Journal of Archaeological Science 37 (2010) 174–177

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Ancient DNA typing of archaeological pig remains corroborates historical records

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ARTICLE INFO

Article history:
Received 7 May 2009
Received in revised form
8 September 2009
Accepted 8 September 2009

Keywords:
Ancient DNA
Provenancing
Lord Howe Island
Phylogeography

ABSTRACT

The recent increase in both the abundance and taxonomic range of DNA sequence data in public repositories makes it possible to determine the maternal origin of lineages of faunal archaeological material by characterizing its mitochondrial DNA. Among the most commonly represented taxa are domesticated animals, for which extensive genetic characterization has revealed high levels of genetic diversity and (in at least some cases) strong phylogeographic clustering. Such information has significant implications not only for characterizing important aspects of the occupation history of archaeological sites, but also in providing novel insights into colonisation history and the scale and scope of trade and exchange networks. This can be done through studying the origins and dispersal of proxy organisms such as commensal and domesticated animals, as well as economically important wild fauna. To illustrate this approach, we compare historical records of maritime movement of people and pigs from two sites on Lord Howe Island, Australia, to phylogeographic results of DNA extracted from pig bones.

1. Introduction

Reconstructing the movement of people, their domestic animals and commensal mammals throughout the world has been successfully attempted using mitochondrial DNA (mtDNA) from modern and ancient sources of DNA (Matisoo-Smith et al., 1998; Larson et al., 2007a; Larson et al., 2007b). For this to be practical, two criteria need to be met. Firstly, a large mitochondrial reference database of a target species must be available. Mitochondrial DNA (mtDNA) is favoured over nuclear DNA for aDNA studies because many more copies of mtDNA are present within cells, thereby increasing the probability of survival and recovery from bones and other organic remnants. In addition, the lack of recombination in mtDNA facilitates phylogenetic and demographic reconstruction using recovered sequences. However, given the rapid rate of post-mortem DNA decay (Hoss et al., 1996; Smith et al., 2003) and the difficulty of amplifying long tracts of aDNA, it is necessary to target a small yet variable portion of the mitochondrial genome for analysis. To meet these conditions, the most common genetic target for aDNA provenancing is the hypervariable region within the mitochondrial control region: a non-coding segment of the mtDNA genome that exhibits a rapid evolutionary rate relative to other mtDNA loci.

Secondly, the genetic signature recovered from the sample must be characteristic of a specific (and preferably restricted) geographic region. Direct correlations between genetic signatures and geographic provenance has been demonstrated for pigs (Larson et al., 2005), cows (Troy et al., 2001), rabbits (Hardy et al., 1995), goats (Naderi et al., 2007), sheep (Guo et al., 2005; Kijas et al., 2006), and to some extent for chickens (Liu et al., 2006) though the phylogeographic signal is less strong in horses (Vila et al., 2001) and dogs (Savolainen et al., 2002). In the case of pigs, a single nucleotide polymorphism is sufficient to place an individual's maternal origin within a specific and circumscribed region of the world (Larson et al., 2005; Larson et al., 2007a; Larson et al., 2007b).

Oceanic islands present an ideal opportunity to test the results of aDNA phylogeography against historical records. Prior to human settlement, oceanic islands were devoid of domestic species. Determining the origins of introduced domestics to these islands using a morphological approach is difficult given the phenotypic similarities between domestic animals derived from different geographical regions.

1.1. Lord Howe Island

Faunal material was excavated at two sites on Lord Howe Island, a small remnant volcanic island located between the Australian east coast and New Zealand in the Tasman Sea (Fig. 1).

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doi:10.1016/j.jas.2009.09.029

