Relict or colonizer? Extinction and range expansion of penguins in southern New Zealand

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Recent human expansion into the Pacific initiated a dramatic avian extinction crisis, and surviving taxa are typically interpreted as declining remnants of previously abundant populations. As a case in point, New Zealand’s endangered yellow-eyed penguin (Megadyptes antipodes) is widely considered to have been more abundant and widespread in the past. By contrast, our genetic and morphological analyses of prehistoric, historic and modern penguin samples reveal that this species expanded its range to the New Zealand mainland only in the last few hundred years. This range expansion was apparently facilitated by the extinction of M. antipodes’ previously unrecognized sister species following Polynesian settlement in New Zealand. Based on combined genetic and morphological data, we describe this new penguin species, the first known to have suffered human-mediated extinction. The range expansion of M. antipodes so soon after the extinction of its sister species supports a historic paradigmatic shift in New Zealand Polynesian culture. Additionally, such a dynamic biological response to human predation reveals a surprising and less recognized potential for species to have benefited from the extinction of their ecologically similar sister taxa and highlights the complexity of large-scale extinction events.

Keywords: Megadyptes antipodes; ancient DNA; New Zealand; palaeontology; Polynesian; extinction

1. INTRODUCTION

Colonization of the Pacific—the ‘final frontier’ of human expansion—has left a trail of vertebrate extinctions readily discernible from archaeological and palaeontological data (Steadman & Martin 2003), providing an accessible system for revealing anthropogenic impacts on indigenous biota (Hurles et al. 2003). Subsistence hunting by early Polynesians is typically implicated in early extinctions (Worthy 1999; Holdaway & Jacomb 2000), and any surviving taxa are usually interpreted as declining remnants of previously abundant populations. With the advent of ancient DNA techniques, we now have a means to test the timing and severity of species and population declines by directly characterizing temporal changes in genetic diversity (Paxinos et al. 2002; Shapiro et al. 2004; Leonard et al. 2007; Valdiosera et al. 2008).

In New Zealand, Polynesian expansion southwards (ca AD 1280), followed by European colonization (AD 1769 onwards), destroyed much of an indigenous biota that was naive to terrestrial mammalian predators (Higham et al. 1999; Wilmshurst et al. 2008). At least 41 per cent of the endemic bird species have become extinct, and 35 per cent of those remaining are now classified as threatened (Worthy & Holdaway 2002). The endangered yellow-eyed penguin (Megadyptes antipodes), also known as hoiho, is one of New Zealand’s most publicized threatened species and is the focus of extensive conservation effort, including strong community involvement. The species is considered taonga (sacred) by the local Māori, is of high economic importance for local tourism industries and has been ecologically well studied over recent decades. The total population of approximately 7000 individuals breeds on the Subantarctic Auckland and Campbell Islands and the southeast coast of the South Island of New Zealand (Marchant & Higgins 1990; McKinlay 2001; figure 1). Previous analysis of the fossil records and anecdotal evidence suggest that this penguin was more abundant and widespread in the past. Consequently, current management assumes that yellow-eyed penguins on the mainland are a declining remnant of the prehistoric population (Worthy 1997; Moore 2001). The presence of penguin bones in archaeological middens from early Polynesian settlers in New Zealand, ancestors of modern Māori, indicates that penguins were subject to human hunting pressure, but to date this finding has not been considered significant. To test for temporal changes in M. antipodes genetic diversity associated with human settlement of New Zealand, we assessed mitochondrial DNA variation of prehistoric, historic and modern samples of yellow-eyed penguin. Based on the results of our genetic analysis, we further performed detailed morphological comparisons between prehistoric and modern Megadyptes bones, which lead us to describe a new penguin species that became extinct only a few hundred years ago and revealed the unsuspected recent range expansion of M. antipodes.
2. MATERIAL AND METHODS

(a) DNA extraction and sequencing

Yellow-eyed penguin blood samples were collected in 2005–2007 by wing venipuncture of the brachial vein from six different locations throughout the species’ breeding range \((n = 15–20\) for each location; M5–M8, M10, M11 in figure 1). DNA was extracted and purified using 40 \(\mu\)g proteinase K in 5 per cent Chelex (Biorad; Walsh et al. 1991). An 813 bp fragment of the first hypervariable region of the mitochondrial control region was amplified using primers: L-Man-CR4 \((5'-\text{CTGTGCACGTCTTTATGACGC-3')}\) and H-Man-CR7 \((5'-\text{GTGCACGTCTTTAAGTAGGTATC-3')}\). PCRs \((15 \mu l)\) containing 0.5 \(\mu\)M of each primer, 0.8 mM dNTPs, 1.5 mM MgCl\(_2\) and 0.75 U Taq polymerase \((\text{Mango Taq, Bioline, Sydney, Australia})\) were amplified for 2 min at 94°C, 35 cycles of 20 s at 94°C, 20 s at 50°C and 1 min at 72°C, followed by 10 min at 72°C. Purified PCR products were sequenced with H-Man-CR7.

