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SHORT COMMUNICATION

Low mtDNA genetic diversity among killer whales around New Zealand

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We report here the genetic diversity of killer whales around New Zealand and compare samples collected in this region (n = 11) with larger geographic databases of mtDNA control region sequences to investigate the relationship of the New Zealand killer whales with more distant populations/ecotypes. Eight variable sites defined four haplotypes, revealing a low mtDNA genetic diversity when compared with other cetacean species and to that observed worldwide for killer whales. The geographic distribution and segregation of haplotypes within New Zealand suggested that this population could be geographically structured. Only one of the New Zealand haplotypes matched with those from other distant regions (the Eastern North Atlantic and Western South Atlantic populations).

Keywords: *Orcinus orca*; killer whale; mtDNA; genetic diversity; New Zealand

Introduction

The killer whale *Orcinus orca* (Linnaeus, 1758) is reported around New Zealand coastal and offshore waters, off subAntarctic islands and the Ross Sea Dependency, Antarctica (Thomas et al. 1981; Baker 1999; Visser 2000; Department of Conservation 2012a,b) (Fig. 1). It is believed that a population of around 100–200 killer whales frequent the main islands of New Zealand (Visser 2000). It has been proposed, although not confirmed, that this population is divided into several subpopulations (Visser 2000). Extensive movements around the main islands of New Zealand have been documented for a few individuals occurring regularly within coastal waters (Visser 1999a, 2000). This might be related with seasonality, as it has been observed in other areas around the world (Baird 2000). Less is known about killer whales around the New Zealand offshore and subAntarctic islands (Kermadec, Chatham, Antipodes, Snares, Auckland and Campbell; Baker 1999; Visser 2000); however, they have been regularly recorded on the Antarctic Ross Sea Dependency (Thomas et al. 1981; Andrews et al. 2008; Ainley et al. 2009; Lauriano et al. 2011).

Four morphological forms or ecotypes of killer whales have been described in the southern hemisphere (referred to here as Types A–D; Pitman & Ensor 2003; Pitman et al. 2011). Differences among the four forms is supported by historical (Mikhalev et al. 1981; Berzin & Vladimirov 1983)

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Supplementary file 1: Table 1. Details of sample collection from killer whales (*Orcinus orca*) in New Zealand waters; **Supplementary file 2:** Figure 1. Photographs of four New Zealand killer whales (*Orcinus orca*) sampled for this genetic analysis (sample codes Oor03NZ07, Oor03NZ08, Oor05NZ12, Oor05NZ13). Photographs of individuals associated to sample Oor97NZ01 can be found in Visser & Fertl (2000).

