplotypes. Each line indicates one mutational step. Reticulations are indicated with dashed or heavy solid lines. RB = Reticulation Break. (b) Nested levels 3 and 4.

**Table 1** Summary of inferences regarding demographic events deduced from clades with significant nested clade values.

Clade	$\chi^2$	Nested clades	$D_c^*$	$D_n^*$	Chain of inference	Demographic event
1-16	$P = 0.06$	Hap2 (INT)	n.s.	349.3, >, $P = 0.07$	1 yes, 2 no, 11 no, 17 no	Inconclusive
		Hap6 (TIP)	n.s.	n.s.		
		Club8 (TIP)	n.s.	n.s.		
		Lov13 (INT)	n.s.	7762.4, >, $P = 0.019$		
		Hap16 (TIP)	n.s.	n.s.		
		Chis968 (TIP)	n.s.	4471.4, <, $P = 0.10$		
		Club7 (TIP)	n.s.	n.s.		
		N(KP)7 (TIP)	n.s.	7115.7, >, $P = 0.07$		
		Ug469 (TIP)	n.s.	n.s.		
		CM1280 (TIP)	n.s.	n.s.		
		A11 (TIP)	n.s.	7562.7, >, $P = 0.05$		
		Ug486 (TIP)	n.s.	n.s.		
		Chow5 (TIP)	n.s.	n.s.		
1-48	$P = 0.007$	I-T	n.s.	n.s.	1 yes, 2 yes, 3 yes, 5 no, 6 too few clades for concordance, 7 yes	Restricted gene flow/dispersal or range expansion/colonization, with some long distance dispersal
		Hap4 (INT)	649.2, <, $P = 0.002$	n.s.		
		CM1256 (TIP)	n.s.	n.s.		
		B453 (TIP)	n.s.	n.s.		
		Og2 (TIP)	n.s.	n.s.		
		Y15 (TIP)	n.s.	4568.4, >, $P = 0.02$		
		Hap8 (TIP)	0.0001, >, $P = 0.08$	n.s.		
1-49	$P = 0.04$	Hap9 (INT)	7255.7, >, $P = 0.03$	n.s.	No inference possible <sup>†</sup>	
		Hap5 (INT)	n.s.	n.s.		
		Lov20 (TIP)	n.s.	n.s.		
2-1	n.s.	I-T	n.s.	n.s.	1 yes, 2 yes, 3 yes, 5 no, 6 too few clades, 7 yes	Restricted gene flow/dispersal or range expansion/colonization, with some long distance dispersal
		1-1 (INT)	n.s.	n.s.		
		1-2 (TIP)	28.2, <, $P = 0.07$	8132.1, >, $P = 0.007$		
		1-3 (TIP)	331.6, <, $P = 0.06$	7249.8, <, $P = 0.01$		
		1-4 (TIP)	0.000, <, $P = 0.05$	6315.9, <, $P = 0.01$		
		1-5 (TIP)	n.s.	n.s.		
		1-6 (TIP)	n.s.	n.s.		
2-6	n.s.	I-T	n.s.	426.0, >, $P = 0.02$	1 yes, 2 yes, 3 no, 4 yes, 9 no	Past fragmentation
		1-22 (INT)	63004.9, >, $P = 0.06$	5504.7, >, $P = 0.0084$		
		1-18 (TIP)	n.s.	n.s.		
		1-28 (TIP)	n.s.	3454.8, <, $P = 0.02$		
		1-17 (TIP)	n.s.	n.s.		
		1-21 (TIP)	n.s.	n.s.		
2-13	n.s.	1-20 (TIP)	n.s.	n.s.	1 yes, 2 no, 11 no, 17 yes, 4 yes, 9 no	Past fragmentation
		I-T	5982.5, >, $P = 0.09$	383.4, >, $P = 0.07$		
		1-47 (INT)	n.s.	6567.7, >, $P = 0.04$		
		1-44 (TIP)	n.s.	n.s.		
2-14 (1-48) (1-49)	n.s.	1-45 (TIP)	n.s.	n.s.	1 yes, 2 no, 11 no, 17 yes, 4 no	Restricted gene flow with isolation by distance
		I-T	n.s.	1155.6, >, $P = 0.04$		
		1-48 (INT)	n.s.	1916.3, >, $P = 0.001$		
3-2 (2-6)	$P < 0.0001$	1-50 (TIP)	n.s.	n.s.	1 yes, 2 no, 11 yes, 12 yes, 13 yes	Range expansion with long distance colonization
		1-55 (TIP)	n.s.	934.6, <, $P = 0.06$		
3-3 (2-13) (2-14(1-48)) (1-49)	$P = 0.005$	I-T	n.s.	878.0, >, $P = 0.01$	1 yes, 2 no I/T	No inference possible <sup>†</sup>
		2-1 (INT)	7700.5, <, $P = 0.002$	8205.9, >, $P = 0.0002$		
		2-6 (INT)	5336.1, <, $P = 0.003$	7953.9, <, $P = 0.001$		
		2-14 (INT)	1673.3, <, $P < 0.001$	3678.4, <, $P = 0.006$		
		2-15 (TIP)	n.s.	2455.5, <, $P = 0.02$		
3-5	n.s.	2-12 (TIP)	671.4, <, $P < 0.001$	6683.8, >, $P < 0.001$	1 yes, 2 yes, 3 no, 4 yes, 9 no	Past fragmentation
		2-13 (TIP)	n.s.	n.s.		
		I-T	n.s.	-2315.4, <, $P < 0.001$		
4-1	$P < 0.001$	2-11 (INT)	n.s.	7056.8, >, $P = 0.04$	1 yes, 2 yes, 3 no, 4 no	Restricted gene flow with isolation by distance
		2-8 (TIP)	n.s.	n.s.		
		I-T	7196.1, >, $P = 0.10$	966.4, >, $P = 0.07$		
		2-16 (TIP)	n.s.	n.s.		
		3-1 (TIP)	5466.1, <, $P = 0.0007$	7366.9, <, $P = 0.0001$		



