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Population structure and intergeneric hybridization in harbour porpoises *Phocoena phocoena* in British Columbia, Canada

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ABSTRACT: We used variation at microsatellite loci and in mitochondrial DNA (mtDNA) to detect and quantify (1) hybridization between the harbour porpoises *Phocoena phocoena* and the Dall's porpoises *Phoconoides dalli* and (2) the genetic population structure of harbour porpoises in British Columbia (BC), Canada. We assayed variation across 262 individuals (204 putative harbour, 44 putative Dall's, 9 putative hybrids, and 5 unidentified porpoises) obtained primarily from strandings and initially identified morphologically. Our assays identified 198 pure (probability ≥0.95) harbour porpoises, 37 pure Dall's porpoises, and 27 individuals of mixed ancestry, and we found that hybrids between the 2 species occurred over a larger geographic range than previously known. Analysis of 8 microsatellite loci was used to estimate a value of F_{ST} between the Strait of Georgia region and waters outside this area that was low and non-significant (F_{ST} = 0.0025, p > 0.25). Cluster-based Bayesian analysis of population structure in harbour porpoises suggested a single genetic population across our sample area. Within the harbour porpoise, there were 50 mtDNA haplotypes that differed from each other by an average of 0.015 substitutions per nucleotide site. Hierarchical AMOVA indicated that there was no significant divergence between the waters within and the waters outside the Strait of Georgia region (Φ_{ST} = −0.022, p > 0.9). Our findings suggest that harbour porpoises inhabiting coastal waters of southern BC constitute a single genetic population, which should be reflected in management decisions. Our data also suggest that these management efforts should take into account occasional hybridization with sympatric Dall's porpoise.

KEY WORDS: Harbour porpoise · Dall's porpoise · Population structure · Hybridization · Species at risk · Microsatellites · D-loop

INTRODUCTION

The targets of biological conservation actions can range from ecosystems to intraspecific populations (Meffe & Carroll 1997). Because financial and socio-economic constraints often make it difficult to protect species over their entire ranges, conservation managers often focus on conserving distinct populations (referred to as 'Distinct Population Segments' under the US Endangered Species Act, or 'Designatable Units' under the Canadian Species at Risk Act (e.g. Taylor et al. 2011, COSEWIC 2013). Thus, in any given case, it is critical to first identify what constitutes a 'population'. Defining these populations, their boundaries and movements is a major goal in conservation genetics (Waples & Gaggiotti 2006, Schwartz et al. 2007). Despite the growing importance of defining population structure in conservation, our understanding of genetic structure remains fragmentary for many marine species, particularly amongst the

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largest creatures on earth — cetaceans (whales, porpoises, and dolphins) — organisms that play a central role in structuring marine ecosystems (Kenney et al. 1997).

More than 50% of cetacean species are classified as 'Data Deficient' (i.e. insufficient data exist to assign a conservation status) by the International Union for Conservation of Nature (IUCN) and 10% are listed as 'Endangered' or 'Critically Endangered' (IUCN 2012). With such scarcity of knowledge, conservation efforts are based on little information — making the protection of cetacean populations extremely challenging.

Harbour porpoise

The harbour porpoise *Phocoena phocoena* is one of the smallest oceanic cetaceans (Hoelzel 2002) and is distributed throughout temperate coastal waters of the Northern Hemisphere (Gaskin et al. 1974, Baird 2003). Throughout the Northern Hemisphere, 3 subspecies have been proposed based on a lack of recent genetic exchange amongst them: *P. phocoena vomerina* in the eastern Pacific Ocean, *P. p. phocoena* in the Atlantic Ocean, *P. p. relicta* in the Black Sea and an unnamed sub-species in the western Pacific Ocean (Rosel et al. 1995a). Some or all of these subspecies comprise a set of spatially structured populations (e.g. Wang et al. 1996, Wang & Berggren 1997, Rosel et al. 1999, Tolley & Rosel 2006, Fontaine et al. 2007, Alfonsi et al. 2012). To date, the majority of studies have focused on northeastern Atlantic and Baltic Sea population groups. By contrast, few studies have directly looked at population structure in the Pacific Ocean (Rosel et al. 1995a, Chivers et al. 2002, Taguchi et al. 2010).

Based on variation in contaminant loads, Calambokidis & Barlow (1991) predicted that several populations of harbour porpoises were present in the northeastern Pacific Ocean. Indeed, Westgate & Tolley (1999) used variation in contaminant loads to infer population subdivision in northeastern Atlantic populations of harbour porpoise. Furthermore, inferences from preliminary mitochondrial DNA (mtDNA) studies suggested that 4 harbour porpoise populations occupy the northeastern Pacific Ocean: one each along the shorelines of California, Washington, British Columbia, and Alaska. Several haplotypes, however, were shared across regions, suggesting potential gene flow or ancestral polymorphism among areas (Rosel et al. 1995a). More recent evidence suggested that there are 4 populations of harbour porpoise between California and Washington, and perhaps only 1 population in British Columbia (Chivers et al. 2002). Chivers et al. (2002), however, noted the possibility that the population structure in British Columbia might in fact be more complex, in view of mtDNA differentiation in a small set of samples between inland waters (i.e. Strait of Georgia) and outer waters (i.e. Vancouver Island) (see Fig. 1). In general, however, levels of differentiation were marginally significant, and the differences were sometimes hard to reconcile geographically. For example, differences in microsatellite DNA allele frequencies were observed between western Vancouver Island and southeast Alaska, but not between the Strait of Georgia and southeast Alaska, which are arguably more spatially isolated from one another (Chivers et al. 2002). Furthermore, sample sizes for the Strait of Georgia were modest (N = 24) and insufficient to examine structure within this region which is densely populated by harbour porpoises. The incomplete knowledge of population structure was cited as an important limitation to effective management of this species, which is listed as a species of 'Special Concern' under the Canadian Species at Risk Act (Fisheries and Oceans Canada 2009).

