JOURNAL OF QUATERNARY SCIENCE (2014) **29**(7) 610–626 ISSN 0267-8179. DOI: 10.1002/jqs.2740

Review

Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects

NICOLAS J. RAWLENCE,[1,2] DAVID J. LOWE,[1]* JAMIE R. WOOD,[3] JENNIFER M. YOUNG,[4] G. JOCK CHURCHMAN,[5] YU-TUAN HUANG[1] and ALAN COOPER[4]

[1]School of Science, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand
[2]Allan Wilson Centre for Molecular Ecology and Evolution, Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[3]Landcare Research, PO Box 40, Lincoln, Canterbury 7640, New Zealand
[4]Australian Centre for Ancient DNA, School of Earth and Environmental Science, University of Adelaide, SA 5005, Australia
[5]School of Agriculture, Food and Wine, University of Adelaide, SA 5005, Australia

Received 1 March 2013; Revised 27 August 2014; Accepted 28 August 2014

ABSTRACT: Palaeoenvironmental DNA (PalEnDNA) is defined as ancient DNA (aDNA) originating from disseminated genetic material within palaeoenvironmental samples. Sources of PalEnDNA include marine and lake sediments, peat, loess, till, ice, permafrost, palaeosols, coprolites, preserved gut contents, dental calculus, tephras, and soils as well as deposits in caves/rockshelters and at archaeological sites. PalEnDNA analysis provides a relatively new tool for Quaternary and archaeological sciences and its applications have included palaeoenvironmental and palaeodietary reconstructions, testing hypotheses regarding megafaunal extinctions, human–environment interactions, taxonomic studies, and studies of DNA damage. Because PalEnDNA samples comprise markedly different materials, and represent wide-ranging depositional and taphonomic contexts, various issues must be addressed to achieve robust, reproducible findings. Such issues include climatic and temporal limitations, the biological origin and state (free versus bound) of PalEnDNA, stratigraphic reliability, sterile sampling, ability to distinguish modern from aDNA signals, DNA damage and PCR amplification, DNA extraction methods, and taxonomic resolution. In this review, we provide a non-specialist introduction to the use of PalEnDNA for Quaternary and archaeological researchers, assess attributes and limitations of this palaeoenvironmental tool, and discuss future prospects of using PalEnDNA to reconstruct past environments. Copyright © 2014 The Authors. *Journal of Quaternary Science* published by John Wiley & Sons Ltd on behalf of Quaternary Research Association

KEYWORDS: ancient DNA; archaeology; environmental DNA; palaeoecology; palaeoenvironmental DNA; PalEnDNA; Quaternary.

Introduction

The use of ancient DNA (aDNA) is becoming an increasingly powerful tool for Quaternary science and archaeology. Since the advent of aDNA-based research a quarter of a century ago (Higuchi *et al.*, 1984; Pääbo, 1985), it has been used to investigate wide-ranging topics, including biogeography (e.g. Mitchell *et al.*, 2014), ecosystem responses to climate change (such as the impact of Holocene climate change on faunal biodiversity: de Bruyn *et al.*, 2011), anthropogenic impact and extinction processes (e.g. Shapiro *et al.*, 2004; Drummond *et al.*, 2005), palaeoenvironments (e.g. Willerslev *et al.*, 2014), human health and disease (e.g. Adler *et al.*, 2013; Krüttli *et al.*, 2014; Metcalf *et al.*, 2014), and human evolution and palaeogenomics (e.g. Green *et al.*, 2010; Meyer *et al.*, 2012; Prüfer *et al.*, 2014). Some of these studies have used discrete materials, such as bone, teeth, leaves and eggshell (Table 1), but aDNA has also been extracted as disseminated genetic material from coprolites, preserved gut contents, dental calculus, sediments (including ice) and soils (Table 2). We term this disseminated genetic material 'palaeoenvironmental DNA' (PalEnDNA). PalEnDNA is becoming more commonly used in attempts to reconstruct past environments (Table 2). However, the first well-known PalEnDNA analysis was only published in 1998 (Poinar *et al.*, 1998) and many issues remain to be addressed to ensure sound and reliable reconstructions of past environments. Most important are the questions relating to the mixed origin(s) of DNA and its state (free versus bound, intracellular versus extracellular) and how these affect DNA extraction efficiency, stratigraphic reliability and the degradation of DNA.

The issues involved with the analysis and interpretation of PalEnDNA are somewhat analogous to those encountered during the early years of radiocarbon (^{14}C) dating and aDNA research. In both these disciplines, methodologies were in their infancy, and replication and self-testing were initially not carried out or made a priority. These deficiencies resulted in the publication of many high-profile claims regarding aDNA that could not be replicated by an independent laboratory. In one such case, Woodward *et al.* (1994) extracted and amplified short (up to 170 bp) fragments of DNA from 80 Ma dinosaur bones. Despite difficulties in identifying a phylogenetic match, Woodward *et al.* (1994) hypothesized the presence of degraded dinosaur DNA. Subsequently, Zischler *et al.* (1995), among others, showed that the putative dinosaur DNA sequences matched human DNA sequences (i.e. human contamination).

In this review, we aim to provide a broad overview of the use of PalEnDNA in Quaternary and archaeological research, to assess attributes and limitations of this palaeoenvironmental

*Correspondence: D. J. Lowe, as above.
E-mail: d.lowe@waikato.ac.nz

Table 1. Summary of sample materials used in aDNA research divided into 'discrete' aDNA and 'disseminated' aDNA. The latter forms the basis of most PalEnDNA research.

Specimens	Examples of use
Discrete fossil or subfossil materials	
Bone	Bunce *et al.* (2009)
Skin/muscle	Cooper *et al.* (1992)
Hair	Gilbert *et al.* (2007)
Feathers	Rawlence *et al.* (2009)
Eggshell	Oskam *et al.* (2010)
Insect cuticles	Thomsen *et al.* (2009)
Teeth	Adler *et al.* (2011)
Plants incl. macrofossils and pollen	Gugerli *et al.* (2005)
Discrete environmental materials	
Coprolites	Poinar *et al.* (1998)
Preserved gut contents	van Geel *et al.* (2008)
Dental calculus	Adler *et al.* (2013)
Disseminated environmental materials	
Sediments (excluding ice)	Willerslev *et al.* (2003)
Ice	Willerslev *et al.* (2007)
Soil	Hebsgaard *et al.* (2009)
Tephra	Haile *et al.* (2007)

tool and to discuss future prospects of using PalEnDNA to reconstruct past environments. To achieve these aims, our review focuses on the following areas:

1. Definitions of aDNA and PalEnDNA
2. Progress in the use of PalEnDNA for Quaternary and archaeological research
3. Climatic and temporal limitations of PalEnDNA-based research
4. Further issues with PalEnDNA-based research
5. Recommendations for future PalEnDNA-based research
6. Prospects for the use of PalEnDNA for palaeoenvironmental reconstruction.

Defining aDNA and PalEnDNA

aDNA is defined as highly degraded, fragmented and chemically modified DNA extracted from historical, archaeological and fossil remains (Fig. 1). Typically, aDNA samples are discrete materials, such as fossil samples of bones, eggshells, feathers or plant remains (e.g. see Gugerli *et al.*, 2005) in contrast to 'environmental' samples (see below). In this definition, aDNA is not defined by age alone but can include archival samples containing degraded DNA. Typically, the size of aDNA fragments is limited to <300 bp, compared with the multiple kilobases standard for modern DNA, unless extraordinary preservation circumstances exist. These circumstances generally feature cold and dry conditions (Orlando *et al.*, 2013) or samples derived from geologically very young materials (e.g. less than around 1000 years old: Rawlence and Cooper, 2013). Most aDNA studies have focused on mitochondrial DNA (mtDNA) for animals, or chloroplast DNA (cDNA) for plants, because these circular genomes exist in multiple copies per cell. In contrast, a single copy nuclear DNA (nDNA) locus has only two copies per cell (inherited from each parent). Consequently, the probability of DNA preservation is generally assumed to be greater for mtDNA and cDNA loci because of their high abundance compared with that of nDNA. As for fragment size, the preservation of large amounts of nDNA is generally limited to situations where highly favourable circumstances exist for preservation (Orlando *et al.*, 2013).

Environmental samples used in aDNA research are those obtained from various sedimentary deposits, including marine and lacustrine sediments, peat, loess, glacial till, ice and frozen materials (permafrost) as well as tephras (loose pyroclastic deposits of explosive volcanic origin including volcanic ash: Lowe, 2011), deposits in caves and rockshelters and archaeological sites, soils, buried soils (palaeosols), coprolites, preserved gut contents and dental calculus (Figs 2 and 3). The optimal materials for sampling and extraction are permafrost (e.g. Lydolph *et al.*, 2005), recently frozen material (e.g. Gould *et al.*, 2010) or desiccated remains (e.g. Wood *et al.*, 2013a, b) mainly because of the exceptional preservation of aDNA provided by such conditions/materials. However, there is potential for many other materials to provide information about the past via aDNA analysis.

The aDNA within environmental samples originates from multiple sources, which can have a significant impact on DNA extraction efficiency (Haile, 2012). The DNA potentially originates from plant material (rootlets, seeds, leaves, pollen), animal waste products (faeces, urine), material that has been shed (skin, hair, feathers, nails) and invertebrate remains (Willerslev *et al.*, 2003; Lydolph *et al.*, 2005; Andersen *et al.*, 2012; Yoccoz *et al.*, 2012). In coprolites, DNA can comprise the defaecator's intestinal biota (e.g. bacteria, parasites), dietary components and environmental sources that arise after deposition (e.g. bacterial and fungal growth) (Goldberg *et al.*, 2009; Jenkins *et al.*, 2012; Tito *et al.*, 2012; Wood *et al.*, 2012a, b, c, 2013a, b).