**Table 1** Continued.

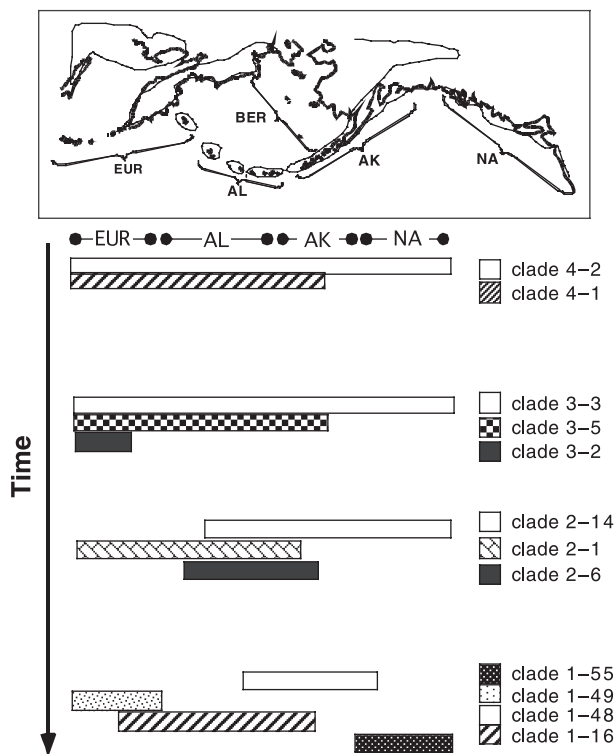
Clade	$\chi^2$	Nested clades	$D_c^*$	$D_n^*$	Chain of inference	Demographic event
(1-16)		3-2 (INT)	8132.9, >, $P < 0.0001$	7817.2, >, $P < 0.0001$		
[3-2(2-6)]		I-T	2666.8, >, $P = 0.0004$	450.3, >, $P < 0.0001$		
4-2	n.s.	3-3 (TIP)	n.s.	n.s.	1 yes, 2 yes,	Restricted gene flow with isolation by distance
[3-3(2-14 (1-48))]		3-4 (INT)	235.7, <, $P < 0.0001$	2888.9, <, $P < 0.0001$	3 no, 4 no	
[2-12(1-49)] (2-13)]		3-5 (INT)	n.s.	6040.6, >, $P = 0.0005$		
Total	$P < 0.0001$	I-T	n.s.	n.s.		
		4-1 (TIP)	7645.5, >, $P = 0.0002$	7015.5, >, $P < 0.00001$	No INT clades	No inference possible†
		4-2 (TIP)	4311.1, <, $P = 0.0004$	6153.8, <, $P < 0.00001$		

TIP, tip clade, INT, interior clade.

Clade numbers within parentheses represent those lower-level clades within the nested series with significant evidence for demographic events. A greater-than or less-than symbol indicates a  $D_c$  or  $D_n$  value that is significantly larger or smaller than expected if haplotypes were distributed randomly.  $P$ -values indicate probability that the  $D_c$  or  $D_n$  estimated from the data were observed by chance. Inferences were drawn from the nested clade analysis key of Templeton (1998). The steps in the chain of inference can be examined by comparison to this key.

\*Results are not significant at  $P < 0.10$ .

†Results of chi-square were significant ( $P < 0.05$ ), but lack of tip clades prevents nested clade inference.



**Fig. 4** A summary of *Eumetopias jubatus* phylogeographic history. Shifts in the geographic distribution of lineages over time are synthesized to present a relative chronology of *E. jubatus* phylogeographic history. The horizontal bars represent the geographic distribution of individual clades within the nested series, and therefore roughly correspond to coalescent events. Clades at the 1-step nested level represent the most recent demographic events, and roughly correspond to four glacial isolates.

**Table 2** Results of analysis of molecular variance among five Steller's sea lion populations derived from the nested clade procedure.