Porpoise hybridization

In addition to the harbour porpoise, the Dall's porpoise *Phocoenoides dalli* also occurs in the inshore waters of British Columbia where the geographic ranges of the 2 species completely overlap (Gaskin et al. 1974, Jefferson 1988). The 2 porpoise species differ in size, colouration, and behaviour (Gaskin et al. 1974, Jefferson 1988), and while sometimes considered members of different sub-families Phocoeninae and Phocoenoidinae (Jefferson 1988), more recent evidence suggests they could be sister species (Rosel et al. 1995b). While the divergence time between these 2 sub-families is still not known, it is believed to have occurred over 3 million years ago (McGowen et al. 2009, Slater et al. 2010).

Hybridization between harbour porpoise and Dall's porpoise has been documented in British Columbia (Baird et al. 1998, Willis et al. 2004) using a combination of morphological evidence, mtDNA, and preliminary nuclear DNA data (inter-simple sequence repeats and ZFX/Y loci), and the hybrids may be reproductively viable (Baird et al. 1998). Hybrids between harbour porpoises and Dall's porpoises most closely resemble the former species morphologically, yet they have been reported to behave much more like Dall's porpoises (Willis et al. 2004),

which makes field recognition of hybrids problematic. Nuclear genetic analysis is necessary to both confirm hybridization and to evaluate whether samples collected from apparent harbour porpoises contain evidence of mixed ancestry. Resolving the extent of hybridization is critical for accurately characterizing the population structure and demographic trends for harbour porpoises.

In this study we revisit population structure of harbour porpoises in British Columbia, and extend previous work by increasing sample sizes and quantifying the extent and distribution of hybrid porpoises in the region through the use of both mtDNA sequence data and nuclear genetic microsatellite markers. Based on the presence of suspected fertile hybrid porpoises inferred from morphological analyses (Baird et al. 1998), we expected to detect backcrosses and advanced generation hybrids using genetic methods. The potential for morphologically cryptic interspecific hybrids creates challenges in defining population and species boundaries and also increases the complexity of establishing meaningful conservation guidelines (Allendorf et al. 2001, Mallet 2005). We focused on the population structure of harbour porpoises in the Strait of Georgia because of the species abundance and because of the numerous anthropogenic threats to harbour porpoises in this area. Given the patterns of population subdivision in other areas of the species' range (Fontaine et al. 2007, 2010, Alfonsi et al. 2012), we expected harbour porpoises to exhibit spatially structured populations.

MATERIALS AND METHODS

Samples and DNA extraction

Skin, muscle, or organ tissue samples from 248 porpoises (190 harbour porpoises *Phocoena phocoena*, 44 Dall's porpoises *Phocoenoides dalli*, 4 unidentified porpoises, 10 hybrid porpoises) were donated by a number of organizations (see Table S1 in the Supplement at www.int-res.com/articles/suppl/n026p001_supp.pdf for a list of sample case numbers and organizations that donated samples). Tissue donations were labelled with suspected species type based on morphological assessment by each collector/organization using gross morphological features to provisionally identify each animal (e.g. height of fin, behaviour), methods which all collectors were very experienced in applying in the field. Most of the samples were collected between May 1992 and May 2012 along the coasts of British Columbia and Washington from stranded carcasses, but a small proportion (<8%) were obtained via biopsy darting from a previous study (Willis et al. 2004). Samples were stored at −20°C in 20% DMSO or 95% ethanol, EtOH. Many agencies exchange samples of tissues, and it is possible that some samples from different agencies may represent duplicates of the same individual porpoises. Consequently, we took extra care to verify sample numbers with all providers to insure that there were no duplicate tissue samples in our analyses. We extracted DNA using standard phenol-chloroform methods (Sambrook et al. 1989).

Microsatellite DNA analysis

Nine tetranucleotide loci were amplified using 9 pairs of primers (Np403, NP404, Np407, Np409, Np417, Np426, Np427, Np428, Np430; Table S2 in the Supplement) designed for the finless porpoise *Neophocoena phocoenoides* (Chen & Yang 2008). Samples were prepared for 2 PCR (polymerase chain reactions) using QIAGEN multiplex kits. Allele size was determined by comparison to a 400 base pair (bp) size standard on a CEQ 8000 (Beckman-Coulter). The PCRs were performed under the following conditions: 95°C for 15 min; 25 cycles of 94°C for 30 s, 57.3°C for 90 s, and 72°C for 60; and 60°C for 30 min. One locus (Np403) was excluded from the analyses as it did not amplify under the multiplex conditions.