Historic toe pad samples were obtained from 55 museum specimens collected between 1888 and 1944 across the breeding range of \textit{M. antipodes} and currently held in 15 museum collections worldwide (see table S1 in the electronic supplementary material). Tissue samples were rehydrated by a 24 hour wash in 1 ml 10 mM Tris-HCl (pH 8.0), and DNA was subsequently extracted using the Chargeswitch Forensic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) or the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) following manufacturers’ instructions. No difference was observed in extraction or amplification success between either of these kits. Two overlapping fragments were amplified using primer pairs L-Man-CR4 \((5'-\text{CTGTGCACGTCTTTATGACGC-3')}\) and H-Man-CR12 \((5'-\text{ACAAACGATAACCACTATG GG-3')}\); 299 bp); and L-Man-CR11 \((5'-\text{GAGTATGTATGAGATTAGCTCC-3')}\) and H-Man-CR14 \((5'-\text{CGGGTTAGTGTATTACAGTG-3')}\); 287 bp), yielding a total of 402 bp. For some samples, a single 444 bp fragment was amplified using primers L-Man-CR4 and H-Man-CR14. Primers H-Man-CR12, L-Man-CR11 and H-Man-CR14 were designed in conserved regions that did not show any polymorphisms in the sequences obtained from modern samples. PCRs \((25 \mu l)\) containing 0.4–0.8 \(\mu\)M of each primer, 0.8 mM dNTPs, 2.0 mM MgCl\(_2\) and 0.5–1.0 U Taq polymerase \((\text{Mango Taq, Bioline})\) were performed as above with cycles increased to 50. Purified PCR products were sequenced with the same primers used for amplification.

A total of 69 prehistoric \textit{Megadyptes} bones from the South Island and the Auckland Islands, New Zealand, were obtained from museum collections (see table S2 in the electronic supplementary material). Morphological descriptions from Worthy (1997) were used for identification of \textit{Megadyptes} bones. All but two bones \((\text{NMNZ S.42156.1 and NMNZ S.42156.2})\) were indirectly dated to AD 500–1700 based on associated archaeological remains. Independence of individual bones was achieved by sampling either the same bone type within a location or bones from different strata within the archaeological site. Bones were sampled using a hand drill and powdered in a Mikro-Dismembrator S \((\text{Sartorius AG, Goettingen, Germany})\). A total of 50–80 mg of bone powder was decalcified in 2 ml 0.5 M filtered EDTA for 24 hours. DNA was extracted using the DNeasy Tissue Kit (Qiagen) following the manufacturer’s instructions with the following modifications: (i) double volumes were used for proteinase K, AL and ATL buffers, and (ii) 2–4 \(\mu l\) of carrier RNA were added to each sample following proteinase K digestion.

![Figure 1. Spatio-temporal genetic relationships and distribution of \textit{Megadyptes} penguins. (a) Prehistoric South Island sequences (\textit{M. waitaha} sp. nov.) are shown in red, \textit{M. antipodes} sequences are shown in blue. Numbers on the main branches in the unrooted Bayesian phylogram represent posterior probabilities and ML bootstrap support. (b) Maps show the distribution of (i) \textit{Megadyptes} before AD 1500, (ii) \textit{Megadyptes} after AD 1800 on the South Island and subantarctic Campbell and Auckland Islands of New Zealand. Sampling sites are indicated with labels for prehistoric (P1–P11) and modern (M4–M11) samples, and prehistoric sites are further split in northern and southern South Island (NSI and SSI, respectively). The number of samples possessing each haplotype varied between 1 and 94 South Island (NSI and SSI, respectively). The number of samples possessing each haplotype varied between 1 and 94.](image)
dNTPs, 2.0 mM MgSO₄, 1 mg ml⁻¹ BSA/RSA and 0.5–1.0 U Taq polymerase (Platinum Taq DNA Polymerase High Fidelity, Invitrogen) were performed with 1 min at 94°C, 50 cycles of 15 s at 94°C, 15 s at 55°C and 30 s at 68°C, followed by 10 min at 68°C. Where necessary, 1 μl of the PCR was used as a template for a second PCR to improve amplification success. PCR products were purified and sequenced with the same primers used for amplification. All sequences are deposited in GenBank (accession numbers FJ391944–FJ391968).