Environmental samples can be lithologically, mineralogically, chemically and biologically variable, containing disseminated, non-discrete material, such as sediment or soil (rather than samples of bone, for example), and represent a wide range of depositional and taphonomic contexts. We advocate the portmanteau abbreviation 'PalEnDNA' to replace several previous terms used for palaeoenvironmental DNA (Fig. 2). Here, PalEnDNA is defined as aDNA originating from disseminated genetic material within palaeoenvironmental samples, and the analysis of PalEnDNA is an exploratory subdiscipline of broader aDNA studies (Fig. 2). Although technically discrete material in one sense, PalEnDNA from coprolites, preserved gut contents and dental calculus originates ultimately from multiple sources. The term 'environmental DNA' (or eDNA) in the context of isolating aDNA from environmental samples (Hebsgaard *et al.*, 2005; see also Bohmann *et al.*, 2014) is not appropriate because eDNA includes modern DNA. 'Sedimentary ancient DNA' (or sedaDNA) (Haile *et al.*, 2009) is used widely and applies to DNA isolated from sedimentary deposits, but this term technically cannot include samples extracted from tephras (which are volcanic/pyroclastic deposits), soils (which form on a wide range of lithologies in addition to sedimentary deposits) or coprolites. 'Lake sediment DNA' (lake sedDNA) obviously applies only to lake sediments (Giguet-Covex *et al.*, 2014). Similarly, 'dirt DNA' (Willerslev and Cooper, 2005; Hebsgaard *et al.*, 2009) applies only to soil-derived aDNA (moreover, the term 'dirt' is a pejorative and non-scientific word meaning excrement). Finally, the use of the term 'fossil DNA' by Coolen and Gibson (2009) with respect to lacustrine sediments is too restricted and easily confused with aDNA isolated from Quaternary fossil material, such as bone. PalEnDNA encompasses all of these sample types.

Progress in the use of PalEnDNA in Quaternary and archaeological research

Since the first publication reporting reproducible PalEnDNA (Poinar *et al.*, 1998, who isolated ground sloth and plant aDNA from a coprolite dated to ca. 20 000 ^{14}C a BP), an increasing number of Quaternary and archaeological studies have incorporated PalEnDNA analyses (Table 2). These

Table 2. A range of PalEnDNA-related publications*. Studies have been organized according to general research area and sample type.

Deposit or material	Target taxa	Age range	Examples (references)
PALAEOECOLOGY: PALAEOENVIRONMENTAL RECONSTRUCTION			
Peat	Plants	155 ka	Suyama *et al.* (1996, 2008)
Permafrost	Bacteria, fungi, bryophytes, plants, invertebrates (insects), vertebrates (mammals, birds)	2 – < 600 ka	Shi *et al.* (1997), Willerslev *et al.* (2003, 2004), Lydolph *et al.* (2005), Vishnivetskaya *et al.* (2006), Johnson *et al.* (2007), Sønstebø *et al.* (2010), Arnold *et al.* (2011), D'Costa *et al.* (2011), Kuzmina *et al.* (2011), Boessenkool *et al.* (2012), Epp *et al.* (2012), Willerslev *et al.* (2014)
Ice	Fungi, protists, plants, invertebrates (insects)	0.3 – < 800 ka	Willerslev *et al.* (1999, 2007), Ma *et al.* (2000)
Lacustrine	Diatoms, plants, invertebrates (crustaceans, copepods)	13 cal ka–modern	Limburg and Weider (2002), Bissett *et al.* (2005), Parducci *et al.* (2005, 2013), Marková *et al.* (2006), Bennett and Parducci (2006), Anderson-Carpenter *et al.* (2011), Xu *et al.* (2011), Magyari *et al.* (2011), Stoof-Leichsenring *et al.* (2012), Gugerli *et al.* (2013), Pedersen *et al.* (2013), Boessenkool *et al.* (2014)
Cave deposit	Plants, invertebrates (insects), vertebrates (mammals, birds)	10.8–0.6 ^{14}C ka	Hofreiter *et al.* (2003b), Willerslev *et al.* (2003), Thomsen *et al.* (2009), Haouchar et al. (2014)
Marine	Invertebrates (foramnifera, radiolarian), plants	≤45 ka	Paffetti *et al.* (2007), Lejzerowicz *et al.* (2013)
Glacial (fluviogravel and moraine)	Plants	4.5–5.2 cal ka	Gould *et al.* (2010)
Soil	Plants, vertebrates (mammals, birds)	5.5 cal ka–modern	Anderson *et al.* (2012), Jørgensen *et al.* (2012b), Wilmshurst *et al.* (2014)
Rodent midden	Plants, vertebrates	10.1 ^{14}C ka	Kuch *et al.* (2002)
Silt, sand, organic deposit (silt-soaked)	Plants		Jørgensen et al. (2012a)
PALAEOECOLOGY: MEGAFAUNAL EXTINCTION			
Permafrost	Vertebrates (mammals, birds)	7.6–11.76 ^{14}C ka	Haile *et al.* (2009)
PALAEOECOLOGY: PALAEODIETARY RECONSTRUCTION			
Coprolite	Plant, invertebrates (parasites), vertebrates (mammals, birds)	32–0.6 ^{14}C ka	Poinar *et al.* (1998, 2001), Hofreiter *et al.* (2000, 2003a), Reinhard *et al.* (2008), Wood *et al.* (2008, 2012a,b,c, 2013a,b)
Intestinal contents	Plants, vertebrates (mammals)	41.9–5.1 ^{14}C ka	Rollo *et al.* (2002), van Geel *et al.* (2008, 2011, 2012, 2014)
Dental calculus (calcified plaque)	Bacteria	Mesolithic, Neolithic, Bronze Age, Medieval	Adler *et al.* (2013), De la Fuente *et al.* (2013), Metcalf *et al.* (2014), Warinner *et al.* (2014)
PALAEOECOLOGY: TAXONOMY			
Coprolite	Vertebrates (mammals)	Late Pleistocene to <3 ^{14}C ka	Poinar *et al.* (2003), Bunce *et al.* (2009), Campos *et al.* (2010)
Hairs in coprolite	Vertebrates (mammals)	13 ka	Clack *et al.* (2012a,b)
ARCHAEOLOGICAL HISTORY			
Cave deposit	Plants, vertebrates (mammals, birds)	12.3 ^{14}C ka	Gilbert *et al.* (2008, 2009), Goldberg *et al.* (2009), Poinar *et al.* (2009), Rasmussen *et al.* (2009), Jenkins *et al.* (2012)
Lacustrine	Bacteria, vertebrates (mammals, fish)	ca. 1.7 cal ka	Matisoo-Smith *et al.* (2008), Madeja *et al.* (2010), Giguet-Covex *et al.* (2014)
Soil	Vertebrates (mammals)	0.9–0.39 ^{14}C ka	Hebsgaard *et al.* (2009)
Gravel, sand, gyttja, peat	Bacteria	10–2.9 ^{14}C ka	Madeja *et al.* (2009)
Coprolites	Bacteria, invertebrates (parasites), vertebrates (mammals, birds)	12.3 ^{14}C ka – 0.6 cal ka	Loreille *et al.* (2001), Iniquez *et al.* (2003a, b, 2006), Kemp *et al.* (2006), Luciani *et al.* (2006), Gilbert *et al.* (2008, 2009), Leles *et al.* (2008), Goldberg *et al.* (2009), Poinar *et al.* (2009), Rasmussen *et al.* (2009), Speller *et al.* (2010), Jenkins *et al.* (2012), Tito *et al.* (2012)
DNA DAMAGE			
Permafrost	All taxa, bacteria	600–10 cal ka	Mitchell *et al.* (2005), Hansen *et al.* (2006)

*A more comprehensive list is given in supporting information Table S1.

studies mainly focused on palaeoecology and archaeology (e.g. Speller *et al.*, 2010; Lejzerowicz *et al.*, 2013), with recent but limited work on tephras (Haile *et al.*, 2007) and soils (e.g. Andersen *et al.*, 2012; Jørgensen *et al.*, 2012b; Wilmshurst *et al.*, 2014).

Palaeoecology

Palaeoecological research using PalEnDNA has included examination of bacteria, animals and plants to reconstruct past environments and evaluate ecosystem change through time (Table 2). Willerslev *et al.* (2007) isolated PalEnDNA from silt-rich ice at the base of the Dye-3 drill core through the Greenland ice sheet (dated to 450–800 ka), and showed that central Greenland supported a diverse flora and fauna, including conifers and butterflies, during a major ice retreat phase – perhaps during Marine Oxygen Isotope Stage 11 (Alley *et al.*, 2010) – before it was subsequently covered in ice more than 2 km in thickness. More recently, Gould *et al.* (2010) extracted PalEnDNA from frozen plant material uncovered by receding glaciers in south-eastern Peru. The material, found at 5200 m elevation and dated to 4500–5200 cal a BP, represents the ice-free vegetation in the area before climatic conditions changed in the mid-Holocene, allowing the extension of glaciers and formation of an ice cap. The study showed that the pre-glacial vegetation was characteristic of wetland environments, which occur predominantly at warmer lower elevations today. Similarly, D'Costa *et al.* (2011) isolated ancient bacterial DNA from frozen sediments from the Dawson City area, Yukon Territory, aged ca. 30 000 cal a BP, and they characterized genes conferring antibiotic resistance to confirm that antibiotic resistance is a natural phenomenon in ecosystems that pre-dates the modern selective pressure of clinical antibiotic use (see also Metcalf *et al.*, 2014; Warinner *et al.*, 2014).

PalEnDNA from coprolites and preserved gut contents has been used to reconstruct palaeodiets of extinct fauna, particularly woolly mammoth, bison and the moa, a large, flightless New Zealand ratite bird (van Geel *et al.*, 2008, 2012, 2014; Wood *et al.*, 2008, 2012a, b, 2013a, b). PalEnDNA from coprolites also contains information about parasite faunas, including those of extinct species. For example, Wood *et al.* (2013a) isolated parasite aDNA and eggs from moa coprolites to show that when moa were hunted to extinction by Polynesians/early Maori, potentially a suite of cryptic co-extinctions in the fossil record was attainable. This research also revealed that parasite abundance could be mapped geographically and that it was largest in lowland moa species, which were at greater population densities.