Identifying hybrids

Data quality was assessed independently for each species by checking for the presence of null alleles, large allele dropout, and deviations from Hardy-Weinberg and linkage equilibrium in MICRO-CHECKER V.2.2.3 (Van Oosterhout et al. 2004) and GENEPOP V.4.1.3 (Raymond & Rousset 1995), respectively. Hybrids were identified using assignment tests in STRUCTURE V.2.3.4 (Pritchard et al. 2000, Falush et al. 2003) and by assessing the posterior probability that each sample would fall into a defined hybrid category using NEWHYBRIDS V.1.1 beta (Anderson & Thompson 2002). Using STRUCTURE, we conducted a test to estimate the admixture/ancestry fraction to each cluster based on all samples of harbour and Dall's porpoises. A total of 204 harbour porpoises, 44 Dall's porpoises, 9 hybrids, and 5 unidentified porpoises were provisionally identified as such using morphology and used in this analysis. The STRUCTURE analysis used 20 independent runs with the fol-

lowing parameters: 100 000 burn-in replicates, 500 000 Markov-chain Monte Carlo replicates and assuming an admixture model with correlated allele frequencies. We tested for the number of putative populations (K) from 1 to 10 using the Evanno et al. (2005) method. The STRUCTURE analysis uses a Bayesian, model-based clustering algorithm to identify how many K exist within a dataset of multilocus allele frequencies such that deviations from Hardy-Weinberg and linkage equilibria are minimized. Individuals within such a dataset are then genetically assigned to 2 or more such genetic populations by calculating the proportion of each individual's genome that is estimated to originate in each population. In STRUCTURE, hybrids were identified using the admixture coefficient, Q, expressed as the proportion of an individual's genome that was identified as that of harbour porpoise (Q_{HP}). Any animal with Q_{HP} of at least 0.95 was identified as a harbour porpoise, while any animal with a $Q_{HP} < 0.05$ was identified as a pure Dall's porpoise and animals with $0.05 < Q_{HP} < 0.95$ were classified as hybrids. We used NEWHYBRIDS to assign a probability that each individual belonged to one of the following multilocus genotypic classes: a pure harbour porpoise (HP × HP), a pure Dall's porpoise (DP × DP), an F1 hybrid (HP × DP), an F2 hybrid (F1 × F1), a backcross with a harbour porpoise (F1 × HP), or a backcross with a Dall's porpoise (F1 × DP). We ran N EWHYBRIDS for 500 000 iterations after 100 000 replicates as a burn-in period. We considered individual porpoises to be hybrids if they had less than a 0.95 probability of being one of the 'pure' parental species, based on output from NEWHYBRIDS.

mtDNA analysis

A ~500 bp region of the mitochondrial D-loop was amplified by PCR for all samples identified using microsatellites as pure harbour porpoise. We used the primers RHD5MF (5′-TAC CCC GGT CTT GTA AAC C-3′) and RHDint (5′-CCT GAA GTA AGA ACC AGA TG-3′) (based on Rosel et al. 1994, Barrett-Lennard 2000). Amplicons were cleaned and purified using a Wizard Prep Kit (Promega) and sent to the Nucleic Acid Protein Service Unit (NAPS Unit, University of British Columbia, Vancouver, BC) to be sequenced using RHD5MF as the sequencing primer. Sequences were visualized and edited in Chromas Lite 2.01 (Technelysium). We inspected all sequences by eye and corrected any ambiguities induced by initial automated base-calling, such as that from the low signal of a few samples. In addition, almost 10% of our samples were sequenced in the reverse direction to verify substitutions, and no inconsistencies were observed between replicate sequences. Mitochondrial DNA sequences were aligned with ClustalW (Thompson et al. 1995). We identified our mtDNA sequences as belonging to either harbour or Dall's porpoise by comparing them to sequences deposited in the GenBank database using BLAST (online searches at http://blast.ncbi.nlm.nih.gov/Blast.cgi?). Analyses of diversity, alternative models of evolution, and phylogeographic tree analyses were conducted using MEGA 5 (Kumar et al. 2008, Tamura et al. 2011). Sequences were deposited in GenBank (for accession numbers see Table S3 in the Supplement).