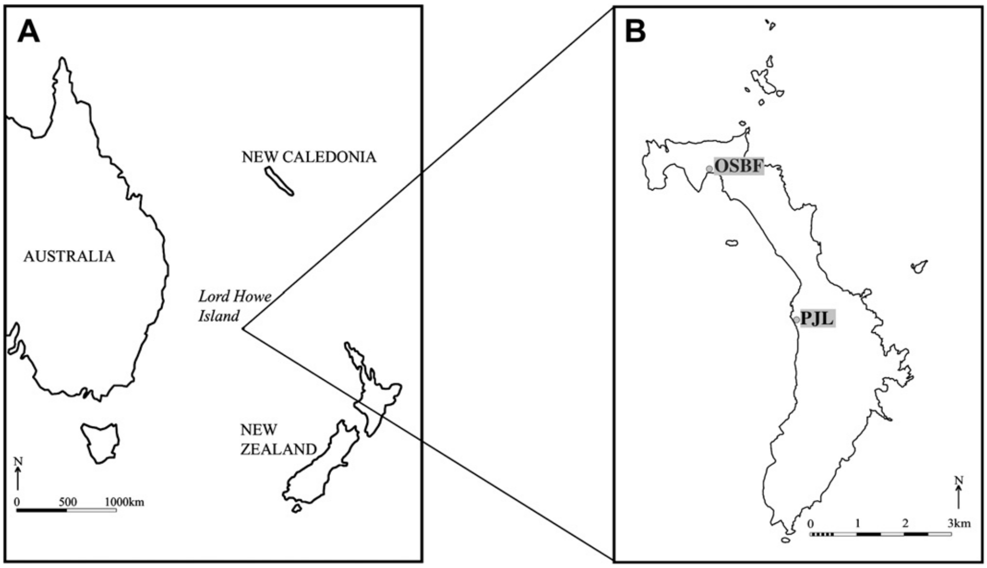

Fig. 1. Two maps depicting (A) the position of Lord Howe Island within the Tasman Sea, and (B) the location of the OSBF and PJL sites on the island.

The island appears to have been uninhabited prior to its discovery in 1788 (Rabone, 1940; Anderson, 2003), but rapidly became a key resupplying destination for passing whaling, sealing, trade and colonial ships. These ships included locally owned colonial vessels that operated around the Tasman Sea, those frequently trading between the colonies and Asia, and long-distance traders and whalers from Britain and the Americas. The ship traffic to the uninhabited island during the first 40 years following its discovery resulted in the release of domesticated pigs (*Sus scrofa*) and goats (*Capra hircus*). As a result of these introductions and ships sporadically resupplying, it is likely that several native species, such as the White Gallinule (*Notornis alba*) and the White-throated pigeon (*Columba vitiensis godmanae*) became extinct or highly endangered by the time permanent settlement began on the island in 1834 (Hutton, 1990).

Originally settled by a small group comprised of three British ex-whalers and their Maori wives and children, Lord Howe was subsequently colonised with the aim of formalising its role as a place of refreshment. Employed by a local Australian whaling firm, the settlers established the island as a supply depot by developing a small scale agricultural industry (Nichols, 2006). Staple crops such as potatoes, onions, cabbages and kumara (*Ipomoea batatas*) were introduced and grown expressly for the ship trade, while local fish, mutton bird (*Puffinus pacificus, P. carneipes*) and feral game were offered for trade as well as providing a significant portion of the local subsistence. Pigs and goats already on the island were incorporated into this enterprise, but whether the initial settlers brought additional pigs and goats along with their fowl (*Gallus* and *Anser*) and dogs (*Canis*), or these stocks were further supplemented by the passing ships is unclear (Rabone, 1940). The supply trade continued on the island until the increased availability of petroleum products in the mid 1850s precipitated a global slump in the whaling industry (Mawer, 1999). By the 1870s the shipping trade was almost finished and the Lord Howe economy was forced to move away from the supply trade in favour of agricultural exports, such as the export to Europe of an endemic palm tree, *Howea fosteriana,* for use as an indoor ornamental plant (Rabone, 1940; Nicholls, 1952).

Archaeological investigations of the settlement landscape of Lord Howe were undertaken as part of doctoral research in 2004 by one of the authors (Kimberley Owens from the Australian National University), and focused on midden materials from two domestic occupation sites, OSBF and PJL (Fig. 1). OSBF is the earliest site on the island, dating from around 1834 to about 1845–50 and was most probably built and occupied by the first settlers who came directly from New Zealand mainland whaling stations. These first settlers may have brought pigs with them from New Zealand or from the Australian colonies. There are also indications that by this time there was already a significant feral pig population established on the island (Nicholls, 1952), which was descended from animals released on at least two occasions by British, American or local Colonial ships for the express purpose of establishing a feral stock which passing ships could reliably draw upon (Rabone, 1940; Nicholls, 1952). Post-cranial elements of pig and goat are poorly represented in the earliest occupation layers of the excavations, suggesting that the bulk of the meat bearing portions of these animals were exported from the island as articles of trade, most likely in a smoked or salted state.

