Mitochondrial and microsatellite DNA analyses of harbour seal population structure in the northeast Pacific Ocean

Theresa M. Burg, Andrew W. Trites, and Michael J. Smith

Abstract: The genetic diversity and population structure of harbour seals (*Phoca vitulina richardsi*) along the coasts of British Columbia and parts of Alaska were investigated using both mitochondrial DNA (mtDNA) and nuclear DNA. A 475-bp fragment of the mitochondrial control region was amplified and sequenced from 128 animals. Sixty variable sites defined 72 mtDNA haplotypes with pairwise nucleotide differences as high as 5%. Fifty-eight haplotypes were represented by a single individual, and shared haplotypes were generally restricted to a small geographic range. Phylogenetic reconstruction revealed two distinct populations comprising (*i*) southern British Columbia and (*ii*) northern British Columbia – southeast Alaska. Furthermore, the order of the clades suggests that the Pacific Ocean was colonized at least twice, 670 000 and 380 000 years ago. Haplotypes from the first invasion are restricted to a small number of seals around southern Vancouver Island. Analyses of five polymorphic microsatellite loci showed significant differences between the populations of southern British Columbia and northern British Columbia – Alaska. Migration rates for males based on microsatellite data (3–22 seals/generation) were higher than those obtained for females from mtDNA data (0.3 females/generation). Combining all the DNA data collected to date suggests that there are at least three populations of harbour seals in the Pacific composed of seals from (*i*) Japan, Russia, Alaska, and northern British Columbia, (*ii*) southern British Columbia and Puget Sound, Washington, and (*iii*) the outer coasts of Washington, Oregon, and California. The data do not support the existence of two subspecies of harbour seals in the Pacific Ocean.

Résumé : Nous avons étudié la diversité génétique et la structure de la population de Phoques communs (*Phoca vitulina richardsi*) le long des côtes de la Colombie-Britannique et d'une partie de l'Alaska par analyse de l'ADN mitochondrial (ADNmt) et de l'ADN nucléaire. Un fragment de 475 pb de la région de contrôle de l'ADN mitochondrial a été amplifié et soumis au séquençage chez 128 phoques. Soixante sites variables ont défini 72 haplotypes d'ADNmt dont les différences entre les paires de nucléotides pouvaient atteindre 5%. Cinquante-huit haplotypes étaient représentés par un seul individu et les haplotypes communs étaient généralement restreints à une région géographique limitée. La reconstruction phylogénétique reconnaît deux populations distinctes, (*i*) l'une du sud de la Colombie-Britannique, (*ii*) l'autre du nord de la Colombie-britannique et du sud-est de l'Alaska. En outre, l'ordre des clades semble indiquer que l'océan Pacifique a été colonisé au moins deux fois, la première il y a 670 000 ans et la seconde il y a 380 000 ans. Les haplotypes de la première invasion prévalent chez un petit nombre de phoques le long de la côte sud de l'Île de Vancouver. L'analyse de cinq locus microsatellites polymorphes a démontré l'existence de différences significatives entre la population du sud de la Colombie-Britannique et celle du nord de la Colombie-Britannique – Alaska. D'après les données sur les microsatellites, les taux de migration des mâles (3–22 phoques/génération) sont plus élevés que ceux obtenus chez les femelles par les analyses d'ADNmt (0,3 femelle/génération). En combinant toutes les données sur l'ADN à ce jour, il semble qu'il y ait au moins trois populations de Phoques communs dans le Pacifique: (*i*) les phoques du Japon, de la Russie, de l'Alaska et du nord de la Colombie-Britannique, (*ii*) les phoques du sud de la Colombie-Britannique et de Puget Sound, Washington et (*iii*) les phoques qui vivent au large des côtes du Washington, de l'Oregon et de la Californie. Les données ne supportent pas l'existence de deux sous-espèces du Phoque commun dans le Pacifique.

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Introduction

There are five recognized subspecies of harbour seals distributed throughout the world's northern oceans. Two of these subspecies, *Phoca vitulina stejnegeri* and *Phoca vitulina richardsi*, inhabit the western and eastern Pacific Ocean, respectively. The eastern Pacific harbour seal inhabits the coastal waters from the Aleutian Islands south to Baja California (Temte et al. 1991).

More than one distinct population of harbour seals may occur in the eastern Pacific based on differences in pelage colouration and pupping times (Stutz 1967; Kelly 1981; Temte et al. 1991). A latitudinal cline in pupping times exists between Baja California (where pupping starts in March), the outer coast of Washington (May), and northern British Columbia and Alaska (June). However, the seals in Puget Sound and around Vancouver Island give birth much later, in July and August (Temte et al. 1991).

Between 1913 and 1969, an estimated 200 000 – 240 000 harbour seals were killed in British Columbia for pelts and bounties. This may have caused a population bottleneck that reduced genetic diversity. However, since their protection in 1970, harbour seals have been increasing in number at rates as high as 12.5% per year, although rates may have slowed in recent years (Olesiuk et al. 1990). In the last 25 years, the number of harbour seals in British Columbia has increased from 9000 to approximately 135 000 and now accounts for almost 50% of the harbour seals in the eastern Pacific (Olesiuk et al. 1990; Pitcher 1990; Barlow et al. 1995; Small and DeMaster 1995).

Increases in the number of harbour seals in British Columbia could be due to the immigration of seals from Alaska and Washington or to an increase in the reproductive rates of harbour seals in British Columbia. If the increase was due to immigration from surrounding areas, harbour seals in British Columbia should have haplotypes similar to those from populations to the north and south. On the other hand, if migration rates are low and an increase in reproductive rates caused the increase in population size, the British Columbia population should contain haplotypes that are not found in the Alaska and Washington populations.

Recent studies investigating population structure and genetic diversity in natural populations have relied heavily on molecular techniques, such as DNA sequencing, allozymes, and restriction fragment length polymorphisms (RFLPs). While the number of studies employing both nuclear and mitochondrial DNA (mtDNA) markers has increased in recent years (Karl et al. 1992; Degnan 1993; Abernathy 1994; Gottelli et al. 1994; Palumbi and Baker 1994; Pope et al. 1996; Moritz et al. 1997; Berube et al. 1998; Brunner et al. 1998; Simonsen et al. 1998), most studies employ only one method (i.e., either mitochondrial or nuclear markers). Greater information can be obtained by simultaneously employing both techniques.

Mitochondrial DNA is useful for molecular analysis, because it is made up of 37 genes that evolve faster than most nuclear genes (Brown et al. 1982). The more slowly evolving regions tend to be useful for phylogenetic studies, while the more rapidly evolving regions may be used for population studies (Stevens et al. 1989; Baker et al. 1993; Árnason and Gullberg 1996). The lack of intermolecular recombination means that the mitochondrial genome is inherited as a single locus. Since mtDNA is maternally inherited in most animals, discrepancies have been found in some phylogenies when both mtDNA and nuclear DNA have been examined. This bias is especially noticeable in animals that exhibit sex-specific dispersal patterns, such as turtles (Bowen et al. 1992), humpback whales (Palumbi and Baker 1994), and white-eyed birds (Degnan 1993).