Population structure analyses

We conducted our analysis of population structure using only those individuals that had a probability ≥0.95 of being a 'pure' harbour porpoise, and again with a 99% threshold value for pure harbour porpoise (N = 183), as identified by either STRUCTURE or NEWHYBRIDS. We did not examine the population structure of Dall's porpoises further, given the very small number of this species that we identified (N = 36) and their scattered distribution in our study area. To identify whether sampling location could be driving population structure in harbour porpoises, we assessed diversity in mtDNA sequences and nuclear microsatellites and analysed them using analyses of molecular variance (AMOVA) in ARLEQUIN V.3.5 (Excoffier et al. 2005), using 2 *a priori* sampling groups: inside waters (Juan de Fuca Strait, Strait of Georgia, and Puget Sound) and outside/northern waters (west of Vancouver Island, Johnstone Strait, and north of Vancouver Island — combined due to low sample sizes) (Fig. 1). The inside/outside waters distinction is a common and important division for studies of population structure in the area (e.g. Beacham et al. 1987, Iwamoto et al. 2004). An AMOVA was used to determine whether population differentiation was greater between or within groups. Because the study area was glaciated until about 10 000 years ago, any population structure that has evolved within the area postglacially was probably driven largely by drift rather than mutation. Consequently, we did not incorporate molecular distances among haplotypes or alleles in the AMOVA analyses. This was supported by permutation analyses conducted in SPAGeDi (Hardy & Vekemans 2002) which showed that variation in allele frequencies via R_{ST} (which invokes the stepwise mutation model) was not significantly different than

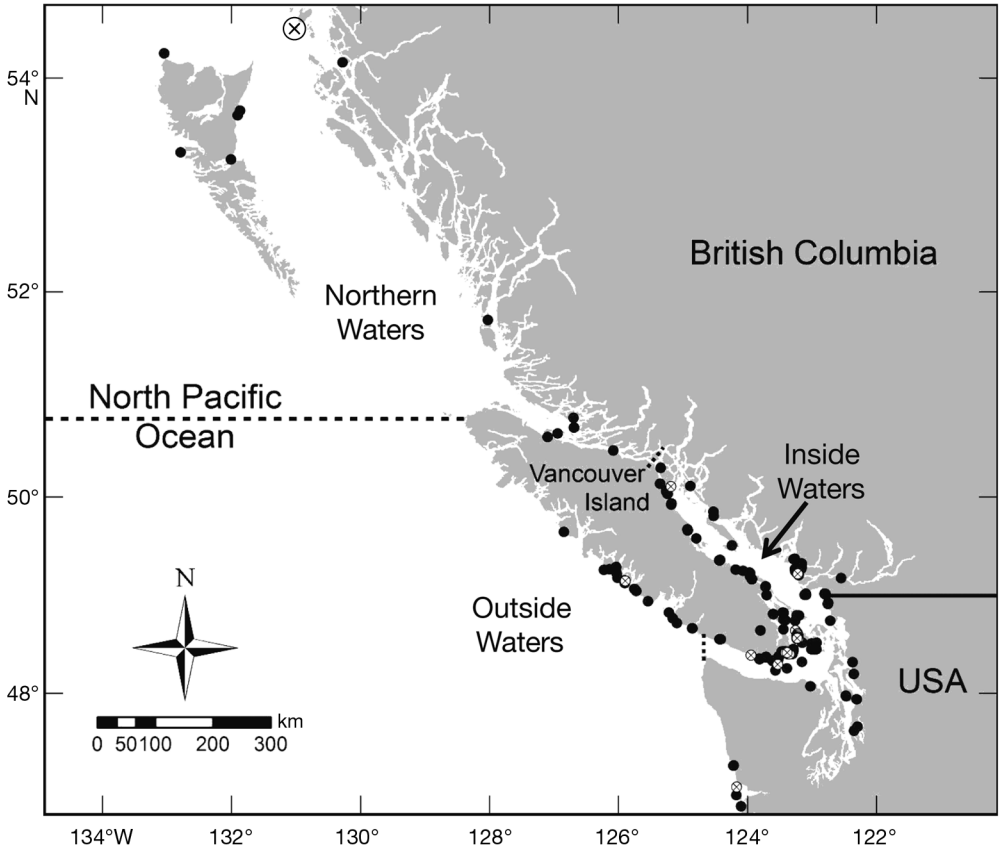

Fig. 1. Sampling locations of the harbour porpoises *Phocoena phocoena* (N = 151, ●) and locations where hybrid porpoises were detected (N = 21, ⊗) along the coast of British Columbia, Canada, and Washington, USA. *A priori* sampling areas (outside, inside, and northern waters) are labelled, and boundaries are indicated by dashed lines. Sample sites shown were included both in mtDNA and/or microsatellite analyses

that using F_{ST} (based on the infinite alleles model). We also used allele frequency contingency tests combined across loci in GENEPOP (Raymond & Rousset 1995) given their relatively high power to test for population differentiation (Ryman et al. 2006).

For all animals classified as harbour porpoise, we used STRUCTURE to estimate the number of putative populations (K) throughout the entire sampling region using the parameters described above, but no prior information on locality.

To augment the analysis of STRUCTURE and to make full use of the final scale geographic coordinate information associated with each sample (cf. Fontaine et al. 2007), we also subjected our data to analysis by Geneland (Guillot et al. 2005). Geneland uses a spatially explicit model to estimate population structure using the Poisson-Voronoi tessellation model to define population boundaries. Coordinates for sampling locations were estimated using the most accurate stranding location information available (Table S4 in the Supplement). We allowed uncertainty in the coordinates based on the longest known daily range movement of harbour porpoises (similar to McAuliffe et al. 2009), as reported via satellite telemetry in Read & Westgate (1997). Geneland was executed using 100 000 iterations, correlated allele frequencies, 10 independent runs and estimating a K from 1 to 10 in R V.2.12.2 (R Development Core Team 2012) using the package Geneland V.4.0.0 with sampling location as Bayesian priors.