The PJL house site dates from the 1860s to about 1920 and an excavation revealed an increased variety and frequency of post-cranial pig, goat and other domesticate skeletal elements, indicating that fewer primary products of these taxa were being traded off the island. By the time this site was established, shipping was on the decline, although there remained ample opportunity for supplementary domestic stock (particularly pigs) to be brought to the island; either as specific imports from the colonies, China or Britain, or as opportunistic trade items with passing ships who had a surplus of livestock but not vegetables (Rabone, 1940; Nicholls, 1952). As shipping decreased further, opportunities for new domestic stock arrivals became fewer and the frequency of ships from outside the local region decreased

2. Materials

Four pig teeth from kitchen midden deposits at OSBF and four pig teeth from domestic underfloor deposits at PJL were collected from occupation levels dating from the 1830s to the 1840s (OSBF) and the 1860s to 1920s (PJL). Both midden and underfloor deposits also contained a wide selection of domestic refuse, including a large variety of faunal remains from domestic and native species, common 19th century British household ceramics and glassware, Scottish clay tobacco pipes, and common beads, buttons and

Table 1
Primers used to amplify pig control region mtDNA (Larson et al., 2007b).

Primers	Sequence	Product length (bp)
Anc f1	5′-CTTTAAAACAAAAAAACCCATAAAAA	125
Anc r1	5′-TTAATGCACGACGTACATAGG	
Anc f2	5′- CGTACATAGCACATATCATGTCA	121
Anc r2	5′-AGAAGAGGGATCCCTGCCAA	
PNG f	5′-TAATCAGCCCATGCTCACAC	104
PNG r	5′-ACTGTGTTAGGGCCTTTGAC	

ammunition. From their history and the known demographics of occupants, these two sites constitute a representative sample of most households on the island spanning from first settlement to the beginning of mature settlement at the beginning of the 20th century.

3. Methods

Appropriate sterile techniques were followed to avoid potential contamination of the samples either with DNA from other samples or from modern humans. DNA was extracted, amplified and sequenced using standard aDNA techniques that have been optimized for bone material (Shapiro et al., 2004), in a dedicated aDNA facility at Oxford University and following established (Cooper and Poinar, 2000) and refined (Gilbert et al., 2005) aDNA protocols.

To assess whether DNA survived in the samples, 2 µl of DNA extract was amplified by PCR using Platinum®*Taq* DNA Polymerase (Invitrogen), a hotstart polymerase, with 50 cycles of 1.5 min initial denaturation at 97 °C, 45 s at 94 °C, 45 s at 56 °C, 1.5 min at 68 °C and a final cycle of 10 min at 68 °C. Primers used are listed in Table 1. Extraction blanks and PCR negative controls were used throughout the experimental procedures (one of each control for every four extracts amplified). All extracts (and blanks) were amplified twice independently with identical results. No negative controls contained any amplification products despite the large number of cycles used, and the potential problem of contamination from modern domesticated animals (Leonard et al., 2007).

DNA extracts from pig bones were first subjected to PCR amplification using the Anc f1/Anc r1 primer pair. These two primers bracket a region of the mitochondrial DNA control region which possesses diagnostic single nucleotide polymorphisms (SNPs) that discriminate not only between European and Asian haplotypes, but also between individual European haplotypes. In order to differentiate between the samples which possessed an Asian signature using the first primer pair, two additional PCRs were carried out using two sets of primer pairs: Anc f2/Anc r2 and PNG f/PNG r. Additional details regarding the differences between all the Asian and European haplotypes are discussed in Larson et al., 2007a and Larson et al., 2007b respectively.

4. Results

4.1. Lord Howe Island pigs

All four pig teeth (GL1093-1096) from the Lord Howe Island site OSBF yielded sequences with the most common domestic European haplotype, ‘A’ (Table 2).

All four pig teeth (GL1097-1100) from the PJL site, possessed separate East Asian haplotypes (Table 2):

GL1097 was identical to four of the most common East Asian domestic pig haplotypes (GX, L, DW, EH) found in specimens from China, Australia, Guam, and Hawaii;

GL1098 possessed a novel haplotype that clustered with other, distinctively East Asian haplotypes;

GL1099 possessed a diagnostic SNP in the amplicon generated by the third primer pair indicating that it was identical to the most common Pacific Clade haplotypes (BX) found in modern and ancient material derived from New Guinea and throughout Near and Remote Oceania, and associated with the dispersal by (Neolithic farmers) of pigs domesticated in South East Asia into Island South East Asia and the subsequent Lapita and Polynesian migrations into Wallacea and remote Oceania; and GL1100 possessed a common East Asian haplotype and was identical to the D and AE haplotypes found in China and in Guam.