Variation source	d.f.	SS	Components	Variation (%)
Among groups	4	70.8	0.28 Va	17.39 $\phi_{ct} = 0.17^*$
Within groups	37	61.5	0.05 Vb	3.24 $\phi_{sc} = 0.04^*$
Within rookeries	287	366.2	1.28 Vc	79.37 $\phi_{st} = 0.21^*$
Total	328	498.5	1.6	

\* $P \leq 0.001$ .

Because the maximum likelihood divergence estimates had consistently narrower confidence intervals (Table 3), they were used to create a chronology of the history of Steller's sea lion lineages (Fig. 5). In general, the pattern of geographic distribution of maternal lineages suggested that three regional groups formed early in the history of the sea lion (Fig. 4), and that the formation of these groups was loosely associated temporally with glacial cycles (Fig. 5). The broad-scale geographic partitioning of lineages (i.e. 3- and 4-step clades) were coincident with two major ice ages, earlier than the LGM, where sea levels were as much as 140 m below current levels (Fig. 5).

Nested clade inference suggested multiple demographic events in the phylogeographic history of *E. jubatus* (Table 1, Fig. 4). In particular, these results indicated that the rookeries in EUR and NA, at the ends of the species' distribution, experienced multiple periods of geographic isolation and expansion. Nested clade ( $D_n$ ) and clade ( $D_c$ ) distances revealed at least three different periods of restricted gene flow between the EUR

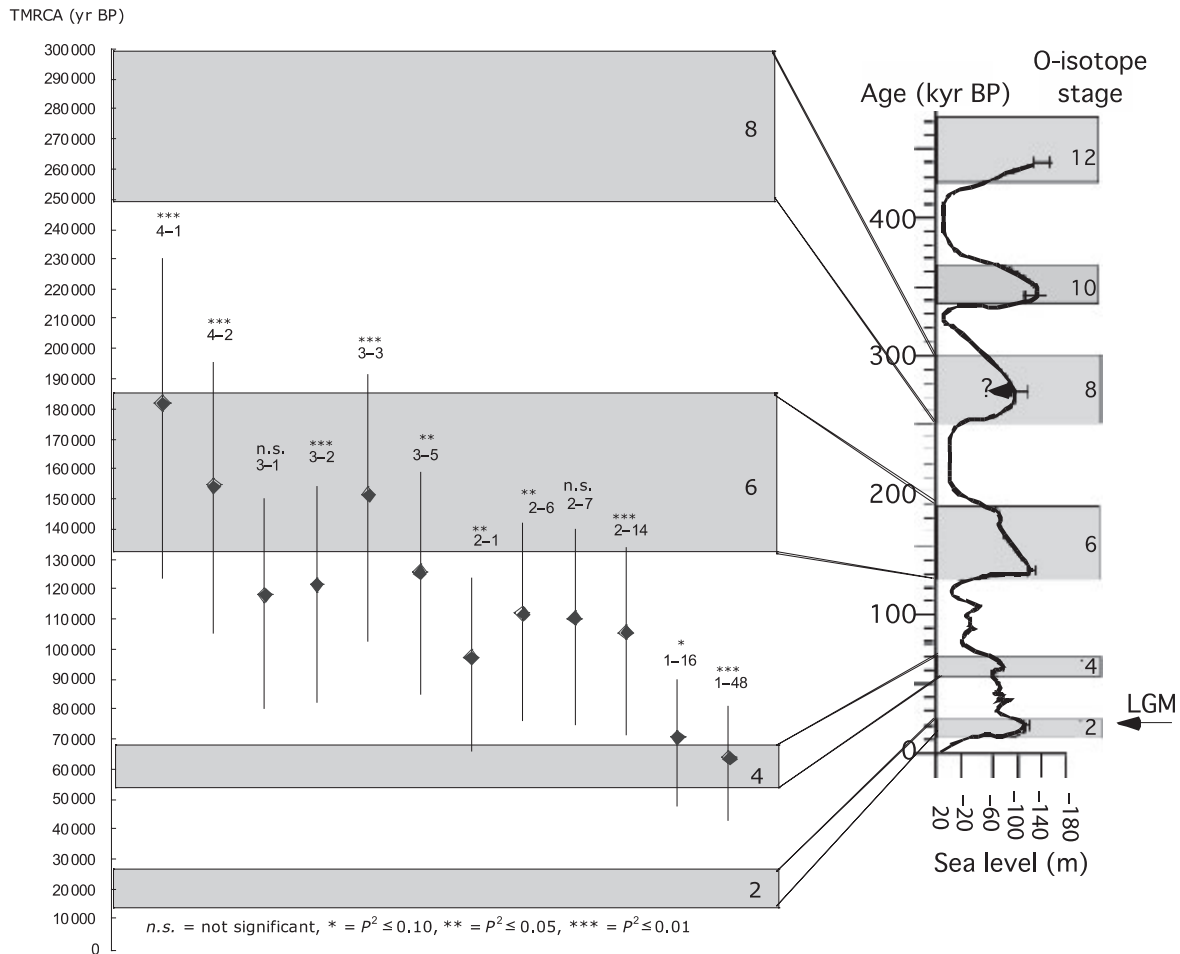
**Table 3** Comparison of TMRCA estimates derived with different methods.

