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Population Structure and Genetic Variability in Northeastern Pacific Killer Whales: Towards an Assessment of Population Viability

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Abstract

Long term studies of killer whales (*Orcinus orca*) in the coastal waters of British Columbia have identified two sympatric non-associating populations: fish-eating *residents* and mammal-eating *transients*. A third group, the *offshores*, frequents the outer continental shelf. The resident population contains two regional subpopulations in British Columbia and is currently listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). In Alaska one additional putative subpopulation of residents and two of transients have been reported. This complex of populations and subpopulations persisting in the absence of obvious dispersal barriers presents a problem to conservation managers who must decide whether subpopulations should be assessed separately or in combination. Clearly, the decisions should rest on an understanding of the discreteness of the subpopulations. Here, we report a molecular study designed to contribute to such an understanding. This study a) characterized each known subpopulation of killer whales genetically, b) compared genetic variability between the subpopulations and c) analysed mating patterns within the resident subpopulations to determine inbreeding levels.

Lightweight pneumatic darts were used to take biopsy samples from 269 individually-identified killer whales off British Columbia and Alaska. Nuclear DNA from the samples was typed at 11 polymorphic microsatellite loci, and the entire mitochondrial D-loop was sequenced. The results were used to construct population phylogenies, assess genetic diversity, calculate fixation indices (*F*-statistics), and conduct paternity analyses. The following findings were key: 1) resident and transient killer whales are reproductively isolated, 2) the division of each into three regional subpopulations is supported genetically, 3) offshores are genetically differentiated from all known resident and transient subpopulations, 4) residents have lower levels of genetic variation than transients, 5) the observation from field studies that residents remain in their natal groups for life is typical of the recent history of the population, 6) despite their lack of permanent dispersal, residents mate outside their natal groups.

One transient subpopulation (the critically endangered *AT1* population of the northern Gulf of Alaska) appears to be genetically isolated from all other subpopulations. Permanent dispersal between the remaining two transient subpopulations is very rare or non-existent, but gene flow mediated by occasional intermatings could not be ruled out. In the resident population, occasional intermatings may occur between the *northern resident* subpopulation (which inhabits central and northern British Columbian waters) and the *Alaska resident* subpopulation (found off the panhandle region of southern Alaska and the Gulf of Alaska coast). Our findings are consistent with the complete genetic isolation of the *southern resident* subpopulation of southern British Columbia and northern Washington. The southern resident subpopulation is of conservation concern because of its small size (less than 85 individuals), a recent decline, and high contaminant loads.

Paternity analysis showed that resident killer whales have strong (presumably behavioural) inbreeding avoidance mechanisms. In all but one instance, pod members

were excluded as possible fathers of calves in the same pod. In the northern resident community, the majority of matings were between individuals from pods belonging to different “acoustic clans”. No paternity matches were made between southern residents and members of the other two resident subpopulations, however, there were several possible matches between the latter two populations.

We recommend that three resident subpopulations, three transient subpopulations, and the offshore population should be recognized as separate stocks or management units for conservation purposes in British Columbia and Alaska.

Résumé

Les études menées à long terme sur l'orque (*Orcinus orca*) dans les eaux côtières de la Colombie-Britannique ont permis d'identifier deux populations sympatriques et distinctes : des *résidents* piscivores et des *migrateurs* dont le régime alimentaire est composé d'autres mammifères. Un troisième groupe, les *hauturiers*, fréquente la marge externe de la plate-forme continentale. La population résidente de la C.B. englobe deux sous-populations de la C.B., considérées comme menacées à l'heure actuelle par le Comité sur le statut des espèces menacées de disparition au Canada (COSEPAC). Une autre sous-population de résidents, dont le statut n'est pas officiel, et deux sous-populations de migrateurs ont aussi été signalées en Alaska. Ce complexe de populations et de sous-populations qui perdure malgré l'absence d'obstacles évidents à la dispersion pose un problème aux responsables de la conservation, qui doivent décider si les sous-populations doivent être évaluées ensemble ou séparément. Il est évident que les décisions devraient reposer sur une connaissance du caractère distinctif des sous-populations. Le présent rapport fait état d'une analyse moléculaire conçue en vue d'acquérir cette connaissance. Elle a permis a) d'établir les caractéristiques génétiques de chaque sous-population d'orque, b) de comparer la variabilité génétique entre les sous-populations et c) d'analyser les patrons d'accouplement au sein des sous-populations résidentes en vue d'établir les niveaux de consanguité.

Des échantillons de tissus ont été prélevés à l'aide de fléchettes pneumatiques légères sur 269 orques au large de la Colombie-Britannique et en Alaska. L'empreinte génétique de l'ADN nucléaire extrait des échantillons a été établie à 11 locus de microsatellites polymorphes, ainsi que la séquence de toute la boucle D mitochondriale. Les résultats ont servi à établir la phylogenèse des populations, à évaluer la diversité génétique, à calculer des indices de localisation (statistiques sur F) et à effectuer des analyses de paternité. Les résultats suivants sont à noter : 1) les résidents et les migrateurs ne s'accouplent pas entre eux, 2) la division des orques en trois sous-populations régionales est justifiée au plan génétique, 3) les hauturiers sont génétiquement différents de toutes les sous-populations connues de résidents et de migrateurs, 4) la variation génétique est moins marquée chez les résidents par rapport aux migrateurs, 5) l'observation sur le terrain que les résidents n'abandonnent jamais leur groupe d'origine est typique de l'histoire récente de la population, et 6) même s'ils n'abandonnent par leur groupe d'origine en permanence, les résidents s'accouplent avec des individus d'autres groupes.

Une sous-population migratrice (soit la population *AT1* du nord du golfe d'Alaska, qui est en grave danger de disparition) semble être génétiquement isolée de toutes les autres sous-populations. Les échanges permanents entre les deux autres sous-populations migratrices sont très rares ou inexistants, mais on ne peut exclure la possibilité d'un flux génétique entre elles par le biais d'accouplements occasionnels. De tels accouplements peuvent se produire entre la sous-population *nordique résidente* (qui fréquente les eaux du centre et du nord de la Colombie-Britannique) et la sous-population *résidente de l'Alaska* (qui fréquente les eaux de l'enclave du sud de l'Alaska et des côtes du golfe d'Alaska). Nos résultats concordent à l'isolation génétique totale de la sous-population *résidente méridionale* du sud de la Colombie-Britannique et du nord de l'État de Washington. On se préoccupe de la conservation de cette sous-population à cause de sa faible taille (ses effectifs se chiffrant à moins de 85 individus), de la baisse récente des effectifs et de sa forte charge en contaminants.

Des analyses de paternité ont révélé que les résidents possèdent des mécanismes d'évitement forts (probablement de nature comportementale) de l'autofécondation. Dans tous les cas, sauf un, les mâles d'une troupe ont été exclus comme père possible des baleineaux de la même troupe. Dans la sous-population nordique résidente, la plupart des accouplements ont eu lieu entre des individus issus de troupes appartenant à différents « clans acoustiques ». Aucun lien de paternité n'a été établi entre les membres de la sous-population méridionale résidente et les membres des deux autres sous-populations résidentes, mais plusieurs possibilités de paternité ont été relevées entre ces deux dernières sous-populations.

Nous recommandons que trois sous-populations résidentes, trois sous-populations migratrices et une population hauturière soient reconnues comme des unités de gestion ou stocks distincts aux fins de conservation en Colombie-Britannique et en Alaska.

Introduction

Killer whales (*Orcinus orca*) are distributed across all major ocean basins but are uncommon throughout most of their range. They reach their highest densities in the north Pacific, north Atlantic, and Southern Oceans. The area most conducive to their study is the western coast of North America from 48-61°N latitude, where they frequent coastal areas protected by offshore islands. In 1970 Fisheries and Oceans research scientist Michael A. Bigg was tasked with estimating the number of killer whales inhabiting the coastal waters of southern British Columbia. Bigg pioneered the application of photo-identification to killer whales, and showed that all individuals could be reliably distinguished by dorsal fin shape, pigmentation patterns, and persistent scars. He found that killer whales were a) far less abundant than generally thought, and b) divided between two sympatric groups (Bigg 1982). These groups, known as *residents* and *transients*, have been referred to variously as types, forms, ecotypes, races, or—the term we use here—populations. In the late 1980's a third population, referred to *offshores*, was identified (Ford et al. 1994). Bigg's findings stimulated great interest in the species and led to an ongoing series of field studies by government, university, and independent researchers. The majority of this work has been conducted in British Columbia, but similar research is now being conducted in both Alaska and Washington State.

