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Research article

Ancient DNA from marine mammals: Studying long-lived species over ecological and evolutionary timescales

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SUMMARY

Marine mammals have long generation times and broad, difficult to sample distributions, which makes inferring evolutionary and demographic changes using field studies of extant populations challenging. However, molecular analyses from sub-fossil or historical materials of marine mammals such as bone, tooth, baleen, skin, fur, whiskers and scrimshaw using ancient DNA (aDNA) approaches provide an opportunity for investigating such changes over evolutionary and ecological timescales. Here, we review the application of aDNA techniques to the study of marine mammals. Most of the studies have focused on detecting changes in genetic diversity following periods of exploitation and environmental change. To date, these studies have shown that even small sample sizes can provide useful information on historical genetic diversity. Ancient DNA has also been used in investigations of changes in distribution and range of marine mammal species; we review these studies and discuss the limitations of such 'presence only' studies. Combining aDNA data with stable isotopes can provide further insights into changes in ecology and we review past studies and suggest future potential applications. We also discuss studies reconstructing inter- and intra-specific phylogenies from aDNA sequences and discuss how aDNA sequences could be used to estimate mutation rates. Finally, we highlight some of the problems of aDNA studies on marine mammals, such as obtaining sufficient sample sizes and calibrating for the marine reservoir effect when radiocarbon-dating such wide-ranging species.

1. Introduction

Marine mammals can live for several decades (some even a century or more, George and Bockstoce, 2008) and have long generation times, a trait that can make studies on changes in genetic diversity through time in response to anthropogenic impacts (such as whaling) and environmental change challenging. Typically, such studies infer historical bottlenecks, founding events, population expansions or range shifts from the genetic signature found in extant populations (see Hoelzel et al., 2002). Ancient DNA (aDNA) studies, on the other hand, offer an opportunity for real-time studies of long-lived species over ecological and evolutionary timescales (O'Corry-Crowe, 2008). The genetic data from aDNA sequences allow tracking a variety of population parameters such as changes in genetic diversity (de Bruyn et al., 2009), past connectivity by using nuclear markers to look at changes in gene flow (Valentine et al., 2008; de Bruyn et al., 2009) and past ecology by combining stable isotope and molecular analysis (e.g. Newsome et al., 2007; Lindqvist et al., 2010).

2. Sources of marine mammal aDNA

In this review we follow the example of Navascués et al. (2010) and define ancient DNA as DNA recovered post-mortem from non-ideal biological material. To date, aDNA studies have been heavily biased towards terrestrial species and in particular the abundant terrestrial megafauna species of the Pleistocene (e.g. Hofreiter et al., 2004; Shapiro et al., 2004; Gilbert et al., 2008; Campos et al., 2010). This bias in the taxa being studied may reflect the relative availability of terrestrial versus marine samples, with sub-fossil remains of terrestrial mammals typically being far more accessible than sub-fossil remains of marine species. However, in locations where large numbers of marine mammal sub-fossil samples have been recovered they are often under-utilized for aDNA work. We suspect that this may arise in part from geneticists working on contemporary marine mammal species being unaware either of the availability of such remains or the potential insights gained by ancient DNA analyses. However, aDNA remains a relatively expensive and risky

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Fig. 1. Mummified seal near Lake Bonney, Antarctica. Photograph: Randy Sliester.

line of research, especially when the data quality assurance and limitations to interpreting the data are considered.

Several thousand sub-fossil bones of terrestrial and marine mammals from the late Pleistocene and early Holocene have been recovered by trawling fishermen and suction-dredging from the Southern Bight of the North Sea (Mol et al., 2006). The marine specimens are thought to originate from inter-glacial periods when sea-level was high (Mol et al., 2006). For example, large numbers of samples of ice-associated or ice-dependent species such as walrus (*Odobenus rosmarus*), beluga (*Delphinapterus leucas*), and harp seal (*Pagophilus groenlandica*) dated to between 25,000 and 45,000 BP have been recovered (Post and Kompanje, 1995; Post, 1999; Mol et al., 2006). Similar findings have been made in southern Scandinavia (Aaris-Sørensen et al., 2010). Although the Southern Bight of the North Sea is exceptional in its sub-fossil record, there are sites throughout the sub-Arctic and Arctic with valuable zooarchaeological data that could also be used in aDNA studies (Dyke et al., 1996; Harington, 2003; Murray, 2008) including more recent middens (a mound or deposit of bones, shells and other refuse) from prehistoric aboriginal human populations (Etnier, 2004; Storå and Ericson, 2004; Newsome et al., 2007; Sommer et al., 2008) as well as animal middens (Magnanou et al., 2009). Additionally, bones found at former whaling stations and aboriginal whaling sites in both the Northern and Southern Hemispheres are a rich data source for aDNA studies (e.g. Rastogi et al., 2004; Lindqvist et al., 2009; Sremba et al., 2010).