Precautions for the analysis of historic and prehistoric DNA were adhered to. Historic sample DNA extractions and PCR set-up were performed inside a UV hood in a laboratory where no contemporary yellow-eyed penguin DNA or vertebrate PCR products have ever been present. Genetic analyses of prehistoric bone samples were all performed at the Australian Centre for Ancient DNA, where extractions and PCR set-up were carried out in a physically isolated, designated ancient DNA laboratory. Contamination was monitored by negative extraction and PCR controls. All historic and prehistoric samples were amplified and sequenced at least twice for both fragments. When conflict was observed among sequences, a third amplification was performed and a majority rule consensus applied (Brotherton et al. 2007). Authenticity of prehistoric sequences was further confirmed by extraction replications, cloning and the use of different primers to amplify fragments within the target region as described in the electronic supplementary material.

(b) Genetic analyses

Sequences were aligned using Sequencher (Schneider 1998) and analyses were restricted to the 402 bp region sequenced for all specimens. Applying the AIC criterion of Modeltest (Posada & Crandall 1998), we obtained HKY+I as most appropriate models of evolution for our dataset. Maximum-likelihood (ML) analyses were performed in PAUP* (Swofford 2003). Model parameters were estimated by a heuristic search, with 100 repetitions of stepwise addition. Using the estimated parameters, node support was calculated with 10000 bootstrap replicates. Bayesian trees were estimated by MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). In two independent runs, with 20 000 000 generations, sampling every 1000th generation and discarding 25 per cent as burn-in. Convergence diagnostics of Bayesian analyses were explored using Tracer (Rambaut & Drummond 2007) and AWTY (Nylander et al. 2008). The topologies of the ML and Bayesian trees were very similar, and therefore only the Bayesian tree is shown in figure 1. The shallow divergence within Megadyptes in relation to other penguin species made accurate model selection through Modeltest and rooting of the trees difficult, and rooted phylogenetic analyses were therefore only performed using a neighbour-joining algorithm with a Kimura-2 distance parameter (see fig. S1 in the electronic supplementary material). Genealogical relationships among samples were reconstructed using a parsimony-based haplotype network with a 94 per cent parsimony criterion in TCS (Clement et al. 2000). Observed genetic divergence (p-distance) was calculated among clades in PAUP* (Swofford 2003). Haplotype and nucleotide diversity indices were determined using DnaSP (Rozas et al. 2003).

(c) Morphometric measurements and analyses

Qualitative osteological comparisons were made for coracid, femur, humerus, tarsometatarsus and tibiotarsus using described terminology from Baumel & Witmer (1993). Morphometric measurements (to the nearest 0.1 mm) of four different bone types (coracid, femur, humerus and tarsometatarsus) were obtained from genetically analysed specimens (complete bones only), 26 contemporary skeletons (collected 1970–1990) and an additional 47 single bones from prehistoric sites (see the electronic supplementary material for a list of specimens), using Vernier calipers. It was unknown whether prehistoric specimens represented single or multiple skeletons, and each bone type was therefore analysed separately. Difference in average bone length among modern and prehistoric samples was determined using ANOVA followed by post hoc analyses (Scheffe) in SPSS v. 16.0. Normality and homoscedasticity assumptions were met and Bonferroni corrections were applied where necessary.

3. GENETIC RELATIONSHIPS

We successfully amplified and sequenced DNA from 100 modern, 43 historic and 42 prehistoric samples. Bayesian, ML and distance analyses all reveal a previously unrecognized and well-supported genetic split among Megadyptes samples. Specifically, all South Island specimens from before AD 1500 (sampling sites P1–P9), with the exception of three specimens, form a well-supported distinct genetic group (figure 1; fig. S1 in the electronic supplementary material). Within this group, a further genetic split is observed between the northern (P1–P3) and southern (P4–P9) South Island samples. None of these prehistoric South Island haplotypes is found in the historic or modern samples. On the other hand, all prehistoric Subantarctic sequences (P10 and P11) cluster with the historic and modern yellow-eyed penguins that now inhabit southern New Zealand and the Subantarctic (M4–M11). Currently, Megadyptes penguins are absent from the northern parts of the South Island (i.e. north of M4). The haplotype network clearly visualizes the substantial divergence between haplogroups, the relatively close relatedness of haplotypes within each group and the presence of two highly common haplotypes (see fig. S2 in the electronic supplementary material). Genetic divergence between the two identified Megadyptes groups was d=2.24–4.23% and diversity indices were found to be low for both the prehistoric South Island penguins (h=0.834 and π=0.009) and the group comprising prehistoric subantarctic and modern penguins (h=0.547 and π=0.004). Based on the observed unique genetic composition and the consistent morphological distinctness (presented below) of the prehistoric South Island penguins, we describe these penguins as a new species.