The collection of killer whale photographs started by Bigg in the early 1970's has been added to every year, and now contains tens of thousands of high quality negatives. Its principal contributors are Department of Fisheries and Oceans personnel and researchers connected with universities or non-governmental organisations such as the Vancouver Aquarium Marine Science Centre. While focused on killer whales off British Columbia, the collection includes the majority of identification photographs taken in recent years in Washington State and southern Alaska, under collaborative arrangements with US researchers. Every identifiable whale in every frame in the collection is entered into a computer database which is updated annually. The primary purpose of the database is to accurately track killer whale individuals and populations over time. It paints a picture of a slow-maturing, slow-reproducing, long-lived species (males and females reach sexual maturity at approximately 13 and 15 years, respectively; the mean generation time is 25 years; at six months of age the life expectancy of resident males and females is 29 and 50 years, respectively; Olesiuk et al. 1990). It also shows that resident, transient and offshore killer whales avoid associating, despite their overlapping distributions.

Characteristics of Resident, Transient and Offshore Killer Whales

Resident killer whales are now thought to prey exclusively on fish. They target runs of salmon, especially chinook (*Oncorhynchus tshawytscha*), but also take a variety of other demersal and midwater species (Ford et al. 1998). While they occasionally harass marine mammals, they have not been seen to eat them, and it is not uncommon to see Dall's porpoises or Pacific white-sided dolphins swimming with them freely. Resident social organization is highly structured. Individuals travel throughout their lives in *matrilines*, comprising a matriarch and her complete lineage (Bigg et al. 1990). Both sexes remain in their natal matriline for life. The absence of dispersal of at least one sex from the natal social group or territory has been described in only one other mammal (the

long finned pilot whale; Amos et al. 1993). Resident matrilines usually contain 4-12 individuals from two to four generations and often travel in association with other matrilines. It is believed that they associate most often with matrilines with which they share recent maternal ancestors (Bigg et al. 1990). Groups of frequently-associating matrilines are known as *pods.* The largest unit of social structure is a set of associating pods that share a common range. Bigg (1982) and subsequent authors referred to this unit as a *community;* we refer to it here as a *subpopulation*. Each resident pod uses a distinct set of stereotyped calls, or *dialect*; pods with related dialects make up an *acoustic clan* (Ford 1991). Subpopulations contain between one and three acoustic clans. Pods associate freely both within and between acoustic clans within their subpopulation but have not been seen to associate with pods from other subpopulations (Bigg et al. 1990).

Transient killer whales live in pods of 1-6 individuals and prey on marine mammals, principally harbour seals, Dall's and harbour porpoises, and Steller and California sea lions (Ford et al. 1998). Less common prey include minke and gray whales, Pacific white-sided dolphins, and sea birds. They have not been seen feeding on fish (Ford et al. 1998). All stranded killer whale carcasses in Alaska and British Columbia that were examined and that had identifiable stomach contents contained either fish or marine mammal remains but not both, supporting the observation that resident and transient diets do not overlap (Ford et al. 1998, Heise et al. in prep.). Studies of transient dialects are at an early stage, and no equivalent of the acoustic clans seen in residents has been identified. As with residents, transient pods sometimes join up and travel together for short periods of time. Three putative populations of transients have been identified along the northwestern coast of North America, based on association patterns (Barrett-Lennard et al. 1995).

Many differences in the behaviour of residents and transients have been described, most of which are probably attributable to their different diets (e.g. Bigg et al. 1987, Morton 1990, Baird et al. 1992, Barrett-Lennard et al. 1996a). For example Baird and Dill (1996) demonstrated that transients hunt seals most efficiently in co-ordinated attacks involving three whales (close to the mean group size they observed), whereas Ford et al. (1998) observed that much larger pods of resident killer whale forage on salmon while dispersed over several square kilometers. Similarly, Barrett-Lennard et al. (1996a) showed that residents produce social calls and echolocation sounds frequently while transients usually travel silently. They noted that this difference is likely accounted for by differences in the hearing sensitivities of fish and marine mammals. Transients often enter small bays and circle rocky islets, while residents generally stay in deeper water and move from headland to headland when following coastlines (Morton 1990).

Members of the *offshore* killer whale population are sighted and photographed only rarely. Little is known about this group other than that it contains at least 200 individuals, is usually sighted 20 km or more off the coast, ranges between California and the southern tip of Alaska, typically travels in groups of 20 or more individuals, does not appear to be divided into subpopulations, and uses echolocation and social calls frequently (Ford et al. 1994). Conspicuous vocal activity characterizes killer whales hunting fish, whereas mammal-hunting killer whales are usually silent (Barrett-Lennard et al., 1996a). We therefore presume that offshores feed primarily on fish (and/or

cephalopods) in the summer, when most recordings have been made. However, we cannot rule out the possibility that they target marine mammals at other times of year.

Subdivision of Resident and Transient Populations.

The resident killer whale population off British Columbia is divided into *southern* and *northern* subpopulations (Bigg et al. 1990, Ford et al. 2000). These groups usually occupy discrete territories (Figure 1) but pods from each are occasionally sighted well within the usual range of the other. For example, two southern resident pods have been seen several times in the spring moving south past northeastern Vancouver Island through a prime foraging area of the northern residents (G.M.E. unpublished data). Similarly, many of the northern residents spent several weeks in the summer of 2000 in the Straits of Georgia and Juan de Fuca, areas normally occupied by southern residents. Pods from the two subpopulations appear to avoid each other during these apparent incursions, and have never been seen to associate. A third putative subpopulation of resident killer whales referred to as the *Alaska residents* inhabits the coastal waters of the northern Gulf of Alaska. Two pods from this subpopulation are frequently seen as far south as the central part of the Alaska panhandle, in an area that slightly overlaps the northern extremity of the range of the northern residents (Figure 1). While pods from the northern and southern resident subpopulations have never been seen in association, there has been one sighting of Alaska resident and northern resident pods in close proximity (Dahlheim et al. 1997), and association between these groups cannot be ruled out. The approximate size of each resident subpopulation is given in Table 1.

All of the transient killer whales off British Columbia are presumed to belong to a single subpopulation, referred to as the *west coast* transients. Members of this subpopulation have been sighted as far south as the central coast of California and as far northwest as Glacier Bay, Alaska. A second putative subpopulation of transient killer whales has been identified in the open waters of the Gulf of Alaska west of Glacier Bay (Barrett-Lennard et al. 1995). This group, referred to as the *Gulf of Alaska* transients, is sighted infrequently and is poorly studied. The western extent of its range is unknown. A third putative subpopulation of transients referred to the *AT1* transients inhabits the waters in and adjacent to Prince William Sound and the Kenai Fjords in the northern Gulf of Alaska. This subpopulation is very small in number and overlaps the range of the Gulf of Alaska transients. It has never been seen to associate with other transients, uses a distinctive set of vocalizations (Saulitis 1993), and specializes on harbour seals and porpoises to a greater extent than the other transient subpopulations. The ranges and sizes of the three transient subpopulations are presented in Figure 2 and Table 1, respectively.

Objectives and History of this Study

This paper reports a comprehensive genetic analysis of population segregation in killer whales off the west coasts of British Columbia and Alaska. Our objectives were to: (1) examine the extent of reproductive isolation between residents and transients, (2) determine the extent of reproductive isolation between putative subdivisions of each population, (3) determine the discreteness of the offshore population of killer whales, (4) compare the levels of genetic diversity in residents and transients, (5) determine whether the observed lack of dispersal of resident killer whales is typical of their recent history,

and (6) examine mating patterns and inbreeding coefficients in the northern resident subpopulation to determine whether inbreeding avoidance behaviours compensate for the lack of dispersal. The study is based on the analysis of DNA from skin biopsies of 269 photo-identified killer whales from British Columbian and Alaskan waters including members of most known resident matrilines. The findings are discussed in the light of their implications for the long-term viability of killer whale populations and subpopulations in British Columbian waters.

The study described here began in 1993 as a PhD project (Barrett-Lennard 2000). It has been supported since October 2000 by the Department of Fisheries and Oceans with funding allocated under the Species at Risk program. The present emphasis is on acquiring and analysing additional DNA samples and conducting preliminary population viability analyses. Since these new analyses were still in progress at the time of writing, most of the findings presented here are from Barrett-Lennard (2000).

Methods

Biopsy Sampling

Skin biopsies obtained from free-ranging killer whales were used as a source of DNA. Biopsy sampling for this study was concentrated in two areas: from northern Vancouver Island to Caamaño Sound, British Columbia (50°45'-53°0'N, 127°0'-129°45'W), and the waters in and around Prince William Sound and Kenai Fjords, Alaska (59°30'-61°0'N, 146°15'-151°0'W). We also biopsied whales near Langara Island (54°14'N, 133°0'W) and in the western Strait of Georgia (49°15'N, 123°42'W). The sampling in Alaskan waters was conducted by C.O. Matkin (North Gulf Oceanic Society, Homer, Alaska), under a collaborative agreement. To locate whales, we visually searched areas where killer whale sightings were common by scanning with binoculars from a 8-m boat and from high points on shore. We also listened for vocalizations with a directional hydrophone. Mariners often reported whale sightings to us by marine radio, helping us to focus searches.