Similar finds have also been made in Antarctica and the sub-Antarctic. Early explorers of the Antarctic continent were surprised to find the mummified remains of seals many kilometres inland and sometimes at high altitudes (Scott, 1905; Wilson, 1907; Fig. 1). Recent explorations have subsequently seen live seals travelling over 100 km inland and at an elevation close to 1000 m (Stirling and Rudolph, 1968). The mummified remains of Antarctic seals, which include the crabeater (*Lobodon carcinophagus*), leopard (*Hydrurga leptonyx*), southern elephant (*Mirounga leonina*) and Weddell (*Leptonychotes weddellii*) seals, vary from twisted remains of skin to near perfect specimens (Péwé et al., 1959; Barwick and Balham, 1967; Dort, 1971; Gordon and Harkness, 1992; Banks et al., 2010). Although to date only one aDNA study of this material has been conducted to our knowledge (de Bruyn et al., 2009), the large number of samples available of the crabeater seal in particular (Péwé et al., 1959; Barwick and Balham, 1967) would be sufficient for several lines of investigation using aDNA studies.

The cold temperature of these high-latitude sites is key to the preservation of aDNA in samples such as sub-fossil tooth and bone or mummified skin over extended time scales. At low-latitude sites with warmer climates, DNA is expected to undergo more rapid post-mortem degradation and samples are therefore usually less suitable for aDNA studies (Hofreiter et al., 2001; Smith et al., 2003; Pääbo et al., 2004).

There is also great scope for applying aDNA methods to more recent samples due to the greater availability of historical samples, which are hundreds rather than thousands of years old. This allows investigation of the demographic and evolutionary history of taxa over the past few hundred years. The interest of marine mammals to taxonomists past and present has meant that many beach-stranded or bycaught cetaceans have been collected and maintained in collections at universities and museums. Several centuries of exploitation of marine mammals including cetaceans, pinnipeds, sirenia, sea otters and polar bears have also provided potential sources of DNA over a time series within which it is possible to address a range of demographic, ecological and evolutionary questions.

DNA has been extracted from historical samples of hair, teeth and bones of several taxa including the pelts of sea otters (Larson et al., 2002; Valentine et al., 2008), whale baleen (Rosenbaum et al., 1997) and mummified skin (Weber et al., 2000). Ethnographic material, which is found in large quantities in the anthropology collections of many museums such as engraved sperm whale (*Physeter macrocephalus*) teeth known as scrimshaw, or baleen used in aboriginal fishing line, nets and other utensils can also yield DNA (Pichler et al., 2001; Foote, unpublished data). The small amount of material needed for the extraction of sufficient DNA using improved extraction methods (e.g. Yang et al., 1998) has greatly reduced the destructiveness of this procedure on valuable museum specimens (Rosenbaum et al., 1997; Pichler et al., 2001; Cipriano and Pastene, 2009).

3. Markers for marine mammal aDNA studies

Mitochondrial DNA is the most widely used marker in ancient DNA studies, including those on marine mammals (see Table 1). As each cell contains hundreds to thousands of copies of the mitochondrial genome, compared to one diploid copy of the nuclear genome, the chances of amplification by the polymerase chain reaction are greatly increased (Binladen et al., 2006; Morin et al., 2007). The age and tissue type can have a dramatic effect on the proportion of mitochondrial to nuclear DNA preserved; e.g. for historical whale baleen the proportion is 40–60-fold higher than in historical bone and tooth samples or fresh preserved soft tissue (Morin et al., 2007).

Mitochondrial DNA is generally assumed to evolve under neutrality, and as such has been used for demographic inference. The diversity of neutrally evolving markers is expected to be proportional to the effective population size, and changes in diversity are therefore assumed to reflect demographic change (e.g. Harrison, 1989; Roman and Palumbi, 2003; Shapiro et al., 2004). However, recent studies have suggested that selective sweeps on the mtDNA could reduce genetic diversity and violate the assumption of neutrality (Bazin et al., 2006; Nabholz et al., 2009). There is evidence that changes in the protein coding genes of the mitochondrial genome of marine mammal species have evolved under positive selection (McClellan et al., 2005; da Fonseca et al., 2008; Foote et al., 2011). Individuals with these changes could be more successful and out-compete those without these changes, leading to a 'selective sweep' during which the variation across mitochondrial genomes without the adaptive mutation is eventually lost or driven to low frequency within the population. This process can result in

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Table 1
Summary of ancient DNA studies on marine mammals.