A					B	
	TMRCAs (10 <sup>6</sup> )	Lower	Upper	ESS	Clade 4-1 Rate (sub/site/my)	TMRCAs (10 <sup>6</sup> )
Clade 4-1						
Combined	0.316	0.157	0.51	256.617	0.015	0.23
G4 Run 1	0.298	0.157	0.475	142.035	0.019	0.182
G4 Run 2	0.334	0.181	0.548	114.582	0.028	0.123
Clade 4-2						
Combined	0.418	0.231	0.622	480.15	0.015	0.195
G4 Run 1	0.41	0.228	0.606	264.079	0.019	0.154
G4 Run 2	0.426	0.231	0.628	216.071	0.028	0.105
Clade 3-1						
Combined	0.302	0.156	0.507	275.547	0.015	0.15
G4 Run 1	0.285	0.139	0.456	168.039	0.019	0.118
G4 Run 2	0.319	0.161	0.53	107.508	0.028	0.08
Clade 3-2						
Combined	0.304	0.16	0.488	276.637	0.015	0.154
G4 Run 1	0.287	0.153	0.439	139.704	0.019	0.121
G4 Run 2	0.32	0.18	0.531	136.933	0.028	0.082
Clade 3-3						
Combined	0.388	0.199	0.599	383.302	0.015	0.192
G4 Run 1	0.385	0.205	0.578	215.236	0.019	0.151
G4 Run 2	0.39	0.194	0.611	168.066	0.028	0.103
Clade 3-4						
Combined	0.228	0.091	0.435	208.879	0.015	0.159
G4 Run 1	0.217	0.085	0.397	100.717	0.019	0.125
G4 Run 2	0.239	0.098	0.455	108.162	0.028	0.085
Clade 2-14						
Combined	0.37	0.165	0.572	440.285	0.015	0.133
G4 Run 1	0.367	0.165	0.547	238.398	0.019	0.105
G4 Run 2	0.373	0.166	0.592	201.886	0.028	0.071
Clade 2-13						
Combined	0.294	0.101	0.504	429.231	0.015	N/A
G4 Run 1	0.29	0.107	0.502	230.278	0.019	N/A
G4 Run 2	0.297	0.097	0.508	198.953	0.028	N/A
Clade 2-1						
Combined	0.274	0.114	0.439	305.444	0.015	0.123
G4 Run 1	0.264	0.123	0.425	164.192	0.019	0.097
G4 Run 2	0.283	0.121	0.469	141.253	0.028	0.066
Clade 2-6						
Combined	0.229	0.075	0.424	214.357	0.015	0.141
G4 Run 1	0.219	0.073	0.398	94.71	0.019	0.112
G4 Run 2	0.239	0.082	0.443	119.646	0.028	0.076
Clade 1-48						
Combined	0.337	0.115	0.566	332.311	0.015	0.081
G4 Run 1	0.333	0.106	0.547	149.513	0.019	0.064
G4 Run 2	0.341	0.116	0.568	182.798	0.028	0.043

(A) *BEAST* estimates of time to the most recent common ancestor (TMRCA) for clades identified by nested clade analysis as having experienced a significant demographic event (Fig. 4, Table 1). Results are from independent MCMC chains (G4 Runs 1, 2) and the average over runs (combined) plus 95% confidence intervals derived from the Bayesian probability distributions (BPD). ESS = Effective Sample Size. (B) TMRCA estimated with the method of Li and Graur (1991) based on the average number of pairwise differences among haplotypes. Dates of divergence were calculated for the 95% CI limits of the estimated rate of substitution of the mitochondrial DNA locus.

and other rookeries (Table 1). In other cases, rookeries in CA were isolated from rookeries from AK east to EUR (Fig. 4, Table 1). Therefore, the nested clade inference key supported the interpretation derived independently from observed patterns of lineage distribution, namely that three groups-Eurasian (EUR),

NA and a central Alaskan/Aleutian (AK + AL) appeared multiple times during the history of the species (4-, 3- and 2-step clades, Fig. 4), with a recent addition of a fourth group that formed from the division of the central group into AK and AL populations (1-step clades, Fig. 4).



**Fig. 5** The chronology of coalescent events derived from the average pair-wise divergence among haplotypes in lineages and the 95% confidence interval of the maximum likelihood estimated rate of substitution. Fluctuations in sea level were derived from Rohling *et al.* (1998) and are presented relative to current sea levels in meters (m). Grey shaded regions represent major periods of glaciation, and lines above and below peaks demarcate the confidence intervals of sea level heights at periods of glacial maxima. LGM is last glacial maximum. TMRCA is time to most recent common ancestor.