Microsatellites are nuclear markers that consist of short tandem repeats, usually 1–5 bp in length, such as $(CA)_n$ or $(ATT)_n$ (Beckmann and Weber 1992). They are found approximately every 10 kb in the eukaryotic genome and are often highly polymorphic (Tautz 1989; Stallings et al. 1991). Polymorphism arises through variation in the number of repeat units present, possibly owing to slipped-strand mispairing (Schlötterer and Tautz 1992). Analysis of nuclear markers such as microsatellites provides both paternal and maternal information. There are several advantages and disadvantages to using microsatellites. Advantages include (*i*) many genetic loci can be analyzed and scored (Hughes and Queller 1993), (*ii*) they provide multiple independent genealogies for population studies, and (*iii*) they undergo high rates of evolution (10^{-4} to 10^{-5}); disadvantages include (*i*) the possibility of null alleles, (*ii*) possible ascertainment bias, and (*iii*) an unclear understanding of mutational process (Callen et al. 1993; Ellegren et al. 1995; Rubinsztein et al. 1995; Crawford et al. 1998).

Molecular studies using mtDNA found that harbour seals from California were different from those in Bristol Bay, Alaska (Stanley et al. 1996), and that seals from Puget Sound, Washington, were distinct from those on the outer coast of the continental United States (LaMont et al. 1996). Both of these studies focused on harbour seal samples from the continental United States (Washington, Oregon, and California) and from one location in northwest Alaska. However, the extent of the population differentiation and the relationship between these different populations and intermediate areas (British Columbia and southern Alaska) is not known.

In this paper, we employed both mtDNA sequence data and microsatellite analyses to examine the phylogeographic structuring of harbour seal populations in British Columbia and parts of Alaska. In addition, we assessed the amount of genetic diversity present in harbour seals in British Columbia with respect to the putative population bottleneck. We also attempted to estimate divergence times between different populations.

Materials and methods

Sample collection and DNA extraction

Whole blood or tissue samples were collected for mtDNA and microsatellite analyses from 128 harbour seals along the coasts of British Columbia and southeast Alaska (Figs. 1 and 2). An additional 94 samples obtained by the Alaska Department of Fish and Game from Icy Bay, Prince William Sound, and Kodiak Island were included in the microsatellite analysis (Fig. 2). Tissue samples were stored in dimethylsulphoxide (DMSO) at room temperature (Amos and Hoelzel 1991), and blood samples were frozen at –80°C. DNA was extracted from whole blood, using the Chelex method (Walsh et al. 1991), or from tissue, using standard protease K/phenol/chloroform extraction (Emmons et al. 1979).

Fig. 1. Sampling sites in the eastern Pacific: Kodiak Island (n = 29), Prince William Sound and Copper River Delta (n = 30), Icy Bay (n = 15), southeast Alaska (n = 30), northern British Columbia (n = 10), Bella Coola (n = 3), and southern British Columbia (South. B.C.) and Vancouver Island (n = 105). The samples from those sites followed by an asterisk were used only for microsatellite analysis with the exception of southeast Alaska, where only 10 of the 30 samples were used for mtDNA analysis. Population divisions based on microsatellite and mtDNA analysis are indicated (Populations 1–3).

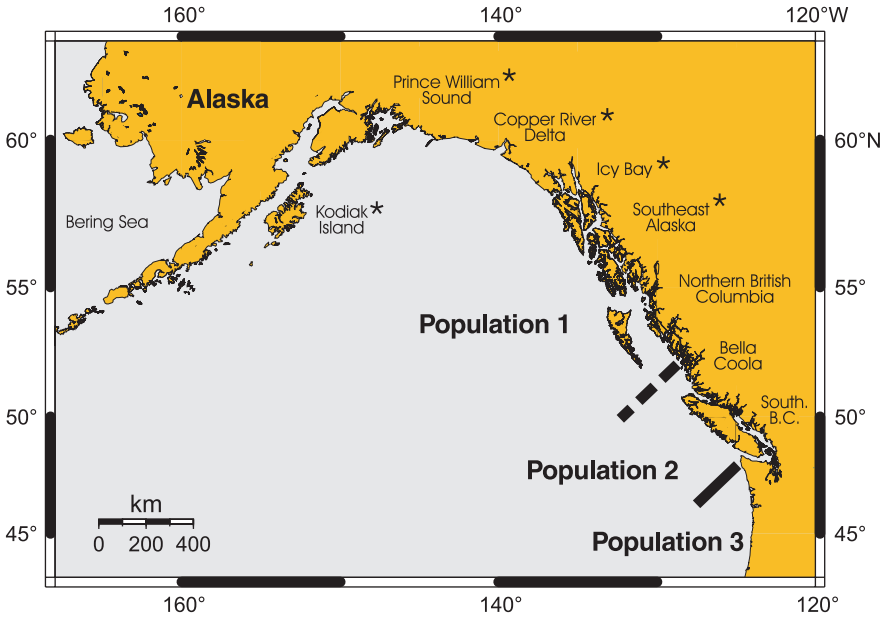
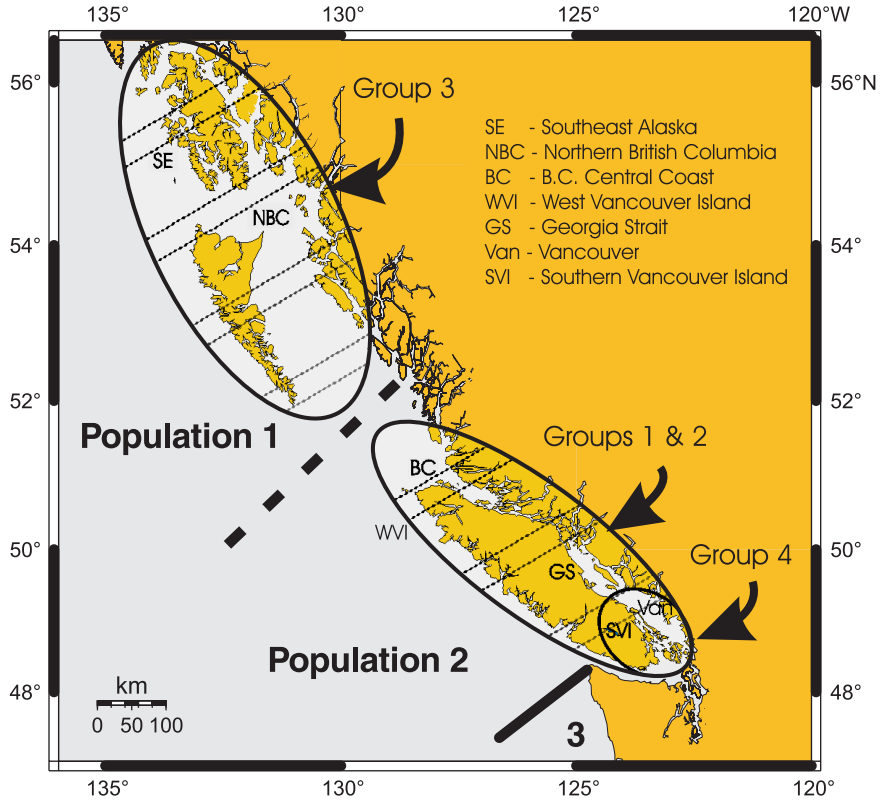


Fig. 2. Distribution of harbour seal groupings in British Columbia based on mtDNA data from individuals collected at the indicated sites. Sample-site codes given in the figure are also used in Figs. 3, 4, 5, and 7.