Finally, we calculated the power we had to detect genetic structure using PowSim V.4.1 (Ryman & Palm 2006) and employing an estimate of effective population size derived using MLNE V1.0 (Wang 2001).

RESULTS

Identifying hybrids

Across all samples of the harbour porpoise there was no evidence of preferential amplification of

short alleles causing an apparent deficiency of heterozygotes, and all but 1 locus were in Hardy-Weinberg equilibrium. The locus that was not in equilibrium (Np426) showed evidence of 1 or more null alleles. Given that exclusion of this locus did not affect our results in any material way and because it was a useful locus when differentiating the 2 species, we retained all 8 loci in subsequent analyses. There was no evidence of deviations from Hardy-Weinberg equilibrium, nor was there any evidence of large allele drop-out or null alleles in the Dall's porpoise *P. dalli* samples. One monomorphic locus (Np407) was especially helpful in discriminating between harbour and Dall's porpoises, as it was fixed for a single allele in harbour porpoise and several alternative alleles were present in the Dall's porpoise and hybrid individuals.

Analysis by STRUCTURE suggested 2 putative groups (K = 2, Dall's and harbour porpoises; Fig. 2; Table S5 in the Supplement), and identified 198 pure harbour porpoises (≥95% harbour porpoise genome, $Q_{HP} \geq 0.95$), 45 pure Dall's porpoises ($Q_{HP} \leq 0.05$) and 19 individuals with mixed ancestry ($0.95 < Q_{HP} > 0.05$). In the STRUCTURE analysis, several hybrids had Q_{HP} values suggestive of their being backcrosses or post-F1 hybrids (i.e. they had $Q_{HP} > 0.50$, but <0.95; Fig. 2). Individuals were also identified as having a variety of mixed ancestry combinations in NEWHYBRIDS (Fig. 2).

The NEWHYBRIDS analysis identified 208 pure (probability ≥ 0.95) harbour porpoises, 37 pure Dall's porpoises, and 17 individuals of apparent mixed ancestry (i.e. probability of being one or other species was <0.95). Of the putative hybrids, NEWHYBRIDS identified many of these individuals as F1 hybrids, whereas others were more likely F2 hybrids, or backcrosses between F1 hybrids and Dall's porpoises (Fig. 2).

Ten samples assigned as 'pure' harbour or Dall's porpoises in the STRUCTURE analysis were not identified as such by NEWHYBRIDS. We categorized all of these individuals as having potential mixed ancestry because (1) we cannot rule out the possibility of many generations of backcrossing, which are more accounted for in NEWHYBRIDS and (2) we wanted to ensure only 'pure' harbour porpoises would be used in the analyses of population structure. Genetically identified hybrid porpoises were distributed throughout the range of the study, but most were identified in the inside waters (Fig. 1). Eighteen of the 27 genetically identified hybrids were mistaken as one or the other parental species based on morphological assessment by experienced observers and a veterinary pathologist. Morphologically identified hybrids all possessed Dall's porpoise mtDNA. Only 30% of genetically identified porpoises had harbour porpoise mtDNA haplotypes, with the remainder having Dall's porpoise mtDNA.

Microsatellite analysis of harbour porpoises

Neither F_{ST} and R_{ST} values, which were highly correlated with one another (r = 0.98), nor allele size made a significant contribution to population differentiation according to the permutation test in SPAGeDi. Therefore, an infinite allele model (and F_{ST}) was used throughout the analyses.

We analysed the microsatellite data for samples in which more than half of the loci amplified (N = 194,