When killer whales were sighted, we photographed as many individuals as possible for positive identification later (using identification catalogues by Bigg et al. 1987, Heise et al. 1992, Ford et al. 1994, Dahlheim 1997, Dahlheim et al. 1997, Ford and Ellis 1999, Matkin et al. 1999b, and unpublished data held by G.M.E.). We then used lightweight pneumatic darts (Barrett-Lennard et al. 1996b) to biopsy individuals that we could identify visually, simultaneously re-photographing those with subtle distinguishing features. These procedures made it possible to avoid darting the same whale more than once, and to match every biopsy sample to an identified whale. We attempted to obtain biopsies from at least one member of every matriline encountered. Adult males were given first priority, followed in order by adult females and juvenile calves of biopsied females. The biopsy samples weighed approximately 0.5 g, one third of which was skin and the remainder subdermal connective tissue and blubber. The skin portion of each biopsy was preserved in dimethlysulphoxide and NaCl (Amos and Hoelzel 1991), and the blubber frozen for contaminant analysis (results in Ross et al. 2000).

Molecular analysis

DNA Extraction

DNA was extracted from the skin samples by digestion with proteinase K, purification with phenol and chloroform, and precipitation with ethanol using standard procedures (Sambrook et al. 1989). Care was taken to prevent cross-contamination by using sterile disposable labware, flame- or acid-sterilizing non-disposable items, and using aerosol-filtered pipettor tips during all procedures. A working solution of each DNA sample was made by diluting a portion of its stock solution in water to a DNA concentration of 50 ng/ul. This working solution was stored at -20°C and was replenished as required from the stock, which was stored at -80°C. DNA extraction and polymerase chain reaction (PCR) preparations were performed in a room off-limits to amplified PCR products.

Mitochondrial DNA

One individual was selected for mtDNA sequencing from each matriline (based on Ford et al. 1994 and Matkin et al. 1999a). PCR was used to amplify the entire D-loop region, which was then sequenced on an Applied Biosystems 377 automated sequencer (see Barrett-Lennard et al. 2000 for detailed procedures). Because the sequence was too long (950 nucleotides) to be resolved in one direction, we ran sequencing reactions from each end of the amplified fragment, and used the approximately 500-base overlap in the sequences of opposite directions to check for errors. As a final accuracy check, we confirmed differences between sequences by manually comparing the chromatographs produced by the automated sequencer.

Microsatellites

We tested 27 primer sets developed for microsatellite analysis in cetacean species (Amos et al. 1993, Buchanan et al. 1996, Richard et al. 1996, Valsecchi and Amos 1996, Hoelzel et al. 1998) for their ability to amplify microsatellite loci in killer whales, using procedures described in Barrett-Lennard (2000). We initially tested each pair of primers on DNA from 40 killer whales that we believed to be distantly related, including resident and transient individuals from both British Columbia and Prince William Sound. The 11 primer pairs that produced clear microsatellite bands on a polyacrlyamide sequencing gel and that revealed at least three different alleles in the test group were used to type all biopsied killer whales. During the routine typing at each of the selected microsatellite loci, samples that failed to amplify or that produced ambiguous bands on the gel were amplified a second and if necessary a third time. We scored the alleles manually by comparison to a reference sequence. As a check, we re-scored each film several days later and compared the two sets of scores. Errors were corrected by this method in approximately 1% of the scores. As an additional check of the consistency of scores, we re-amplified a minimum of 5% of the samples at each locus and scored them two more times. No differences were found between scores in the first and second amplification.

Data analysis

Mitochondrial DNA: Population Comparisons

We inferred historical relationships among the haplotypes using a branch-and-bound search algorithm to find optimal trees based on a maximum-likelihood criterion (Swofford et al. 1996); calculations were performed using PAUP* version 4.0b2a, (Swofford 1998). The maximum likelihood analysis used nucleotide frequencies and transition/transversion ratios based on the sequences. We repeated the analysis on 100 bootstrapped versions of the data to determine support for the tree topology.

Microsatellite DNA (A): Population Comparisons

We grouped the data based on population subdivisions suggested by observational data (Bigg et al. 1990, Ford et al. 1994, Barrett-Lennard et al. 1995), the mitochondrial analysis described above, or both. The offshores were treated as a seventh subpopulation. Using microsatellite genotypes from the group with greatest sample size, we tested each locus for evidence of heterozygote deficiency using Guo and Thompson's (1992) Markov chain method as implemented in GENEPOP (Raymond and Rousset 1995). An unbiased estimate of gene diversity (H_e) was calculated for each locus in each subpopulation using Nei and Roychoudhury's formula (in Nei 1987). To compare gene diversities between residents and transients, we used a nested two-way ANOVA, with population and locus as factors and with subpopulations nested within populations. We also calculated Weir and Cockerham's (1984) estimators of Wright's *F*-statistics for the subpopulations using the program FSTAT 2.8 (Goudet 1995). Wright's *F*-statistics are described in more detail below. To determine 95% confidence intervals for the estimates, we performed 1000 bootstraps by resampling among loci.

We calculated Nei's standard genetic distance D_s (Nei 1972) between all putative subpopulations using MICROSAT 1.5 (Minch et al. 1995). D_s does not assume any particular mechanism of mutation, unlike measures that assume that mutation occurs in a stepwise fashion (e.g. $\delta\mu^2$, Goldstein et al. 1995). Stepwise mutation-based measures are expected to be linear with respect to time at phylogenetic time scales, whereas D_s is a more appropriate measure when divergences have taken place recently and genetic drift, not mutation, is the main force creating differentiation (Goldstein et al. 1995, Paetkau et al. 1997). The genetic distance matrix was used to construct a neighbour-joining (Saitou and Nei 1987) tree, using the NEIGHBOR subroutine in PHYLIP 3.5c (Felsenstein 1993). To determine support for the tree topology we used MICROSAT to bootstrap the allele frequency data 1000 times by resampling among loci and to calculate distance matrices for each bootstrapped data set. NEIGHBOR and CONSENSE subroutines in PHYLIP were then used to determine the percentage of bootstraps supporting each part of the tree.

Microsatellite DNA (B): Parental Exclusions

Bigg et al. (1987, 1990) and subsequent authors inferred maternal relationships based on repeated sightings of identified whales in close association. Most inferences were based on calf/adult female associations, but some were based on associations between adults. Here, we performed parentage tests for each sampled individual whose putative mother

(based on Bigg et al. 1990, Ford et al. 1994, and Ford et al. 2000) was also sampled. We first checked the microsatellite genotypes for matches between the individual and its putative mother. Matches were considered to have failed if the two whales did not have at least one allele in common at each of the 11 loci. We then searched for matches between individuals whose putative mothers were not excluded in the first test, and all males that were of reproductive age when the individual was conceived. As male killer whales reach sexual maturity between 10.5 and 15.5 years (Olesiuk et al. 1990) and gestation lasts approximately 17 months (Walker et al. 1988), we considered males to be candidate fathers if they were born 12 or more years before the individual and were not known to have died more than two years prior to the its birth. Paternity candidates were considered to be possible fathers if they possessed each of the individual's alleles that could not be attributed to its mother.

To test whether mate choice in resident killer whales is contingent on pod, clan, and/or subpopulation membership, we compared group memberships of offspring and their matching candidate fathers. The method is conceptually similar to an *F*-statistic based test, but differs in that it reveals contemporary rather than historical patterns. Offspring that matched more than one candidate father were excluded from the tests unless all of the matching candidates came from the same group as each other. Log-likelihood ratio contingency tests (Zar 1996) were used at the subpopulation and clan levels to determine whether the ratio of genotype matches within groups to matches between groups was independent of the ratio of genotype mismatches within groups to mismatches between groups. A different approach was needed at the pod level, as random mating would result in relatively few intra-pod paternities. Since pods by definition swim together at least half the time, we assumed that half of the calves would result from intra-pod matings if mate choice was independent of pod membership. We used a binomial test (Zar 1996) to estimate the probability of the observed numbers of intra-pod and extra-pod paternal matches under this assumption.

An alternative approach to the strict exclusion method of parentage testing used here involves estimating the likelihood of parentage based on genotype similarities (e.g. Marshall et al. 1998, Goodnight and Queller 1999). This has the advantage of identifying which of two or more non-excluded father candidates is more likely to be the true father of an offspring, and reduces the probability of incorrectly excluding true fathers because of genotyping errors or mutation. While useful in many applications, higher-than-average likelihood-of-parentage values are necessarily assigned to close relatives of offspring because of genetic correlations resulting from common descent, which makes the method problematic for distinguishing between closely related and unrelated candidate parents. We therefore did not use it here and instead used genotyping checks to minimize false exclusions and focused on offspring with single candidate fathers or sets of fathers from a common group.