Mitochondrial DNA amplification and sequencing

Two PCR primers, WKT115 (5′-ATGACCCTGAAGAA(G/A)G-AACCAG-3′) and WKT283 (5′-TACACTGGTCTTGTAAACC-3′), were used to amplify a 520-bp product containing a portion of the tRNA threonine and proline and part of the control region (LaMont et al. 1996). PCR was performed using Ultratherm polymerase (BioCan Scientific) according to manufacturer's instructions. Amplification consisted of one cycle of 95°C for 60 s, 52°C for 60 s,

and 72°C for 90 s; 25 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s; and one final cycle of 94°C for 30 s, 52°C for 30 s, and 72°C for 5 min. The PCR product was sequenced using the direct PCR sequencing kit from USB Amersham.

Mitochondrial DNA sequence analysis

Several different phylogenetic approaches were used to determine the phylogeographic relationship among the 128 harbour seals sampled from British Columbia and southeast Alaska. The sequences were manually aligned using both the ESEE (Cabot and Beckenbach 1989) and SeqApp (Gilbert 1992) programs. Individuals with identical sequences were removed and assigned to a shared haplotype (given a letter designation). Pairwise distances were calculated using Nei's distances, and neighbor-joining trees were constructed using published harbour seal (*Phoca vitulina vitulina*) and grey seal (*Halichoerus grypus*) sequences (Árnason and Johnsson 1992; Árnason et al. 1993) as outgroups. Maximum-likelihood and maximum-parsimony analyses were also conducted, using the programs PHYLIP (Felsenstein 1989) and PAUP (Swofford 1991), respectively. In addition, a minimum-spanning tree (Rohlf 1993) was constructed to resolve the relationship between the different haplotypes. Mitochondrial F_{ST} estimates were calculated using AMOVA (Excoffier et al. 1992).

Microsatellite isolation

A genomic library was constructed using approximately 100 µg of total genomic DNA digested with *Sau*3A. Fragments between 200 and 600 bp were isolated from low melting point agarose gel (Thuring et al. 1975), ligated into the *Bam*HI site of pUC18, and transformed into *Escherichia coli* DH5α.

Positives were isolated using a biotin labelled ($(AC)_{12}$) probe (University Core DNA Services, Calgary, Alta.) according to Glenn (1995). Primers were designed using Oligo 4 (Rychlik 1992), minimizing self-complementarity and primer–dimer formation.

Two microsatellites were successfully isolated through screening of the genomic library, one of which was polymorphic (TBPv2a, 5′-CTCTCCCATCCTCATATTA-3′ and TBPv2b, 5′-GTACTACCCAATATAGAGAC-3′). An additional five microsatellite primers were obtained, four from Goodman (1997) and one from R. Slade (University of Queensland, Australia). PCR conditions for SGPv and BG primers are described in Gemmell et al. (1997).

Microsatellite allele detection

One member of a primer pair was end-labelled using [γ^{32}P]ATP. DNA amplification was performed in a 10-µL cocktail consisting of approximately 100 ng DNA template, 2–4 pmol of each primer, 200 µM dNTP, 0.5 mM $MgCl_2$, 1× PCR buffer, and 0.2 U Ultratherm polymerase (BioCan Scientific) in a GTC genetic thermocycler (GL Applied Research Inc.). Amplification consisted of an initial 2-min denaturation at 94°C, followed by 7 cycles of denaturation at 94°C for 60 s, annealing at 48°C for 60 s, and extension at 72°C for 60 s. An additional 25 cycles consisted of denaturation at 89°C for 40 s, annealing at 48°C for 40 s, and extension at 72°C for 40 s. PCR products were electrophoresed in 6% denaturing TBE (1× TBE buffer : 90 mM Tris-borate, 2 mM EDTA (pH 8.0)) acrylamide gels for 2–4 h, depending on the size of the PCR product (Glenn 1995).

Statistical analysis of microsatellite variation

The alleles were sized and scored using a sequencing reaction as a size ladder. Some samples from key individuals were amplified and the resulting PCR product run on each gel as markers to ensure consistent scoring of the alleles.

The permutation approach was used to detect significant differences in allele frequency distribution (Raymond and Rousset 1995). The fixation index (F_{ST}) and inbreeding coefficient (F_{IS}) were also calculated, according to Hartl and Clark (1980). R_{ST}, a measure of population subdivision for microsatellites equivalent to F_{ST}, was calculated. Heterozygosity and probability of identity were calculated according to Nei and Roychoudhury (1974). Deviations from Hardy–Weinberg equilibrium at each locus in each geographic location were examined using Fisher's exact test (Guo and Thompson 1992). Migration rates were estimated using F_{ST} (Weir and Cockerham 1989), private alleles model (Slatkin 1985; Slatkin and Barton 1989), and R_{ST} (Slatkin 1995).