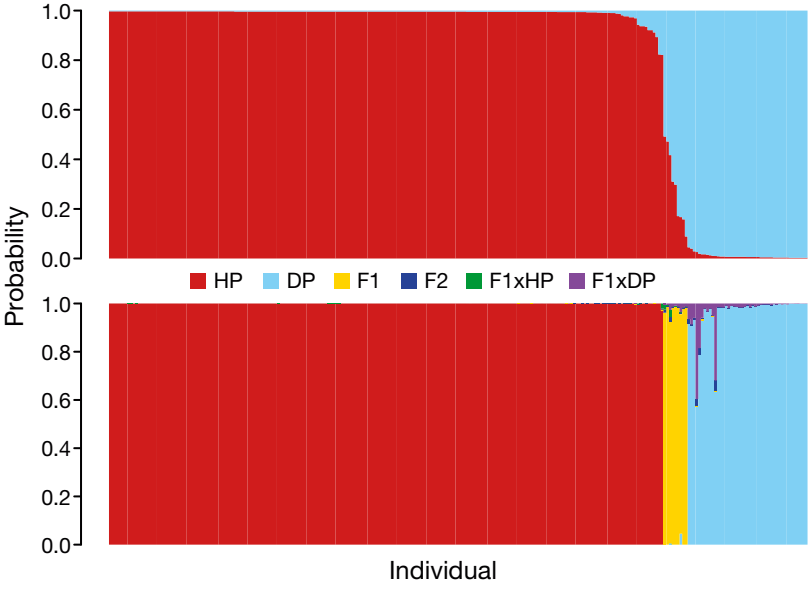

Fig. 2. Graphic output of genetic assignments of individuals according to the programs STRUCTURE (upper panel) and NEWHYBRIDS (lower panel) for 262 harbour, Dall's, and hybrid porpoises. Each individual is represented by a thin vertical bar, with colours in the upper panel depicting probability (p) of ancestry in either harbour porpoise (*Phocoena phocoena*; red) or Dall's porpoise (*Phocoenoides dalli*; light blue) in the STRUCTURE analysis. In the NEWHYBRIDS analyses (lower panel), the colours represent the probability of each individual being 1 of 6 genotypes: pure harbour porpoise (HP), pure Dall's porpoise (DP), an F1 hybrid (F1), an F2 hybrid (F2), a backcross to a harbour porpoise (F1 × HP), or a backcross to a Dall's porpoise (F1 × DP)

'pure' harbour porpoises as identified in both STRUCTURE and NEWHYBRIDS). Microsatellite diversity varied considerably amongst loci, with observed heterozygosity (H_o) ranging from 0.36 to 1.0 and the number of alleles at each locus ranging from 1 to 12 (Table 1). Seven of 28 pairwise tests between loci showed signs of linkage disequilibrium; however, only 1 comparison remained significant after Bonferonni correction (Np404/Np426).

The analysis by STRUCTURE suggested that variation across samples was best represented by a single population of harbour porpoises as the highest log-likelihood was consistently associated with K = 1 (Fig. S1). Geneland also suggested a single population (N = 167, K = 1) (Fig. S2, both figures in the Supplement at www.int-res.com/articles/suppl/n026p001_supp.pdf), and there was no support for population differentiation from the analysis in GENEPOP (χ^2 = 15.122, df = 14, p = 0.37). Almost all of the variation in allele frequencies was found within rather than among any regional groupings (inside [N = 134] vs. outside waters [N = 25]) when examined with an AMOVA (F_{ST} = 0.0025, p = 0.25 ± 0.02). When all analyses were repeated using a 99% threshold for 'pure' species, the same results were obtained as the original analyses using the 95% threshold (Tables S6–S11, Fig. S3, all in the Supplement).

Analysis using MLNE estimated an effective population size of 10 000 individuals; and using this we determined that our allele frequency data had adequate power (0.85) to detect genetic structure with F_{ST} values of >0.01, but only an estimated power of 0.13–0.26 to detect genetic structure for F_{ST} values closer to those seen in our survey (F_{ST} = 0.0025).

Table 1. Variation at 8 microsatellite loci assayed in the harbour porpoise *Phocoena phocoena*. H_e: expected heterozygosity; H_o: observed heterozygosity

Loci	No. of alleles	H_e	H_o	Allele size (range, bp)
Np404	6	0.6105	0.5926	134–150
Np407	1	0.0000	0.0000	186
Np409	3	0.4895	0.4844	221–229
Np417	12	0.7762	0.8000	128–176
Np426	6	0.4018	0.3579	103–116
Np427	7	0.6656	0.6667	178–194
Np428	8	0.7476	0.7713	110–134
Np430	3	0.0941	0.0855	144–168

Table 2. Nucleotide and haplotype diversity of 500 base pairs of mtDNA D-loop sequences from 151 harbour porpoises *Phocoena phocoena*, 125 of known location. Data are means ± SD

	Nucleotide diversity	Haplotypic diversity	Sample size (N)
Three sampling locations			
Northern waters	0.0112 ± 0.0076	1.0000 ± 0.1265	5
Outer waters	0.0107 ± 0.0062	0.8242 ± 0.0977	14
Inside waters	0.0109 ± 0.0059	0.8954 ± 0.0232	106
Two sampling locations			
Outside/North	0.0107 ± 0.0060	0.8713 ± 0.0725	19
Inside waters	0.0109 ± 0.0059	0.8954 ± 0.0232	106
All samples			
Single population	0.0105 ± 0.0056	0.8896 ± 0.0217	151

mtDNA variation in harbour porpoises

Forty-four unique mtDNA control region haplotypes were defined by 42 variable sites, identified from 125 'pure' harbour porpoise samples from known geographic locations. There were 53 different haplotypes identified as harbour porpoise mtDNA, including individuals from both known and unknown locations. Only 6 haplotypes were found in samples from >1 sampling region. The 6 harbour porpoise haplotypes were found in both 'inside' and 'outside' regions (Table S3). Because there were very few shared haplotypes, haplotype diversity exceeded 0.8 in each defined subpopulation, but nucleotide diversity was much lower at 0.01 (Table 2). The best evolutionary model under the corrected Akaike's information criterion (AICc) (i.e. having the lowest AICc score) was a Tamura-Nei (TN) model including a gamma distribution and invariant sites (TN93 + G + I). The average genetic distance amongst harbour porpoise haplotypes was 0.015 (SE = 0.003), and that between harbour and Dall's porpoise was 0.204 (SD = 0.026). The resulting tree using the TN genetic distance and both a finless porpoise (GenBank Accession Number HQ108437.1) and a Dall's porpoise (GenBank JX475429, this study) as outgroups showed little to no resolution of major groupings (e.g. divergent clades with >50% bootstrap support, tree not shown), so we did not consider phylogeographic-level structure further. The results of the AMOVA suggested that no differentiation existed between outside/northern waters (N = 19) and inside waters (N = 106, Φ_{ST} = –0.022, p = 0.97 ± 0.01).

