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Thorough assessment of DNA preservation from fossil bone and sediments excavated from a late Pleistocene–Holocene cave deposit on Kangaroo Island, South Australia

Dalal Haouchar[a], James Haile[a], Matthew C. McDowell[b], Dáithí C. Murray[a], Nicole E. White[a], Richard J.N. Allcock[c], Matthew J. Phillips[d], Gavin J. Prideaux[b], Michael Bunce[a,e,*]

[a] Australian Wildlife Forensic Services and Ancient DNA Laboratory, School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia
[b] School of Biological Sciences, Flinders University, Bedford Park, SA 5042, Australia
[c] LotteryWest State Biomedical Facility: Genomics, School of Pathology and Laboratory Medicine, The University of Western Australia, Nedlands, WA 6009, Australia
[d] School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, QLD 4001, Australia
[e] Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, WA 6845, Australia

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ABSTRACT

Fossils and sediments preserved in caves are an excellent source of information for investigating impacts of past environmental changes on biodiversity. Until recently studies have relied on morphology-based palaeontological approaches, but recent advances in molecular analytical methods offer excellent potential for extracting a greater array of biological information from these sites. This study presents a thorough assessment of DNA preservation from late Pleistocene–Holocene vertebrate fossils and sediments from Kelly Hill Cave Kangaroo Island, South Australia. Using a combination of extraction techniques and sequencing technologies, ancient DNA was characterised from over 70 bones and 20 sediment samples from 15 stratigraphic layers ranging in age from >20 ka to ~6.8 ka. A combination of primers targeting marsupial and placental mammals, reptiles and two universal plant primers were used to reveal genetic biodiversity for comparison with the mainland and with the morphological fossil record for Kelly Hill Cave. We demonstrate that Kelly Hill Cave has excellent long-term DNA preservation, back to at least 20 ka. This contrasts with the majority of Australian cave sites thus far explored for ancient DNA preservation, and highlights the great promise Kangaroo Island caves hold for yielding the hitherto-elusive DNA of extinct Australian Pleistocene species.

1. Introduction

Islands have long provided a natural laboratory for the study of evolutionary processes because evolutionary changes on them are often magnified, simplified and therefore more readily interpretable (e.g., Darwin and Wallace, 1858; MacArthur and Wilson, 1967; Losos and Ricklefs, 2010). The study of genetic variation on islands also has a long history (Lomolino et al., 1989; Van der Geer et al., 2010). However, ancient DNA (aDNA) analyses applied to stratified, dated faunal successions can add a temporal context, allowing the ebb and flow of genes, species and communities to be assessed, particularly in combination with more traditional analyses of vertebrate and plant macrofossils and pollen. A necessary prerequisite for aDNA research is adequate biomolecular preservation. Cave systems represent an ideal environment for palaeontological investigations as they often contain relatively complete and undisturbed stratigraphic deposits that harbour several environmental proxies (White, 2007; Butzer, 2008) that have been subjected to minimal temperature and humidity fluctuations; conditions that favour DNA persistence (Stone, 2000). Such caves represent archives of well-preserved Quaternary vertebrate assemblages (Prideaux et al., 2007, 2010), with the ability to preserve invaluable repositories of past biodiversity. All samples (bones and sediments) analysed in this study were obtained directly from Kelly Hill Cave (KHC), Kangaroo Island (KI) with the aim of conducting a

* Corresponding author. Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, WA 6845, Australia.
E-mail address: michael.bunce@curtin.edu.au (M. Bunce).

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thorough assessment of DNA preservation in KHC to determine whether genetic data could enhance temporal information about faunal change on KI. Moreover, as part of this study the preservation of plant DNA obtained directly from sediments was assessed (Haile et al., 2007; Willerslev et al., 2007) with the aim to provide palaeovegetation data to complement fossil data.

In this study a combination of techniques such as a relatively new bulk-bone method (Murray et al., 2013) and High-Throughput Sequencing (HTS) technology was used in order to capture aDNA from a variety of samples collected from KHC. Also we show how the addition of a palaeontological molecular perspective may complement existing morphology based studies allowing identification of osteologically absent and cryptic species, and the investigation of genetic change over time. These results are overlayed upon the palaeogeographic history of KI, which provides a model context for studying mainland–island interactions. This is pertinent to KHC, because it contains an excellent Late Quaternary vertebrate fossil assemblage (Pledge, 1979) that records the response of Australian native fauna to both the Last Glacial Maximum (LGM) and isolation of KI caused by rising sea levels at 8.9 ka (McDowell, 2013).