Microsatellite DNA (C): F-statistic-based inference of mating patterns

Wright's (1951) fixation indices *F*is, *F*st and *F*it were used to analyze the partitioning of genetic variation. The fixation indices are measures of standardized variances in allele frequencies that detect departure from Hardy-Weinberg equilibrium caused by biased inbreeding, biased outbreeding, or population subdivision and drift. The subscripts i, s

and t refer to individual, subpopulation and total population, thus *F*is detects inbreeding in individuals relative to their subpopulation, *F*st detects inbreeding in subpopulations relative to the total population (providing a measure of population subdivision), and *F*it detects inbreeding relative to the total population. *F*st values range from 0 in panmictic populations to 1 in populations made up of subpopulations that are fixed for different alleles. In contrast, *F*is and *F*it can range between –1 and 1, indicating maximal outbreeding and inbreeding respectively. Sugg et al. (1996) provide a useful review of the interpretation of *F*-statistics in socially structured populations. Calculations were performed according to the formulae of Weir and Cockerham (1984), as implemented in the program FSTAT 1.2 (Goudet 1995). Confidence intervals were determined by bootstrapping 1000 times, using the 11 microsatellite loci as resampling units.

Results

Biopsy samples

We obtained biopsy samples from 261 identified killer whales off British Columbia and southern Alaska, and obtained tissue from the stranded carcasses of eight additional identified individuals. The sampled whales were from 111 known matrilines and included offshores and members of each resident and transient subpopulation. In addition, colleagues kindly supplied tissue samples from four killer whales from the Atlantic ocean. The population, clan and pod membership of the sampled whales are listed in Table 2.

Northern and southern residents, AT1 and west coast transients, and offshores were each monomorphic and had different haplotypes. Alaska residents had two haplotypes, one matching southern residents and the other northern residents; pod members always shared a single haplotype, but pods with different haplotypes were frequently seen in close association. The Gulf of Alaska transients also had two haplotypes, one found in all samples from three pods, the second in both samples from a single pod. Two haplotypes were found in Atlantic whales. One was from a whale that stranded in southern Brazil, the other from two whales captured near Iceland and one that stranded in western France. An unrooted maximum likelihood phylogram based on the D-loop sequence data is presented in Figure 3. The transient subpopulations were an outgroup to all others, including Atlantic whales. Barrett-Lennard (2000) constructed a similar tree using the same data with the addition of a Risso's dolphin (*Grampus griseus*) as an outgroup, and found that in 96% of the bootstrap trees at least one of the two killer whale groups (transients and non-transients) was monophyletic.

Microsatellite DNA: Population Comparisons

We amplified all 269 DNA samples from Pacific killer whales at 11 loci microsatellite loci. Heterozygous individuals of both sexes were scored, and none of the 11 loci were sex-linked. The number of alleles per microsatellite locus in the resident, transient, and offshore populations ranged between 3 and 20, with a mean of 7.8 (Table 3). Tests for heterozygote deficiency in the largest putative subpopulation sampled, the northern

residents, were negative for all 11 loci, with p values ranging between 0.27 and 0.91. Gene diversity (H_e) levels are presented in Table 3. *He* was significantly greater in transients than residents ($F_{1,50} = 12.66$, $p < 0.001$). Gene diversity in the small sample of offshores was similar to the residents but was not compared statistically.

Estimates of Wright's *F*-statistics for all seven putative subpopulations, for the three resident subpopulations, and for the three transient subpopulations are presented in Table 4. Here, the F_{st} estimates reveal strong segregation between offshores, residents, and transients and weaker subdivision within the resident and transient assemblages. The F_{is} estimates provide no evidence that inbreeding occurs within the subpopulations. Pairwise F_{st} values are presented in Table 5, and show less segregation between the Alaskan residents and northern residents than between either subpopulation and the southern residents. Similarly, less segregation exists between the Gulf of Alaska and west coast transients than between either subpopulation and the AT1's. Figure 4 is a neighbour-joining phylogram of the seven subpopulations based on their genetic distances and shows a clear separation of residents and transients with the offshores occupying intermediate positions.

Paternity matches within and between clans, pods and subpopulations

Subpopulations

A total of 103 resident offspring belonging to 3 subpopulations were screened for possible fathers against all sampled resident males that were alive and mature when they were conceived—an average of 53 males per calf. Twenty one of them matched a single father and an additional 18 matched a set of males that were all from the same subpopulation. Of the 39 matches, 33 (85%) were with candidate fathers from the same subpopulation as the offspring. All of the matches between subpopulations were between the northern residents and Alaska residents (but few southern residents were sampled). The hypothesis that mating is independent of subpopulation membership was rejected (log-likelihood ratio test, $p < 0.0001$). No matches were found when the same set of resident offspring were screened for possible matches with transient and offshore males.

Clans

Paternity tests were conducted between 69 northern resident calves and males that were alive and mature when they were conceived (an average of 31 males were screened per calf). Seventeen offspring matched a single candidate father and six matched a set of males that were all from the same clan. Of these 23 offspring, 5 matched males from their own clan and 18 matched males from clans other than their own. The proportion of inter-clan matches (0.78) was higher than the proportion of inter-clan paternity tests (0.54), and the hypothesis that mate preferences are independent of clan membership was rejected (log-likelihood ratio test, $p = 0.014$). This exercise was not repeated for the southern residents, which have only a single clan. In the Alaska residents, 33 calves were tested against an average of 25 males each. Of these, three offspring had a single matching candidate father and two matched a set of male clan-mates; of these five matches three were within and two between clans, and the hypothesis that mating preferences are independent of clan membership was not rejected (log-likelihood ratio test, $p = 0.377$).

Pods

Of the 69 northern resident calves referred to above, 17 matched a single father candidate and two matched a set of males from the same pod. Of these 19 matches, all but one were between pods. The binomial probability of obtaining this result if mating is equally frequent within and between pods is <0.0001. In the Alaska residents, three individuals matched a single candidate and one matched a set of males that were all from a single pod. All matches were between pods. The probability of obtaining this result if mating was equally likely within and between pods was 0.062. This analysis was not performed on southern residents, because only one mother-calf pair was sampled, and the calf did not match any males. When the analysis was repeated on all three resident subpopulations combined, 24 out of 25 matches were between pods.

F-statistic-based inference of mating patterns

Partitioning of genetic variance within and between pods was analysed at acoustic clan and subpopulation levels. Two clans among the northern residents were analysed; one (*A*) contains 10 pods and the other (*G*) contains four. The third northern resident clan (*R*) contains only two pods and was not included. The results of this analysis are presented in Table 6. *F*is values were significantly less than zero in all but one analysis, indicating more heterozygosity than expected if matings were random and consistent with a pattern of biased outbreeding. The exception was the analysis of clans within the Alaska resident subpopulation, in which *F*is did not differ significantly from zero. Estimates of *F*it for pods within clans and for clans within the northern resident subpopulation were negative, consistent with biased outbreeding, although in only one comparison was the result supported statistically. On the other hand, the positive *F*it for clans within the Alaska resident community is consistent with the paternity analysis, which suggested that mating is more common within clans in that subpopulation. Positive *F*st values indicated significant partitioning of genetic variance among pods in A-clan, among pods in the entire northern resident subpopulation, among clans in the northern resident community, and among subpopulations in the entire resident population. This partitioning presumably reflects allelic correlations arising from lack of dispersal of individuals.

Table 7 presents pairwise *F*st for all of the acoustic clans within the northern, southern, and Alaska residents. *F*st values were lower for pairs of clans from the same subpopulation than for pairs from different subpopulations. Pairs that included J-clan, the single southern resident clan, had the highest *F*st's. Interestingly the J-clan / AD-clan pair had the highest *F*st estimator, despite the fact that they have the same mitochondrial D-loop haplotype (see Figure 3).

Discussion

This study builds on earlier genetic studies of killer whales in the northeastern Pacific (summarized in Stevens et al. 1989 and Hoelzel et al. 1998), but differs from them in the following ways: the number of samples was several times greater than in earlier studies, all killer whales were positively identified (so that all of the molecular results could be

used to characterize groups rather than to assign unknown individuals to groups), four of the six subpopulations analysed here had not been compared previously, as many matrilines as possible were represented (previous studies used multiple samples from a small set of matrilines), and the length of mitochondrial DNA sequenced and the number of microsatellite loci typed were much greater than in earlier studies. Our findings have six significant conservation implications that are expanded on below.

1. Resident and transient killer whales are reproductively isolated.

Individuals classified *a priori* as resident or as transient had no mitochondrial haplotypes in common, and there were many more fixed mitochondrial differences between the two populations than among their subpopulations (Figure 3). Similar results were reported by Stevens et al. (1989) and Hoelzel et al. (1998), based on much smaller sample sizes. Since the classifications were made independently of any genetic comparisons and our samples were large, the result indicates that female migration between the two forms has been extremely rare for many generations. Comparisons of mitochondrial and nuclear microsatellite DNA—inherited from mothers only and from both parents, respectively—are often used to test for sex-biased dispersal. In this case, however, the general patterns are similar: the microsatellite phylogram (Figure 4) preserves the separation of residents and transients, pairwise *F*st values (Table 4) are much higher between resident and transient subpopulations than between subpopulations of a common population, and several loci have population-specific alleles. These results suggest that neither sex disperses at an appreciable rate between populations. As such, the two populations meet Moritz's (1994) definition of evolutionarily significant units.