DISCUSSION

Hybridization between harbour and Dall's porpoises

Our results substantiate previous evidence of harbour × Dall's porpoise hybridization, and support the hypothesis that reproductively viable hybrids are capable of backcrossing with either parental species, with a higher tendency of backcrossing with Dall's porpoise (Baird et al. 1998, Willis et al. 2004). We have identified hybrids from a much larger geographic range than was covered by previous specimens and sightings. Further, our data suggest that hybridization is most common in the inside waters (Fig. 1), but further research is required to determine if this reflects a genuine pattern or a bias in the sampling efforts between inside and outside waters. For instance, if there is a fitness disadvantage in hybrid porpoises (Burke & Arnold 2001), strandings may be biased towards hybrids, and, thus, sampling of such strandings may represent a positive bias when estimating the frequency of hybrids. Such a bias could, however, only be identified by sampling a large number of live animals. By using opportunistic stranded carcasses, our study was limited to samples that were found reported and collected. Much of the coastline of British Columbia is remote and uninhabited, especially for the populations from the outside waters; therefore, our samples were not evenly distributed across the sampling area, and outside waters were significantly underrepresented.

Although cetacean hybridization is not uncommon (Sylvestre & Tasaka 1985), there may be inherent behaviours of either parental species that facilitate hybridization. For instance, the aggregating behaviour of harbour porpoises could provide an explanation for the occurrence of hybrids. While coercive mating is known to occur in some cetaceans (Scott et al. 2005), there is no direct evidence of it occurring in porpoises. If harbour porpoises do practice coercive mating, female Dall's porpoise near harbour porpoise aggregations could easily be engaged by male harbour porpoises. Such coercive mating might provide one way whereby female mate preference might be suppressed, leading to hybridization. This could also explain why first generation hybrids more frequently result from a male harbour porpoise mating with a female Dall's porpoise. Because these hybrid calves are then raised by a Dall's mother, they will most likely associate with Dall's porpoises and thus should be more likely to mate with another Dall's porpoise.

With increasing access to and cost effectiveness of genetic analyses, it is now feasible to set up a genetic monitoring program for cetaceans. Such programs are important to help understand the occurrence and long-term trends in hybridization, as well as the trends in genetic diversity (e.g. Schwartz et al. 2007). Monitoring changes in genetic diversity can help identify changes in population size or potential threats to populations. This can be of great aid to conservation management decisions as it may highlight changes or threats to populations that are not always evident from field surveys. Conservation efforts can be greatly aided by information from by-catch studies highlighting how parental species may be affected by increased rates of unidirectional hybridization and contribute to multispecies genetic monitoring programs (Hewitt 1988, Allendorf et al. 2001).

Population structure of harbour porpoises

Analysis of both nuclear microsatellites and mitochondrial DNA sequence variation failed to detect significant population subdivision within harbour porpoise, suggesting that along the coastline of southern British Columbia the species comprises a single, panmictic population. These results contrast with those from other areas within the global range of the harbour porpoise (e.g. Wang et al. 1996, Wang & Berggren 1997, Rosel et al. 1999, Chivers et al. 2002, Tolley & Rosel 2006, Fontaine et al. 2007, 2010, Wiemann et al. 2010, Alfonsi et al. 2012, De Luna et al. 2012). In particular, Chivers et al. (2002) suggested more subdivision of harbour porpoises within the same region that we studied. Chivers et al. (2002), however, did not account for the possibility of hybrids, which could have inflated differentiation. Additionally, some areas studied by Chivers et al. (2002) differed in mtDNA, but not microsatellites, and genetic differentiation was patchy and was not always associated, with increasing spatial isolation between the areas sampled. Further, the majority of differences observed by Chivers et al. (2002) were between broader geographic scale regions that were defined *a priori* based on management boundaries set by areas of low porpoise density. In contrast, our analysis, at least within the Strait of Georgia, employed much higher sample sizes, yet STRUCTURE analyses indicated a lack of subdivision within this area. Larger sample sizes of porpoises from outside waters may reveal evidence of differentiation from porpoises from inside waters. In addition, it has been suggested that stranding records may underestimate

the levels of population structure as all populations might not be represented equally (Bilgmann et al. 2011). Similar caveats as we have applied to the estimation of hybridization from stranding samples, therefore, also apply to our estimates of population subdivision in harbour porpoise.