Results

Mitochondrial DNA analysis

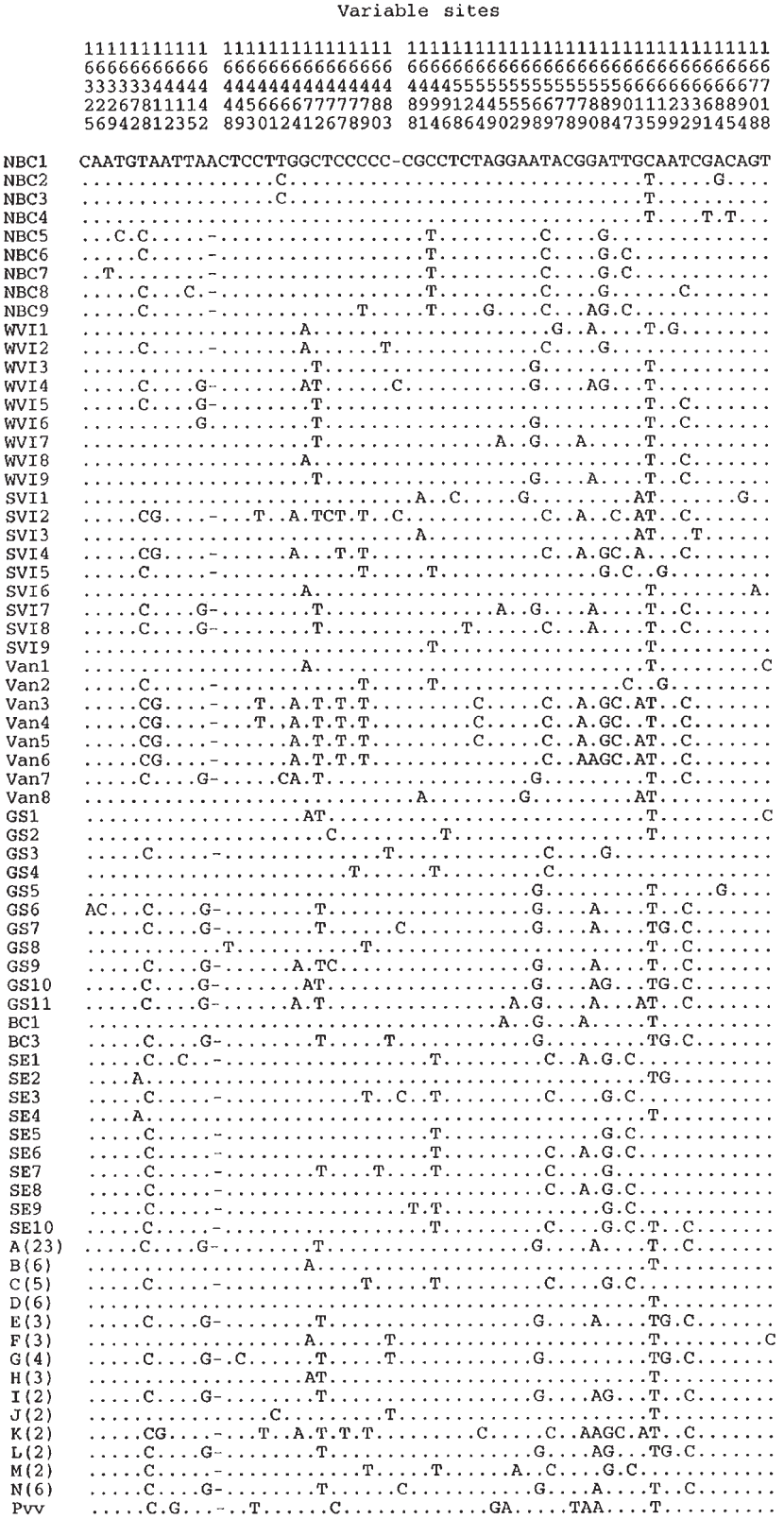
A 475-bp fragment spanning positions 16 254 – 16 774 of the published harbour seal mitochondrial genome (Árnason and Johnsson 1992) was sequenced from 128 harbour seals from British Columbia and southeast Alaska. Within this region, 60 variable sites defined a total of 72 mtDNA haplotypes (Fig. 3). This includes two insertion deletion events, one between positions 16 447 and 16 448 and the other between positions 16 484 and 16 485. Most of the 72 haplotypes were unique to single animals. Only 14 were shared between two or more individuals (Figs. 4 and 5). Twelve of the 14 shared haplotypes were found around Vancouver Island and the adjacent mainland (hereinafter referred to as southern British Columbia), while the other two shared haplotypes, C and D, were found in seals from both southern British Columbia and northern British Columbia – Alaska. The most frequent haplotype, A, represented 18% of the individuals analyzed, while the next most frequent haplotypes, B, D, and N, were found in only 4.7% of the harbour seals sampled (Fig. 3).

Nei's distances between haplotypes ranged from 0.026 to 5%. In general, haplotypes within the same geographic area were more similar to each other (0.008–0.0096%) than to those haplotypes from other geographic areas (0.0186–0.024%), with one notable exception. A group from southern Vancouver Island (Vancouver, Victoria, and Nanaimo; Group 4) containing seven haplotypes formed a distinct clade separated from the other seals in southern British Columbia by a minimum of eight nucleotide substitutions (0.037%) (Fig. 5).

The neighbor-joining tree suggests four primary groupings: one group comprising northern British Columbia and southeast Alaska (Group 3; Fig. 1, Population 1), two sympatric groups in southern British Columbia (Groups 1 and 2; Fig. 1, Population 2), and a group from southern Vancouver Island (Group 4; Fig. 1, Population 2) (Fig. 4). Bootstrap values show that these groupings are strongly supported at the base of each clade, but resolution of terminal branching order is poor. The four major groupings were consistently reproduced using different phylogenetic approaches, including maximum likelihood and maximum parsimony.

The minimum-spanning tree better illustrates the division between these four groups (Fig. 5). Group 1 contains seals mainly from southern British Columbia, but also contains six unique haplotypes and one shared haplotype that are found in seals from northern British Columbia and Alaska (Group 3). Furthermore, the haplotypes in Group 1 form an

Fig. 3. Aligned control region sequences from 128 harbour seals, showing variable sites and insertion–deletion events (–). The 14 most common haplotypes (letters A–N) are also given. Each of the 44 unique haplotypes found in a single harbour seals is identified by a letter code (corresponding to the sampling sites shown in Fig. 2) followed by a number. Variable sites within the 475-bp fragment spanning positions 16 254 – 16 774 of the published mitochondrial genome (Pvv) are numbered according to the published harbour seal sequence (Árnason and Johnsson 1992).



articulating network in which several alternative pathways exist between some animals.

Analysis of the groupings using AMOVA indicates a F_{ST} of 0.662 between the southern British Columbia and northern British Columbia – Alaska groups ($P < 0.001$).

Microsatellite analysis

DNA from the 118 seals sampled in British Columbia and the 104 seals from Alaska was analyzed using seven microsatellite loci. Five of the seven microsatellites used in this study were found to be polymorphic, having 4–12 alleles per locus (Fig. 6) and an average heterozygosity of 47%. Genetic differentiation was tested using GENEPOP (Raymond and Rousset 1995) (Table 1). The only significant population structuring detected using microsatellites was that between southern British Columbia and northern British Columbia – Alaska ($P < 0.001$). Four microsatellite loci, TBPv2, SGPv9, SGPv11, and BG, showed significant differences between the southern British Columbia region and northern British Columbia – Alaska (P values of <0.001, 0.002, 0.02, and 0.03, respectively). Two of the loci, SGPv10 and SGPv11, further subdivided the northern British Columbian – Alaskan group. The SGPv11 locus divides the harbour seal populations into three significantly different populations (Fig. 1): (*i*) western Alaska (sampling sites, Kodiak Island and Prince William Sound), (*ii*) central and southeastern Alaska to northern British Columbia (sampling sites, Copper River Delta, Icy Bay, southeast Alaska, and northern British Columbia), and (*iii*) southern British Columbia (sampling sites, southern British Columbia and Vancouver Island) ($P < 0.001$).