4. SYSTEMATIC PALAEOONTOLOGY

Sphenisciformes Sharpe, 1891

Spheniscidae Bonaparte, 1831

Megadyptes Milne-Edwards, 1880

Megadyptes antipodes (Hombron & Jacquinot 1841)

Megadyptes waitaha sp. nov.

(a) Etymology

From Waitaha (Māori): the first Polynesian tribe that occupied much of the South Island, New Zealand, before they were displaced by Ngāti Māmoe, who in turn were later dominated by Ngāti Tahu.
Dunes has been widely studied and has been dated to the Late Holocene, between 600 and 1500 years BP (Worthy 1998; Duncan et al. 2002).

(d) Paratypes
CM AV11995, right femur, complete. CM AV16258Z, right femur, complete. CM AV34941, left femur, complete.

(e) Referred material
Referred material includes all specimens from the northern South Island to Codfish and Stewart Island, just south of the South Island, that are listed in table S2 and methods of the electronic supplementary material.

(f) Diagnosis
Megadyptes waitaha bones are slender and smaller than those of M. antipodes and differ for a range of characters described below. Megadyptes waitaha further forms a distinct genetic group based on hypervariable region I (HVI) of the mitochondrial control region. Genetic divergence from M. antipodes in HVI mtDNA is 2.24–4.23 per cent with the following fixed character states (character for M. waitaha/character for M. antipodes, position corresponding to Eudyptes chrysocome mitochondrial genome sequence, GenBank accession number AP009189): T/C (15829), A/G (15855), G/T (15910), T/G (16006) and A/G (16072).

(g) Description and comparisons
Megadyptes waitaha bones are distinguished from M. antipodes as follows: (i) femur: lacks a prominent vascular foramen in the fossa poplitea; linea intermuscular caudalis more pronounced; crista trochanteris shorter and narrower; impressiones obturatoriae squarer and more pronounced; condylus medialis less robust; (ii) tibiotarsus: crista cnemialis more pointed; (iii) tarsometatarsus: crista medialis hypotarsi more flattened; cotyla lateralis laterally less prominent; crista lateralis hypotarsi less pronounced; medial foramina vascularia proximalia more heavily occluded plantaroproximally; medial margin more concave, giving whole bone more slender appearance; (iv) coracoid: facies articularis sternalis less robust; dorsal facies articularis sternalis less robust; medial process above acohumeral surface (neck) between processus acrocoracoideus and processus glenoidalis smaller and less ventrally curved; processus glenoidalis more robust; narrower coracohumeral surface (neck) between processus glenoidalis and processus acrocoracoideus; foramina prococoroideus absolutely and relatively larger; (v) humerus: impression coracohumeralis more superficially deeper, especially proximally; ventrally located secondary fossa within fossa pneumotricipitalis deeper and orientated more anterior-ventrally; sulcus transversus dorsal pit relatively deeper; ventral bit shallower; sulcus tendinii musculus humerotricipitalis (sela-moid groove) deeper; and the proximal trochlear process caudally bounding the humerotricipital sulcus is more pointed and bent ventrally near tip.

Bones from M. waitaha are significantly smaller than bones from M. antipodes (figures 2 and 3; table S3 in the electronic supplementary material). There is, however, no size differentiation between M. waitaha bones from the northern and the southern South Island of New Zealand (figure 3). The similar size of northern and southern populations of M. waitaha occurred over a geographical range greatly exceeding the distance from the South Island.
but also against other species such as the overlooked groups: (factor ANOVAs showed significant differences among the decades of human settlement (Nagaoka 2001). This finding is consistent with the fact that large-bodied species within just small game and fish observed in stratified middens reflects the transition from big game (including large penguins) to hunting in prehistoric New Zealand (Holdaway & Jacomb 2007). We suggest that a similar extinction–colonization process such as that observed in Megadyptes might also explain the previously reported arrival of an Australian Eudyptula minor lineage in southern New Zealand (Banks et al. 2002; Overeem et al. 2008).