Archaeology

aDNA has been used in a variety of archaeological contexts but the use of PalEnDNA is an underdeveloped but emerging area of archaeological research. Currently, PalEnDNA has been used to examine broad archaeological topics, such as past human and farming activities including animal domestication (Hebsgaard *et al.*, 2009; Speller *et al.*, 2010; Giguet-Covex *et al.*, 2014), ancient human health (Leles *et al.*, 2008; Tito *et al.*, 2012; Adler *et al.*, 2013; Warinner *et al.*, 2014), whether it is possible to use bacterial indicators of 'human presence' (Matisoo-Smith *et al.*, 2008; Madeja *et al.*, 2009, 2010, cf. the use of faecal sterol molecular markers by D'Anjou *et al.*, 2012) and the settlement chronology of the Americas (Gilbert *et al.*, 2008; Jenkins *et al.*, 2012) (Table 2). Further research could utilize PalEnDNA (in the absence of an archaeological record) to examine the introduction of food crops and animals and to evaluate the environmental impacts of humans.

Hebsgaard *et al.* (2009) analysed a soil profile from a field adjacent to a Norse farm in Greenland dated to 1000–1400 AD to investigate past farming activities and to track the relative proportions of cattle, sheep and goat aDNA at the site. The study showed that cattle decreased in relative abundance between 1180 and 1260 AD, reflecting abandonment of the farm but occasional use by other Norse farmers for livestock grazing. Similarly, Speller *et al.* (2010) investigated turkey domestication using aDNA from bones and PalEnDNA from coprolites sourced from archaeological sites in the south-western USA dating from 200 BC to 1800 AD. Their study showed that turkeys were domesticated twice in North America before European contact.

Tito *et al.* (2012) used PalEnDNA from human coprolites (sourced from archaeological sites in south-western USA, Mexico, and Chile) to examine the ancestral human distal gut microbial community assemblage and human health, and examined several approaches to address issues with post-depositional changes in microbial content. Similarly, dental calculus (calcified plaque) on the teeth of ancient humans has also been used to examine changes in the human oral

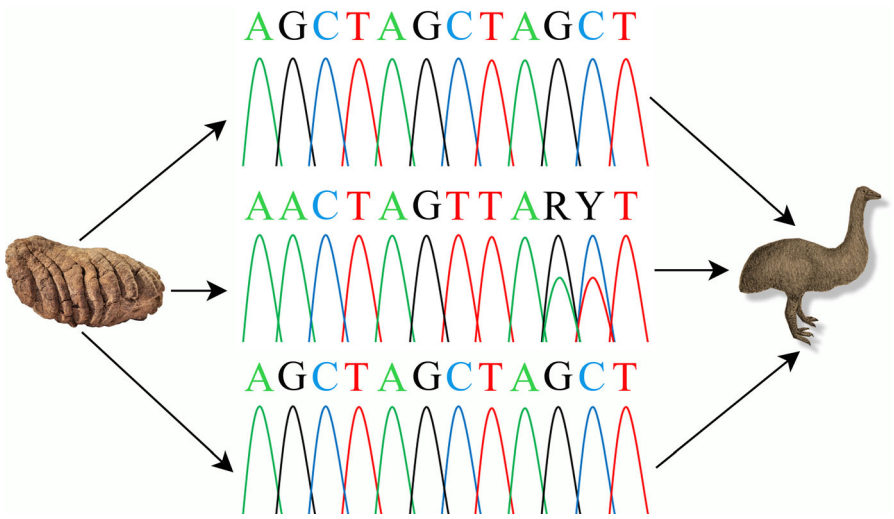

Figure 1. Schematic diagram of ancient DNA (aDNA) sequences obtained from a moa (Aves: Dinornithiformes) coprolite. The three sequences represent the same mitochondrial DNA (mtDNA) region used to identify moa coprolites to species. Because there are multiple mtDNA genomes per cell, each fragment has been independently amplified from a different genome. The peaks represent one of the four DNA bases, adenine (A), guanine (G), cytosine (C) and thymine (T). aDNA is highly degraded, fragmented and chemically modified. One common type of DNA damage is miscoding lesions, which are post-mortem base pair modifications leading to the incorporation of incorrect bases during DNA amplification. The middle sequence has characteristic miscoding lesions (unlike the upper and lower DNA sequences) represented by G–A and C–T transitions or by the ambiguous bases R (G or A) and Y (C or T). Ambiguous bases result from varying levels of DNA damage on each of the mitochondrial genomes.

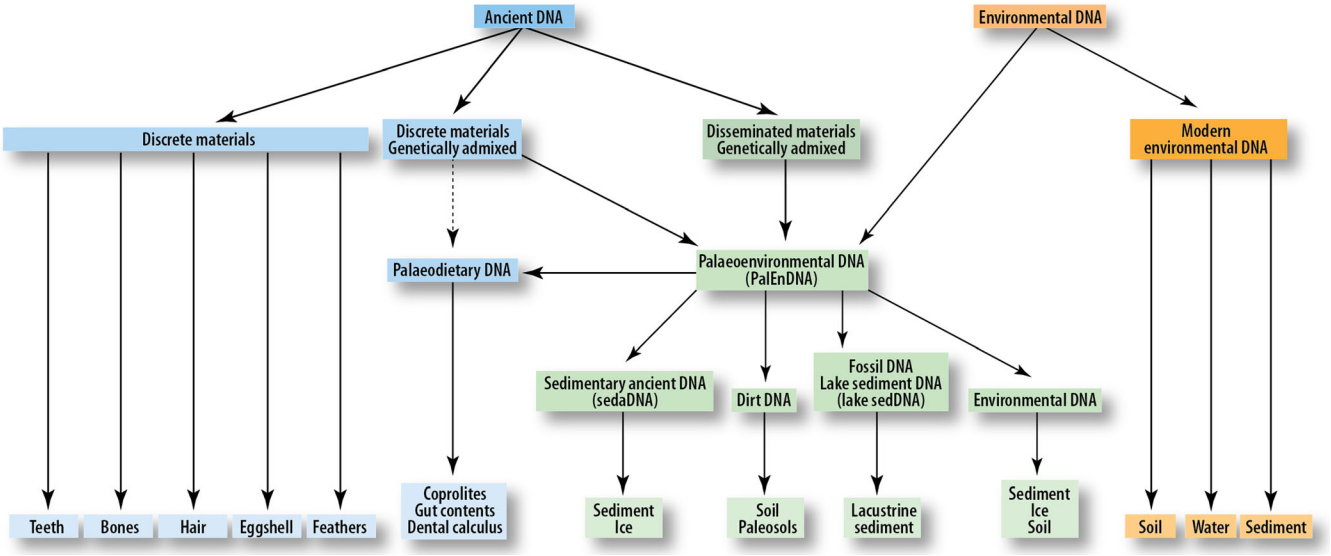

Figure 2. Hierarchy and relationships of the main descriptive terms and sampling materials for PalEnDNA research in comparison with terms used for modern environmental DNA research and aDNA research focusing on discrete samples. We suggest that some terms used previously, including dirt DNA, environmental DNA (in the context of aDNA derived from sediment), sedimentary ancient DNA, lake sediment DNA and fossil DNA, are ambiguous or unnecessary and should be abandoned, and 'palaeoenvironmental DNA' (PalEnDNA) used instead. Studies on coprolites, preserved gut contents and dental calculus fall under PalEnDNA but, as effectively 'discrete' rather than 'disseminated' materials per se, they additionally occupy an intermediary position (see also Table 1).

Figure 3. Examples of deposits that potentially contain PalEnDNA. Clockwise from top left: permafrost comprising frozen organic-rich loess and ice wedges (Yukon Territory, Canada); three split cores of organic-rich lake sediment containing well-preserved tephra layers dating from ca. 15 600 to ca. 7000 cal a BP (NI, New Zealand) (photo from Lowe, 1988, p. 133, with permission from Taylor and Francis; age on layer labelled Mamaku Ash is ca. 8000 cal a BP); moa coprolite (SI, New Zealand); preserved dental calculus (denoted by arrow) on ancient human molar tooth (photo courtesy of Julien Soubrier and Laura Weyrich, ACAD); multisequal soil and palaeosols developed on five Holocene tephra beds (NI, New Zealand) (see McDaniel *et al.*, 2012); and peat, with a thin, mid-Holocene tephra layer showing as a white layer (NI, New Zealand) (photo from Alloway *et al.*, 2013, p. 288, with permission from Elsevier).

microbiome and diet over time, and host immunity (Adler *et al.*, 2013; Metcalf *et al.*, 2014; Warinner *et al.*, 2014). Dental calculus holds a major advantage for microbial study because the bacterial DNA is calcified in place before death (e.g. Fig. 3), minimizing subsequent taphonomic alteration of community signals that complicate coprolite analysis. Both the coprolite and the dental calculus studies suggested that ancestral human microbial communities are a close match to those from modern rural or hunter-gatherer communities, and that cosmopolitan lifestyles have led to significant changes in the human microbiome.

The use of PalEnDNA from sediments as an indicator of human presence is controversial (e.g. Matisoo-Smith *et al.*, 2008; Madeja *et al.*, 2009, 2010). PalEnDNA from lake sediments sampled from Round Lake, northern New Zealand (Matisoo-Smith *et al.*, 2008), revealed bacterial sequences below the Taupo tephra (dated at 232 ± 10 AD: Hogg *et al.*, 2012) that were a 98–99% match to human faecal bacteria (e.g. *Prevotella* spp.). From this match, the authors suggested human presence in New Zealand before the currently accepted earliest settlement date of ca. 1280 AD (Higham *et al.*, 1999; Hogg *et al.*, 2003; Wilmshurst *et al.*, 2008; Lowe, 2011). However, as admitted by Matisoo-Smith *et al.* (2008), *Prevotella* can also be found in animals' and insects' guts that utilize anaerobic digestion, thereby compromising its use as an indicator of human presence unless species-level identifications are possible.