Order	Species	Maximum age of samples (YBP)	Markers	References
Cetacea	Bowhead whale	51,000	mtDNA	Rastogi et al. (2004); Borge et al. (2007); McLeod et al. (2008, 2010)
	Bottlenose dolphin	1,400	mtDNA microsatellite	Nichols et al. (2007)
	Killer whale	<200	mtDNA	Morin et al. (2007); Foote et al. (2009)
	Longman's beaked whale	<100	mtDNA	Dalebout et al. (2003)
	North Atlantic right whale	<400	mtDNA	Rosenbaum et al. (1997, 2000); Rastogi et al. (2004); McLeod et al. (2008, 2010)
	Northern bottlenose whale	<100	SRY	Gowans et al. (2000)
	Sperm whale	<100	mtDNA	Pichler et al. (2001)
Sirenia	Steller's sea cow	>300	mtDNA	Rainey et al. (1984); Ozawa et al. (1997)
Carnivora				
Suborder Pinnipedia	Northern fur seal	2,425	mtDNA	Pinsky et al. (2010)
	Northern elephant seal	1,000	mtDNA	Weber et al. (2000)
	Guadalupe fur seal	>300	mtDNA	Weber et al. (2004)
	Southern elephant seal	7,087	mtDNA	de Bruyn et al. (2009)
	Walrus	<100	mtDNA	Lindqvist et al. (2009)
Suborder Fissipedia	Sea otter	<450	mtDNA, microsatellite	Larson et al. (2002); Valentine et al. (2008)
	Polar bear	>100,000	mtDNA	Lindqvist et al. (2010)

a genetic signature similar to a population bottleneck even if there has been no change in effective population size. It is therefore advisable to undertake statistical tests for neutrality of mutations against selective sweeps (e.g. Fu, 1997), or use two non-linked markers, when making inferences about demography using genetic diversity as a proxy (Ballard and Whitlock, 2004; Alter and Palumbi, 2009).

One of the most commonly used types of nuclear DNA marker in marine mammal population genetics of contemporary populations is the short-tandem-repeat or microsatellite. These are repetitive sequences of nucleotide pairs or triplets that vary in length depending upon the number of repeats. This variability has made them the marker of choice in contemporary population genetics. However, the length, low copy number, and difference in length between alleles of most microsatellite loci can make them difficult to amplify reliably. In particular, attempts to amplify microsatellite loci from degraded DNA samples can result in 'allelic dropout'. Multiple single-tube PCRs for the same loci for each sample can greatly reduce the bias from allelic drop out and misidentification of heterozygotes as homozygotes in degraded DNA samples (Taberlet et al., 1996; Allentoft et al., 2011). However, this greatly increases both the time and financial costs of the project and may explain why few marine mammal aDNA studies have utilized microsatellite loci. An alternative type of nuclear DNA marker to microsatellites are single nucleotide polymorphisms (SNPs) (Morin et al., 2004). The amplified fragment can therefore be much shorter than for microsatellite loci, reducing PCR failure rate, and each fragment can be the same length for all alleles, reducing the probability of allelic dropout (Morin and McCarthy, 2007). This principle also applies to other markers, such as Y-chomosomal markers, which are used to molecularly identify the sex of a specimen. Gowans et al. (2000) found that 1100 bp long ZFY–ZFX regions of the sex chromosomes were unsuitable for use on degraded samples of northern bottlenose whale (*Hyperoodon ampullatus*). They found that a short fragment (147 bp) of the Y-chromosome-specific SRY loci was more reliable and amplified more successfully in degraded and historic samples. Similarly, a real-time PCR assay targeting a 105 bp region of ZFY–ZFX has been shown to be effective for sex identification in most cetaceans and has high success rates for modern and historical samples (Morin et al., 2005, unpublished data).