There is no reason to suppose that residents and transients are reproductively incompatible (that is, separated by post-mating isolating mechanisms). Both have crossed with Icelandic whales in captivity (whale identities from Hoyt 1984, mating records from Duffield et al. 1995) and produced fertile offspring. Since residents and transients are sympatric, their genetic separation must be maintained by positive assortative mating. Mating preferences could be based on culturally or genetically inherited behaviours that distinguish residents and transients, such as those associated with foraging (e.g. Morton 1990, Barrett-Lennard 1996a, Ford et al. 1998) or communication (Ford 1991). They could also be influenced by subtle differences in phenotype (see Bigg et al. 1987, Baird and Stacey 1988). However, it seems unlikely that individual mating preferences alone could account for the near complete genetic isolation of the two populations. We argue that the social cohesion of subpopulations is likely the most important factor in the isolation of residents and transients.

2. The subdivision of resident and transient populations into at least three regional subpopulations is supported genetically.

The finding of fixed mitochondrial differences between the northern and southern residents effectively rules out substantial female-mediated gene flow between them (Figure 3). The microsatellite analysis (Tables 4 and 5, Figure 4) showed that they are also strongly differentiated at nuclear loci, indicating that male-mediated gene flow is also small at best. Members of the two populations are believed to come into acoustic

and perhaps visual contact at least occasionally, indicating that their reproductive isolation results from behavioural or social factors rather than physical separation.

The Alaska residents have two mitochondrial DNA haplotypes, one in common with the residents and the other with the northern residents, suggesting that they share relatively recent maternal ancestors with both groups. Their microsatellite genotypes indicate relatively weak separation from the northern residents and much stronger separation from the southern residents, as reflected in the *F*st values in Table 5 and the bootstrap values in Figure 4. These patterns may reflect contemporary patterns of gene flow, with occasional matings taking place between the Alaska residents and northern residents but few matings between either population and the southern residents, or they may reflect historical associations and founding events. The only observation of possible association between resident subpopulations was a sighting of two pods of Alaska residents in close proximity to two northern resident pods (Dahlheim et al. 1997). In contrast, pods from the same subpopulation are seen associating and intermingling frequently. The three resident subpopulations meet Moritz's (1994) definition of management units.

The general pattern of genetic differentiation among transient subpopulations is similar to that of residents. The west coast transients and AT1's each have a single unique mitochondrial haplotype and two unique haplotypes were found in the Gulf of Alaska transients. The four transient haplotypes cluster with each other with stronger bootstrap support than with the haplotypes of any other population (Figure 3). At the same time, the fixed haplotypic differences between transient subpopulations suggest that female dispersal between them is rare at best. We note, however, that our sample of Gulf of Alaska transients is small, and it is possible that AT1 or west coast haplotypes will be discovered in the Gulf of Alaska transients with more sampling effort. The microsatellite-based pairwise *F*st estimate for the west coast and Gulf of Alaska transient subpopulations is relatively low (Table 5), evidence that their separation is either incomplete or has occurred recently. The separation of both groups from the small AT1 transient subpopulation appears to be older and/or more complete. The isolation of the AT1's appears likely to result in its extinction, as it presently has only 10 members and has not reproduced sucessfully since 1984 (Matkin et al. 1999b). The 3 transient subpopulations meet the definition of management units.

3. Offshores are genetically differentiated from all known resident and transient subpopulations.

Residents and offshore killer whales probably share more recent maternal ancestors with each other than either does with transients, based on their similar mitochondrial haplotypes and position on the mitchondrial tree in Figure 3. In contrast, microsatellite loci group offshores and transients (Figure 4). This situation is consistent with several possible scenarios of historical and contemporary gene flow (see Barrett-Lennard 2000). In any case, by Moritz's (1994) criteria, offshores are an evolutionarily significant unit containing a single identified management unit.

4. Residents have lower levels of genetic variation than transients.

We found higher levels of mitochondrial DNA variation in transients than in residents (four haplotypes in three subpopulations and two haplotypes in three subpopulations,

respectively). Microsatellite DNA diversity was also significantly higher in transients than in residents. There are several plausible explanations for these differences. First, the subpopulation size estimates in Table 1 may accurately reflect resident numbers, but underestimate transient numbers. Transients are more difficult to census than residents (Ford and Ellis 1999), and many west coast and Gulf of Alaska transients may remain uncatalogued (the AT1 population is well-studied and confined in distribution, and is unlikely to contain unidentified members; Matkin et. al. 1999b). Second, transient subpopulations may also be less closed to gene flow than residents, and their genetic diversity may be augmented by occasional matings with either offshores or unknown subpopulations of killer whales. Finally, the patterns could result from historical contingencies— recent bottlenecks or founder effects—that have been more frequent and/or more severe in residents than transients. We rule out a fourth explanation below, that residents mate within their natal groups, and are therefore significantly more inbred than transients. It is especially noteworthy that the southern resident subpopulation, presently listed as threatened because of its small size and recent declines, has the lowest gene diversity of all subpopulations including the AT1's, which only contain 10 individuals.

5. The observation that resident killer whales do not disperse from their natal groups is typical of the recent history of the population.

One of the most striking findings to emerge from nearly 30 years of field studies of resident killer whales is the absence of dispersal of members of either sex from their natal pods (Bigg et al. 1990, Ford et al. 2000). This pattern may be a general characteristic of resident killer whales. Alternatively, it could be a pattern that only occurs when resource competition is relaxed and subpopulations are growing, as they have been for most of the the past three decades (Olesiuk et al. 1990, Matkin et al. 1999a). The fact that the Alaska resident subpopulation contains two mitochondrial DNA haplotypes provided an opportunity to examine this question. One of the haplotypes is fixed in one of the two acoustic clans in the subpopulation, the second is fixed in the other clan. Pods of the Alaska residents associate independently of clan membership, so individuals are in frequent social contact with members of other clans. There is little nuclear DNA differentiation of the two clans, and paternity analysis indicates that inter-clan matings are common (see below). If females dispersed between pods even rarely, the observed relationship between clan membership and mitochondrial haplotype would break down. We conclude therefore that successful dispersal by female residents has not occurred for many generations. Mitochondrial comparisons cannot detect historical trends in male dispersal, but can identify males that have themselves dispersed between subpopulations. We found no cases of males carrying a mitochondrial haplotype different from that of the rest of their pod among the Alaska residents and conclude therefore that male dispersal has been absent or rare for at least four decades.

6. Resident killer whales outbreed without dispersing.

All but one test of within-pod paternity in resident killer whales was rejected in this study. The rarity of intra-pod matings was supported by the *F*-statistics. If mating takes place between but not within pods, then pod members should be more heterozygous than if they mated randomly within pods, and a negative *F*is is expected. Here, *F*is was

negative for resident pods within the two clans and one subpopulation tested (Table 6). The clustering of maternal relatives in pods is expected to cause pods to be genetically differentiated despite the apparent inter-pod mating system. This differentiation is expected to be reflected in positive *F*st values. Statistical support for *F*st > 0 was strong in two of the three analyses. These results suggest that non-dispersing resident killer whales avoid inbreeding at least as effectively as they would if they dispersed from their natal groups.

One of our most striking findings is that most breeding occurs between and not within acoustic clans in the northern residents. This conclusion followed from the paternity analysis, in which most calf/candidate male matches were between clans. It is also consistent with the negative *F*is estimator from the clan/subpopulation analysis which indicates that clans are more heterozygous than would be expected if mating were random (Table 6). Since northern resident clans are significantly differentiated from each other (*F*st >0, Table 6), clan exogamy (outside mating) is expected to increase average heterozygosity beyond the level that would result from pod exogamy alone. As resident killer whale subpopulations have small effective population sizes in a genetic sense (c. 70 individuals, Barrett-Lennard 2000), such a mating system is likely of significant selective value in preventing inbreeding.

In contrast to the northern residents, we found no evidence of acoustic clan exogamy in the Alaska residents. Our sample of calves in this group with matching fathers was small (five), but in three cases the matches were within the clan. In addition, we did not detect excess heterozygosity indicative of exogamy within the clans in this subpopulation (i.e., *F*is was not significantly less than 0, Table 6). The Alaska resident clans are large, containing an average of more than 180 individuals compared to 70 and 80 for the northern residents and southern residents, respectively. Each of the Alaska resident clans contains groups of pods with substantially different (although not discrete) repertoires (Yurk et al. in prep). It is therefore likely that females can use dialect differences to avoid consanguineous matings in this subpopulation at the sub-clan as opposed to the clan level.