## Discussion

### Predictions 1 and 2: Genetic structure of female lineages and glacial refugia

One attribute of the nested clade approach is its ability to infer historical processes from the phylogenetic association of lineages that otherwise do not display a definitive geographic pattern (Templeton, 1998, 2004). In the case of Steller's sea lion, inferences derived from the nested pattern of haplotypes revealed multiple vicariant events, corresponding to either the formation or degeneration of populations associated with glacial refugia (Table 1, Fig. 4). In particular, our results support the association of breaks in the distribution of genetic variation along the coast of NA, including the Gulf of AK and British Columbia, that are congruent with ice-free islands during the LGM, notably Kodiak Island, the

Alexander Archipelago and the Queen Charlotte Islands (Fig. 4). In addition, breaks in the distribution of genetic variation suggest that EUR and AL Island rookeries represent remnants of two independent glacial refugia. The exact locations of these refugia are unknown, but they were likely small, ice-free areas in these regions, similar to those off the coast of British Columbia and the Gulf of AK. There are several other lines of evidence supporting an effect of glacial refugia on the structuring of female lineages of Steller's sea lion. First, the derivation of population boundaries was performed with simultaneous incorporation of haplotype frequency and evolutionary history in the nesting procedure. We argue that this is a powerful, objective approach to defining populations without the need for *a priori* assumptions of either the number or geographic boundaries of populations. Furthermore, basic predictions of coalescence theory lead to similar conclusions regarding the genetic

structure of maternal lineages among geographic localities, i.e. the change in distribution of lineages over time can be interpreted without reference to the nested clade inference key as evidence for the formation of populations at different geographic scales throughout the history of the species (Fig. 4). Yet the most compelling evidence is found in the similar patterns of population structure of Steller's sea lion and other taxa (Fig. 1), including harbour seals and sea otters, that provide additional support for the presence of refugia somewhere within these two regions (Cronin *et al.*, 1993; Bickham *et al.*, 1996; Westlake & O'Corry-Crowe, 2002). In total, we suggest that the geographically extensive sampling of Steller's sea lion rookeries and the congruent patterns of population structure derived from studies of multiple taxa independently validate the presence of multiple insular refugia and their importance in shaping the phylogeographic history of taxa in the North Pacific and Bering Sea.

The divergence between Steller's sea lion populations generally followed a pattern of IBD, with the greatest amount of divergence between rookeries in EUR and NA. This is not unexpected given the relatively early separation of these clades at the 4-step nested level (Fig. 4). However, it is possible that patterns of IBD arise in response to limited dispersal between regions that resulted in the accumulation of genetic divergence over time, and it is not necessary to invoke glacial vicariance as the explanation for patterns of population structure. Nevertheless, the pattern of temporal progression from 4-step to 1-step clades (Fig. 4) suggests that the boundaries of EUR and NA isolates were somewhat ephemeral, and that AL and AK populations formed and persisted following the expansion and divergence of EUR and NA isolates. The pattern of IBD seems more consistent with a scenario of re-colonization of AL and AK regions from EUR and NA refugia following glacier recession, which is consistent with the presence of multiple insular refugia.

### Prediction 3: dates of population divergence and glacier chronology

Estimates of coalescence times suggest a demographic history of *E. jubatus* that predates the Wisconsin glaciation (Fig. 4, Table 3). These results are consistent with several previous studies that attributed population structuring of Arctic species to demographic events older than the LGM. For example, patterns of genetic subdivision of the rock ptarmigan are presumably the result of events occurring prior to the LGM (Holder *et al.*, 2000; Brunhoff *et al.*, 2003). Klicka & Zink (1998) examined the evolution of NA songbirds and proposed that the 35 species of birds in their study underwent speciation prior to the late Pleistocene. Given that *E. jubatus* split from *Z. californianus* approximately 2 000 000 years ago, it is not unreasonable to expect *E. jubatus* populations to

currently bear a signature of the effects of glacial cycles that are much older than the Wisconsin glaciation.

The distribution of ice sheets and the duration of the glacial cycles occurring earlier in the Pleistocene are not as well understood as the Wisconsin glaciation. There is strong evidence, however, for changes in sea level associated with glacial cycles dating back to the early Pleistocene (Rohling *et al.*, 1998). Over the last 450 000 years, the sea levels have been as much as 150 m below current levels (Rohling *et al.*, 1998). Many areas of the coastline that are now submerged by seawater were exposed during glacial periods, and served either as corridors for the dispersal of plants and animals (e.g. BER) or as isolated refugia. The fragmentation and expansion of *E. jubatus* coincides with periods of ice sheet advance and retreat, namely the long glacial cycles between 150 000 and 300 000 years ago (Fig. 5, Table 2). This chronology of events is consistent with a scenario of multiple, independent fragmentations by advancing glaciers during interglacial stades, followed by isolation and divergence in several refugia until the ice receded. If it is assumed that contemporary patterns of genetic variation bear the signature of both historic and current processes, one could infer that the effects of insular refugia on population structure represent more recent, fine-scale events in the history of *E. jubatus*, compared to the formation of older and larger glacial refugia associated with the NA mainland. Concordance between the distributions of insular refugia proposed in this study and similar studies of other biota provide additional evidence for the existence of these smaller refugia, despite the inability of phylogenetic methods to detect their formation.