4. Phylogenetic studies of marine mammals using aDNA

One of the earliest uses of aDNA sequences was for phylogenetic studies, whereby sequences of extinct species are included in molecular phylogenies to test taxonomic relationships that had previously been hypothesized based upon morphological characteristics alone. Although the first proliferation of sequences from extinct species in the 1980s and 1990s originated predominantly from terrestrial species such as the quagga (*Equus quagga quagga*; Higuchi et al., 1984), marsupial wolf (*Thylacinus cynocephalus*; Thomas et al., 1989), mammoth (*Mammuthus primigenius*; Höss et al., 1994; Hagelberg et al., 1995) and cave bear (*Ursus spelaeus*; Hänni et al., 1994), with Steller's sea cow they did include at least one marine mammal species. Steller's sea cow (*Hydrodamalis gigas*) was a giant Sirenian first discovered in 1741 in the North Pacific, but following two decades of unsustainable harvest, is thought to have become extinct by 1768 (Turvey and Risley, 2006). The phylogenetic position of the Steller's sea cow within the Sirenia and the Tethytheria was determined first with immunological data (Rainey et al., 1984) and then by sequencing of the mitochondrial *cytochrome b* gene (Ozawa et al., 1997). These studies revealed that the divergence of the Steller's sea cow from the dugong (*Dugong dugon*) was almost as ancient (22 MYA) as the split between the manatees (*Trichechus* spp.) and the dugong. Prior to this study, it was thought that the sea cow and dugong had split more recently than the dugong and the manatees.

Ancient-DNA protocols have also been used on historical museum samples to identify the species of the sample and confirm taxonomic identification and status of extant species. For example, Longman's beaked whale (*Indopacetus pacificus*) had until recently rarely been observed in the wild due to its distribution being limited to a remote pelagic habitat. Prior to a study by Dalebout et al. (2003), only two specimens had been described. Phylogenetic analyses of short (130–409 bp) mitochondrial DNA (mtDNA) control region and *cytochrome b* fragments showed that the sequences obtained from four additional museum specimens grouped with the holotype of this species, thereby tripling the known number of specimens of this species. In cases where there are putative but cryptic subspecies/subtypes based on morphology or other phenotype data, molecular identification can be used to assign these museum specimens to subtype (see examples in the following

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sections). In some cases putative cryptic subspecies have been shown to be geographic outliers rather than distinct taxonomic units. For example, the Laptev Sea walrus *O. rosmarus laptevi* is one of three described walrus subspecies. However, aDNA analysis combined with morphometrics did not support this taxonomic status, instead suggesting that the Laptev walrus was the westernmost population of the Pacific walrus *O. rosmarus divergens* (Linqvist et al., 2008).

There are important considerations when applying phylogenetic or population genetic analyses to aDNA datasets such as using appropriate methodology for utilizing heterochroneous data (see Navascués et al., 2010 for a review) and assessing if apparent mutations are artefacts arising from post-mortem damage (see Hofreiter et al., 2001). These caveats are generally applicable to both terrestrial and marine datasets. However, a consideration relevant specifically to studies of marine species is that radiocarbon dating of marine-derived carbon is complicated by the so-called Marine Reservoir Effect (MRE). Marine-derived ^{14}C carbon has an extended mean residence time relative to terrestrial ^{14}C, meaning that the oceans are depleted in ^{14}C, which leads to an offset of ^{14}C age between marine- and terrestrial-derived carbon (Ascough et al., 2006). Additionally, local climatic and oceanographic variables lead to local variation in ^{14}C age within the marine environment (Ascough et al., 2006, 2009) and changes in the offset over time (Bondevik et al., 2006). Therefore, although samples may be collected from a localized area, the large ranges of many marine mammal species mean they are likely to have integrated the reservoir age from a number of water masses (Mangerud et al., 2007). The only study that we could find which considered these factors in detail used whales caught at known dates to calibrate pelagic ^{14}C reservoir ages, assuming the measured value from the whales to be a mean for the waters within their range (Mangerud et al., 2007). This problem becomes important when samples used for ancient DNA studies are dated to put them in a temporal context and even more so if the ages of the sequences are used to estimate the nucleotide substitution rate of the genetic locus investigated (e.g. Ho et al., 2007, 2011).

5. Estimating changes in genetic diversity due to anthropogenic exploitation

Using museum collections for aDNA analysis has the potential to be informative for species conservation and management (Etnier, 2004; Leonard, 2008). Ancient DNA studies can help establish baselines of pre-exploitation or pre-environmental degradation of genetic diversity and geographic range. Given sufficient samples along a timeline and across a geographic range, aDNA studies allow reconstructing responses to past environmental change through the investigation of changes in genetic variation across space and time (Roy et al., 1994; Leonard, 2008). In this section we review case studies that applied ancient DNA techniques to obtain data that were informative for the management of extant marine mammal populations.