In contrast to outbreeding at the pod and clan levels, the paternity analysis suggests that mating between resident subpopulations is at most rare. This is consistent with the *F*st values in Tables 4 and 5, as described under point 2 above. Nevertheless, neither the paternity analysis nor the fixation index analyses entirely rule out mating between resident subpopulations. Indeed, the fact that mating occurs between pods in the absence of permanent dispersal suggests that mating between subpopulations in the absence of dispersal is not out of the question. Additional research, ideally combining paternity analysis and gene frequency analysis as employed here, will be necessary to resolve this pattern with certainty.

CONCLUSIONS: CONSERVATION IMPLICATIONS

Killer whales are long-lived, slow-reproducing animals (the life expectancy of 6 month old males and females is approximately 29 and 50 years, respectively; intrinsic population growth rate is approximately 2.9%/yr; Olesiuk et al. 1990). Their populations are therefore slow to respond numerically to changes in reproductive and mortality rates, even when the long-term consequences of those changes could be profound. In addition, because populations are small, short-term trends are driven by stochasticity in the timing of birth and deaths. As a consequence, the assessment of underlying population trends takes many years. These aspects of the biology of killer whales mean that the impact of factors such as contaminant and noise pollution, reductions in food supply, and human disturbance could take years to be detected even *with* comprehensive monitoring.

The assessment and conservations of northeastern Pacific killer whales is further complicated by the fact that they live in at least three distinct populations, two of which are subdivided into at least three genetically differentiated subpopulations. The subpopulations range in size from 10 to over 360 individuals, which would be considered very small in most species (Lynch et al. 1995). Small population size increases the risk of population extinction from environmental or demographic stochasticity. This risk is likely lower in killer whales than in other species because mortality rates are low (Olesiuk et al. 1990) and population fluctuations are of relatively low amplitude (G.M.E. unpublished data). A second risk factor in small populations is that high inbreeding levels will cause mean fitness to decline. The results presented in this paper suggest that residents avoid consanguineous matings remarkably well. This may well be a specific adaptation to small population sizes, selected for by the cost of inbreeding depression. In any case, it suggests that resident killer whale populations are probably genetically viable at smaller population sizes than most other species. Nevertheless, low genetic variability may decrease reproductive rates and increase the vulnerability of small populations to environmental hazards such as disease and pollution. The relatively low genetic variation of the southern residents is cause for concern since the population is known to have high contaminant levels (Ross et al. 2000). Furthermore, its effective population size has declined substantially in the last 5 years which is expected to accelerate its loss of genetic variability in the future. The mating patterns of transients are unknown but their higher levels of genetic diversity suggest that their subpopulations are no less viable from a genetic standpoint than those of residents.

In theory, small populations can be “rescued” demographically (by immigration) or genetically (by intermating). However, there is no reason to believe that either would occur in depleted killer whale subpopulations. Indeed, the AT1 transient subpopulation of Alaska has declined to 10 individuals and has not produced a surviving calf since 1984 despite having reproductive-aged females and potential mates in the overlapping Gulf of Alaska transient subpopulation. Its extirpation seems a virtual certainty. If, as our results suggest, mating occurs between (and not within) resident pods from the same subpopulation, then the females in the largest of the three pods in the southern resident subpopulation (L-pod) have only a single mature male with which to mate. That male (J01) is one of the oldest resident males known. Additional mates will probably become

available as subadult males mature over the next few years, however, the apparent shortage of males at present illustrates the vulnerability of the southern resident subpopulation for demographic reasons as well as (potentially) for genetic reasons.

The northern resident population numbers approximately 214 individuals, and has been increasing slowly for many years. As mentioned earlier, it is possible that it experiences some gene flow with the Alaska resident subpopulation, which would help it maintain relatively high genetic diversity. It is therefore more likely to be robust to environmental change and demographic factors that might put it as risk (such as chance generations with few females) than the southern residents. The west coast transient subpopulation is believed to be similar in size to the northern residents, and may experience limited gene flow with the Gulf of Alaska transients. It is therefore also probably intrinsically more robust than the southern residents. However, it should be noted that its population trend is unknown and it has extremely high contaminant levels (Ross et al. 2000), and therefore warrants close monitoring.

Recommandations

1) Three populations units of northeastern Pacific killer whales should be recognized as distinct populations or *evolutionarily significant units* (ESU's): resident, transient, and offshore killer whales.

2) Seven population units of northeastern Pacific killer whales should be considered *stocks* or *management units* (MU's) for conservation purposes: the southern, northern and Alaska residents (Figure 1), the west coast, Gulf of Alaska and AT1 transients, (Figure 2), and the entire offshore population. Four of these MU's have been sighted in British Columbian waters to date.

3) The southern resident subpopulation of British Columbia is of conservation concern because of its small size and recent declines. Determining the discreteness of this subpopulation is hampered by the small number of DNA samples that have been acquired from it to date. Priority should be placed on increasing the number of samples and using them in paternity analyses to determine whether all calves are fathered by members of the subpopulation. This information should then be incorporated into a viability analysis of the southern resident population.

4) The analysis of the offshore population is also based on a small set of samples. Priority should be placed on acquiring and analysing more samples from this group.

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Table 1. Identity and estimated size of known killer whale populations and subpopulations in the northeastern Pacific.

Subpopulation	Size	Source
Northern resident	214	Ford et al. 2000
Southern resident	82	Ford et al. 2000
Alaska resident	360*	Matkin et. al. (1999b)
West Coast Transient	219*	Ford and Ellis (1999)
Gulf of Alaska Transient	60 *	Ford and Ellis (1999)
AT1 Transient	10	Matkin et al. (1999b) [¥]
Offshore[§]	200*	Ford and Ellis (1999)

Subpopulations that have been sighted in Canadian waters are underlined.
* Subpopulations that have not been completely enumerated. [¥] Matkin et al. 1999b list 11 individuals in the AT1 subpopulation, however one member has subsequently died.
[§] The offshore group is a population rather than a subpopulation.

Table 2. Population, subpopulation, clan and pod identity of biopsied whales analysed in this study.

Population	Putative Subpopulation[1]	Acoustic Clan	Pod
Resident (216)	Southern resident (8)	J (8)	J1 (7)
			L1 (1)
	Northern resident (126)	A (75)	A1 (17)
			A4 (10)
			A5 (15)
			B1 (8)
			C1 (8)
			D1 (4)
			H1 (5)
			I1 (1)
			I2 (3)
			I18 (4)
		G (34)	G1 (7)
			G12 (7)
			I11 (14)
			I31 (6)
		R (17)	R1 (15)
			W1 (2)
	Alaska resident (82)	AB (44)	AB (14)
			AG (3)
			AI (6)
			AJ (12)
			AN (8)
			AX (1)
		AD (38)	AD5 (4)
			AD16 (4)
			AE (15)
			AK (12)
			AS (3)
Offshore (7)	[British Columbia & SE Alaska] (7)		
Transient (46)	West coast transient (30)		
	Gulf of Alaska transient (8)		
	AT1 transient (8)		
Atlantic (4)	[West coast of France] (1)		
	[Iceland] (2)		
	[Southern Brazil] (1)		

Numbers of samples in each category in round brackets. Pod designations based on Heise et al. (1992), Ford et al. (1994), Ford and Ellis (1999), Matkin et al. (1999b). Acoustic clan designations for British Columbian residents from Ford et al. (1994). Offshore killer whales identified by G.M.E. based on unpublished data. Tissue samples from identified carcasses were provided by D. Bain (two offshore samples) and D. Nagorsen (L1 pod sample). Icelandic, French and Brazilian samples provided by C. Wright, A. Collet, and E. Secchi respectively. [1]Sampling location is given in square brackets when subpopulation is unknown.

Table 3. Gene diversities and total number of alleles at 11 microsatellite loci in seven subpopulations of killer whales from Alaska and British Columbia.

Subpop.[2]	FCB4	EV37	FCB12	417	KW2M	FCB17	FCB5	EV1	464	FCB11	415	Mean
SR	0.473	0.384	0.648	0.000	0.627	0.142	0.560	0.362	0.142	0.473	0.560	0.398
NR	0.718	0.550	0.421	0.277	0.399	0.229	0.499	0.432	0.443	0.510	0.612	0.463
AR	0.545	0.692	0.337	0.234	0.533	0.486	0.494	0.371	0.501	0.577	0.631	0.491
OFF	0.704	0.670	0.264	0.142	0.473	0.264	0.528	0.660	0.264	0.637	0.660	0.479
WCT	0.792	0.733	0.419	0.437	0.815	0.577	0.736	0.711	0.664	0.683	0.742	0.664
GAT	0.879	0.705	0.663	0.358	0.810	0.489	0.758	0.800	0.753	0.780	0.716	0.701
AT1	0.686	0.543	0.699	0.568	0.000	0.503	0.503	0.000	0.523	0.607	0.000	0.421
Alleles[1]	20	9	6	3	8	4	6	7	6	8	9	7.8
Ref.[3]	Buch.	Val.	Buch.	Schl.	Hoel.	Buch.	Buch.	Val.	Schl.	Buch.	Schl.	