2. Kangaroo Island

Kangaroo Island lies at the entrance to Gulf St Vincent in South Australia (Fig. 1), and is the third-largest land-bridge island in Australia (4405 km^2), with a length of 145 km and width of 55 km at its widest point (Abbott, 1974; Hope et al., 1977; Lampert, 1981; Twidale and Bourne, 2002) (Fig. 1). It is geologically continuous with the adjacent Fleurieu Peninsula, but was isolated by glacial erosion during the Late Carboniferous and Early Permian (Belperio and Flint, 1999). Today it is separated from Yorke Peninsula by Investigator Strait, a 50-km stretch of 30–35 m deep water (Fig. 1).

During the late Pleistocene, sea levels were 120 m lower than at present (Chappell and Shackleton, 1986; Yokoyama et al., 2001) and KI was connected to the mainland. Global warming during the early Holocene caused a rapid rise in sea-level, isolating KI from the mainland at 8.9 ka (Belperio and Flint, 1999; Bradley, 1999; Cutler et al., 2003). Prior to its isolation, gene flow was presumably continuous between the mainland KI which supported a species-rich fauna (Abbott, 1974; McDowell, 2013). Once isolated, the newly marooned organisms would have become more susceptible to genetic drift; island floras and faunas tend to be diverse after initial isolation but subsequently suffer elevated selection pressures, loss of genetic diversity and elevated rates of extinction (Diamond, 1972; Foufopoulos and Ives, 1999; Stiller et al., 2010).

Despite the loss of genetic diversity caused by island living, many species that have become extinct or endangered on the mainland find refuge on islands due to relaxed competition and reduced predation pressures (Lister, 2004). KI retains the largest proportion of uncleared native vegetation of any southern Australian agricultural district. In addition KI remains free of rabbits and foxes that have had such a catastrophic impact on mainland biota (Robinson and Armstrong, 1999) enhancing its conservation importance. Biodiversity management can be complimented by aDNA analyses and assessments of the fossil record, providing parameters such as population sizes, levels of gene flow and population relatedness (Ramakrishnan et al., 2005; Leonard, 2008; De Bruyn et al., 2011).

2.1. Study site

The KHC complex on KI (35.83° S, 137.33° E) is ideally suited to explore biomolecule preservation as it has an already well-studied and well-dated palaeontological record that spans the terminal late Pleistocene to the middle Holocene (McDowell, 2013). In addition numerous surveys of the island's modern flora and fauna have also been made (Robinson and Armstrong, 1999).

KHC is the focus of an ongoing palaeontological project that investigates how climate change and isolation due to sea level rise has affected the fauna of KI (McDowell et al., 2013). To date, a fauna rich in mammals, birds, reptiles, frogs and land-snails has emerged

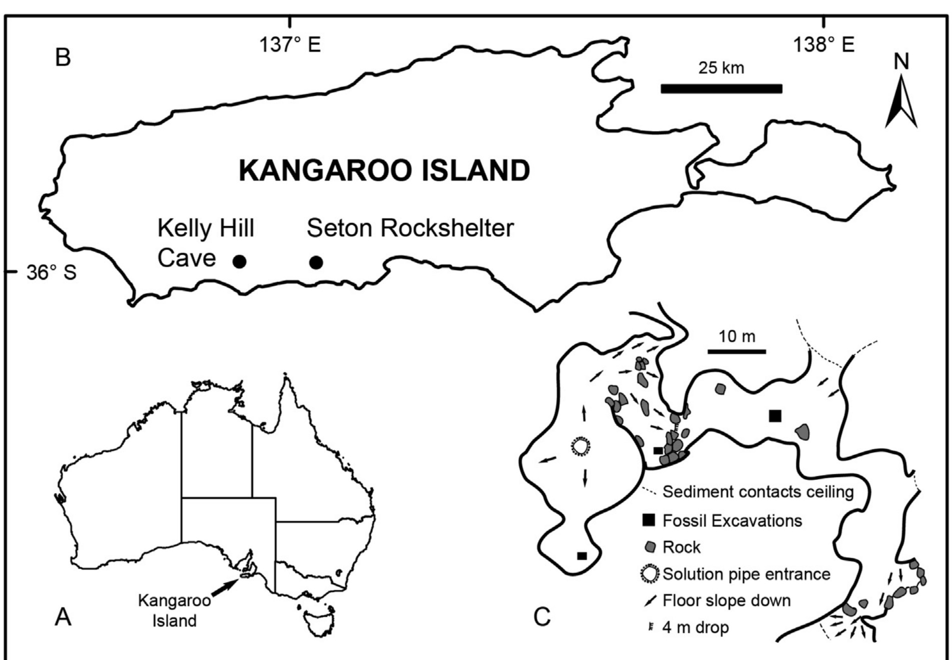

Fig. 1. A. Location of Kangaroo Island relative to the Australian mainland. B. Location of Kelly Hill Cave and Seton Rock Shelter, Kangaroo Island. C. Relevant map section of Kelly Hill Cave showing the location of modern solution pipe entrances, fossil excavation and the blocked palaeo-entrance through which excavated sediments and bones entered the cave (McDowell, 2013).