Several studies on marine mammal species have investigated changes in genetic diversity using historical or ancient samples. A number of these studies found changes in genetic diversity during periods of high exploitation. For example, Weber et al. (2004) found, based on historical samples, that the Guadalupe fur seal (*Arctocephalus townsendi*) shows a loss of genetic diversity in mtDNA genotypes associated with 18th and 19th century commercial sealing, consistent with a signature of a genetic bottleneck previously found in modern samples from extant populations (Bernardi et al., 1998). Similarly, the northern elephant seal (*Mirounga angustirostris*) is known from census data to have undergone a severe bottleneck due to 18th and 19th century commercial sealing, but subsequently rebounded from a low of approximately 20 individuals in 1892 to over 175,000 individuals today (Hoelzel, 1999). Impacts of this population bottleneck include low variation at all genetic markers analysed to date and a loss of fitness as inferred by increased fluctuating asymmetry and variability of quantitative traits in post-bottleneck skulls compared with pre-bottleneck ones (Hoelzel, 1999). Two separate studies found only two mtDNA genotypes in samples from over 150 post-bottleneck individuals, collected from colonies in central and southern California (Hoelzel et al., 1993; Weber et al., 2000). In contrast, four mtDNA genotypes were found in just five pre-bottleneck samples, indicating much greater genetic diversity before commercial harvesting (Weber et al., 2000). The aDNA analysis of just a few samples in these case studies provided confirmation of the loss of mtDNA diversity due to a population bottleneck, which had previously only been inferred from the genetic signature from modern populations.

Another pinniped species, the northern fur seal (*Callorhinus ursinus*), appears to have been more robust against a loss of genetic diversity over a prolonged period of exploitation despite losing most of its former breeding range (Pinsky et al., 2010). Using sub-fossil samples of harvested specimens collected from archaeological sites along the entire US Pacific coast, Newsome et al. (2007) used stable isotope analysis to show that both northern and southern sites contained unweaned pups and therefore were likely breeding colonies. Further, they found differences in life history strategies, based on isotopic values, between the northern and southern sites, with pups being nursed for up to three times as long in northern regions (Newsome et al., 2007). The contraction of the breeding range to the more northerly sites was concurrent with the loss of the alternative southern life history strategy. It could thus be expected to have been concurrent with a loss of genetic diversity. However, genetic diversity appears to have been maintained despite this loss of breeding range, and the life history variation likely resulted from behavioural plasticity (Pinsky et al., 2010), although the study could not exclude a potential loss of allelic variation at nuclear loci that may be responsible for the original behavioural variation. A lack of population structure and high dispersal rate, plus the use of Arctic refugia are all hypothesized to have led to the resilience in this species against loss of genetic diversity, at least with regard to mitochondrial DNA (Pinsky et al., 2010).

The sea otter (*Enhydra lutris*), once widely spread around the North Pacific rim, was also commercially hunted during the 18th, 19th and 20th centuries until a ban on hunting in 1911. Analyses of both mtDNA and nuclear microsatellites of one pre-fur-trade and five modern populations found substantial loss of diversity in the microsatellite alleles (Larson et al., 2002). The authors of this study suggested that this loss of genetic diversity could result in inbreeding depression. Following the extinction of numerous otter populations, there have been attempts to reintroduce sea otters from the Aleutian Islands to Alaska, British Columbia, Washington, Oregon and California during the past century. While the northern reintroductions have been successful, the Oregon reintroductions did not become established (Valentine et al., 2008). Analysis of the mtDNA control region of otter samples dated to between 175 and 2000 years old from two locations in Oregon indicated that the pre-fur-trade otter populations along the Oregon coast grouped genetically with the native California populations (subspecies *E. lutris nereis*) rather than the Alaskan populations (subspecies *E. lutris kenyoni*) that had been used to restock the area (Valentine et al., 2008). The historical Oregon sea otters were also morphologically more similar to the southern subspecies (Lyman, 1988) and these morphological differences between the two subspecies may, at least partially, be adaptive. Valentine et al. (2008) concluded that re-stocking from the California populations, which may be more adapted to the Oregon environment, could be more

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successful than previous relocations of otters from Alaska to Oregon.

