MOLECULAR ECOLOGY

Molecular Ecology (2009) **18**, 5207–5217 doi: 10.1111/j.1365-294X.2009.04407.x

Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations

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Abstract

Ecological divergence has a central role in speciation and is therefore an important source of biodiversity. Studying the micro-evolutionary processes of ecological diversification at its early stages provides an opportunity for investigating the causative mechanisms and ecological conditions promoting divergence. Here we use morphological traits, nitrogen stable isotope ratios and tooth wear to characterize two disparate types of North Atlantic killer whale. We find a highly specialist type, which reaches up to 8.5 m in length and a generalist type which reaches up to 6.6 m in length. There is a single fixed genetic difference in the mtDNA control region between these types, indicating integrity of groupings and a shallow divergence. Phylogenetic analysis indicates this divergence is independent of similar ecological divergences in the Pacific and Antarctic. Niche-width in the generalist type is more strongly influenced by between-individual variation rather than within-individual variation in the composition of the diet. This first step to divergent specialization on different ecological resources provides a rare example of the ecological conditions at the early stages of adaptive radiation.

Keywords: Atlantic, ecotype, killer whale, *Orcinus orca*, Phylogenetics

Received 12 July 2009; revision received 22 September 2009; accepted 23 September 2009

Introduction

Adaptive radiation is the process of diversification from a single ancestral form into a variety of ecological or geographic niches to produce new ecologically specialized forms (Gavrilets & Losos 2009). Ecological diversification leads to divergence in morphological and other phenotypic traits through phenotype plasticity and genetic divergence resulting in resource or trophic polymorphisms, that may represent the incipient stages of speciation (Smith & Skúlason 1996). Theoretical approaches have modelled mechanisms that allow this process to occur in allopatry or sympatry where the absence of geographic barriers to gene flow should erode differentiation (e.g. Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2003). These theoretical models are being increasingly supported by empirical evidence from a range of studies, which identify ecological factors as the driver of speciation (e.g. Funk *et al.* 2006). Phylogenetic tests combined with studies of the micro-evolutionary processes of ecological diversification at its early stages can provide a useful approach to investigating the influence of ecology on evolutionary divergence (e.g. Huber *et al.* 2007; Steinfartz *et al.* 2007; Wolf *et al.* 2008).

Killer whales (*Orcinus orca*) are widely distributed throughout the world's oceans (Forney & Wade 2007), however the worldwide genetic diversity of killer whales, based on mtDNA control region sequences, is low and consistent with a historical bottleneck followed by rapid expansion (Hoelzel *et al.* 2002). Despite this

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low genetic diversity several disparate types or forms of killer whale have been characterized through behavioural observations (Ford *et al.* 1998; Pitman & Ensor 2003), tooth wear (Caldwell & Brown 1964; Dahlheim *et al.* 2008), morphometric measurements (Pitman *et al.* 2007) and stable isotope measurements (Herman *et al.* 2005). Three types have been described in the North Pacific; a marine-mammal eating 'transient' type, a fish-eating nearshore 'resident' type and an 'offshore' type also thought to be pisciverous (Ford *et al.* 1998; Herman *et al.* 2005). Phenotypic differences between types are mostly plastic, behavioural traits (e.g. Barrett-Lennard *et al.* 1996; Foote & Nystuen 2008), with only subtle morphological variation in fin shape and pigmentation patterns qualitatively described to date (Baird & Stacey 1988). Morphological differences in both quantitative and qualitative traits such as body length and pigmentation patterns are much greater between three Antarctic types (Pitman & Ensor 2003; Pitman *et al.* 2007) despite relatively low levels of sequence variation in the mtDNA control region (LeDuc *et al.* 2008). Observations suggest that the larger Antarctic type A killer whales specialize in predating minke whales, Antarctic type B killer whales are seal-eating specialists and Antarctic type C are a pisciverous dwarf form (Pitman & Ensor 2003; Krahn *et al.* 2008).

Here we provide evidence for the occurrence of two sympatric, morphologically disparate types in the North Atlantic characterized using two correlates of feeding behaviour: nitrogen stable isotope analysis and measurements of apical tooth wear; both of which have been shown to differ substantially between North Pacific types (Caldwell & Brown 1964; Herman *et al.* 2005; Dahlheim *et al.* 2008).

Materials and methods

Sample collection

Epidermal samples were obtained from necropsy from stranded animals or remote biopsying of free-ranging animals (e.g. Palsbøll *et al.* 1991) concurrent with behavioural observations or stomach contents in the case of some stranded samples. Sample storage varied, with some samples wrapped in aluminium foil and frozen at -20 °C on collection without preservative, others were stored in 20% dimethyl sulphoxide (DMSO) saturated with salt or 70% ethanol. Tooth and bone samples were obtained from museum and private collections using the sampling methodology of Morin *et al.* (2006). To ensure that no duplicate samples were included if a specimen was incomplete, only clearly labelled samples were included. A total of 125 samples from across the North Atlantic were used in this study. Sixty-four were epidermis samples used only to generate a reference library of DNA sequence variation to target regions of maximum sequence variation in tooth and bone samples. The remaining 61 samples were those for which we had body length measurements and/or data on tooth wear and consisted of tooth or bone from 50 individuals, tooth or bone and epidermis from three individuals and epidermis only from eight individuals.