The observed heterozygosity ranged from 44 to 81% (average 66%) for the five polymorphic microsatellite loci, while the expected heterozygosity within each population varied between 51 and 87% (average 72.5%). The southern British Columbia population had a slightly lower overall heterozygosity than the northern British Columbia – Alaska population. The total observed heterozygosity was lower than the overall expected heterozygosity.

The probability of identity (i.e., the probability that two individuals drawn at random from the same population have identical genotypes at all loci) was 1 in 10 000 for seals from southern British Columbia and 1 in 50 000 for seals from northern British Columbia – Alaska at the five polymorphic loci examined.

The proportion of microsatellite genotypes observed in the two areas was compared with Hardy–Weinberg proportions (HWP) using Fisher's exact test (Guo and Thompson 1992). Highly significant departures ($P < 0.001$) from HWP were found in 2 of 10 comparisons (Table 1). Null alleles were detected at high frequencies (12%) for the SGPv11 locus. Overall F_{ST} values were small (F_{ST}, 0.08; R_{ST}, 0.011).

Table 1. Statistical probability of genetic differentiation using GENEPOP and Hardy–Weinberg proportions (HWP) between northern British Columbia – Alaska (nBC/AK) and southern British Columbia (sBC) populations.

		HWP	
Locus	*P*	nBC/AK	sBC
TBPv2	<0.001	0.789	0.437
SGPv9	0.002	0.419	0.722
SGPv10	0.67	0.651	0.895
SGPv11	0.02	0.009	0.031
BG	0.03	0.225	0.297
Overall	<0.001		

Discussion

Morphometric versus genetic population structuring

Historical population segregation based on pelage-pattern differences (Stutz 1967; Kelly 1981) are not supported by genetic data. Pelage differences suggested that the population of the Queen Charlotte Islands was separate from the population at Glacier Bay, while all the loci examined, both mitochondrial and nuclear, indicate that they are one population. However, both molecular data and pelage patterns suggest that the population of Vancouver Island is different from those of Glacier Bay and the Queen Charlotte Islands.

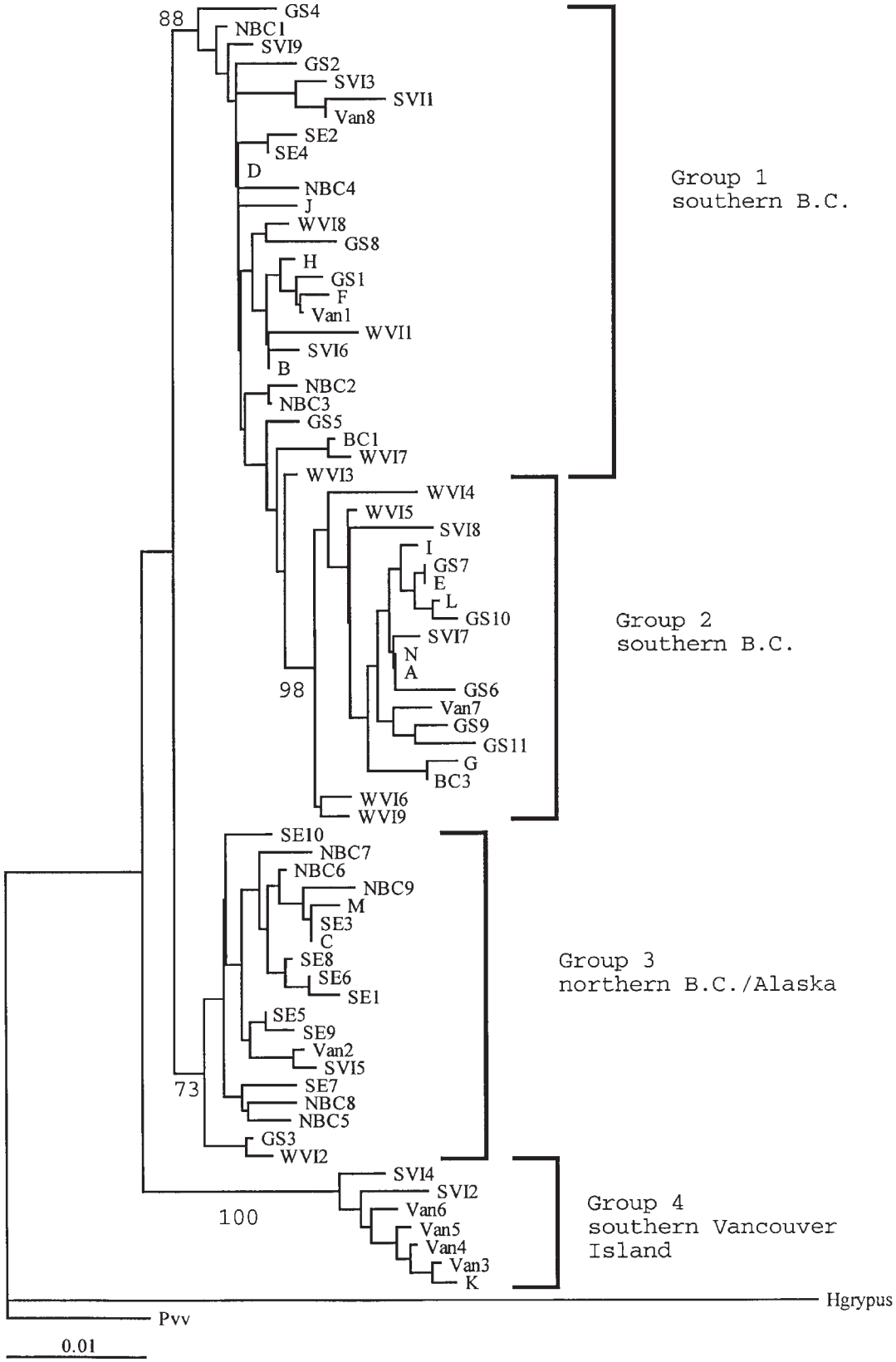
The genetic differentiation of the populations found in British Columbia and Alaska is supported by a difference in pupping times, however. Upon further examination of all of the available harbour seal mtDNA data to date (Bickham and Patton 1994; LaMont et al. 1996; Stanley et al. 1996), there appear to be three main populations in the Pacific (Fig. 1): (*i*) Japan, Russia, Alaska, and northern British Columbia (Group 3, Population 1), (*ii*) southern British Columbia (Groups 1, 2, and 4, Population 2) and Puget Sound (with two sympatric lineages), and (*iii*) the outer coasts of Washington, Oregon, and California (Population 3). A fourth group comprising a small number of seals from Puget Sound and southern Vancouver Island (Group 4, Population 2) is also apparent (Figs. 1 and 7). The differences in pupping times roughly correspond to these three populations in the eastern Pacific, but do not explain why Group 4 contains seals from Puget Sound and southern British Columbia. However, this last group could be explained by multiple colonizations of southern British Columbia and Puget Sound by seals from different maternal lineages.