However, it is important to note that confidence intervals associated with estimates of intraspecific divergence times are large, and that there is lack of concordance among estimates derived from different methods. This is not unexpected given the intraspecific lineage sorting process that can be complicated by demographic factors such as population growth, expansion and substructure within regions due to reproductive and dispersal behaviours (Ball *et al.*, 1998; Hoelzer *et al.*, 1998). Nevertheless, results of this study are compelling given the degree of congruence among the structuring of multiple taxa with overlapping distributions, similar dispersal capabilities and ties to terrestrial breeding localities. Even if the dates of divergence of Steller's sea lion are loosely associated with glacier advance, the pattern of population structure supports a region-wide series of phenomena that have shaped the history of multiple taxa before the LGM.

In summary, the pattern of diversification of female lineages of Steller's sea lions appears to correlate with the glacial advances and retreats during the Pleistocene, from approximately 60 000 to 180 000 years BP (Fig. 5). Four populations, ostensibly derived from distinct glacial refugia, are recognized including NA, AK, AL and EUR.

In a previous study of the population genetics of this species, Baker *et al.* (2005) examined control region sequences from 1568 pups taken from 50 rookeries from throughout the range of the species. That study, which focused primarily on population genetic analyses using haplotype frequencies, resolved three genetically distinct stocks. An eastern stock ranges from northern California to southeastern AK and equates to NA in this study. An Asian stock includes rookeries in the Sea of Okhotsk, Kuril Islands and Kamchatka Peninsula and equates to EUR in this study. And, a western stock ranges from Prince William Sound to the Commander Islands and equates to AK and AL in this study. The addition of the cytochrome *b* dataset and the nested clade analysis performed in this study provide an evolutionary framework that increases our understanding of the dynamic nature of population structure of this endangered species.

## Acknowledgments

The National Marine Fisheries Service provided funding for this study. We thank V. Burkanov and D. Calkins (coordinators for the collecting trips in Russia) and T. Gelatt and K. Pitcher of the Alaska Department of Fish and Game. All specimens were humanely treated and collected under Marine Mammal Protection Act permit 782-1532-02. We gratefully acknowledge A. Rambaut and A. Drummond for help with BEAST analysis and editing xml input files. Thanks to D. Honeycutt and C. Matson who provided detailed editorial comments and R. Trujillo who provided some of the DNA sequence data.