Studies on tephras and soils

Although several aDNA studies have utilized tephras as an age-equivalent dating method through tephrochronology (e.g. Chan *et al.*, 2005; D'Costa *et al.*, 2011; Letts *et al.*, 2012), so far only Haile *et al.* (2007) have reported PalEnDNA from tephra deposits. Their study found moa DNA within two discrete tephra layers in two adjacent New Zealand caves, which Haile *et al.* (2007) suggested originated either from trampling of faeces or feathers, or the incorporation into the tephras of previously deposited aDNA-bearing cave sediment (Fig. 4).

Under some circumstances the deposition of tephra can increase the probability of fossil preservation. Pyroclastic flows are emplaced at high temperatures (ca. 150–700 °C) (e.g. Banks and Hoblitt, 1981; McClelland *et al.*, 2004; Hudspith *et al.*, 2010), rendering such deposits temporarily sterile (e.g. Smith, 1985; Clarkson *et al.*, 1988; VanderHoek and Nelson, 2007). Burial through rapidly accumulating tephra-fall, especially in proximal localities, is also likely to increase the preservation potential because any biological material is quickly isolated from biogeochemical processes active at the land surface (Ponnamperuma *et al.*, 1967; Fridriksson, 1987; Allen *et al.*, 2005). Consequently, it has been hypothesized that tephra, where present, could provide sterile control layers between deposits containing PalEnDNA – for example, Haile *et al.* (2007) assumed there would be no aDNA within tephra layers. However, soils developed on tephra deposits tend to promote excessively long root growth (e.g. Lowe and Palmer, 2005; Hewitt, 2010; McDaniel *et al.*, 2012) that penetrate through layers, and the assumption of sterility is thus questionable.

Few studies have explicitly examined PalEnDNA from soils or palaeosols (Table 2) (e.g. Hebsgaard *et al.*, 2009; Jørgensen *et al.*, 2012b; Yoccoz *et al.*, 2012; Wilmshurst *et al.*, 2014). Jørgensen *et al.* (2012b) analysed PalEnDNA extracted from soil developed on nunataks (rock outcrops) in southern Greenland, together with modern and historical botanical survey data, to examine changes in plant species composition since the Holocene Thermal Maximum (ca. 5500 cal a BP). The research showed that significant changes in species composition occurred with anthropogenic climate change in contrast to previous episodes of natural climate change. In an investigation on pollen and aDNA in soil cores from an offshore island, Tawhiti Rahi, the largest of the Poor Knights Islands group in northern New Zealand, Wilmshurst *et al.* (2014) found that even though better taxonomic resolution was possible with pollen analysis compared with short fragments of aDNA, the aDNA provided a very useful complementary dataset by confirming the local presence of certain taxa. Moreover, Wilmshurst *et al.* (2014) found no sign of post-depositional

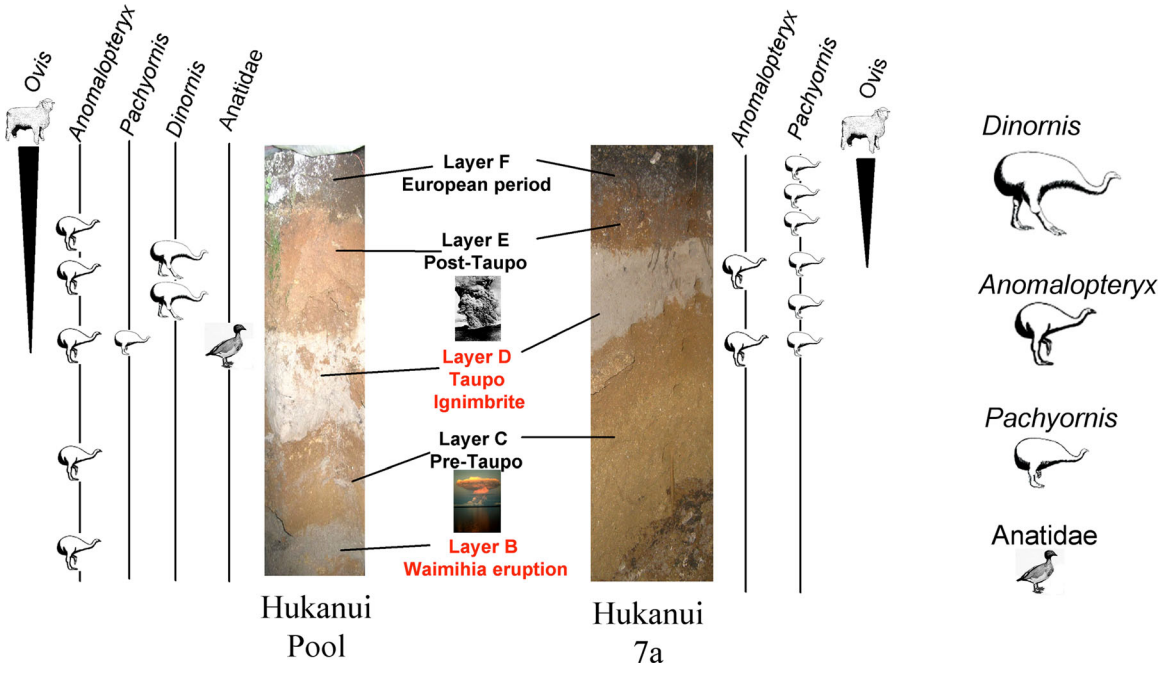

Figure 4. Evidence for DNA leaching in two different stratigraphic sections (approximately 300 m apart) at Hukanui cave sites, eastern North Island, New Zealand. The two named tephras are layer D, Taupo ignimbrite (erupted 232 ± 10 AD) (Hogg *et al.*, 2012) and layer B, Waimihia tephra (erupted 3401 ± 108 cal ka BP) (Lowe *et al.*, 2013). Reproduced from Haile *et al.* (2007, p. 984, with permission from Oxford University Press).

reworking and mixing with modern material in pre-human samples.

Despite these studies showing the utility of soils and palaeosols for PalEnDNA analysis, such materials are usually only examined from permafrost or cave/rockshelter settings (e.g. Lydolph *et al.*, 2005; Haile *et al.*, 2007; Johnson *et al.*, 2007) rather than from extensive soils from open (non-frozen) landscapes. By nature, soils comprise a highly complex and varied ecosystem (Churchman and Lowe, 2012) and can be grouped at the highest taxonomic level into orders (with numerous sub-orders, groups, and lower taxa) (e.g. Ahrens and Arnold, 2012) defined by characteristics such as different amounts and types of clay minerals, which potentially affect DNA binding capacity, DNA preservation and DNA extraction efficiency (Lloyd-Jones and Hunter, 2001; Herrera and Cockell, 2007; Young *et al.*, 2014). For example, modifications to DNA extraction methods are commonly required for soils with high concentrations of humic acids and certain types of clay minerals (e.g. allophane, a nanocrystalline aluminosilicate clay with extremely high surface areas and variable charge: Churchman and Lowe, 2012) that promote phosphate, and therefore potentially DNA, adsorption (e.g. Herrera and Cockell, 2007; Rai *et al.*, 2010; Huang *et al.*, 2014).

Climatic and temporal limits of PalEnDNA-based research

The theoretical limit of aDNA survival under ideal conditions, such as in permafrost and ice, is ca. 1 million years (Lindahl, 1993a, b; Allentoft *et al.*, 2012). However, the current empirical limit is up to 400–800 ka for PalEnDNA from ice and permafrost (Willerslev *et al.*, 2007) and around the same age for bone (Orlando *et al.*, 2013). In general, DNA preservation is site specific and heavily influenced by the thermal history of the material (Lindahl, 1993a, b; Smith *et al.*, 2001, 2003; Sawyer *et al.*, 2012). The highest success rate for PalEnDNA isolation is achieved with specimens from frozen (Gould *et al.*, 2010), arid, or temperate areas rather than hot and humid areas (Poinar *et al.*, 1998; Thomsen *et al.*, 2009; Haouchar *et al.*, 2014). The current upper ages for replicable results from warm to cool, wet sites (including those containing lacustrine and marine deposits) are ca. 10 000 and ca. 45 000 years, respectively (Bissett *et al.*, 2005; Paffetti *et al.*, 2007; Anderson-Carpenter *et al.*, 2011; Lejzerowicz *et al.*, 2013), compared with ca. 32 000 years for dry sites (Poinar *et al.*, 1998). Hot and humid locations do not favour DNA preservation, although small amounts may still be isolated if the microsite conditions are suitable (Larson *et al.*, 2007) and appropriate precautions against exogenous contamination are taken (Thomson *et al.*, 2014a,b). New *in vitro* DNA repair mechanisms and single-stranded genomic library methods have the potential to increase the empirical limit of DNA detection to include mid-Quaternary remains (see Meyer *et al.*, 2012).

Issues with PalEnDNA-based research

DNA extraction

PalEnDNA can exist as either intracellular DNA or extracellular DNA. Extracellular DNA can be either free from the matrix (non-metabolized and metabolized via invertebrates and bacteria), or bound to humic acids, minerals (including clay), proteins and sugars (Alvarez *et al.*, 1998; Poinar *et al.*, 1998; Arnold *et al.*, 2011). The form of PalEnDNA within a sample may have a significant effect on DNA extraction efficiency. Humic acids and some clay minerals (e.g. allophane) have a strong binding capacity for DNA, therefore inhibiting recovery of DNA from such materials (Alvarez *et al.*, 1998; Saeki *et al.*, 2010; Huang *et al.*, 2012, 2014). Saeki *et al.* (2010) showed that <2% of adsorbed DNA could be released from humic acids. There has been limited testing of commonly used PalEnDNA extraction methods to determine the relative efficiency and taxonomic biases (e.g. Willerslev *et al.*, 2003; Xu *et al.*, 2011; Haile, 2012; Kuch and Poinar, 2012; Taberlet *et al.*, 2012b). van Geel *et al.* (2012) showed that fast commercial and slower in-house extraction methods of mammoth intestinal contents resulted in a different but complementary taxonomic coverage for plant species. In contrast to PalEnDNA, it is well known within the soil microbiological field that different DNA extraction methods can result in an over- or under-representation of specific bacterial phyla, with extraction efficiency and taxonomic coverage forming important aspects of experimental design (e.g. Holmsgaard *et al.*, 2011; Knauth *et al.*, 2013; Young *et al.*, 2014).