[1]Total number of alleles in all seven subpopulations. [2] Subpopulations abbreviations as follows: SR, southern residents; NR, northern residents; AR, Alaska residents; OFF, offshores; WCT, west coast transients; GAT, Gulf of Alaska residents. For testing for *F*st differences from zero, multi-locus genotypes were permuted among subpopulations 10,000 times. [3]The original reference describing each locus, abbreviated as follows: Buch.: (Buchanan et al. 1996); Val.: (Valsecchi and Amos 1996); Hoel.: (Hoelzel et al. 1998); Schl.: (Schlötterer et al. 1991).

Table 4. Weir and Cockerham (1984) estimators of *F*-statistics combined over 11 microsatellite loci for killer whale subpopulations from Prince William Sound, Alaska and British Columbia†.

	*F*is	*F*st	*F*it
all subpopulations [7]	-0.014 (-0.049 — 0.022)	0.205 (0.140 — 0.269)	0.194 (0.114 — 0.276)
resident subpopulations [3]	-0.019 (-0.056 — 0.020)	0.088 (0.032 — 0.146)	0.070 (0.003 — 0.127)
transient subpopulations [3]	0.004 (-0.096 — 0.086)	0.167 (0.088 — 0.241)	0.170 (0.073 — 0.236)

† Subpopulations as listed in Table 1. Round brackets indicate ninety-nine percent confidence intervals for each estimator; square brackets the numbers of subpopulations in each analysis.

Table 5. Weir and Cockerham (1984) estimators of *F*st combined over 11 microsatellite loci for each pair of sampled subpopulations of killer whale from Prince William Sound and British Columbia. The probabilities that the statistics were not greater than zero, based on permutation tests, were less than 0.001 in every case.

Residents	NR	0.144					
	AR	0.187	0.076				
Offshores	OFF	0.321	0.278	0.305			
Transients	WCT	0.229	0.278	0.259	0.153		
	GAT	0.226	0.251	0.234	0.182	0.065	
	AT1	0.429	0.430	0.399	0.422	0.224	0.290
		SR	NR	AR	OFF	WCT	GAT
			Residents		Offshores	Transients	

Abbreviations as in Table 3. For testing for *F*st differences from zero, multi-locus genotypes were permuted among subpopulations 10,000 times.

Table 6. Overall *F*is, *F*st, and *F*it estimators for resident killer whale pods, acoustic clans, and subpopulations from the northeastern Pacific Ocean, based on 11 microsatellite loci.

indiv-iduals	sub-group	total group	F_{is}	F_{st}	F_{it}
105	pod [10]	A- clan	-0.107 (-0.163 — -0.048)	0.066 (0.045 — 0.086)	-0.034 (-0.087 — 0.017)
76	pod [4]	G- clan	-0.414 [-0.247 — -0.013)	0.025 [-0.008 — 0.065)	-0.113 [-0.220 — 0.007)
214	pod [16]	NR sub-population	-0.112 [-0.138 — -0.087)	0.062 [0.048 — 0.082)	-0.043 [-0.078 — -0.004)
214	clan [3]	NR sub-population	-0.064 [-0.095 — -0.031)	0.027 [0.012 — 0.043)	-0.035 [-0.073 — 0.007)
360+	clan [2]	AR sub-population	0.025 [-0.073 — 0.119)	0.008 [-0.003 — 0.020)	0.033 [-0.069 — 0.126)
656+	subpop.[3]	all residents	-0.019 [-0.056 — 0.020)	0.088 [0.032 — 0.146)	0.070 [0.003 — 0.127)

A- and G-clans are two acoustic clans of the northern residents. NR and AR refer to the northern resident and Alaska resident subpopulations, respectively. The *individuals* column lists the approximate number of individuals in each corresponding total group. Square brackets contain the number of subgroups in the corresponding total groups. Numbers in round brackets are 99% confidence intervals, obtained by bootstrapping the loci. Single or double underlining indicates that the entire 99% confidence interval of the fixation index is below or above zero, respectively.

Table 7. Pairwise *F*st estimators based on 11 microsatellite loci for six acoustic clans of killer whales from the northern resident, southern resident, and Alaska resident subpopulations from British Columbia and Alaska. The estimators were significantly greater than zero ($p<0.001$ in every case except AD/AB, for which $p=0.035$).

Northern	G	0.023				
	R	0.023	0.052			
Southern	J	0.141	0.173	0.175		
Alaska	AB	0.070	0.072	0.078	0.177	
	AD	0.101	0.099	0.096	0.208	0.008
		A	G	R	J	AB
			Northern		Southern	Alaska

A, G, R, J, AB, and AD are the names of acoustic clans (see Table 2). Values in boxes are *F*st estimators for clans within subpopulations. The arrow indicates the AD/J pair, which are allopatric but have identical mitochondrial D-loop haplotypes.

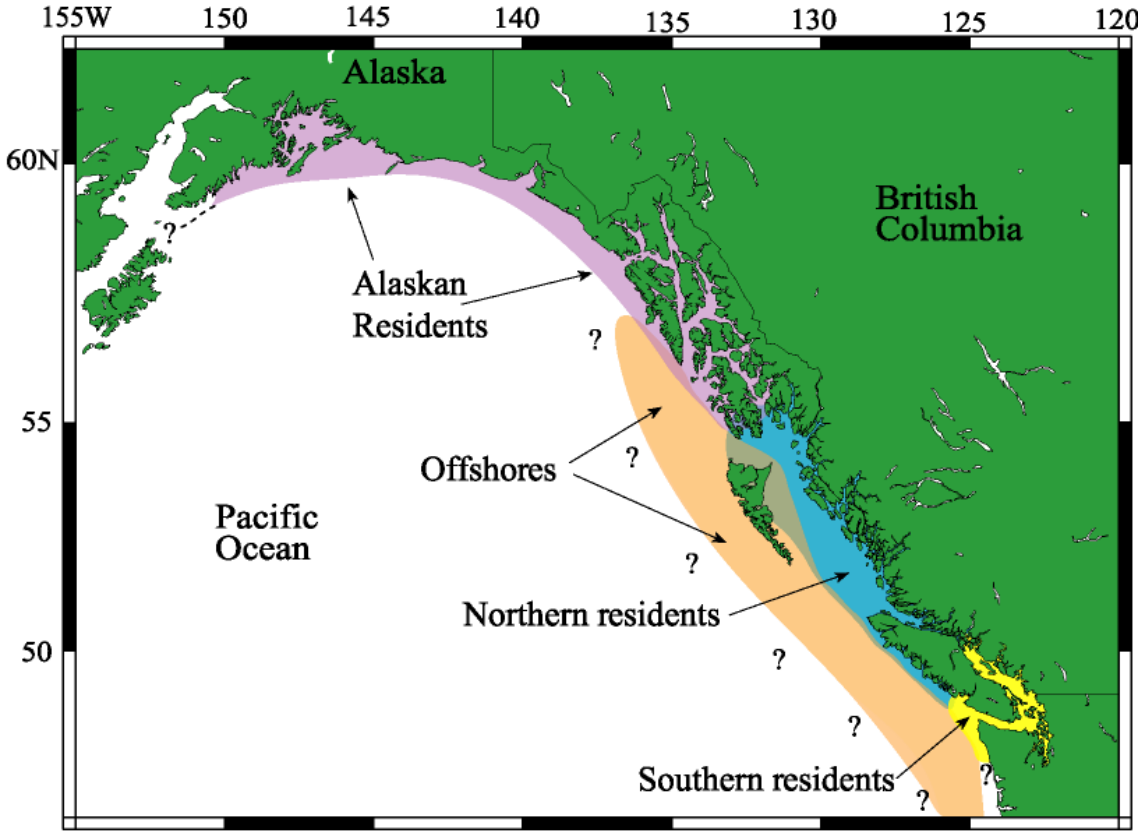

Figure 1. Approximate ranges of the offshore population and resident subpopulations of killer whales in the northeastern Pacific Ocean, based on Matkin et al. 1997, Matkin et al. 1999b, and Ford et al. 2000. Intermediate colours indicate regions of overlap.