Comparison of western and eastern Pacific mtDNA haplotypes failed to differentiate between the two subspecies *P. v. stejnegeri* and *P. v. richardsi*. The haplotypes from the western Pacific clustered with those from northern British Columbia and Alaska (Fig. 7). The number of samples from the western Pacific ($n = 38$) (Stanley et al. 1996) is small compared with the number from the eastern Pacific ($n = 252$) (LaMont et al. 1996; Stanley et al. 1996; this study), but there is no evidence to support the proposed subspecies. Temte et al. (1991) noted that the pupping time of *P. v. stejnegeri* is similar to that of *P. v. richardsi* above 50°N (Fig. 1, south of the Bella Coola sampling site) and roughly coincides with the north–south split that was found in both the mtDNA and microsatellite data. A more comprehensive sampling of the western Pacific needs to be undertaken before any changes can be made to the subspecies status of harbour seals in the Pacific.

Geographic population subdivisions

Mitochondrial and nuclear DNA analyses both support the separation of eastern Pacific harbour seals into two populations comprising southern British Columbia and northern

Fig. 4. Neighbor-joining tree, based on Nei's distances, constructed for a 475-bp region of the mitochondrial control region from 128 harbour seals. Bootstrap values for the major groups are indicated at the branch points. Published sequences used for outgroups are from an eastern Atlantic harbour seal (Pvv) (Árnason and Johnsson 1992) and a grey seal (Hgrypus) (Árnason et al. 1995). For sample codes, see Fig. 2.



British Columbia – Alaska (Fig. 2). Microsatellite analysis resulted in slightly different population structuring than mtDNA analysis (Fig. 2). The separate maternal lineages found in southern British Columbia (Groups 1, 2, and 4) comprise one population based on microsatellite data. However, the basic north–south population split that was detected using the mtDNA (Fig. 4) was also evident with the microsatellite DNA (Figs. 1 and 2).

While significant differences exist for both mitochondrial and microsatellite DNA in terms of population structure, F_{ST} statistics vary 10-fold. Estimates for mtDNA are 0.662, while for microsatellites the F_{ST} equivalent is 0.080. If one cautiously uses these to estimate migration rate (Nm), the resulting values, 0.3 and 3 seals/generation, are quite different. This could be interpreted two ways. On the one hand, the difference might be due to differences in mutation rates between microsatellites and mitochondrial DNA (Scribner et al. 1994). On the other hand, male-biased migration can often lead to contrasting results from mtDNA and nuclear DNA. If males are migrating more than females, one should find that the phylogeny based on mtDNA is more structured than that based on nuclear DNA, and migration rates from nuclear genes would be more than twice those estimated from mtDNA. Higher rates of male migration are common in many marine species (e.g., Bowen et al. 1992; Karl et al. 1992; Palumbi and Baker 1994). Telemetry studies suggest that harbour seals are nonmigratory and have small home ranges, although some long-range movements have been observed (Olesiuk et al. 1990). Satellite-telemetry data from a small number of harbour seals has yet to show a significant difference between the movements of males and females. However, it is unlikely that migration rates between southern and northern British Columbia as low as 1 female and 20 males every 30 years (based on Nm from mtDNA and microsatellite data, respectively) could be estimated from telemetry studies, unless a large number of animals were monitored over a long period of time.

Sequence divergence between the different maternal lineages suggests that there were two separate invasions of the Pacific Ocean by harbour seals from the Atlantic Ocean. The first invasion occurred approximately 0.67 million years ago (MYA) and is presently represented by a small number of harbour seals in Japan and southern Vancouver Island (Group 4). The second invasion occurred over 0.38 MYA and resulted in the southward colonization of the Pacific as shown by the relative positioning of the mtDNA groups. The three lineages (Groups 1, 2, and 3) from the last invasion are the most widely distributed in terms of haplotypic diversity and geographic distribution. Japan and Alaska were colonized first by harbour seals entering the Pacific Ocean through the Bering Sea. Maternal lineages from Alaska then moved south to colonize southern British Columbia. The southern British Columbia group, in turn, colonized the coast from Washington to California.

The estimated time of divergence between the northern and southern populations based on microsatellite data is about 9000 years ago (Goodman 1997). This timing roughly corresponds to the end of the last glaciation, which ended approximately 10 000 – 15 000 years ago (Clague 1989).

The difference in divergence times between mitochondrial and nuclear DNA could be due to differences in the modes of inheritance and mutation rates of the two DNA molecules. Microsatellites are biparentally inherited and, as a result, undergo recombination. mtDNA, on the other hand, does not undergo intermolecular recombination, and resulting phylogenies represent colonization events and other separations in populations that occurred in the distant past. Microsatellites often show more recent separations, as past separations or population differences are masked by recombination and back mutation of the microsatellite alleles (DiRienzo et al. 1994). This means mutations in microsatellites are more likely to produce an allele that is already present in the population (and therefore go undetected), unlike mtDNA, in which a mutation often results in a new haplotype being formed.

Effects of glaciation on population structuring

One possible explanation for the reduced size of Group 4 (southern Vancouver Island – Puget Sound) is that glaciation or some other large-scale event severely reduced the first group of invading harbour seals in the North Pacific. Further glaciation 14 000 – 18 000 years ago (Clague 1989) probably resulted in some mixing between the groups along the western coast of North America, which had originated during the first and second invasions, when animals moved to the refugia on the Queen Charlotte Islands (Population 1), in the Brooks Range (western Vancouver Island, Population 2), and south of Washington state (Population 3), which were unglaciated (Fulton 1989). Populations 1 and 2 (northern British Columbia – Alaska and southern British Columbia) appear to have remained separated since the last glaciation, which ended 10 000 –11 000 years ago in the eastern Pacific (Clague 1989), and are now reproductively isolated by temporal variation in pupping times as discussed earlier (Temte et al. 1991).

The dividing line between northern and southern populations in British Columbia occurs somewhere north of Vancouver Island and south of the Queen Charlotte Islands for both mtDNA and microsatellites (Figs. 1 and 2). Additional samples from the central areas of British Columbia's central coast (Fig. 1) are required, to determine more accurately where this division occurs. A similar north–south split in populations is observed in marine invertebrates, fish, and mammals in the eastern Pacific (Okazaki 1984; Wilson et al. 1987; Varnavskaya and Beacham 1992; Taylor et al. 1994; Bickham et al. 1995, 1998a, 1998*b*; Cronin et al. 1996; Taylor et al. 1996; Arndt and Smith 1998; Byun et al. 1998). With the exception of two mammals (Steller sea lions and sea otters), the splits occur south of the Queen Charlotte Islands and north of Vancouver Island, the same area in which the harbour seal populations are split. This can be explained by the presence of glacial refugia in each area.