Similar studies to investigate the loss of genetic diversity due to whaling have been conducted on historical samples of two of the most heavily exploited baleen whale species, the bowhead whale (*Balaena mysticetus*) and the North Atlantic right whale (*Eubalaena glacialis*). Both species were thought to have been equally targeted by Basque whalers during the 16th and 17th centuries. However, two recent studies that conducted molecular analyses on bones collected from a comprehensive search of 16th and 17th century Basque whaling ports found that all but one bone, including those previously identified as from right whales based on osteology, were molecularly identified as being from bowhead whales (Rastogi et al., 2004; McLeod et al., 2008). The authors concluded that the bowhead whale, rather than the North Atlantic right whale, was the main target of Basque whalers. Therefore, it is expected that, if at all, bowhead whales but not northern right whales may have undergone a loss of genetic diversity from Basque whaling (McLeod et al., 2008). In line with these results, genotyping of the single right whale bone identified by McLeod et al. (2008) using 27 microsatellite loci and comparison with the alleles for these loci in the extant population also suggests little or no loss of diversity (McLeod et al., 2010). All the alleles present in the historical sample were found in the extant population, and heterozygosity was similar for the historical bone and the modern samples (McLeod et al., 2010). Comparison of mtDNA diversity of late 19th and early 20th century museum specimens with samples from 84% of the extant population also suggests that loss of genetic diversity due to modern 20th century whaling has been modest (Rosenbaum et al., 2000). Although bowhead whales appear to have been the primary target of Basque whalers, Borge et al. (2007) found little change in levels of genetic diversity of the mtDNA throughout the Holocene. As in the case of the northern fur seal, the lack of population structure and the high dispersal rate in bowhead whales (McLeod, 2008) may have helped reduce the loss of diversity.

6. Investigating genetic change during periods of environmental change

Loss of genetic diversity can also be caused by habitat loss and environmental change. Ancient-DNA studies can be applied to look at past responses to climatic variation and to help understand how different taxa may respond to ongoing directional climate change (e.g. Shapiro et al., 2004; Drummond et al., 2005; Campos et al., 2010). A recent study used DNA extracted from mummified southern elephant seal skins found on a beach in the Antarctic to reconstruct the demographic history of this former breeding colony (de Bruyn et al., 2009). Comparing the mtDNA haplotypes with those for other locations suggested that this Antarctic breeding colony had been founded by ancestors from the sub-Antarctic island of Macquarie during the retreat of glaciers and sea ice at the Ross Sea Embayment 7500–8000 YBP. Following this founding event, changes in diversity suggest that the newly established breeding colony rapidly expanded and became independent from other colonies. Interestingly, the authors detected a signal of one-way migration back from the Antarctic colony to the sub-Antarctic colony at Macquarie Island some 1300 YBP, when the glaciers and sea ice subsequently started to re-encroach on this breeding colony. However, despite this back migration, much of the new genetic diversity generated by the founding event and subsequent expansion was lost (de Bruyn et al., 2009). In addition to the demographic insights, this case study also provides a useful insight into how mobile species with large ranges may be able to adapt to current changes in climate by habitat tracking (see next section).

In terrestrial mammals there is little evidence of habitat tracking during past episodes of climate change. Instead, phylogeographic studies tracking changes in haplotype distribution over time using aDNA and comparisons of pre- and post-glacial genetic diversity suggest that only a few relict populations of formerly widespread species survived in refugia during glaciations (Hofreiter et al., 2004; Dalén et al., 2007; Hofreiter and Barnes, 2010). However, in contrast to terrestrial habitats, the low cost of movement (Williams, 1999) and few geographic barriers may facilitate habitat tracking in the marine environment during environmental change. Ancient-DNA studies using samples from across large spatial and temporal scales, such as those from the North Sea, which was covered by seasonal sea ice during the last ice age, offer the opportunity to investigate the influence of past glaciations on the phylogeography of extant populations of marine species. Unfortunately, despite samples being available, with the exception of the study by de Bruyn et al. (2009), no data on this question have so far been published to the best of our knowledge. However, such studies could provide useful insights into the potential for marine mammal species to respond to future climate change.

Studies have also used sequences from historical samples to infer more recent range shifts, contractions or extinction of local populations. Stranding data for marine species offer a long-term measure of relative occurrence over time (Hart et al., 2006), although such presence-only data cannot conclusively demonstrate the absence of a species (Tingley and Beissinger, 2009). However, stranding data combined with molecular techniques can provide important insights into historical ranges. For example, Morin et al. (2006) sequenced a diagnostic fragment of the mtDNA control region from killer whale (*Orcinus orca*) museum specimens that had stranded or were harpooned along the Pacific coast of North America during the past two centuries. As mtDNA haplotypes are fixed in many killer whale populations in this region, the sequence was to some extent informative of the population of origin. The distribution of haplotypes was mostly consistent with the range of current populations. However, a haplotype associated with a population from British Columbia was found in California (Morin et al., 2006).

Nichols et al. (2007) analysed bottlenose dolphin (*Tursiops truncatus*) bones recovered from a Saxon settlement on the Humber River estuary in England and compared a short mtDNA sequence and microsatellite alleles with those from current populations. Both the mtDNA and microsatellite data suggested that the Humber samples were from a population distinct from the extant ones that had been sampled in waters around the UK or neighbouring waters. The authors concluded that this result was best explained by local population extinction during the past few hundred years (Nichols et al., 2007). They argued that these findings may indicate a more general decline of a bottlenose dolphin meta-population. The excellent collections at UK and other European museums offer the possibility to measure the past genetic diversity in this species to further investigate this possible decline.