DNA extraction, PCR amplification and sequencing

DNA was extracted from epidermis using the Qiagen DNeasy (Qiagen DNeasy) kit following the manufacturer's guidelines. DNA was extracted and purified from approximately 0.01–0.19 g (sample dependent) of powdered bone or tooth following Yang *et al.* (1998). Blank extractions were included every five samples to monitor for contamination. Given polymorphism was found in the first 400 bases (see results), we limited the PCR amplification of the tooth and bone samples to the 5′ fragment of the mtDNA control region using primers H16498 (5′-CCT GAA GTA AGA ACC AGA TG-3′) and L15812 (5′-CCT CCC TAA GAC TCA AGG AAG-3′; Zerbini *et al.* 2007), plus an additional non-overlapping 130 bp fragment of the mtDNA control region which contains polymorphic nucleotide sites diagnostic of each of the North Pacific types using primers DH6 (5′-AAA TAC AYA CAG GYC CAG CTA-3′) and DL5 (5′-CCY CTT AAA TAA GAC ATC TCG- ATG G-3′; Morin *et al.* 2006). The amplicons correspond to nucleotide positions 001-426 and 502-632 of the mtDNA control region as per LeDuc *et al.* (2008).

Each 25 μL PCR contained 1 μL extracted DNA, 1× PCR buffer, 1 mM $MgCl_2$, 400 nM of each primer, 0.1 mM mixed dNTPs and 0.1 μL AmpliTaq Gold enzyme (Applied Biosystems). PCR amplifications were performed using an MJ Thermocycler with a 4 min activation step at 95 °C, followed by 50 cycles of 95 °C for 20 s, 54 °C for 20 s, 72 °C for 20 s, followed by a final extension period of 72 °C for 7 min. To guard against the incorporation of erroneous data derived from DNA damage or contamination, the PCR amplification and sequencing process was replicated twice for each sample. Furthermore PCR negative controls without DNA were included for every three samples amplified to monitor for contamination during the PCR set up. All DNA extraction and PCR set up of archived museum samples was performed in a dedicated clean laboratory. The amplified PCR products were purified using an Invitek PCRapace purification kit (PCRapace, Invitek). Purified products were sequenced in both directions using the PCR primers and ABI sequencing chemistry by the Macrogen commercial sequencing service (Macrogen).

Genetic data analysis

The generated sequences were aligned against previously published sequences for the same genetic region (Hoelzel *et al.* 2002; LeDuc *et al.* 2008) using Clustal_W as implemented in the software package GENEIOUS 4.6 (Drummond *et al.* 2009).

Phylogenetic relationships based on the sequence data were estimated using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. ML phylogenetic analysis of the sequence data was performed using PHYML 3.0 (Guindon & Gascuel 2003). The best-fit model of nucleotide substitution was selected by comparing the GTR (Generalized Time Reversible), HKY85 (Hasegawa *et al.* 1985), JC69 (Jukes & Cantor 1969), K80 (Kimura 1980), and TN93 (Tamura & Nei 1993) models and selecting the model that gave the highest log-likelihood. The transition/transversion ratio, the proportion of invariable sites, the gamma distribution and the starting tree, estimated using a BIONJ algorithm (Gascuel 1997), a variant of the neighbour-joining algorithm (Saitou & Nei 1987), were also estimated by PHYML 3.0. The reliability of the optimized tree was estimated using the approximate Likelihood Ratio Test method with Shimodaira-Hasegawa-like support (Anismova & Gascuel 2006). BI phylogenetic analysis of the sequence data was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) using the GTR + gamma model as selected above. Four independent Monte Carlo Markov chains (MCMC) were run simultaneously for 1 500 000 generations with the current tree and parameter values sampled every 100 generations. Convergence was judged to have occurred when the standard deviation of split frequencies was <0.01 after 1 500 000 generations resulting in 15 000 trees. The potential scale reduction factor (PSRF) was 1.0 for all parameters. The majority-rule consensus tree was summarized from 11 250 trees, as 3750 (25%) were discarded as burn-in. Support for clades is expressed as posterior probabilities. The phylogenies were rooted with an outgroup sequence of false killer whale (*Pseudorca crassidens*) from GenBank (Accession no. EF601220).

Tooth wear measurement

The degree of apical tooth wear (TW) was quantified as an index based on the methodology of Labrada-Martagón *et al.* (2007):

$$\mathrm{TW} = \sum qk_q n$$

where q is the degree of tooth wear (0 = none; 1 = wear up to quarter of the crown height; 2 = wear up to half the crown height; 3 = wear of more than half the crown height), k_q is the number of teeth worn to level q, and n is the total number of teeth. Broken teeth were excluded from the analysis.

Stable isotope analysis

Tooth, bone & muscle samples were used for stable isotope analysis. As in most cases only small quantities (<1 g) of material were sampled to minimize damage to specimens, we were unable to remove the inorganic carbon by decalcification with HCl (see Mendes *et al.* 2007). For 15 samples we had sufficient material (>5 g) to decalcify with HCl (0.5N) and measure δ^{15}N and δ^{13}C values both decalcified and untreated portion. Approximately 0.4 mg of treated sample and 2.5 mg of untreated sample were placed in tin capsules and nitrogen and carbon isotope analyses performed simultaneously using continuous-flow isotope ratio mass spectrometry. Replicate measurements of internal laboratory standards (gelatine, two isotopically disparate alanines, and tryptophan) indicate a precision of 0.1‰.