## References

- Abbot, R.J., Smith, L.C., Milne, R.I., Crawford, R.M.M., Wolff, K. & Balfour, J. 2000. Molecular analysis of plant migration and refugia in the arctic. *Science* **289**: 1343–1346.
- Baker, A.R., Loughlin, T.R., Burkanov, V., Matson, C.W., Trujillo, R.G., Calkins, D.G., Wickliffe, J.K. & Bickham, J.W. 2005. Variation of mitochondrial control region sequences of Steller sea lions, *Eumetopias jubatus*: the three-stock hypothesis. *J. Mammal.* 2006.
- Ball, R.M., Neigel, J.E. & Avise, J.C. 1998. Gene genealogies within the organismal pedigrees of random-mating populations. *Evolution* **44**: 360–370.
- Barnes, I., Mathews, P., Shapiro, B., Jensen, D. & Cooper, A. 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. *Science* **295**: 2267–2270.
- Bickham, J.W., Loughlin, T.R., Calkins, D.G., Wickliffe, J.K. & Patton, J.C. 1998. Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. *J. Mammal.* **79**: 1390–1395.
- Bickham, J.W., Patton, J.C. & Loughlin, T.R. 1996. High variability for control-region sequences in a marine mammal: implications for conservation and biogeography of Steller sea lions (*Eumetopias jubatus*). *J. Mammal.* **77**: 95–108.
- Bohonak, A.J. 2002. *IBD* (isolation by distance): a program for analyses of isolation by distance. *J. Hered.* **93**: 153–154.
- Brunhoff, C., Galbreath, K.E., Fedorov, V.B., Cook, J.A. & Jaarola, M. 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. *Mol. Ecol.* **12**: 957–968.
- Byun, S.A., Koop, B. & Reimchen, T.E. 1997. North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution* **51**: 1647–1653.
- Clague, J.J. 1989. Quaternary geology of the Queen Charlotte Islands. In: *The Outer Shores* (G.G.E. Scudder & N. Gessler, eds), pp. 65–74. University of British Columbia, Vancouver.
- Clarke, T.E., Levin, D.B., Kavanaugh, D.H. & Reimchen, T.E. 2001. Rapid evolution in the *Nebria gregaria* group (Coleoptera: Carabidae) and the paleogeography of the Queen Charlotte Islands. *Evolution* **55**: 1408–1418.
- Clement, M., Posada, D. & Crandall, K.A. 2000. tcs: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657–1660.
- Congdon, B.C., Piatt, J.F., Martin, K. & Friesen, V.L. 2000. Mechanisms of population differentiation in marbled murrelets: historical versus contemporary processes. *Evolution* **54**: 974–986.
- Cook, J.A., Bidlack, A.L., Conroy, C.J., Demboski, J.R., Fleming, M.A., Runck, A.M., Stone, K.D. & MacDonald, S.O. 2001. A phylogeographic perspective on endemism in the Alexander Archipelago of southeast Alaska. *Biol. Conserv.* **97**: 215–227.
- Cowan, I.M. 1989. Birds and mammals of the Queen Charlotte Islands. In: *The Outer Shores*. (G.G.E. Scudder & N. Gessler, eds), pp. 175–186. University of British Columbia, Vancouver.
- Crandall, K.A. & Templeton, A.R. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**: 959–969.
- Cronin, M.A., Bodkin, J.L., Ballachey, B.E., Estes, B. & Patton, J.C. 1996. Mitochondrial-DNA variation among subspecies and populations of sea otters (*Enhydra lutris*). *J. Mammal.* **77**: 546–557.
- Cronin, M.A., Spearman, W.J., Wilmot, R.L., Patton, J.C. & Bickham, J.W. 1993. Mitochondrial DNA variation in chinook and chum salmon detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. *Can. J. Fish. Aquat. Sci.* **50**: 708–715.
- Drummond, A.J., Nicholls, G.K., Rodrigo, A.G. & Solomon, W. 2002. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics* **161**: 1307–1320.
- Drummond, A.J. & Rambaut, A. 2003a. BEAST. VER. 1.0.3. <http://evolve.zoo.ox.ac.uk/>
- Drummond, A.J. & Rambaut, A. 2003b. TRACER. VER. 1.0.1. <http://evolve.zoo.ox.ac.uk/>
- Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
- Fedorov, V.B., Goropashnaya, A.V., Jaarola, M. & Cook, J.A. 2003. Phylogeography of lemmings (*Lemmus*): no evidence for postglacial colonization of Arctic from the Beringian refugium. *Mol. Ecol.* **12**: 725–731.
- Fedorov, V.B. & Stenseth, N.C. 2002. Multiple glacial refugia in the North American Arctic: inference from phylogeography of the collared lemming (*Dicrostonyx groenlandicus*). *Proc. Royal Soc. Lond B* **269**: 2071–2077.
- Flagstad, O. & Roed, K. 2003. Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Mol. Ecol.* **57**: 658–670.