7. Investigating ecology and evolution

Further ecological and evolutionary inferences can be made when aDNA studies are combined with those of stable isotopes and/or morphometric measurements (e.g. Leonard et al., 2007; Richards et al., 2008). Phylogenetic reconstruction of sequences obtained from historical museum specimens using aDNA protocols demonstrated that killer whale (*O. orca*) specimens from the Northeast Atlantic belong to two distinct clades (Foote et al., 2009). The two lineages also differed in body length, tooth count, niche width based on Nitrogen N^{15} isotopic ratios, and tooth-wear (Fig. 2). Foote et al. (2009) suggested that the types were genetically,

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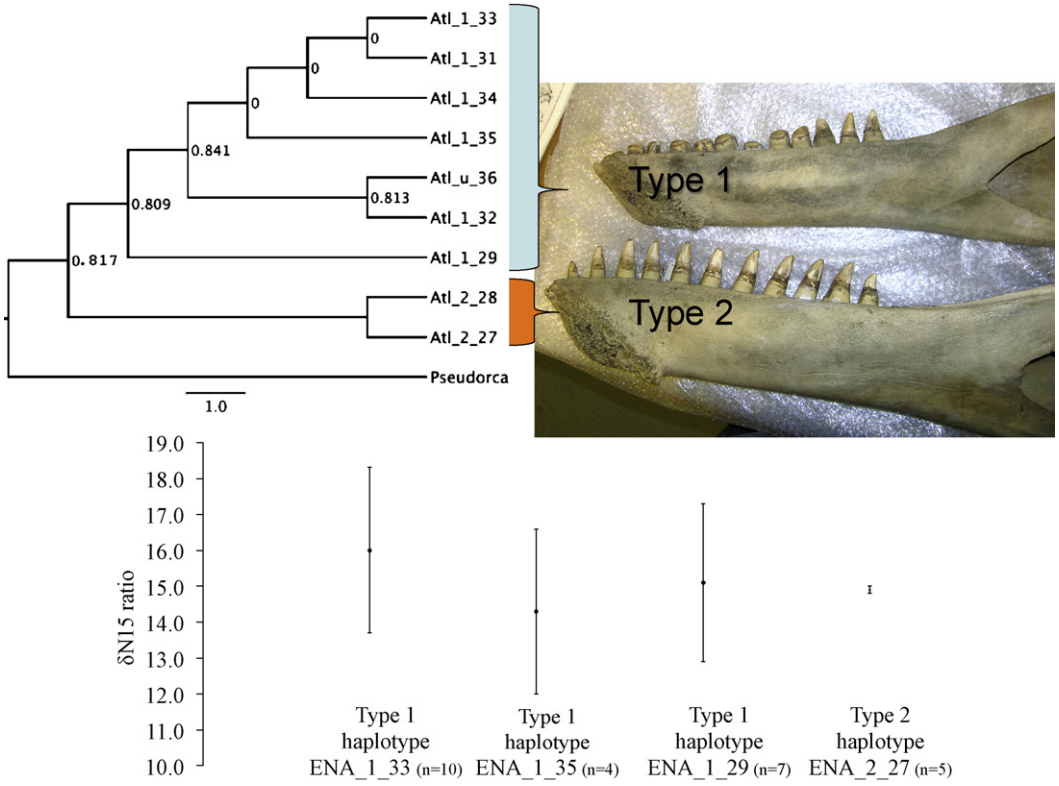

Fig. 2. Differences in apical tooth wear, tooth count, and niche width based on $\delta^{15}N$ stable isotope values between two sympatric, but genetically divergent lineages (type 1 and type 2) of North Atlantic killer whale (adapted from Foote et al., 2009).

morphologically and ecologically disparate enough that they should be considered separate 'Evolutionary Significant Units' (ESUs; see Ryder, 1986).

Using high-throughput sequencing techniques to generate the complete mitogenome of a polar bear jaw dated to between 110,000 and 130,000 years old, Lindqvist et al. (2010) were able to show that this sample was phylogenetically positioned at the branching point between polar bears and brown bears. By incorporating stable isotope measurements to infer diet, they could furthermore show that polar bears were hunting on the Arctic sea ice already at this early stage of their evolution. These examples show that there is indeed added scientific value in combining ancient DNA with stable isotope studies.