Geographical origin

It is generally assumed that PalEnDNA reflects a local signal, with animals required to be physically present to leave genetic traces, and the physical conditions of a site (e.g. size of cave entrance), organism behaviour and physiology (e.g. urine content, biomass) dictating which species are able to be detected (Lydolph *et al.*, 2005; Haile *et al.*, 2007; Andersen *et al.*, 2012). Lydolph *et al.* (2005) isolated ancient fungal DNA from Siberian permafrost, and showed that mammalian coprophilous fungi were present in samples containing mammalian DNA, suggesting that the PalEnDNA originated from animal faeces and was local in origin. Haile *et al.* (2007) showed that the DNA of small moa was only found in small rockshelters, whereas DNA of moa of all sizes was found in large rockshelters, also suggesting a local origin in this instance. Andersen *et al.* (2012) found similar results to those of Lydolph *et al.* (2005) and Haile *et al.* (2007) in European zoological parks. Animal DNA distribution was governed by behaviour and use (e.g. trails and latrine sites), and was absent from sites not frequented. For plants, Yoccoz *et al.* (2012) showed that plant DNA from sediment and soil reflected the taxonomic diversity of the local underground plant biomass (i.e. roots).

Recently, it has been argued that regional processes may also be responsible for some PalEnDNA. For example, a significant component of PalEnDNA can originate from long-distance dispersal (e.g. wind-blown pollen) and post-depositional reworking (Arnold *et al.*, 2011; Reyes *et al.*, 2011). Jørgensen *et al.* (2012a) found that although plant macrofossils (and the aDNA extracted from them) represented a local signal, pollen represented a regional and reworked signal. Ancient DNA can be isolated from individual pollen grains (Parducci *et al.*, 2013), meaning regional and reworked pollen signals could influence palaeonenvironmental reconstructions based on PalEnDNA. In reality, the geographical origin of PalEnDNA is probably site- and taxon-specific, and PalEnDNA is likely to reflect a mixture of local, regional and reworked signals.

Stratigraphic reliability

Stratigraphic reliability can be compromised by post-depositional reworking and DNA leaching or migration, and can cause intermixing of modern and ancient genetic signals and seriously affect the robustness of palaeoenvironmental

reconstructions. Such intermixing can occur through the movement of water, active bacterial or fungal growth (e.g. Tito *et al.*, 2012), and bioturbation, especially in marine and lacustrine sediments and in soils.

Post-depositional reworking occurs when material from one stratigraphic layer or position is incorporated into another layer or position of a different age. This reworking has been shown to occur during glacial–interglacial transitions and in soils via pedogenesis (including multiple processes, such as leaching of solutes, migration of clay or organic matter in suspension, and soil mixing by biota or by shrink–swell or freeze–thaw processes), through mass movement events on slopes, or by mixing of materials from the act of coring itself. Reworking may partly explain differences between taxonomic assemblages reconstructed from plant macrofossil, pollen and PalEnDNA (Arnold *et al.*, 2011; Reyes *et al.*, 2011; Jørgensen *et al.*, 2012a). Post-depositional reworking poses considerable problems for PalEnDNA analyses when deposits without good stratigraphic control are chosen for analysis. For example, Haile *et al.* (2009) isolated mammoth and horse aDNA from palaeosols developed on loess adjacent to the Yukon River, Alaska, dated to ca. 10 500 cal a BP which was 3000–5000 cal years younger, and 8 m higher, in a stratigraphic succession than the last fossilized instances of these species in the Alaskan–Yukon region. Haile *et al.* (2009) therefore concluded that there was no upward reworking at the site because of the absence of mammoth and horse aDNA in control samples taken from adjacent sites. However, it is difficult to rule out reworking of older upstream deposits in settings such as a floodplain. In a separate study, Arnold *et al.* (2011) isolated mammoth aDNA from permafrost dating to ca. 5000 cal a BP, long after mammoth became extinct in the region. Dating of permafrost sediments containing mammoth aDNA using both ^{14}C for organic fractions and optically stimulated luminescence (OSL) for inorganic fractions showed significant mismatches between the ages obtained (^{14}C: ca. 19 000–5000 cal a BP; OSL: mean age 5700 a BP). These results suggest that extensive reworking can occur even in some permafrost situations, and reinforces the point that an understanding of geomorphological or pedogenic processes, and stratigraphic control, is critical for successful PalEnDNA research.

DNA leaching and migration (hereafter referred to as DNA leaching) occur when stratigraphically younger DNA is transported through a sequence into older layers or vice versa (Fig. 4). DNA leaching may be partly a function of animal behaviour (e.g. nature of latrine sites, population density), physiology (e.g. mammalian versus avian, urine volume and form), the amount of net water movement (e.g. degree of soil saturation at a latrine site) and soil properties (e.g. grain-size distribution, pore size distribution, hydraulic conductivity: Andersen *et al.*, 2012). The extent of DNA leaching is heavily debated (e.g. Gilbert *et al.*, 2008; Rasmussen *et al.*, 2009; cf. Poinar *et al.*, 2009) and its prevalence is unknown. Currently, DNA leaching has been documented with mammalian-derived DNA in seasonally wet sites and possibly coprolites (Haile *et al.*, 2007; Andersen *et al.*, 2012; Jenkins *et al.*, 2012). Haile *et al.* (2007) found that in temperate New Zealand caves, modern sheep DNA had leached into pre-Polynesian layers containing extinct avian species. In contrast, Hebsgaard *et al.* (2009) found no evidence for DNA leaching in a wet open site in Greenland, suggesting this phenomenon may not be a universal concern in seasonally wet sites in cold environments.

In contrast to wet sites, it has been hypothesized there is no DNA leaching in permafrost, recently frozen sediments, in some dry cave deposits, or in saturated marine or lacustrine sediments (Willerslev *et al.*, 2004; Lydolph *et al.*, 2005; Hansen *et al.*, 2006; Gilbert *et al.*, 2008; Anderson-Carpenter *et al.*, 2011; Jenkins *et al.*, 2012; Giguet-Covex *et al.*, 2014). However, DNA leaching must be tested critically in each site and situation to enable it to be ruled out or otherwise (e.g. Haile *et al.*, 2007; Jenkins *et al.*, 2012; Giguet-Covex *et al.*, 2014).

DNA leaching can also occur when environmental DNA 'leaches' into coprolites from the surrounding matrix (Jenkins *et al.*, 2012). Jenkins *et al.* (2012) excavated a Camelidae coprolite from Paisley Cave, Oregon, with a macrofossil age of 12 125 ± 30 ^{14}C a BP, which was found to have a water-soluble fraction age of 11 315 ± 25 ^{14}C a BP, a difference of around 800 ^{14}C years. Three human coprolites were excavated in close proximity to the Camelidae coprolite but these had concordant macrofossil and water-soluble fraction ^{14}C ages. Jenkins *et al.* (2012) suggested that DNA leaching had occurred in the Camelidae coprolite but that any DNA leaching was limited spatially, stratigraphically and in volume, and that any wetting events (to enable dissolution and leaching to occur) were rare.

DNA damage

PalEnDNA preservation is not uniform across the environment and is heavily influenced by geological age, climate and depositional site characteristics. In addition, PalEnDNA is degraded by hydrolytic and oxidative attack, and bacterial metabolism. As a result, aDNA is heavily fragmented, degraded and chemically modified (Fig. 1). Four general types of damage can occur: (i) fragmentation, (ii) abasic sites (missing DNA bases), (iii) crosslinking (condensation reactions between DNA and proteins or sugars) and (iv) miscoding lesions (base pair modifications leading to the incorporation of incorrect bases during DNA amplification) (Fig. 1) (Pääbo *et al.*, 2004; see also Rizzi *et al.*, 2012). Fragmentation, abasic sites and crosslinking inhibit the amplification of aDNA, whereas miscoding lesions result in erroneous sequences that can have a significant impact on taxonomic resolution.

Although these types of damage have been well characterized for homogeneous samples, such as bone (e.g. Brotherton *et al.*, 2007), data relating to DNA damage in heterogeneous palaeoenvironmental samples are limited. The available studies suggest that PalEnDNA from permafrost is significantly more damaged than DNA from marine sediments of similar age, with crosslinking and miscoding lesions potentially a dominant type of damage (Hansen *et al.*, 2006; Orlando *et al.*, 2013). In contrast, Corinaldesi *et al.* (2008) found that in marine sediments enzyme activity that degrades DNA was high compared with processes that lead to abasic site damage. Anderson-Carpenter *et al.* (2011) suggested that miscoding lesions occur rapidly after deposition of plant material in lacustrine settings, whereas Wood *et al.* (2012a) showed that miscoding lesions were present in highly conserved plant cDNA *rbcL* sequences isolated from coprolites. However, given the absence of individual (discrete) specimens in PalEnDNA, it is difficult to tell the difference between taxonomic diversity and miscoding lesions, which may lead to an overestimation of taxonomic diversity. To overcome such difficulties, results can be replicated or analysis can be limited only to sequences that match 100% to reference sequences (e.g. Willerslev *et al.*, 2007). However, these methods are not foolproof because a 100% match could still be generated with a sequence containing miscoding lesions. Additionally, the use of computer programs that can account for DNA damage during data analysis are available (e.g. Munch *et al.*, 2008a, b; Taberlet *et al.*, 2012a).