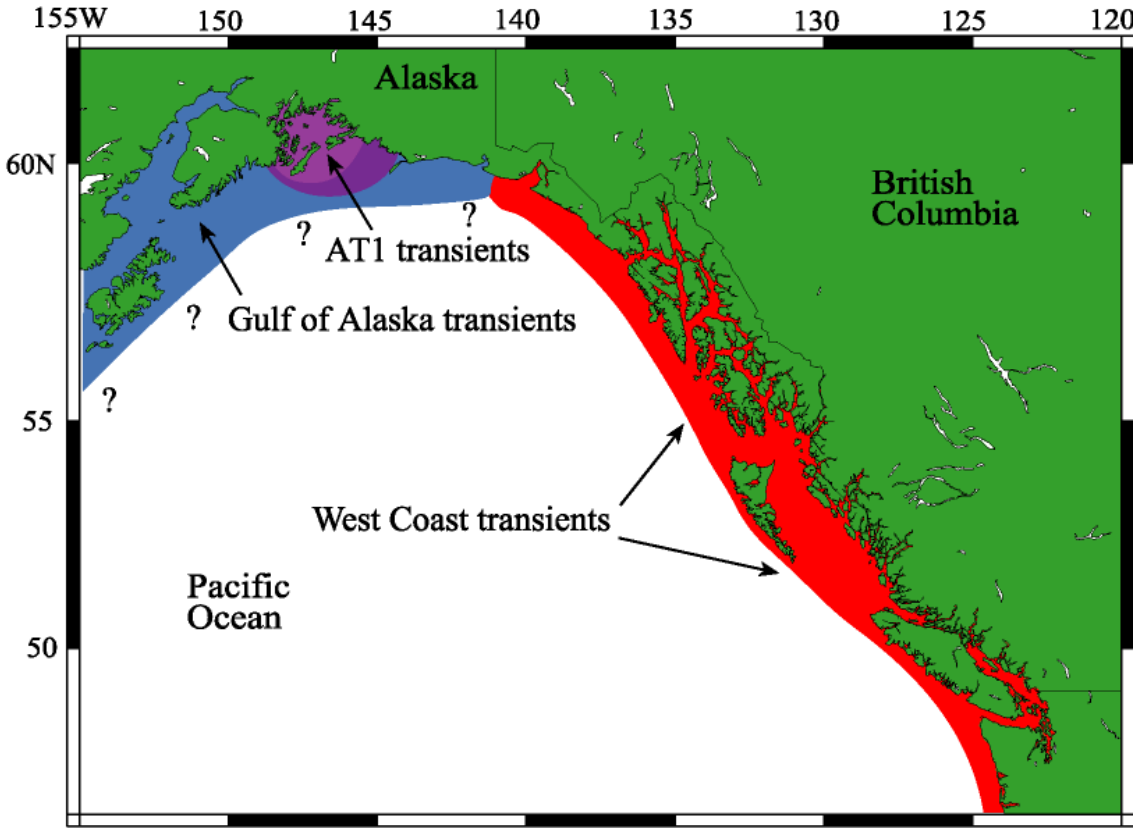

Figure 2. Approximate ranges of transient killer whale subpopulations in the northeastern Pacific Ocean, based on Barrett-Lennard et al. 1995, Ford and Ellis 1998, Matkin et al. 1999b. The western extent of the range of the Gulf of Alaska transients is conjectural—most sightings have been made between Kodiak Island and Prince William Sound. Intermediate colour indicates region of overlap.

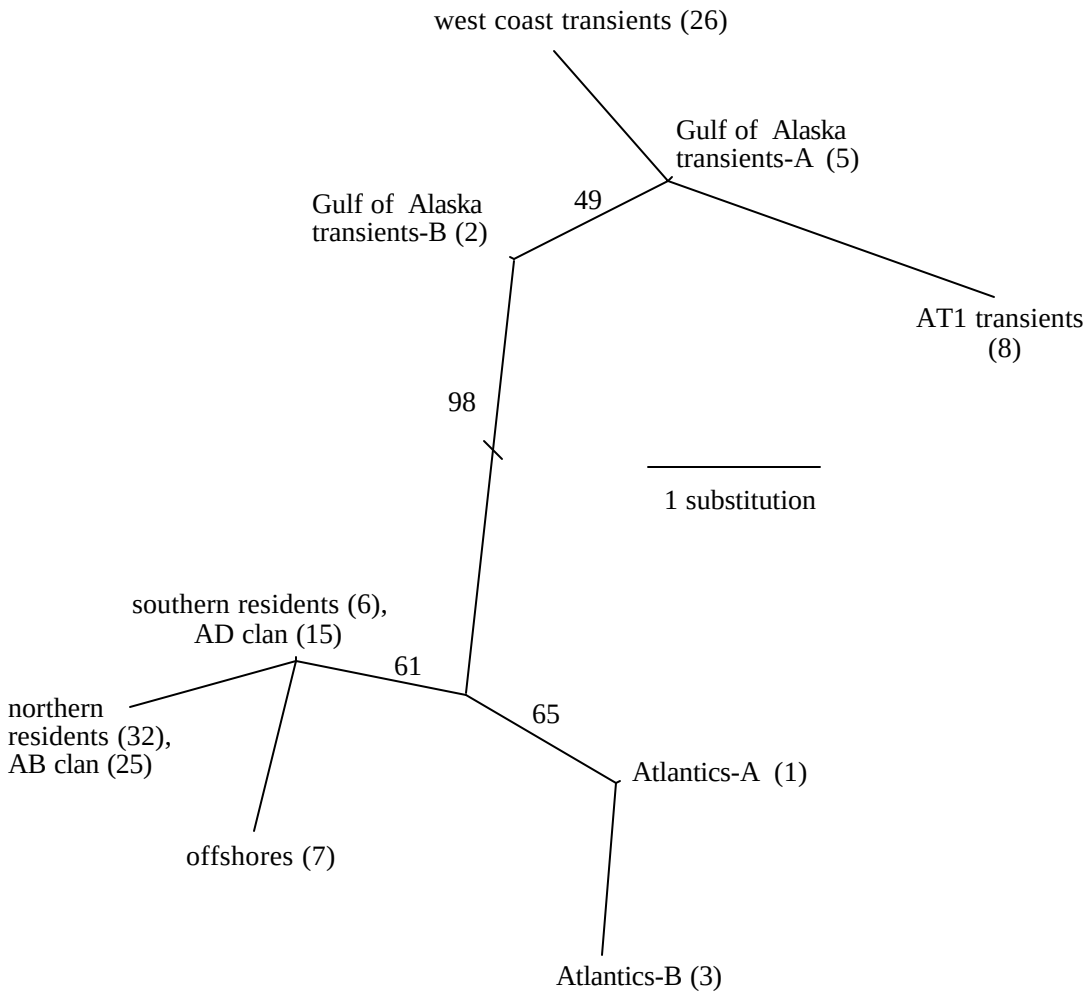

Figure 3. Maximum likelihood phylogram based on seven Pacific and two Atlantic killer whale mitochondrial D-loop haplotypes. The numbers on branches indicate percentage bootstrap support (see methods). The number of whales sequenced with each haplotype is shown in brackets. AB and AD refer to two acoustic clans of Alaska residents (see Table 2). The suffixes A and B indicate two different haplotypes from the same subpopulation or, in the case of the Atlantics, the same ocean. The length of the longest branch was reduced by half in this drawing (slash mark).

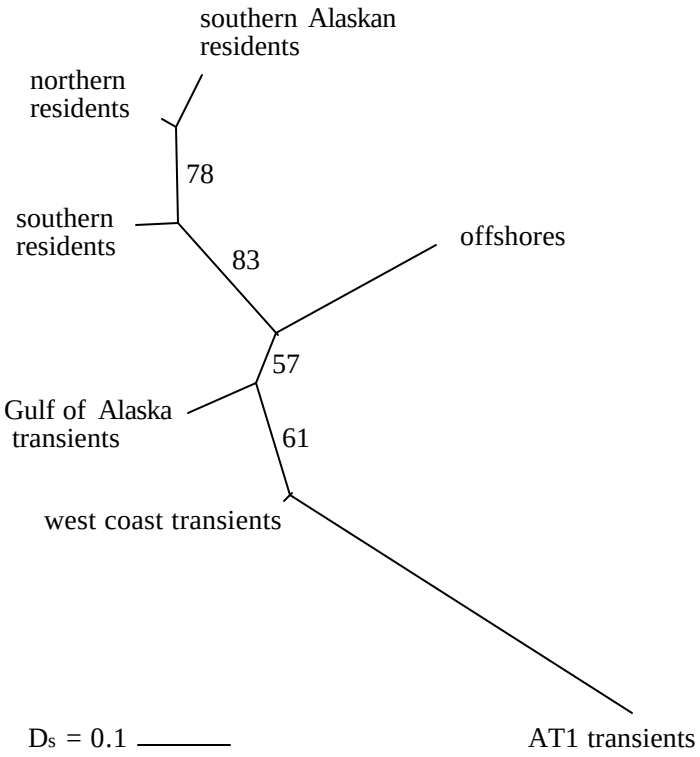

Figure 4. Unrooted neighbour-joining phylogram for Alaskan and British Columbian killer whales based on 11 microsatellite loci, using Nei's standard genetic distances. The numbers give percentage bootstrap support. When the offshore population was removed, support for the resident/transient separation was 97%. Atlantic killer whales were not included in this analysis because of their small sample size.