Offspring that are still nursing on their mother's milk produced by catabolism of her own tissues will effectively be feeding at a higher trophic level than her (Mendes *et al.* 2007; Newsome *et al.* 2009), and were therefore excluded when comparing mean δ^{15}N or variance between haplotypes. Ontogenetic shifts in δ^{15}N in Pacific killer whales suggest a post-weaning age of 4 years (Newsome *et al.* 2009). From length measurements of Norwegian killer whales, individuals of length greater than 410 cm had four or more dentine growth layers (Christensen 1984) and should be of post-weaning age. Individuals of 410 cm or less (n = 8) for which we obtained δ^{15}N values were thus excluded from inter-haplotype δ^{15}N comparison.

Differences in trophic level between haplotypes were measured by comparing mean δ^{15}N using a 1-way ANOVA. Variance in δ^{15}N values was taken as a measure of trophic niche width (Bearhop *et al.* 2004) and homogeneity of variance was compared between haplotypes using a variance ratio test (F-test).

Results

Tooth wear, morphological and genetic divergence

We classified 37 sub-adult or adult specimens with tooth wear TW > 0.5 as 'Type 1'. In contrast five sub-adult or adult specimens exhibited little (TW < 0.5) or no apical tooth wear and were classified as 'Type 2' (Fig. 1). The degree of apical tooth wear in type 1 specimens appears to progress at relatively similar rates for at least the three haplotypes for which we had several samples (Fig. 1d). During behavioural observations and