The presence of the Pacific Clade haplotype on Lord Howe is interesting since no domestic pigs on either New Zealand nor Australia have yet to be shown to possess a Pacific signature, despite the ubiquity of Pacific Clade pigs in New Guinea and in the islands within the Near and Remote Pacific.

5. Discussion

5.1. Lord Howe Island

The finding that two sites, lying just 8 km apart, contain pigs with exclusively Asian or European haplotypes is surprising, especially since the material culture of both settlements is essentially identical. Of course, with such a small sample size, the difference could merely be the result of chance (founder effect) rather than a conscious choice made by the settlers. An alternative explanation is that the European haplotypes, which are found only at the earlier OSBF site, originated from ships that had recently resupplied in the colonies. Although early shipping to the island included American and British whaling vessels that had come from across the Pacific and Indian oceans, most would have resupplied in colonial ports prior to visiting Lord Howe, which at

Table 2
Showing haplotype designations of pig samples from Lord Howe Island. For haplotype designations see Larson et al., 2007a,b.

Site	Sample Code	Elements	Notes	Extraction No.	Haplotype Designation	Geographic Region of Origin	GenBank Accession No.
OSBF (1830s–1840s)	J5/I5/2-3/011	M1/M2	Maxillary - worn	GL1093	A	European	EU826005
OSBF (1830s–1840s)	J5/2-3/067	Premolar	Maxillary - worn	GL1094	A	European	EU826006
OSBF (1830s–1840s)	J5/2-3/083	Premolar	Mandibular premolar P4?	GL1095	A	European	EU826007
OSBF (1830s–1840s)	J5/2-2/132	Canine	Female	GL1096	A	European	EU826008
PJL (1860s–1920s)	2/3/012	Incisor	Mandibular	GL1097	GX, L, DW, EH	Asia (China, Australia, Guam, Hawaii)	EU826009
PJL (1860s–1920s)	PJ2/1/5/019	Premolar	Mandibular	GL1098	–	East Asia	EU826010
PJL (1860s–1920s)	A/3/035	Mandible	inc dP4, M1	GL1099	BX	Asia (Pacific)	EU826011
PJL (1860s–1920s)	A2/3/075	Premolar	Maxillary	GL1100	D, AE	East Asia (China, Guam)	EU826012

that time were still likely to have had almost exclusively European descended stock.

In contrast, by the time of the establishment of PJL, there would have been sufficient time for any later stock introductions from other sources to have become established on the island; either as new additions to feral populations, or as husbanded animals. Further, although shipping was on the decline by this point, what shipping there was typically came directly from all over the Pacific, rather than via colonial ports as captains were keen to avoid the mainland ports and the risk of losing scarce crew to the lure of the Australian and New Zealand goldfields (Rabone, 1940; Nicholls, 1952), providing ample opportunity for the introduction of Asian pigs. The far-reaching nature of Lord Howe's maritime network and consequent opportunities for stock recruitment are exemplified by the stories of two of settlers who arrived during this time, including the man who settled PJL, Perry Johnson.

Perry Johnson was an emancipated American slave who had come to the island as a crewman on an American whaler, the *Will o' the Wisp* which would have had an extended and convoluted voyage throughout the Pacific as was the norm for American whaling vessels at this time (Rabone, 1940; Nicholls, 1952; Mawer, 1999). Similarly, Nathan Thompson of the American whaler *Belle* disembarked and settled at Lord Howe with three Gilbert Island (Kiribati) women who had been picked up during his ship's previous voyaging around the Pacific, which also included recorded stops in Vanuatu and New Caledonia (Rabone, 1940; Nicholls, 1952; Nichols, 2006). All modern pigs sampled from Vanuatu possess Pacific Clade haplotypes and thus it is possible, that the Pacific Clade pig on Lord Howe island may have been picked up here (Lum et al., 2006; Larson et al., 2007a).

6. Conclusions

The examples taken from Lord Howe Island show the ability to discover the provenance of individuals of any species that has a well-characterised phylogeographic signal and for which a mtDNA reference has been created. This materially increases the amount of archaeological information that can now be obtained from skeletal material, and is perhaps of particular interest to those studying recent human settlement of oceanic islands. This level of detailed interpretation, currently inaccessible using standard zooarchaeological analyses, has been revealed a picture which in this case correlates particularly well with the island's historical record.

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