Conclusions

Using either mtDNA or microsatellites exclusively demonstrates the northern and southern split in the harbour seal populations, but neither alone reveals the multiple colonizations or the effect of the last glaciation on population structure. Furthermore, the difference in migration rates between male and female harbour seals would have gone undetected if only one technique had been used. The lack of strong genetic partitioning may result from the preglacial populations

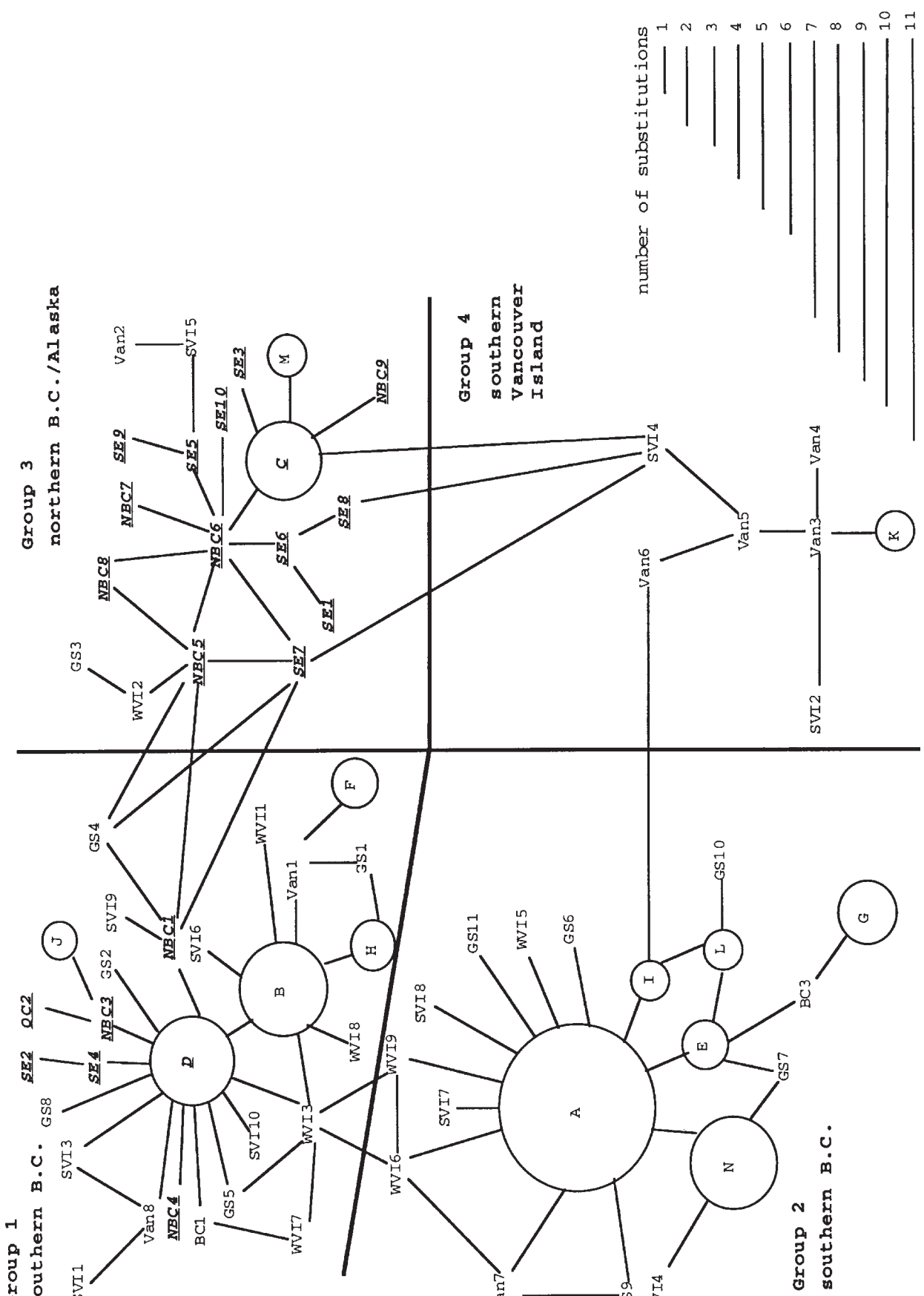
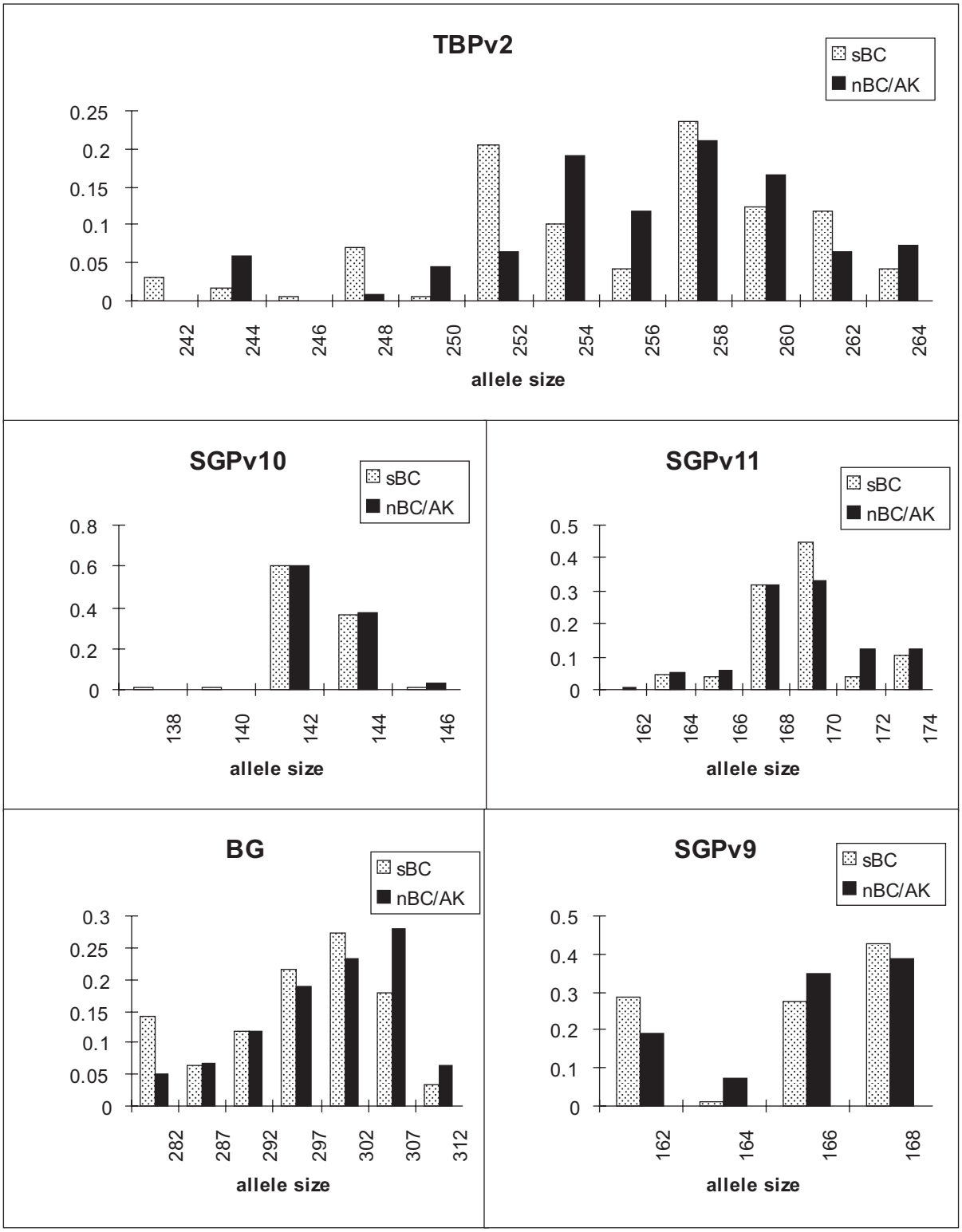