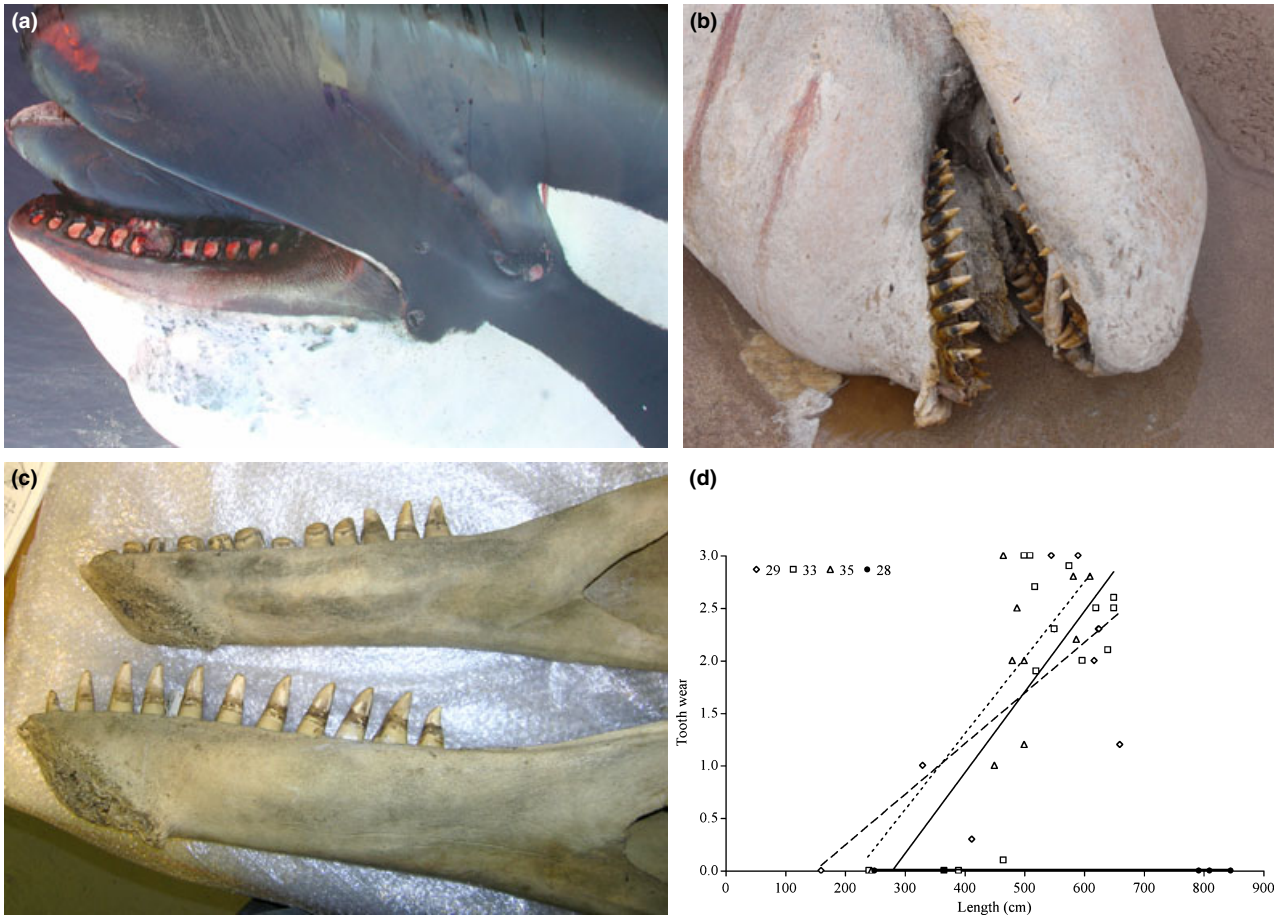

Fig. 1 Variation in the degree of apical tooth wear in North Atlantic killer whales (a) an adult Type 1 specimen with severe apical tooth wear (TW = 3.0), (b) an adult Type 2 specimen with no apical tooth wear (TW = 0.0). (c) A comparison of an adult Type 1 mandible and an adult Type 2 mandible showing differences in apical tooth wear and tooth count. (d) Apical tooth wear progresses with increasing body size at a similar rate in three Type 1 haplotypes (29, 33, 35), there is no evidence of apical tooth wear in even the largest Type 2 samples (haplotype 28), which are up to 185 cm larger than the largest Type 1 specimen. Photo credits (a) Rob Deaville, ZSL/UK Cetacean Strandings Investigation Programme (b) Robert Murray, (c) Andrew Foote.

necropsies we noted apical tooth wear in killer whales feeding on mackerel and herring (see Supporting Information). One specimen (sample 49) previously reported to have worn teeth (McHugh *et al.* 2007) had teeth that had become worn subsequent to being broken rather than the progressive wear seen in the other specimens and teeth wear and so was not classified as either type based on tooth wear.

From our 125 samples we successfully amplified and sequenced the variable region of the mtDNA control region of 122 samples, including 57 of our 61 samples for which we had body length measurements and/or data on tooth wear, and identified 11 unique haplotypes (Table 1). Sequences generated from the epidermis samples of the mtDNA control region (989 bp) indicated that only the first 409 bp contained any variation (Table 1). Four haplotypes (27, 29, 33, 34) had been previously published (Hoelzel *et al.* 2002; see Supporting Information), whilst seven were novel to this study. As noted above, all the polymorphic nucleotide sites were found in the 5′region (Table 1). The GTR model gave the highest log-likelihood (−1848.89) and had the following parameters: gamma shape parameter = 0.4; a proportion of invariable sites = 0.81; equilibrium base frequencies of 0.30, 0.24, 0.14 and 0.32 for A, C, G and T nucleotides respectively; and GTR relative rate parameters of 419.6, 66887.3, 833.7, 1.0, 31512.2 and 1.0 for A-C, A-G, A-T, C-G, C-T and G-T substitutions respectively. The most divergent sequences (27 & 36) differed by only 0.7%. These findings are consistent with previous results showing low mtDNA diversity and divergence in this species (Hoelzel *et al.* 2002; LeDuc *et al.* 2008).

Type 1 constituted seven haplotypes (29–35), which included the haplotypes we also identified in samples collected from free-ranging killer whales feeding upon

Table 1 Polymorphic nucleotide sites in the mitochondrial DNA control region of 62 North Atlantic killer whales. *n* indicates the total number of individuals with that haplotype. Each haplotype consisted of just one ecotype, however some individuals were calves or the teeth were missing and so were not assigned to type. The number in parentheses indicates the number of independent sampling events, e.g. when more than one sampled individual had simultaneously stranded this would be considered a single sampling event

			Nucleotide site										
Haplotype	*N*	Type	9	83	112	122	158	205	276	278	304	326	409
27	2 (2)	2	A	T	C	T	C	C	A	C	A	A	A
28	7 (5)	2	—	.	.	.	.	.	.	T	.	.	.
29	13 (13)	1	.	.	.	.	.	.	G	T	.	.	.
30	1	1	—	.	.	.	.	.	G	T	G	.	G
31	1	1	—	.	.	C	.	.	G	T	T	.	.
32	1	1	—	.	.	.	.	T	G	T	T	.	.
33	20 (20)	1	—	.	.	.	.	.	G	T	T	.	.
34	1	1	—	C	.	.	.	.	G	T	T	.	.
35	9 (9)	1	—	.	.	.	.	.	G	T	T	G	.
36	1	u	—	.	T	.	T	T	G	T	T	.	.
37	1	u	—	.	.	.	.	.	.	T	.	.	G

herring around Iceland and Norway and mackerel in the North Sea (A.D. Foote, University of Aberdeen, unpublished data). Type 2 constituted just two haplotypes (27 & 28). There was a single fixed difference of a base substitution between the two ecotypes (Table 1), less than the variation within type 1 haplotypes and comparable to the difference between Antarctic type B and type C killer whales (LeDuc *et al.* 2008). The divergence between the type 1 and type 2 haplotypes was independent from the divergences between the North Pacific and Antarctic types (Fig. 2). Two haplotypes (36 & 37) could not be assigned to either type due to a lack of data on adult tooth wear, but based on the diagnostic polymorphic site, haplotype 36 would be classified as type 1 and haplotype 37, sampled from an individual predating a sperm whale in the Gulf of Mexico, would be classified as type 2. Five of the seven type 1 haplotypes and haplotype 36 fell within a well-supported monophyletic clade in both the BI and ML phylogenies (Fig. 2). The other type 1 haplotypes (29 & 30) had an unresolved polytomic relationship with the type 2 haplotypes, North Pacific resident and offshore haplotypes and an Antarctic type A haplotype in the BI phylogeny (Fig. 2a). The ML phylogeny partially resolved this polytomy and placed the type 2 haplotypes within a well-supported monophyletic clade containing most of the Antarctic haplotypes and most closely clustering them with a subset of Antarctic type A haplotypes (Fig. 2b).