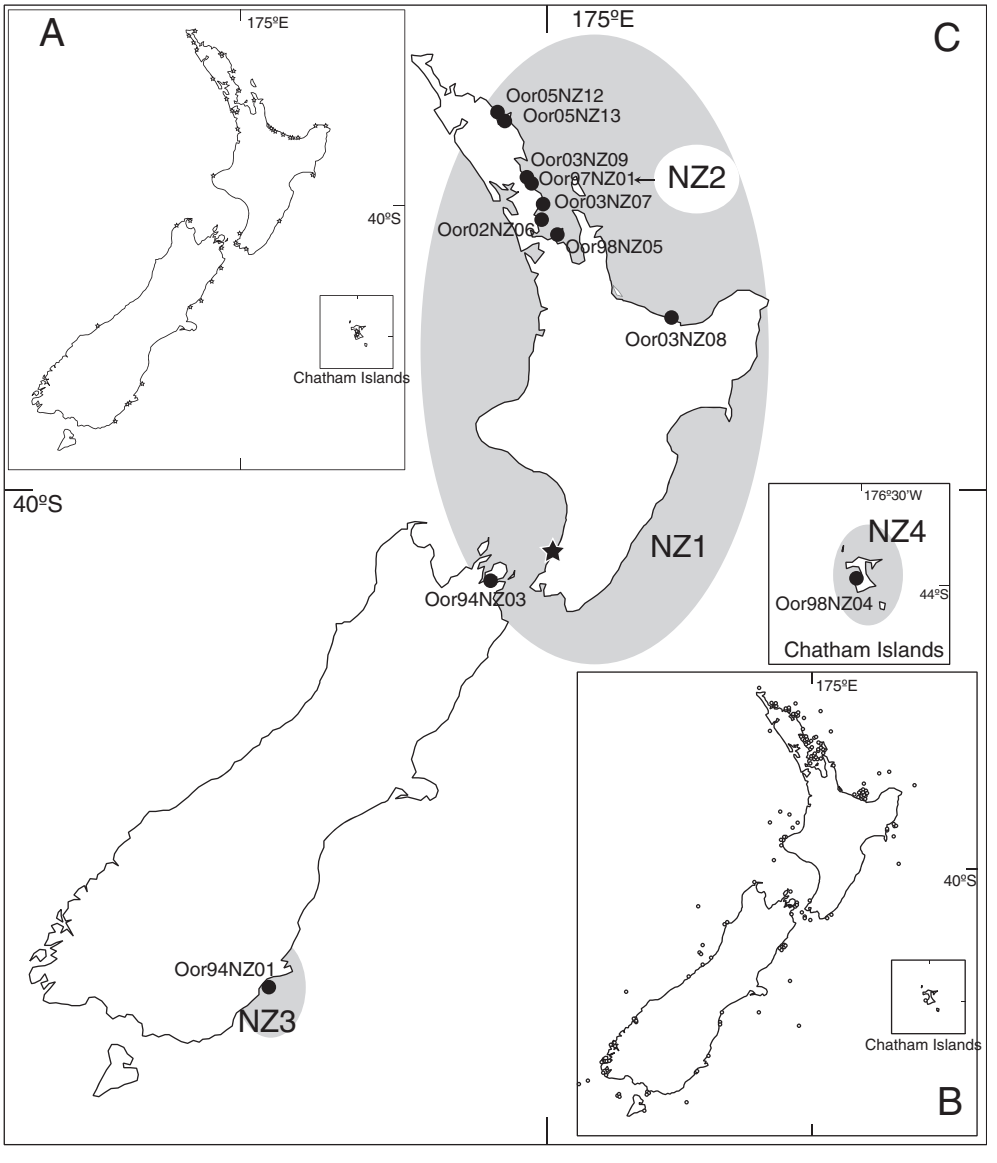

Figure 1 The distribution of killer whales (*Orcinus orca*) around New Zealand. **A**, Strandings (from 1915 to 2009; Department of Conservation 2012b). **B**, Sightings (from 1985 to 2009; Department of Conservation 2012a). **C**, Location of samples collected around New Zealand utilised in this analysis, including their haplotype assignment (sample code associated to the database of New Zealand Cetacean Tissue Archive, University of Auckland). Location of collection for Type D killer whale from Foote et al. (2013) is shown with a filled star.

and contemporary (Pitman et al. 2007) morphological data, diet specialisation (Berzin & Vladimirov 1983) and genetic analysis (LeDuc et al. 2008; Morin et al. 2010; Foote et al. 2013). New Zealand is the only place in the southern hemisphere where three out of the four forms of killer whales have been reported (Visser 1999b; Pitman & Ensor 2003; Pitman et al. 2011; Foote et al. 2013). As these

southern hemisphere forms have been described only recently, little is known about their distribution or movements.

It is believed that dependence on local prey contributes to non-random distribution and population differentiation among killer whales in other parts of the world, resulting in different foraging specialists. In the North Pacific, there is reduced gene flow among three different ecotypes: fish-eating 'residents'; marine mammal-eating 'transients'; and 'offshore' feeding on marine fish and, possibly, other offshore prey (Hoelzel et al. 1998; Ford et al. 2000; Hoelzel et al. 2007; Krahn et al. 2007; Dahlheim et al. 2008; Morin et al. 2010; Pilot et al. 2010). Benthic foraging of stingrays by killer whales has been reported to occur commonly in New Zealand, but seems uncommon elsewhere (Visser 1999a; Duignan et al. 2000).

Previous genetic analyses have included a small number (n = 5) of New Zealand killer whale samples for worldwide phylogeography studies, identification of putative forms/species and estimation of divergence times among types (Hoelzel et al. 2002b; Morin et al. 2010; Foote et al. 2013). Here, we report the genetic diversity of killer whales around New Zealand, and compare samples collected in this region with larger geographic databases (Hoelzel et al. 2002b; Morin et al. 2010; Foote et al. 2013) to investigate the relationship of the New Zealand killer whales with more distant populations/ecotypes.