Recommendations for future PalEnDNA-based research

Several recommendations concerning field and laboratory work, and data analysis, are provided here to guide researchers in the use of PalEnDNA and to help ensure palaeoecological reconstructions are robust (Fig. 5). It is important to note that potential complexities vary at every field site, and hence requirements need to be addressed individually according to local conditions. It is also important to appreciate that even when a genetic result passes all suggested tests, the default hypothesis should still be that it could have resulted from contamination, as is true for all criteria of authenticity used for aDNA (e.g. Cooper and Poinar, 2000; cf. Gilbert *et al.*, 2005).

Fieldwork

Site selection and stratigraphy

Careful site selection and reliable stratigraphy are essential because post-depositional reworking and DNA leaching can reduce the robustness of palaeoenvironmental reconstructions. Arnold *et al.* (2011) recommended that high-energy environments and those with very rapid deposition should normally be avoided. Rather, low-energy environments, where DNA is perhaps more likely to remain intracellular, should be targeted. To control for DNA leaching, sites with effectively no net water movement, such as permafrost, recently frozen sites, lacustrine or marine sediments, and some dry cave deposits, provide possibilities to minimize these processes. Even at sites such as these, past hydrological conditions may have resulted in DNA leaching, and hence over long periods the potential for this phenomenon is usually regarded as high unless demonstrated otherwise.

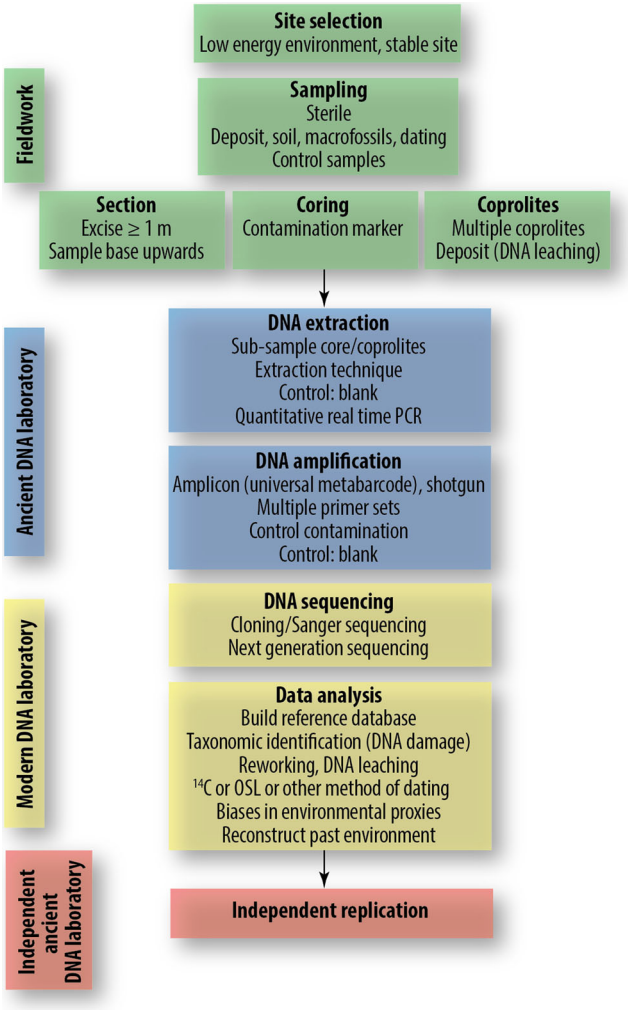

Figure 5. Recommended PalEnDNA-based research workflow summary to help ensure robust palaeoecological reconstructions are obtainable.

Detailed examination of the stratigraphic features of deposits at an exposure or in an excavation, and associated fieldwork over a wider region, can provide an indication of stratigraphic reliability because the identification of a consistent pattern indicates a degree of replication. Tephrostratigraphy (where available) can also be used to assess stratigraphic integrity, but not necessarily the reliability of the PalEnDNA record with regard to post-depositional reworking and DNA leaching. D'Costa *et al.* (2011) sampled aDNA sequences from Late Pleistocene permafrost sediments (mainly frozen loess) immediately overlain by a geochemically distinctive, thick tephra layer (up to 80 cm in thickness), the Dawson tephra aged ca. 30 000 cal a BP, at Bear Creek east of Dawson City in the Yukon Territory. The presence of the intact tephra layer and cryostratigraphic features demonstrated that the permafrost had not thawed since the time of deposition and so, in the absence of fluid leaching, the site represented an ideal source of uncontaminated and, through tephrochronology, securely dated aDNA (D'Costa *et al.*, 2011).

Additional control samples should be taken for plant macrofossil and pollen analysis, and OSL and ^{14}C dating (if in range), or other methods where appropriate, such as (U−Th)/He or U−Pb dating (e.g. Danišík *et al.*, 2012; Sirocko *et al.*, 2013; Coffey *et al.*, 2014), to help assess the stratigraphic reliability of PalEnDNA.

Recent studies have shown that PalEnDNA, macrofossils and microfossils have complementary overlapping datasets, potentially allowing distinction between local, regional and re-worked signals in some sites (Jørgensen *et al.*, 2012a). OSL dating of quartz grains within sediment can potentially date the inorganic, and hence bound, DNA fraction. In contrast, ^{14}C dating can potentially date the organic and unbound DNA fraction. The same sort of split applies to macrofossil versus water-soluble-fraction ^{14}C dates on coprolites (Jenkins *et al.*, 2012). Mismatched reliable dates would suggest that post-depositional reworking has occurred, as was the case with mammoth aDNA dating to 5000 cal a BP in permafrost (Arnold *et al.*, 2011), or that there is some problem with dating procedures as demonstrated, for example, with the application of OSL on loess–tephra sequences in New Zealand and north-west Canada (Lowe *et al.*, 2010; Demuro *et al.*, 2013). For bulk sediment and soil, radiocarbon dates should generally be viewed as range finders rather than precise (or accurate) age estimates. The only study where PalEnDNA has been directly dated is that of Willerslev *et al.* (2007), where silty ice at the base of a Greenland ice core containing PalEnDNA was dated using a combination of ^{10}Be/^{36}Cl isotope ratios, single-grain luminescence measurements, amino acid racemization combined with basal ice temperature modelling, and phylogenetic tree-branch length estimates for the age of the PalEnDNA sequences. For studies utilizing coprolites, samples of associated deposits should be collected and analysed in conjunction with those of the coprolites to search for signs of potential DNA leaching (e.g. Jenkins *et al.*, 2012). However, this practice is not applicable if a significant amount of the deposit is composed of disaggregated coprolites (e.g. Poinar *et al.*, 2001; Willerslev *et al.*, 2003; Hofreiter *et al.*, 2003b; Haile *et al.*, 2007; Wood *et al.*, 2008, 2012a) because there will be overlapping genetic

signals arising from both the coprolites and the associated deposits.

Modern control samples, including analyses of water and surface deposits, can be used to test for the presence of aDNA, which could indicate post-depositional reworking (Haile *et al.*, 2009). Ancient control samples, including intervening purportedly 'sterile' layers (e.g. tephra deposits) between stratigraphic layers of interest, should also be included to test for the presence of DNA leaching and post-depositional reworking.

Sterile sampling

Results from PalEnDNA research, and aDNA research in general, are highly prone to errors because of contamination from exogenous modern DNA. As noted earlier, there are numerous examples of studies where contamination has led to erroneous results, such as those involving amber-preserved insects (Cano *et al.*, 1992; Austin *et al.*, 1997), Permian salt crystals (Vreeland *et al.*, 2000; Park *et al.*, 2009) and even some Neanderthal fossils (Green *et al.*, 2006, 2009; Wall and Kim, 2007). Contamination can arise contemporaneously with the geological processes that resulted in the incorporation of DNA into a deposit, during post-depositional reworking or leaching (as discussed previously), during sampling, and during laboratory work. To minimize the risk of contamination in the field, several precautions should be taken (Willerslev *et al.*, 2004). Sampling should normally be conducted in a sterile manner, which involves the wearing of protective clothing, such as gloves, facemask or a body suit, and using sterilized equipment (Fig. 6). When undertaking coring, a recognizable genetic tracer can be used to determine how far exogenous contamination can penetrate the retrieved core (Willerslev *et al.*, 2003, 2007; Hebsgaard *et al.*, 2009; D'Costa *et al.*, 2011). When sampling sections (e.g. road cuttings) or pit faces, the sampling surface ideally should be incised at least 1 m to minimize the possibility of sampling deposits containing modern DNA (Willerslev *et al.*, 2004), although the depth of incision remains arbitrary and untested. Sampling of sections (outcrops) should also be conducted from the base upwards to avoid contamination of older layers by younger materials (Haile *et al.*, 2009). Regardless of sampling technique, parallel (duplicate) samples should be taken to examine intra-site variation, and from nearby sites to examine inter-site variation. Importantly, for coprolites, Wood *et al.* (2012a) showed that multiple samples from the same deposit should be analysed to help ensure palaeodietary reconstructions are not distorted by single defaecation events or seasonal bias. For instance, James and Burney (1997) examined the diet of the extinct moa-nalos from Hawaii, and found that pollen analysis of the coprolites indicated deposition in spring alone.

Laboratory work

Because of the degraded nature of PalEnDNA, and the ease with which samples can be contaminated, the DNA extractions and polymerase chain reaction (PCR) setup – the method used to amplify aDNA to workable concentrations – must be conducted in a physically isolated, dedicated aDNA laboratory with large numbers of controls and independent replication where necessary (see Cooper and Poinar, 2000). Unlike radiocarbon dating, there are very few commercial aDNA laboratories (e.g. Lakehead University Paleo-DNA Laboratory), and hence Quaternary and archaeological researchers wanting to incorporate aDNA into research projects will need to collaborate with staff at existing laboratories. Table S2 (supporting information) provides a list of some of the aDNA laboratories around the world in which PalEnDNA research has been conducted within the last 5 years.