Molecular evolution can also be investigated using aDNA datasets. This has been applied to at least one marine mammal species. Evolutionary rates can be estimated using phylogenies, pedigree data and from aDNA sequences of C^{14}-dated samples. However, recent studies have found order-of-magnitude differences between short-term (<1 MY) mutation rates estimated using pedigree-based or aDNA data and long-term substitution rates estimated using species-level phylogeny-based data (Parsons et al., 1997; Lambert et al., 2002; Santos et al., 2005; Ho et al., 2007). For example, the short-term mutation rate of the mtDNA control region of the bowhead whale estimated using the aDNA dataset of Borge et al. (2007) is approximately 2×10^{-7} substitutions per site per year (Ho et al., 2007), compared with a long-term phylogeny-based substitution rate of approximately 2×10^{-8} (Rooney et al., 2001). However, subsequent data randomization analyses suggest there may not be sufficient temporal information to support rates estimated from the bowhead whale dataset (Ho et al., 2011). Given the relatively large number and the temporal range of samples sequenced in the bowhead whale aDNA study by Borge et al. (2007), it may be that very large aDNA datasets, spanning timescales of 10,000s of years are needed to reliably estimate evolutionary rates of species with slow molecular clocks such as baleen whales.

Ho et al. (2005) suggested that this time dependency of mutation rates is due to short-term transient mutations that will in the long-term eventually be removed by processes such as purifying selection. Therefore, genetic studies need to use an appropriate substitution rate for the timescales over which an analysis is conducted (Ho et al., 2005; Henn et al., 2009). For example, recent estimates of historical pre-whaling population sizes of minke (*Balaenoptera acutorostrata*), fin (*Balaenoptera physalus*) and humpback whale (*Megaptera novaeangliae*) based on current genetic diversity were an order-of-magnitude greater than previously estimated based on whaling catch records (Roman and Palumbi, 2003). However, these analyses used phylogenetic-based estimates of the mutation rate, which, as noted above, are likely to be an order-of-magnitude lower than mutation rates over the timescales for which they were estimating demographic change. Mutation rates estimated from moderate sized datasets of aDNA sequences (e.g. Ho et al., 2007) would be more appropriate for such studies investigating change over short timescales. This demonstrates both another important use for aDNA sequences and an important consideration for those using aDNA sequences to investigate phylogenetics or demographic change.

Genomic studies on marine mammals are just beginning to emerge. For example, recent studies have utilized mitogenomics of a global dataset of killer whales generated by high-throughput sequencing methods to test different evolutionary questions on divergences and adaptation (Morin et al., 2010; Foote et al., 2011). The first complete genome of a marine mammal, the bottlenose dolphin, has been sequenced, albeit currently at low coverage. Even so, the field of contemporary marine mammal genomics has a lot of catching up to do with the burgeoning field of palaeogenomics (Hofreiter, 2008). Complete mitogenome sequences of ancient terrestrial species are becoming relatively common, and

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mitogenome phylogenies have been produced for several Pleistocene taxa (Gilbert et al., 2008; Willerslev et al., 2009; Stiller et al., 2009; Briggs et al., 2009), as have several nuclear genomic investigations (Noonan et al., 2005; Poinar et al., 2006; Green et al., 2006; Miller et al., 2008). Recently the first complete nuclear genome sequences of ancient hominids were published (Green et al., 2010; Rasmussen et al., 2010; Reich et al., 2010). With new protocols and ever-greater sequencing power becoming available, palaeogenomics may allow real-time tracking of evolutionary changes due to selection at the molecular level for marine mammals. However, the long generation times of many marine mammal species mean that for evolutionary change to be detectable, very strong selection would have had to have led to rapid change over relatively few generations. It would also only be possible for species for which large sample sizes and time series of specimens were available.

8. Summary

The purpose of this review was to highlight the variety and importance of aDNA studies that have been and could in future be applied to marine mammals. We have shown that there are sample sets available that would allow the type of population genetics investigations that have become increasingly common for terrestrial species. A number of studies have gained great insights by sequencing the mtDNA from only a small number of samples, and recently the first larger scale studies have started to appear. We predict that these studies will become increasingly common in the near future, leading to greater insights into past migrations, demographics and generally population developments of marine mammals, as testified by the few studies already published. These studies have proven particularly valuable when combined with comparisons with contemporary populations. Ancient-DNA analyses can provide a means of validating hypotheses based on the genetic 'footprint' of past demographic or evolutionary events in contemporary populations by investigating real time changes in populations from the time period under investigation. The rapid evolution of high-throughput sequencing technology is also facilitating the first wave of paleogenomic studies, which we feel certain will soon include marine mammal species.

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