Materials and methods

A total of 11 samples were available for analysis (Fig. 1, SF1). One sample was collected in the Chatham Islands, eight on the North Island and two on the South Island of New Zealand. Nine skin samples were collected from stranded whales. Four samples were collected from solitary live stranded animals, which were refloated and returned to the sea, and five were collected from dead stranded individuals. Two samples were collected from wild-ranging killer whales in the Bay of Islands, North Island, using a biopsy system (Krützen et al. 2002). A comparison of post ocular patches and dorsal fin photographs of three of the refloated stranded killer whales and the two wild-ranging killer whales indicated that they corresponded to different individuals. One sample included in our analysis was included previously in a published study (Hoelzel et al. 2002b). All samples were stored in 70% ethanol at −15 °C and archived in the New Zealand Cetacean Tissue Archive at the University of Auckland (Thompson et al. 2013).

Genomic DNA was extracted using a standard phenol/chloroform extraction protocol (Baker et al. 1994). Symmetrical amplification of the mtDNA control region was performed via the polymerase chain reaction (PCR Saiki et al. 1988) following standard protocols (Palumbi 1996). Two overlapping 800 base pair (bp) fragments of the mtDNA control region were amplified using the primers light-strand tPro-whale M13Dlp1.5 and heavy-strand Dlp8G (Garrigue et al. 2004), and the primers Dlp7 (5′-CCYCTTAAATAAGAC ATCV-CAATGG-3′) and M13-t-phe (5′-TGTAAAACGA CGGCCAGTTANNCATTTTCAGTGYWTTGCT TT-3′). Thermocycle profiles consisted of initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 40 s, and extension at 72 °C for 40 s. A final extension period at 72 °C for 10 min was included. PCR products were cleaned with Exo-SAP-IT (USB) and sequenced in both directions with BigDye™ terminator chemistry on an ABI3 100 DNA sequencer (Applied Biosystem), using the same amplification primers as sequencing primers. Sequencher™ (version 4.1.2, Genes Codes Co.) was utilised for the alignment and assembly of the overlapping sequences. The program MacClade version 4.0 (Maddison & Maddison 2000) was used to identify variable sites and unique haplotypes. Genetic diversity was estimated at haplotype (h) and nucleotide (π) levels using Arlequin version 3.5.2.1 (Excoffier et al. 2005). Molecular identification of sex was based on amplification of *sry* fragment following the protocol of Gilson et al. (1998) and confirmed by field morphological observations of dorsal fin (Heyning & Dahlheim 1988).

Sequences were compared to the worldwide sample datasets reported by Hoelzel et al. (2002b) and Morin et al. (2010), including also the recent

sequence of a Type D killer whale obtained by Foote et al. (2013), to identify shared haplotypes. Morin et al. (2010) and Foote et al. (2013) complete mitochondrial genome data was trimmed to match the consensus region used in our analysis. In addition to the previous dataset, a sequence from Colombia (sample provided by S. Caballero), one from Chile (sample provided by J. Acevedo) and seven from the Japan and Korea meat markets were included in our analysis for comparisons (for more details on Japan and Korea markets surveys see Baker et al. 1996, 2000, 2006).

Results

The full length of the mtDNA control region (915 bp) was sequenced from seven of the individual New Zealand killer whales and nearly complete lengths from three other individuals (SF1). One of the samples could be sequenced for only the first 582 bp of the control region. The molecular sex identification confirmed the sex identification in the field of all the samples: nine males and two females. A total of eight variable sites defined four haplotypes (Table 1).

Among the 11 individuals, the most common haplotype (NZ1) was represented by eight individuals from the North Island and the northern tip of the South Island (Fig. 1). The other three haplotypes (NZ2, NZ3 and NZ4) were represented by only one individual each. Two of these were observed in the southern part of the South Island, the Chatham Islands and one in the North Island, within the range of the common NZ1 haplotype.

Given the finding of only four haplotypes and the relatively small sample size, the mtDNA diversity observed in the New Zealand population was low compared with other cetacean species (New Zealand killer whales: $\pi = 0.0018 \pm 0.0013$ and $h = 0.491 \pm 0.175$; see Pichler & Baker [2000] and Hoelzel et al. [2002a]) for values of genetic diversity on cetacean species) and to that observed at worldwide level for killer whales ($\pi = 0.0052 \pm 0.0031$, Hoelzel et al. 2002b) and some regional populations of odontocetes.

The New Zealand killer whale mitochondrial sequences obtained here were compared to

Table 1 Variable sites of New Zealand killer whales (*Orcinus orca*) haplotypes compared to haplotype ANTRS from Hoelzel et al. (2002b) representing a Type A killer whale and Morin et al. (2010) southern hemisphere killer whales Types A, B and C haplotypes. Numbers indicate the position in relation to the beginning of the control region 5′ end.