Ancient DNA analyses are proving to be an extremely valuable tool in wildlife conservation, providing an ability to directly characterize temporal changes to the Subantarctic islands, and thus the geographical distance between the M. waitaha and M. antipodes populations in prehistoric times.

5. DISCUSSION
(a) Human-mediated extinction of a new penguin species
Genetic and morphological analyses reveal a previously unrecognized penguin species in the Megadyptes genus. None of the haplotypes of this species are found in any of the historic or modern samples analysed, indicating that M. waitaha no longer survives. The presence of its bones in archaeological context implies that its extinction was probably caused by overexploitation (Jones et al. 2008). This finding is consistent with the fact that large-bodied species were particularly vulnerable to extinction by hunting in prehistoric New Zealand (Holdaway & Jacomb 2000; Duncan & Blackburn 2004). Indeed, the marked transition from big game (including large penguins) to small game and fish observed in stratified middens reflects the population decline of the larger species within just decades of human settlement (Nagaoka 2001). This previously described 'blitzkrieg' was obviously not only directed against the well-known moa (Diamond 2000), but also against other species such as the overlooked penguin we describe here. It is thus likely that M. waitaha became extinct within a few hundred years of human settlement in New Zealand. The recognition of two species in Megadyptes reveals an original taxon distribution similar to that of Eudyptes, which displays noticeable speciation within the genus (Jouventin et al. 2006), including different species inhabiting the South Island and Subantarctic islands of New Zealand.

The phylogeographic split between northern and southern South Island samples of the extinct M. waitaha is concordant with biogeographic disjunctions observed around an upwelling zone at latitude 42° S in a number of coastal invertebrate taxa in New Zealand (e.g. Apte & Gardner 2002; Ayers & Waters 2005). This upwelling and associated longitudinal change in currents and water temperature may have also presented a barrier to gene flow for M. waitaha. Currently, M. antipodes does not breed above 43° S, although occasional vagrants are found as far north as New Zealand’s North Island (Marchant & Higgins 1990).

(b) Recent range expansion of the yellow-eyed penguin
Our findings demonstrate that yellow-eyed penguins are not a declining remnant of a previous abundant population, but instead went through a recent range expansion following the extirpation of M. waitaha. Only three of the prehistoric penguin specimens on the South Island were identified genetically and morphologically as M. antipodes. These specimens probably represent non-breeding vagrants from the Subantarctic, as now commonly occurs with Eudyptes species. Therefore, it seems almost certain that the entire extant yellow-eyed penguin population on the South Island is derived from a Subantarctic stock.

The rapid replacement of M. waitaha by M. antipodes suggests that competition between the two species previously prevented M. antipodes from expanding northwards. The successful expansion of M. antipodes into the South Island, prior to the increase of European settlers and their commensals in the late 1800s and soon after the anthropogenic extinction of M. waitaha, may imply that a paradigmatic shift in Māori culture took place. Indeed, it has been suggested that cultural change (including new forms of resource monitoring and conservation) in Māori culture may have developed from the early sixteenth century, possibly forming the basis of modern Māori environmental management (Anderson 2002). Alternatively, the archaeological record shows a marked lack of coastal South Island village sites from the early sixteenth century, in the period following the extinction of big game, suggesting a local temporary reduction of the human population (Anderson & Smith 1996). Environmental changes such as the severe decline in populations of sea lions (Phocarctos hookeri), known predators of penguins, might also have facilitated M. antipodes colonizing the South Island (Childerhouse & Gales 1998; Lalas et al. 2007). We suggest that a similar extinction–colonization process such as that observed in Megadyptes might also explain the previously reported arrival of an Australian Eudyptula minor lineage in southern New Zealand (Banks et al. 2002; Overeem et al. 2008).
Complexity of large-scale extinction events

Our study reveals a new level of biogeographic and ecological complexity potentially associated with large-scale extinction events that afflicted, for example, the Pacific prehistoric avifauna and North American Pleistocene megafauna. Whereas conventional wisdom suggests that surviving species—like their extinct counterparts—suffered major genetic and ecological declines (Hofreiter 2007), we propose that in some instances native species benefited from the extinction of their ecologically similar sister taxa. For example, we suggest that this extinction—expansion interaction might have had a particularly strong influence on seabird distributions: as numerous colonies went extinct (Steadman 1995), newly vacated habitats would have facilitated rapid range expansion in this highly mobile group of species, as in Pterodroma nigripennis, for example (Worthy & Holdaway 2002). Such dynamic anthropogenic processes may turn out to be far more common and important than previously understood.

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