Fig. 5. Minimum-spanning tree based on pairwise nucleotide differences between mitochondrial haplotypes. Samples from northern British Columbia – Alaska are indicated in bold face. The number of nucleotide substitutions is relative to the branch length connecting the haplotypes. Circles indicate a shared haplotype, with the size of the circle indicating the relative numbers of seals that share that haplotype. For sample codes, see Fig. 2.

Fig. 6. Microsatellite allele distributions for five polymorphic loci (TBPv2, SGPv10, SGPv11, BG, and SGPv9) in southern British Columbia (sBC) and northern British Columbia – Alaska (nBC/AK) harbour seal populations.



mixing during the last period of glaciation, or from the large number of unique haplotypes, as has been found in humpback whales (Baker et al. 1993), which may reduce the ability to detect population subdivision. Thus, a combination of glaciation, large population numbers, continuous distribution, and male-biased migration may prevent the finer-scale detection of distinct genetic populations of harbour seals in the eastern Pacific.

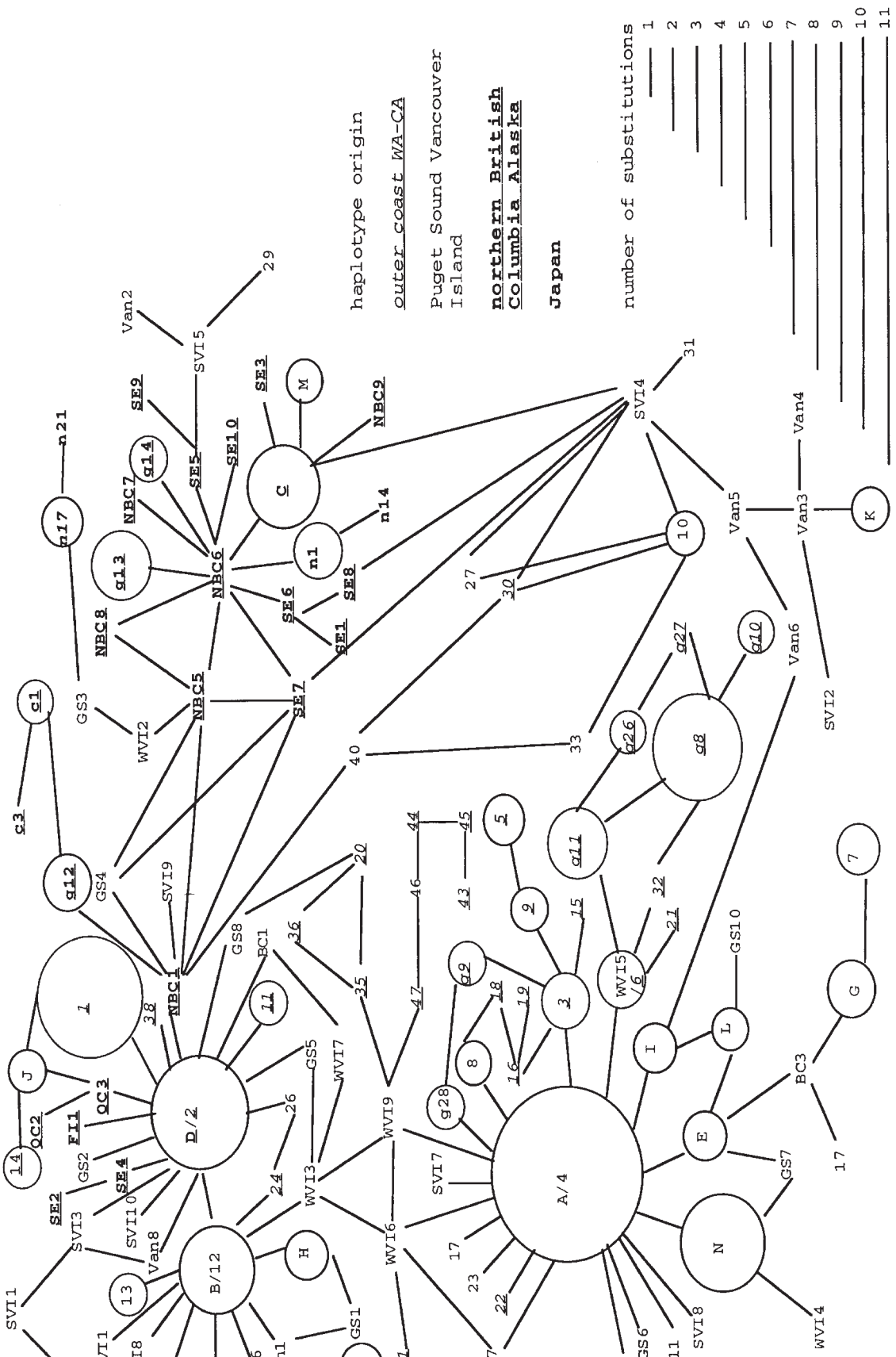


Fig. 7. Minimum-spanning network based on all published mtDNA data to date (LaMont et al. 1996; Stanley et al. 1996, this study). Geographic origin of the haplotypes is indicated in the legend (for sample codes, see Fig. 2). Samples from LaMont et al. (1996) are numbered (e.g., 30) and those from Stanley et al. (1996) are given a lowercase letter followed by a number (e.g., c1).

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