The distribution of total body length of the two types showed bimodality *sensu* Schilling *et al.* (2002). Males were judged to be adults when over 600 cm in length based upon body length measurements for males with greater than 18 tooth dentine growth layers (Christensen 1984). Mean body length of type 1 adult males (630 cm) differed from the mean body length of type 2 adult males (816 cm) by 186 cm, greater than twice the common standard deviation (84 cm). This is consistent with bimodality of body size found in Norwegian whaling catch statistics (see Pitman *et al.* 2007), which indicated a maximum size of 6.5 meters for killer whales taken on the Norwegian herring grounds, but catches from across the NE Atlantic included individuals as large as 9 meters. We found differences in the mean number of teeth in the lower jaw (type 1, mean ± SD = 12 ± 0.44; type 2 mean ± SD = 11 ± 0; Fig. 1c) and pigmentation pattern (see Supporting Information).

Ecological divergence

The C/N ratios of treated samples ranged between 3.2–3.5 within the range expected for unaltered collagen (DeNiro 1985; Ambrose 1990). Removing the inorganic carbon had little or no effect on $\delta^{15}N$ measurements, but at least some of the untreated samples provided inaccurate $\delta^{13}C$ values (see Supporting Information). We therefore used only $\delta^{15}N$ values from both treated and untreated samples. In total 39 bone and tooth samples dating between 1844–1997 and one freeze-dried muscle sample from 2008 were analyzed in this way.

The mean $\delta^{15}N$ values between the four haplotypes (three type 1 haplotypes and one type 2 haplotype), for which >2 samples were measured, were not significantly different, ($F_{3,23} = 0.780$, $P = 0.517$; Table 2), suggesting no distinction in trophic position between type 1 and type 2. However the variance in $\delta^{15}N$ values, which provides a comparable estimate of trophic niche width (Bearhop *et al.* 2004), was significantly larger for