Figure 6. Undertaking sterile fieldwork in PalEnDNA research. The Holocene tephra–palaeosol section was cleaned back nearly 1 m to expose a fresh face for sampling, which has been conducted using sterile equipment. Sampling was undertaken from the base upwards to avoid contamination of older samples with younger material. Note the protective gear, including gloves, disposable body suit and face mask.

Subsampling

When working with sediment cores, the outside 1–3 cm should be removed because this may have been exposed to exogenous contamination in the field during coring (Willerslev *et al.*, 2004). For previously collected cores without a contaminant tracer, this trimming procedure is recommended to ensure 'sterile' samples are obtained for PalEnDNA analysis. Sub-samples should be taken from the centre of the core, and from the base to the top of the core, to avoid contamination of older layers by younger DNA. This protocol also applies to coprolites, where the outer layer should be irradiated by UV light and removed so that only 'sterile' inner materials are sampled (Wood *et al.*, 2012a).

DNA extraction

A key target of PalEnDNA research is an efficient DNA extraction method regarding DNA concentration, fragment size, purity (e.g. absence of humic acids and tannins that can inhibit the amplification of aDNA) and taxonomic coverage (see van Geel *et al.*, 2012), with reproducible differences between sites, deposits, and origin and form of DNA (Young *et al.*, 2014). Currently, PalEnDNA extraction methods are neither efficient nor consistent across these categories, and only limited testing of their efficacy and consistency has been performed (e.g. van Geel *et al.*, 2012; Wales *et al.*, 2014).

There are relatively few commonly used DNA extraction methodologies that are specific for environmental DNA (including PalEnDNA). These include the use of kits that are commercially available (e.g. MoBio, Norgen, Macherey & Nagel), and specialized within-laboratory methods (Willerslev *et al.*, 2003; Haile, 2012; Kuch and Poinar, 2012; Taberlet *et al.*, 2012b). These methods have been trialled on a relatively limited number of samples and are generally based around the targeting of intracellular DNA, although Taberlet *et al.* (2012b) targeted extracellular DNA using a phosphate buffer. The wide range of materials analysed in PalEnDNA research, and their depositional and taphonomic contexts,

will have a significant impact on extraction efficiency. For example, allophane can bind 95–99% of available DNA, with <2% of DNA in an extractable form (Saeki *et al.*, 2010). Humic acids, which are representative of organic matter in soils, also have a strong binding affinity for DNA with a 2% release rate (Saeki *et al.*, 2010). For research questions comparing taxonomic diversity between samples, a consistent DNA extraction method will be sufficient. However, when the aim is to explore the taxonomic diversity within a single sample, the most efficient DNA extraction method, or a combination of multiple extraction methods, would help to maximize taxonomic coverage (e.g. van Geel *et al.*, 2012). Differing taxonomic coverage between plant macrofossils and PalEnDNA indicates that the commonly used extraction methods are not particularly efficient at extracting all DNA from a sample (e.g. Wood *et al.*, 2012a).

Library preparation

PCR uses primers specific to genetic markers to amplify aDNA to workable concentrations. PalEnDNA samples are nearly always genetically admixed, and potentially contain ancient and modern inputs. Thus, the choice of genetic marker will depend on the target of interest, the required taxonomic resolution and the extent of DNA degradation. There are two approaches to obtaining PalEnDNA data from environmental samples: amplicon sequencing and shotgun sequencing. Amplicon sequencing uses 'universal' metabarcode genetic markers. These metabarcodes are highly variable, allowing taxonomic discrimination, but they are flanked by regions conserved across multiple taxa. Commonly used metabarcodes in PalEnDNA research are listed in Table S3. However, many metabarcodes have resolution problems. The short plant *rbcL* (h1a/h2a) and *trnL* (p6-loop) metabarcodes for aDNA can only be resolved to the family–order level (and, more rarely, to genus or species) because of the small fragment size (Willerslev *et al.*, 2003; Taberlet *et al.*, 2007; Sønstebø *et al.*, 2010) (cf. longer *rbcL* and *trnL* metabarcodes can resolve to genus and species level, but are not usually suitable for aDNA research because of their size). van Geel *et al.* (2012) recommended that multiple, increasingly specific, genetic markers are used in a tiered approach to avoid biases towards different plant taxa. The use of universal metabarcodes also increases the chances of amplifying exogenous contamination. It is possible to avoid specific types of contaminating sequences through the use of blocking primers, which were originally developed to prevent the amplification of defaecator DNA in dietary analysis of stomach contents (Vestheim and Jarman, 2008; Rasmussen *et al.*, 2009; Boessenkool *et al.*, 2012; Calvignac-Spencer *et al.*, 2013), improving the detection of rare DNA sequences. Boessenkool *et al.* (2012) used blocking primers to prevent the amplification of human DNA from permafrost samples, allowing the amplification of aDNA specifically from rare extinct mammalian taxa, including woolly rhino. An additional approach includes sequencing negative extraction and PCR controls, followed by the removal of DNA sequences found in the negative controls from the PalEnDNA sequence datasets.

In contrast to targeted amplicon sequencing, shotgun sequencing can be used to provide a random survey of PalEnDNA within a sample, regardless of taxa and genetic marker (Tringe *et al.*, 2005). The results, however, will be dominated by bacterial and human DNA sequences unless approaches are taken to block their amplification.

The amount of DNA sequence data that can be obtained from PalEnDNA samples has taken a technological leap forward over the past decade. Next-generation sequencing (NGS) provides orders-of-magnitude greater amounts of sequence data than traditional (Sanger) methods, and has brought about a revolution in aDNA research to the extent that entire genomes of extinct hominins can now be reconstructed (e.g. Meyer *et al.*, 2012). The power of the approach lies in the vast numbers of sequences that are generated in parallel, providing significantly greater sequencing coverage and depth. These in turn allow a detailed analysis of DNA damage, and hence NGS can provide a valuable complement to the replication of results. Consequently, there is a significant (growing) demand for bioinformatics tools to analyse such quantities of data (Knapp and Hofreiter, 2010).

Data analysis

Reference sequence database and taxonomic identification

For all taxa, the construction of a reference sequence database to facilitate taxonomic resolution is usually essential. Gould *et al.* (2010) could only identify 50% of plant *trnL* sequences to a specific taxon because of the small size of the *trnL* metabarcode and the number of available reference sequences on GenBank, a comprehensive public database of nucleotide sequences and supporting bibliographic and biological annotation (see www.ncbi.nlm.nih.gov/genbank/). Local databases can be created to include only the reference sequences of interest, which will reduce the computational power required for data analysis. For example, Sønstebø *et al.* (2010) constructed a database of complete plant *trnL* sequences from 842 modern Arctic species, which has been used in subsequent studies by Boessenkool *et al.* (2012) and Jørgensen *et al.* (2012a). Curated online databases are also available for PalEnDNA analysis, including GREENGENES for bacterial 16S rRNA gene sequences, UNITS for fungal internal transcribed spacer (ITS) sequences, and SILVA for 18S rRNA gene sequences.

Several phylogenetic methods have been developed to determine the taxonomic affinity of PalEnDNA sequences but a full description is beyond the scope of this review. Briefly, the methods can be divided into comparisons against reference databases (e.g. GenBank) or phylogenetic analyses, such as Bayesian-based approaches (e.g. Haile *et al.*, 2007; Wood *et al.*, 2012a, 2013a, b), including those that can take into account DNA damage, especially miscoding lesions and how they affect taxonomic identification (e.g. Munch *et al.*, 2008a, b).

Complementary nature of PalEnDNA and biases of different environmental proxies

Multidisciplinary and multiproxy approaches to Quaternary and archaeological research have been shown to improve palaeoenvironmental reconstructions (e.g. Newnham *et al.*, 1995; Birks and Birks, 2006; Birks *et al.*, 2010; Jørgensen *et al.*, 2012a; Wood *et al.*, 2012a; Parducci *et al.*, 2013; van Geel *et al.*, 2014). aDNA analyses, including PalEnDNA studies, should not be viewed as replacing more traditional techniques, such as studies of plant macrofossils and pollen, but rather as a complementary tool generating both overlapping and separate results (e.g. Wood *et al.*, 2012a; Pedersen *et al.*, 2013; Boessenkool *et al.*, 2014). For example, comparisons of PalEnDNA analysis with pollen records from the same sediment cores from two volcanic crater sites in the Albertine Rift, eastern Africa, showed that plant diversity determined from aDNA analyses improved vegetation reconstructions based on pollen records by revealing additional

taxa and by enhancing taxonomic resolution (Boessenkool *et al.*, 2014). Furthermore, the two measures together (PalEnDNA and pollen) enabled vegetation changes at different geographical scales to be distinguished, with PalEnDNA mainly reflecting local vegetation, whereas pollen represented a wider provenance area.

Similarly, Parducci *et al.* (2013), working on lake sediments, concluded that the use of metabarcoding provided a complementary, but not an alternative, tool to pollen analysis for investigating past flora. In addition, metabarcoding can provide a local signal from the vegetation in the absence of (sub)fossil evidence, but has limited capacity to detect all taxa, regardless of their abundance. Parducci *et al.* (2013) suggested that metabarcoding should be followed by pollen analysis and the use of species-specific primers to provide the most comprehensive signal from the environment (see also van Geel *et al.*, 2012, and commentary by Gugerli *et al.*, 2013). In another multi-faceted study, Wood *et al* (2012a) combined analysis of PalEnDNA, pollen and plant macrofossils from coprolites to reconstruct the habits and habitats of New Zealand's extinct upland moa comprehensively (Fig. 7), and Wood *et al.* (2013b) used a parallel approach to reconstruct ecological niche partitioning among four sympatric species of moa in the Dart River Valley, South Island, New Zealand. In these studies, a high abundance of Poaceae was detected from pollen analysis but only single Poaceae DNA sequences were detected, suggesting that Poaceae pollen was incidentally ingested while feeding.