- Fleming, M.A. & Cook, J.A. 2002. Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Mol. Ecol.* **11**: 795–807.
- Gorbics, C.S. & Bodkin, J.L. 2001. Stock structure of sea otters (*Enhydra lutris kenyoni*) in Alaska. *Mar. Mammal Sci.* **17**: 632–647.
- Grosswald, M.G. & Hughes, T.J. 2002. The Russian component of an Arctic ice sheet during the last glacial maximum. *Quaternary Sci. Rev.* **21**: 121–146.
- Hellberg, M.E. 1994. Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution* **48**: 1829–1854.
- Heusser, C.J. 1989. North Pacific coastal refugia—the Queen Charlotte Islands in perspective. In: *The Outer Shores*. (G.G.E. Scudder & N. Gessler, eds), pp. 91–106. University of British Columbia, Vancouver.
- Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.
- Hoelzer, G.A., Wallman, J. & Melnick, D.J. 1998. The effects of social structure, geographical structure, and population size on the evolution of mitochondrial DNA: II. Molecular clocks and lineage sorting period. *J. Mol. Evol.* **47**: 21–31.
- Holder, K., Montgomerie, R. & Friesen, V.L. 1999. A Test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). *Evolution* **53**: 1936–1950.
- Holder, K., Montgomerie, R. & Friesen, V.L. 2000. Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. *Mol. Ecol.* **9**: 1265–1278.
- Hundertmark, K.J., Bowyer, R.T., Shields, G.F. & Schwartz, C.C. 2003. Mitochondrial phylogeography of moose (*Alces alces*) in North America. *J. Mammal.* **84**: 718–728.
- Kavanaugh, D.H. 1989. The ground-beetle (Coleoptera: Carabidae) fauna of the Queen Charlotte Islands. Its composition, affinities and origins. In: *The Outer Shores* (G.G.E. Scudder & N. Gessler, eds), pp. 131–176. University of British Columbia, Vancouver.
- Klicka, J. & Zink, R.M. 1998. Pleistocene effects on North American songbird evolution. *Proc. Royal Soc. Lond B* **266**: 695–700.
- Kumar, S., Tamura, K., Jakobsen, I.B. & Nei, M. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics* **17**: 1244–1245.
- Kumar, S., Tamura, K. & Nei, M. 1993. MEGA: Molecular Evolutionary Genetic Analysis. ver. 1.01.
- Lacourse, T., Mathewes, R.W. & Fedje, D.W. 2003. Paleocology of late-glacial terrestrial deposits with in situ conifers from the submerged continental shelf of western Canada. *Quaternary Res.* **60**: 180–188.
- Li, W.-H. & Graur, D. 1991. *Fundamentals of Molecular Evolution*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Maddison, W.P. & Maddison, D.R. 1992. MACCLADE: Analysis of phylogeny and character evolution. ver. 3.0.
- Maniatis, T., Fritsch, E.F. & Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Mann, D. & Hamilton, T.D. 1995. Late Pleistocene and Holocene paleoenvironments of the north Pacific coast. *Quaternary Sci. Rev.* **14**: 449–471.
- Mann, D.H. & Peteet, D.M. 1994. Extent and timing of the last glacial maximum in southwestern Alaska. *Quaternary Res.* **42**: 136–142.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Pielou, E.C. 1991. *After the Ice Age: the Return of Life to Glaciated North America*. University of Chicago Press, Chicago.
- Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Mol. Ecol.* **14**: 817–818.
- Posada, D., Crandall, K.A. & Templeton, A.R. 2000. GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Mol. Ecol.* **9**: 487–488.
- Rambaut, A. 2003. RHINO. ver. 1.2. <http://evolve.zoo.ox.ac.uk/>
- Raum-Suryan, K.L., Pitcher, K.W., Calkins, D.G., Sease, J.L. & Loughlin, T.R. 2002. Dispersal, rookery fidelity, and metapopulation structure in Steller's sea lions (*Eumatopias jubatus*) in an increasing and decreasing population. *Science* **18**: 764–766.
- Repenning, C.A. 1976. Adaptive evolution of sea lions and walruses. *Syst. Zool.* **25**: 375–390.
- Rohling, E.J., Fenton, M., Jorissen, F.J., Bertrand, P., Ganssens, G. & Caulet, J.P. 1998. Magnitudes of sea-level lowstands of the past 500,000 years. *Nature* **394**: 162–165.
- Rozas, J. & Rozas, R. 1999. DNASP VERSION 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**: 174–175.
- Rutter, N. 1995. Problematic ice sheets. *Quatern. Int.* **28**: 19–37.
- Schneider, S., Kueffer, J.M., Roesslo, D. & Excoffier, L. 1997. ARLEQUIN: An exploratory population genetics software environment. ver. Version 1.0.
- Smith, C.T., Nelson, R.J., Wood, C.C. & Koop, B.F. 2001. Glacial biogeography of North American coho salmon (*Oncorhynchus kisutch*). *Mol. Ecol.* **10**: 2775–2785.
- Stanley, H.F., Casey, S., Carnahan, J.M., Goodman, S., Harwood, J. & Wayne, R.K. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Mol. Biol. Evol.* **13**: 368–382.
- Swofford, D.L. 1999. PAUP\* Phylogenetic Analysis Using Parsimony (\*and other methods). ver. 4.
- Tamura, K. & Nei, T. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**: 512–526.
- Templeton, A.R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* **7**: 381–397.
- Templeton, A.R. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. *Mol. Ecol.* **13**: 789–809.
- Templeton, A.R., Boerwinkle, E. & Sing, C. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics* **117**: 343–351.
- Templeton, A.R., Crandall, K.A. & Sing, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619–633.
- Templeton, A.R. & Sing, C.F. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* **134**: 659–669.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. CLUSTAL W, improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680.

- Tremblay, N.O. & Schoen, D.J. 1999. Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. *Mol. Ecol.* **8**: 1187–1198.
- Trujillo, R.G., Loughlin, T.R., Gemmell, N.J., Patton, J.C. & Bickham, J.W. 2004. Variation in microsatellites and mtDNA across the range of Steller sea lion, *Eumetopias jubatus*. *J. Mammal.* **85**: 338–346.
- Waddell, P.J., Penney, D. & Moore, T. 1996. *Extending Hadamard conjugations to model sequence evolution with variable rates across sites. Information and Mathematical Sciences Report. Series B: 96/11.* Massey University, New Zealand.
- Weir, B.S. & Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
- Westlake, R.L. & O'Corry-Crowe, G.M. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. *J. Mammal.* **83**: 1111–1126.
- Wynen, L.P., Goldsworthy, S.D., Insley, S.J., Adams, M., Bickham, J.W., Francis, J., Gallo, J.P., Hoelzel, A.R., Majluf, P., White, R.W.G. & Slade, R.W. 2001. Phylogenetic relationships within the eared seals (Otariidae: Carnivora): implications for the historical biogeography of the family. *Mol. Phylogenet. Evol.* **21**: 270–284.
- Yang, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. *Trends Ecol. Evol.* **11**: 367–372.
- Zink, R.M., Rohwer, S., Drovetski, S., Blackwell-Rago, R.C. & Farrell, S.L. 2002. Holarctic phylogeography and species limits of three-toed woodpeckers. *Condor* **104**: 167–170.

Received 06 May 2005; revised 29 July 2005; accepted 08 September 2005