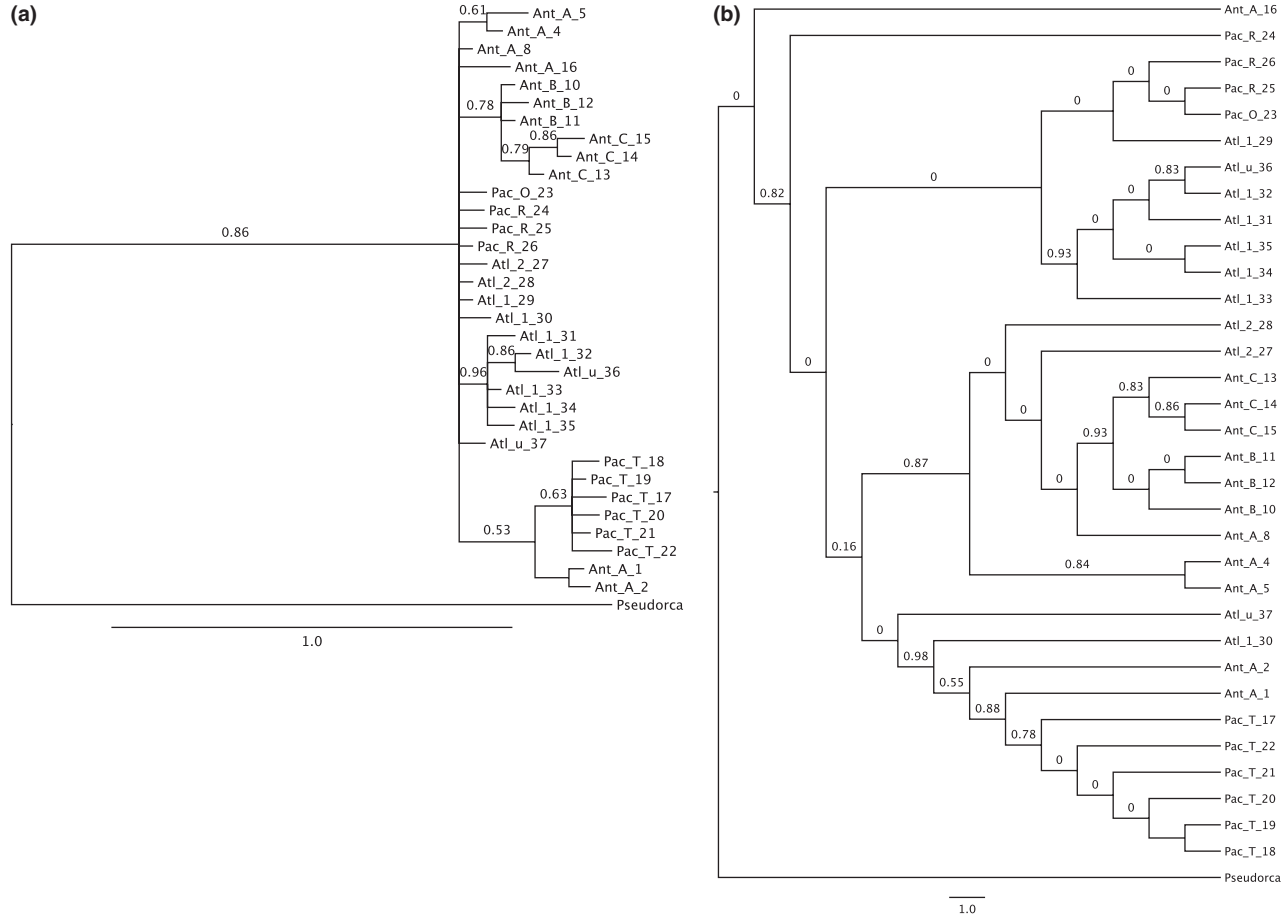

Fig. 2 Phylogenies of killer whale types estimated using (a) Bayesian Inference (BI) and (b) Maximum Likelihood (ML) methods. Node labels indicate prior probabilities for the BI tree and aLRT branch-support numbers for the ML tree. Taxa labels indicate the region (e.g. Atl for North Atlantic; Ant for Antarctic; and Pac for North Pacific), ecotype (e.g. 1 for type 1; 2 for type 2; u for unknown; T for transient; R for resident; O for offshore; A for type A; B for type B; and C for type C) and haplotype.

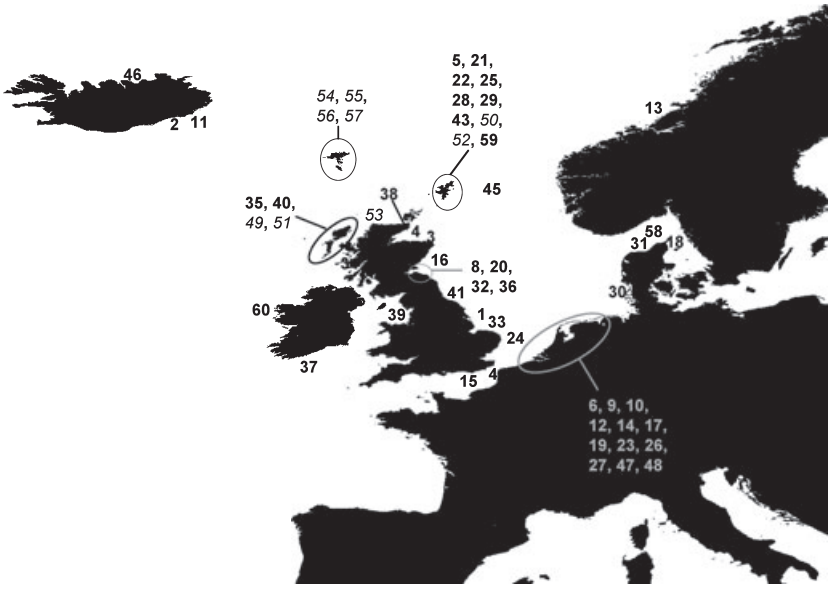

Fig. 3 Map of Northeast Atlantic showing the locations from where the *Orcinus orca* samples originated. Numbers correspond to Table 3. Samples with the two haplotypes associated with type 2 are shown in italics. Samples 7, 44, 61 & 62 originated from the Northwest Atlantic and their positions are therefore not show.

Table 2 Mean $\delta^{15}N$ stable isotope values (and SD) for North Atlantic killer whale haplotypes indicate trophic position. Variance indicates trophic niche width. *n* indicates number of individuals, the number in parentheses indicates the number of independent sampling events

Haplotype	Type	*n*	Mean	SD	Range	Variance
28	2	5 (4)	+14.9	0.3	14.6–15.3	0.1
29	1	7 (7)	+15.1	2.2	12.7–17.9	4.8
33	1	11 (11)	+16.0	2.4	12.7–20.1	5.7
35	1	4 (4)	+14.3	2.3	11.9–17.1	5.5

each of the type 1 haplotypes than for the type 2 haplotype (33/28: $F_{4,10} = 74.27$, $P < 0.001$; 35/28: $F_{3,4} = 71.08$, $P < 0.001$; 29/28: $F_{4,6} = 62.51$, $P < 0.001$; Table 2). There was no significant difference in the variance in $\delta^{15}N$ values between the three type 1 haplotypes (33/35: $F_{3,10} = 1.05$, $P = 0.900$; 33/29: $F_{6,10} = 1.19$, $P = 0.869$; 35/29: $F_{3,6} = 1.14$, $P = 0.814$; Table 2).

Discussion

Our data on tooth wear, $\delta^{15}N$ values and mtDNA sequences reveal ecological divergence at two taxonomic levels in North Atlantic killer whales. Firstly, differences in tooth wear indicate strong ecological contrast between two subsets of haplotypes, and $\delta^{15}N$ values indicate type 1 to be a generalist while the type 2 appears to be highly specialized. These differences are correlated with differences in morphological traits such as length, tooth count and pigmentation patterns, but only shallow divergence in mtDNA sequence. Secondly, our high $\delta^{15}N$ variances within each haplotype for the generalist type, suggest consistent individuality over a lifetime in the relative proportions of prey types consumed (Bearhop *et al.* 2004), indicating an ecological gradient within this type. All adult specimens with haplotypes 29–35 had significant tooth wear indicating that they share a common foraging method, e.g. suction-feeding on whole herring or mackerel. However, the range in $\delta^{15}N$ values suggests that subsets of individuals are additionally persistently feeding at a higher trophic level. This is consistent with observational data of small subsets of naturally marked identified individuals belonging to the Icelandic and Norwegian herring-eating populations persistently predating seals (Bisther & Vongraven 2001; Foote *et al.* in press).