High levels of structure in other areas such as the eastern North Atlantic and adjacent areas likely stem from the fact that such studies spanned a much larger geographic range (up to 10 000 km of marine waters), included multiple populations in highly distinct environments, and focused on what are thought to be 2 subspecies (e.g. Rosel et al. 1995a, Fontaine et al. 2007). In fact, even across large areas of the eastern Atlantic and Black Sea, at most 2 to 3 genetic populations were identified and F_{ST} values across much of the North Atlantic area were very low (<0.001; Fontaine et al. 2007). By contrast and despite the complex geography of the southern British Columbia coastline, the relatively small area that we surveyed and the fact that this area was glaciated until as recently as about 8000 to 10000 years ago are key factors that have constrained the evolution of detectable population subdivision in harbour porpoises.

The combined set of samples in our study show levels of genetic diversity comparable to those of single populations from other studies (i.e. Rosel et al. 1999, Chivers et al. 2002, Wiemann et al. 2010, De Luna et al. 2012). Some of these previous studies used the same mtDNA region that we employed (D-loop), and most had a slightly higher number of microsatellite alleles. The tetranucleotide microsatellite loci assayed in our study had slightly fewer alleles than were present in other studies using dinucleotide loci isolated from other cetacean species (Valsecchi & Amos 1996). Therefore, the choice of mtDNA region and microsatellite markers in our study should have a power for detecting subdivision similar to that in the other studies of harbour porpoise. Nonetheless, while F_{ST} values calculated with more samples from our area (particularly in outside and northern waters) or more loci might prove statistically significant, they likely would not have approached the relatively high levels (~0.05) reported by De Luna et al. (2012) for the eastern Atlantic.

Population structure of marine mammals in British Columbia is not well understood for most species. For species in which population genetic studies have been undertaken, the patterns of population structure have a variety of driving forces. The killer whales *Orcinus orca* in British Columbia belong to at least 4 separate populations that are maintained by culturally transmitted social behaviour, foraging behaviour, and food preferences, while occupying overlapping geographic regions (Hoelzel et al. 1998, Barrett-Lennard 2000, Baird 2001). By contrast, harbour seal *Phoca vitulina* population structure appears to reflect colonization from different refugia following the last glaciation (Burg et al. 1999).

While fine-scale differences in population structure may be influenced by many local factors, population structure at a broad scale across the North Pacific Ocean exhibits many similarities in different species. For instance, there is evidence for 2 genetically distinct Steller sea lion *Eumetopias jubatus* populations: one in the Aleutian Islands stretching over to Japan and a second from southeastern Alaska down to northern California (Hoffman et al. 2006). Dall's porpoises are characterized by 3 populations in the western, central, and northeastern Pacific (Escorza-Treviño & Dizon 2000). At a broad scale, many species exhibit similar patterns of population structure, but these population borders are influenced by trophic level, social and mating patterns, and prey resource availability, seasonality, and distribution, and are rarely identical. Consequently, extending the boundaries of our study region or including more samples from northern British Columbia may reveal more than one genetic population of harbour porpoises.

The processes that seem to have resulted in a single genetic population of harbour porpoises in southern British Columbia are unknown. Individual range size of harbour porpoises is still uncertain; however, limited satellite tagging data have recorded maximum daily movements of 60 to 100 km (Read & Westgate 1997, Sveegaard et al. 2011). Harbour porpoises are relatively solitary animals, and it is possible that they travel long distances along the coastline in British Columbia and do not mate in consistent locations. Alternatively, some aspect(s) of harbour porpoise biology and behaviour may actually favour panmixia. Throughout their range, harbour porpoises are typically found in very small groups of 1 to 3 individuals. In British Columbia, however, large aggregations have occasionally been reported with some group sizes exceeding 200 individuals. More than 60 of these aggregations of >50 animals have been reported to the BC Cetacean Sightings Network over the past 10 yr, occurring all along the coast throughout the year, but peaking in frequency around the mating season of May through September (Hall 2011, C. Birdsall, BC Cetacean Sightings Network, pers. comm. 2012). The causes of this social behaviour are unknown, but, as in many other cetacean aggregations, it could be associated with peaks in prey availability or could also serve to increase mating oppor-

tunities (Calambokidis et al. 2002, Canning et al. 2008, Hall 2011). Harbour porpoise densities are relatively low in British Columbia, possibly due to high rates of predation by Bigg's (transient) killer whales (Ford et al. 1998), which may cause them to roam widely in search of mates and result in higher levels of gene flow across the region. Satellite tagging of multiple individuals in these groups during the mating season may provide insight into dispersal patterns and help explain the apparent lack of genetic structure in southern British Columbia.

CONCLUSIONS

Our study provides independent, genetic confirmation of hybridization between harbour and Dall's porpoises, and thus stresses the importance of screening for potential hybrids when studying harbour porpoises anywhere within their range overlap with Dall's porpoises. Our results illustrate the need for increased understanding of hybridization and introgression between cetaceans, especially in terms of research on what drives hybridization and the relative fitness of porpoise hybrids. Our study also suggests that a single population of harbour porpoises inhabits the waters off British Columbia and northern Washington, a larger geographic area than in many other parts of the species' range. These results fill an information gap identified by Fisheries and Oceans Canada, support the current management plan of a single population, and provide baseline data that will enable future detection of long-term changes to genetic diversity within the population (Schwartz et al. 2007).

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