Code	62	86	98	107	113	267	273	280	281	301	389	406	454	455	476	500	523	536	908
ANTRS*	G	C	G	T	T	C	A	T	C	A	T	A	T	C	C	C	T	A	C
AntA1**	.	.	A	.	.	.	.	C	T	.	C	.	.	.	.	.	.	.	.
AntB1**	.	.	A	.	.	.	.	C	T	.	.	.	.	.	T	.	.	G	.
AntC3**	.	.	A	.	.	.	.	C	T	.	.	.	.	.	T	.	.	G	T
Type D**	.	.	A	C	C	.	G	C	T	G	C	G	.	T	.	.	C	.	.
NZ1***	.	.	A	.	.	.	G	C	T	T	.	.	.	.	.	.	.	.	.
NZ2†	A	.	A	.	.	.	G	C	T	T	.	.	.	.	.	.	.	.	.
NZ3††	.	T	A	.	.	T	G	C	T	.	.	.	C	T	.	T	.	.	.
NZ4†††	.	.	A	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.

*ANTRS haplotype reconstructed from Hoelzel et al. (2002b); **Genebank accession numbers. AntA1: GU187217, AntB1: GU187215 and AntC3: GU187207 from Morin et al. (2010) and Type D: KF164610 from Foote et al. (2013); ***Matched with haplotype WSPNZ1 in Hoelzel et al. (2002b) and SWPUNZ haplotype in Morin et al. (2010) datasets; †Matched with haplotype WSPNZ2 in Hoelzel et al. (2002b) dataset; ††Genebank accession number KC465959; †††Genebank accession number KC465960.

haplotypes from other geographic populations around the world. Two of the four New Zealand haplotypes (NZ3 and NZ4) did not match with other haplotypes from either datasets (sequences were deposited in GenBank accession no. KC465959 and KC465960, respectively). Our NZ1 haplotype corresponded to one of the haplotypes from New Zealand (WSPNZ1) previously reported by Hoelzel et al. (2002b) and one (SWPUNZ) reported by Morin et al. (2010). Our NZ2 haplotype matched with one haplotype from Hoelzel et al. (2002b); WSPNZ2), for the reason that this individual (Oor97NZ01) was included in both studies. The only New Zealand haplotype shared with any other region around the world was NZ1. Based on the available sequence length this haplotype is shared with whales from the Eastern North Atlantic and Western South Atlantic populations. It is possible that this identity is the result of a weak phylogeographic signal in the killer whale mtDNA control region, or of recent common ancestry of these extensively separated populations, as has been recently shown by Morin et al. (2010).

Discussion

The geographic distribution and segregation of the New Zealand mtDNA haplotypes obtained here suggest that this population could be geographically structured. The mostly non-overlapping distribution of the four haplotypes suggests some degree of segregation that may be the result of a reduced number of matrilineal groups and some degree of local philopatry. A more intensive and wide-ranging sampling is needed to assess this, together with the incorporation of demographic data into the analysis.

An alternative explanation for the observed geographic distribution of haplotypes could be that the different haplotypes represent the different morphological forms or ecotypes of killer whales reported in New Zealand (Visser 1999b, 2000; Pitman & Ensor 2003; Pitman et al. 2011). Photographic records indicate that five of our New Zealand samples (Oor97NZ01, Oor03NZ07, Oor03NZ08, Oor05NZ12, Oor05NZ13; SF Fig. 1), which included both NZ1 and NZ2 haplotypes, were collected from the type of killer whales found worldwide (Pitman & Ensor 2003). It is not possible to rule out that the more geographically and genetically distant killer whales from Chatham Islands and South Island represent different forms as no photographic records were available. However, two specific fixed differences reported for Types B and C killer whales (in positions 476 [T] and 536 [G] of the mtDNA control region; Morin et al. 2010), were not present in New Zealand killer whales. This suggests that these individuals did not correspond to either of these forms (SF Table 1). The comparison of New Zealand haplotypes with the recently reported sequence of a Type D killer whale (Foote et al. 2013) also confirmed that our samples did not represent this poorly described form.

Supplementary files

Supplementary file 1: Table 1. Details of sample collection from killer whales (*Orcinus orca*) in New Zealand waters.

Supplementary file 2: Figure 1. Photographs of four New Zealand killer whales (*Orcinus orca*) sampled for this genetic analysis (sample codes Oor03NZ07, Oor03NZ08, Oor05NZ12, Oor05NZ13).

Photographs of individuals associated to sample Oor97NZ01 can be found in Visser & Fertl (2000).

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