The low variance of $\delta^{15}N$ values for type 2 specimens indicates a narrow niche width and suggests shared dietary specialization. Baleen from a minke whale was found in the stomach of one individual that we sampled (Eschricht 1866) and baleen whales, such as minke or fin whale, are approximately one trophic level less than type 2 specimens (Born *et al.* 2003; Das *et al.* 2003), assuming a isotopic enrichment of +3.4 per trophic level (Post 2002). Other cetaceans may therefore be the main prey of type 2 killer whales.

The sampling of our two types overlapped both geographically (Fig. 3; Table 3) and temporally indicating these different forms are sympatric. Type 1 specimens were found from Norway to Newfoundland (Fig. 3; Table 3). Our type 2 specimens were less widely spread having been sampled from Scotland and the Faeroe Islands. However, over 100 whales with body length greater than found for any type 1 specimens (700–850 cm), but within the body length range of type 2 specimens, were taken from across the North Atlantic by whalers during the last century (Pitman *et al.* 2007). This suggests type 1 and type 2 killer whales are both more widely distributed than the range over which our samples were obtained from.

The low genetic differentiation between North Atlantic killer whale haplotypes suggests a relatively rapid and recent divergence in phenotype. The apparent trophic diversification of type 1 individuals, and between types 1 and 2 is based on traits ($\delta^{15}N$ values and tooth wear) indicative of ecological segregation within a single generation. However our samples of both ecotypes were collected over a period of 165 years (mid-1800s–2008), at least 11 killer whale generations (based on a generation time of 15 years; Olesiuk *et al.* 1990). Reproductive isolation and phenotype divergence have been shown to evolve in fewer than 13 generations in wild populations with divergent ecology (Hendry *et al.* 2000). Additionally the mtDNA control region is just a single neutral loci and rapid phenotype divergence with little or no mtDNA sequence divergence has been noted previously in species with ecologically divergent forms (Grant & Grant 2008; Losos 2009).

The phenotypic differences in pigmentation, tooth count and body length could be due to phenotypic plasticity but would need to have a genetic basis to be maintained (Schluter 2000). Although our understanding of the ecology of killer whales in the Antarctic and North Atlantic is still far from complete, there does appear to be some initial evidence of parallel dietary specialization and body size that should be further explored. Such matching of phenotypic and ecological differences between two ocean basins would provide evidence for a genetic basis under directional selection, as oppose to simply phenotypic plasticity. However additional criteria, such as ruling out chance, would need to be met before such observational data would make a compelling case for a process such as character displacement (Schluter 2000). Although the two types described here are found in sympatry over a localized area of the North Atlantic (Fig. 3), the extent of this

Table 3 List of samples used in this study. Full institute names are given in the Supporting Information online, — indicates this information was not available

Sample No.	Location	Institute	Haplotype	Sex	Length (cm)	Tooth wear	δ15N (‰)
1	England	NHM	33	F	240	0.0	19.2
2	Iceland	ZMA	33	M	334	—	18.1
3	Scotland	NMS	33	—	366	0.0	16.5
4	Scotland	NHM	33	F	390	0.0	14.5
5	Scotland	NMS	33	M	465	0.1	16.3
6	Netherlands	ZMA	33	F	500	3.0	—
7	Greenland	Cop	33	F	510	3.0	12.7
8	Scotland	NMS	33	F	518	2.7	—
9	Netherlands	RMNH	33	F	520	1.9	17.8
10	Netherlands	ZMA	33	F	550	2.3	19.2
11	Iceland	HWM	33	F	550	—	13.4
12	Netherlands	NMR/RMNH	33	M	575	2.9	—
13	Norway	NHM	33	M	597	2.0	12.8
14	Netherlands	RMNH	33	M	600	—	20.0
15	England	NHM	33	M	620	2.5	15.9
16	Scotland	NHM	33	M	640	2.1	17.9
17	Netherlands	RMNH	33	M	650	2.5	—
18	Kattegat Sea	Cop	33	M	650	2.6	16.1
19	Netherlands	RMNH	33	—	—	1.6	18.2
20	Scotland	NMS	33	M	—	—	15.4
21	Scotland	SAC	34	F	345	1.0	—
22	Scotland	NMS	35	M	244	0.0	—
23	Netherlands	ZMA	35	F	450	1.0	—
24	England	NHM	35	F	480	2.0	15.3
25	Scotland	SAC	35	F	488	2.5	—
26	Netherlands	ZMA	35	F	500	2.0	—
27	Netherlands	RMNH	35	F	500	1.2	—
28	Scotland	SAC	35	M	587	2.2	—
29	Scotland	SAC/NMS	35	M	610	2.8	12.9
30	Denmark	Cop	35	F	582	2.8	11.9
31	Denmark	Cop	35	F	465	3.0	17.1
32	Scotland	NMS	31	F	—	2.8	13.3
33	England	NHM	32	F	—	0.9	12.9
34	Scotland	NMS	29	F	160	0.0	17.3
35	Scotland	NHM	29	M	330	1.0	15.3
36	Scotland	NMS	29	F	412	0.3	—
37	Ireland	UCC	29	F	545	3.0	—
38	Scotland	SAC/NMS	29	F	550	2.3	12.7
39	England	IOZ	29	M	590	3.0	—
40	Scotland	NMS	29	M	617	2.0	12.7
41	England	NHM	29	M	624	2.3	—
42	England	NHM	29	M	625	2.3	17.7
43	Scotland	NHM/NMS	29	M	660	1.2	14.1
44	Newfoundland	NMS	29	M	—	1.0	16.1
45	North Sea	UOA	29	M	—	3.0	—
46	Iceland	NMR	29	F	—	—	17.9
47	Netherlands	Ecomare	—	M	550	1.3	—
48	Netherlands	NMR/RMNH	—	M	578	1.0	—
49	Scotland	SAC/NMS	27	F	610	—	17.0
50	Scotland	NMS	27	M	—	0.4	15.5
51	Scotland	SAC	28	—	249	0.0	—
52	Scotland	NHM	28	M	792	0.1	14.9
53	Scotland	SAC	28	M	845	0.1	15.3
54	Faeroes	Cop	28	M	810	0.1	14.8
55	Faeroes	Cop	28	—	—	0.0	14.8
56	Faeroes	Cop	28	—	—	—	14.6

Table 3 *Continued*

Sample No.	Location	Institute	Haplotype	Sex	Length (cm)	Tooth wear	δ15N (‰)
57	Faeroes	Cop	28	—	365	0.0	14.7
58	Denmark	Cop	36	F	322	0.0	16.5
59	Scotland	NMS	—	M	366	0.0	—
60	Ireland	NHM	—	—	—	—	—
61	Greenland	Cop	30	—	—	0.6	18.0
62	Gulf of Mexico	SWFSC	37	M	—	—	—

ocean basin means we cannot rule out divergence during allopatry and subsequent secondary contact. However, the individuality in the diet of type 1 provides a mechanism by which morphologically distinct types could evolve in sympatry from a common ancestor (see Bolnick *et al.* 2003).

Sympatric, disparate killer whale types have now been documented in the three areas with greatest density (Forney & Wade 2007); the Northeast Atlantic, North Pacific and the Antarctic. Our phylogenies suggest that these niche segregations have arisen from multiple origins. Theory predicts that evolutionary branching can occur most easily along environmental gradients (Doebeli & Dieckmann 2003). Foraging specialization appears to be the environmental gradient in each case of evolutionary branching in killer whales that has allowed for rapid and disparate diversification. However for this diversification to be considered as incipient speciation requires evidence of reproductive isolation between types, which is so far lacking for Atlantic and Antarctic types, other than that inferred through the disparity of phenotypic traits. Maintaining biological diversity is a key goal for conservation and management bodies and identifying at risk 'Evolutionary Significant Units' (ESUs) can assist with this (Allendorf & Luikart 2007). Our genetic data alone are not currently sufficient to establish either type as an ESU; however the data on phenotypic disparity, which suggest individuals may not be 'exchangeable' between types (see Crandall *et al.* 2000), should also be considered and strongly suggest each type should be classified as an ESU.

Multiple rapid and extensive ecological divergences have been noted in a number of species with short generation times (e.g. Grant & Grant 2008; Losos 2009) but here we document a rare example of multiple rapid ecological divergences in a long-lived mammal.

Acknowledgements

We would like to thank the curators of the collaborating institutions for facilitating the use of specimens from their collections (see Supporting Information for full details). Neil Anderson, Stuart Angus, Rob Deaville, Mary Harman, Simon Ingram, Don O'Driscoll, Robert Murray and Emer Rogan generously provided photographs. We would like to thank John Durban, Bob Pitman, Rick LeDuc, Carlos De Luna Lopez and Kelly Robertson for useful discussions and for advice on sampling, and members of the Piertney lab group, University of Aberdeen and the Ancient DNA and Evolution group, University of Copenhagen for assistance in the lab. This manuscript benefited from comments from the editor and two anonymous referees. Funding was provided by Carnegie Trust for the Universities of Scotland, the Marie Curie Actions 'GENETIME' grant fund, the Danish National Science Foundation 'Skou' award program, the Marine Directorate of the Scottish Government, the National Environment Research Council, Scottish Natural Heritage and an Aberdeen University 6th Century Scholarship to Andrew Foote.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 (a) $\delta^{15}N$ values of decalcified and untreated samples from 15 individuals. (b) $\delta^{13}C$ values of decalcified and untreated samples from 15 individuals.

Fig. S2 (a) Type 1 killer whales; the eye patch has a parallel orientation and the anterior end is in front of the blowhole (photo credit Andrew Foote). This pigmentation pattern was found in photographic data of killer whales from Norway, Iceland, Shetland and the North Sea and in photographs of eight stranded type 1 individuals sampled in this study. (b) Type 2 killer whales; the eye patch has an angular orientation and the anterior end is behind the blowhole (photo credit Lewis Drydale, HWDT). This pigmentation pattern was found in a small community of individuals on the west coast of Scotland, and individuals off the Azores (Karin Hartman; Annette Scheffer unpublished data) and one type 2 individual sampled for this study.

Fig. S3 A free-ranging killer whale photographed feeding on mackerel in the North Sea exhibits severe apical tooth wear in the front teeth (photo credit Harriet Bolt).

Table S1 Table of haplotypes

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