Plant macrofossils represent a local signal (potentially more regional in some environmental settings, such as those involving alluvial deposits) but they are affected to some degree by taphonomic biases. Hard parts such as seeds, wood and leaf cuticles preserve better than soft parts such as fruit and flowers in coprolites (Wood, 2007). Pollen represents a local to regional signal but can be devalued by taxonomic resolution issues (Anderson-Carpenter *et al.*, 2011). For example, *Coprosma* and Poaceae pollen in New Zealand can only be identified to genus and family level, respectively, despite each group being relatively species-diverse. Pollen analyses also suffer from quantification problems with differences in pollen production and dispersal variability between species biasing palaeovegetation reconstructions made from pollen-count data (Wood *et al.*, 2012a). PalEnDNA has further biases associated with differential extraction efficiencies (van Geel *et al.*, 2012; Wales *et al.*, 2014) and taxonomic resolution. Importantly, DNA-based species identifications are not quantitative either, partly because of the DNA extraction method or because of the sequence coverage from NGS. Additional proxies that could be included are geographical distribution data and historical botanical checklists (Hofreiter *et al.*, 2003a; Jørgensen *et al.*, 2012a; Wood *et al.*, 2012a; Wilmshurst *et al.*, 2014).

Replication

A key aspect of aDNA research is replication, either internally (within the laboratory) or externally (by another laboratory). Willerslev *et al.* (2007) considered PalEnDNA sequences to be genuine (reliable) only after they were independently replicated and a 100% match to reference sequences was attained. However, independent replication is commonly not used, encouraged by a misconception that the sequencing depth generated by NGS approaches is a suitable substitute for contamination tests. This approach was shown when Green *et al.* (2006) published 1 million base pairs of Neanderthal nuclear DNA using NGS, but subsequently 10–78% of the data were suggested to be contamination from modern human DNA (Wall and Kim, 2007; Green *et al.*, 2009).

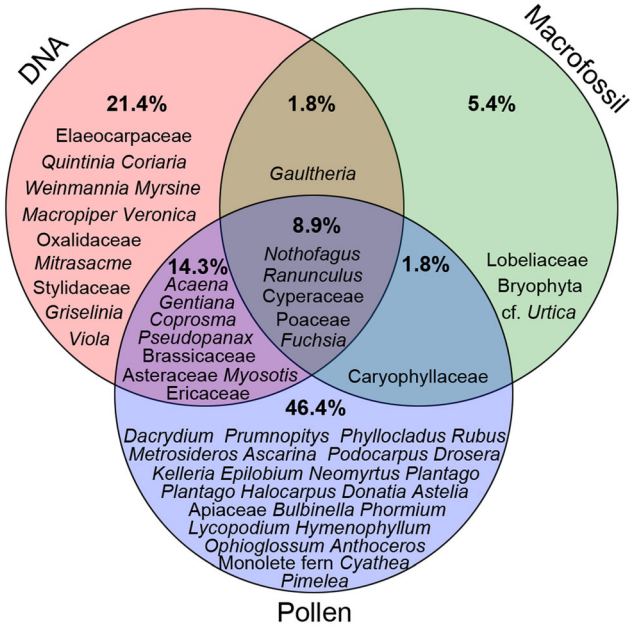

Figure 7. Plant taxa detected in eight upland moa coprolites using three different diet proxies (aDNA, plant macrofossils, pollen). Each proxy revealed plant taxa not detected by the others, reinforcing the conclusion that the proxies are complementary and that a multiproxy approach is needed for gaining maximum palaeodietary information (Jørgensen *et al.*, 2012a; Wood *et al.*, 2012a). Figure from Wood *et al.* (2012a, p. 10).

For PalEnDNA, putative taxa with low abundance and heterogeneous distributions, especially in independent samples from the same layer or deposit, may not be replicated because of drop-out (failure to amplify) and stochastic variation (Willerslev *et al.*, 2007; Haile *et al.*, 2009). In lieu of independent replication, macrofossil- and pollen-derived data may provide an additional means of verification (Wood *et al.*, 2012a; Fig. 7).

Prospects: using PalEnDNA for Quaternary palaeoenvironmental reconstruction

PalEnDNA, defined as aDNA extracted from disseminated (non-discrete) genetic material from environments of the past, forms the basis of an emerging and exciting sub-discipline of aDNA research. The combination of PalEnDNA studies with the analysis of plant macrofossils and pollen and other proxies is providing a powerful means to reconstruct past environments more comprehensively (e.g. Jørgensen *et al.*, 2012a; Boessenkool *et al.*, 2014). PalEnDNA researchers are also beginning to obtain a greater understanding of both the power and the limitations of the technique (e.g. Munch *et al.*, 2008a,b; Boessenkool *et al.*, 2012; van Geel *et al.*, 2012), meaning that more robust reconstructions are possible. However, scientists undertaking Quaternary and archaeological studies who want to utilize the PalEnDNA technique need to plan prudently and to evaluate field procedures and sample collection techniques, as noted earlier.

For PalEnDNA-based research to become a more firmly established technique, and for its value as a tool for palaeoenvironmental reconstruction to be properly assessed, the issues discussed in this review need to be addressed, including the form of PalEnDNA, and the efficiency and taxonomic coverage of DNA extraction methods. The field would benefit

from an in-depth understanding of DNA damage and degradation rates in PalEnDNA, and procedures to distinguish between true taxonomic diversity and miscoding lesions. Laboratory and analytical methods to determine the level of mixing of ancient and modern DNA in samples also need developing.

The incorporation of PalEnDNA into Quaternary and archaeological research programmes requires careful planning of research questions and of field and laboratory work – rather than simply being an extra 'add-on' to conventional stratigraphic studies – and will require modifications to existing fieldwork protocols. To advance the field of PalEnDNA-based research, metadata relating to the type of deposit, or soils, such as stratigraphy, soil horizonation, mineralogy and chemical properties (e.g. pH) may need to be included. Multiple proxies, replicable stratigraphies and reliable dating methods can be used to help determine the stratigraphic reliability of PalEnDNA assays and to increase taxonomic resolution in conjunction with the construction of DNA sequence reference databases. Publication of failures for given DNA extraction methods is also critical so that success/failure rates can be calculated for given deposits or soils, environments and time periods.

In conclusion, the field of PalEnDNA research remains young and the reliability of signals extracted from a wide variety of sedimentary or volcanic deposits, soils and palaeosols for palaeoecological or archaeological research still needs to be fully assessed. However, the potential is considerable, and PalEnDNA-based research is set to grow rapidly. It will be important for Quaternary and archaeological researchers, together with geochronologists and various other specialists, such as bioinformaticians, to be actively involved in guiding and developing the field.

Supporting Information

Additional supporting information can be found in the online version of this article:

Table S1. A more comprehensive list of PalEnDNA publications to supplement Table 2 in the main article.
Table S2. A selection of ancient DNA laboratories that have published PalEnDNA research within the last 5 years.
Table S3. Universal metabarcoding genetic markers and PCR primers used in PalEnDNA-based research.

Acknowledgements. Funding for this research was provided by the New Zealand Marsden Fund (contract UOW1006), administered by the Royal Society of New Zealand, and by the Australian Research Council. We are grateful to editor Antony Long for inviting us to contribute this review and for his encouragement and comments. Five anonymous reviewers are also thanked for their comments, which helped us to improve the paper markedly. We appreciated discussions with several colleagues who read early drafts. Maria Gehrels, Julien Soubrier and Laura Weyrich provided two photographs in Fig. 3, Max Oulton drafted Figs 2 and 5, and Ray Cursons, Heng Zhang and Bruce Clarkson provided useful information or support.

Abbreviations. ACAD, Australian Centre for Ancient DNA; aDNA, ancient DNA; NGS, next-generation sequencing; NI, North Island; OSL, optically stimulated luminescence; PalEnDNA, palaeoenvironmental DNA; PCR, polymerase chain reaction; SI, South Island.

References

Adler CJ, Haak W, Donlon D, *et al.* 2011. Survival and recovery of DNA from ancient teeth and bones. *Journal of Archaeological Science* **38**: 956–964.

Adler CJ, Dobney K, Weyrich LS, *et al.* 2013. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nature Genetics* **45**: 450–455.

Ahrens RJ, Arnold RW. 2012. Soil Taxonomy. In *Handbook of Soil Sciences*. 2nd edn Vol. 1, Huang PM, Li Y, Sumner ME (eds). CRC Press: Boca Raton; 31.1–31.13.

Allen MF, Crisafulli CM, Morris SJ, *et al.* 2005. Mycorrhizae and Mount St. Helens: story of a symbiosis. In *Ecological Responses to the 1980 Eruption of Mount St. Helens*, Dale VH, Swanson FJ, Crisafulli CM (eds). Springer: New York; 221–231.

Allentoft ME, Collins M, Harker D, *et al.* 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings of the Royal Society of London Series B* **279**: 4724–4733.

Alley RB, Andrews JT, Brigham-Grette J, *et al.* 2010. History of the Greenland Ice Sheet: paleoclimatic insights. *Quaternary Science Reviews* **29**: 1728–1756.

Alloway BV, Lowe DJ, Larsen G, *et al.* 2013. Tephrochronology. In *The Encyclopaedia of Quaternary Science*. 2nd edn, Vol. 4, Elias SA, Mock CJ (eds). Elsevier: London; 277–304.

Alvarez AJ, Khanna M, Toranzos GA, *et al.* 1998. Amplification of DNA bound on clay minerals. *Molecular Ecology* **7**: 775–778.

Anderson-Carpenter LL, McLachlan JS, Jackson ST, *et al.* 2011. Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. *BMC Evolutionary Biology* **11**: 30.

Andersen K, Bird KL, Rasmussen M, *et al.* 2012. Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular Ecology* **21**: 1966–1979.

Arnold LJ, Roberts RG, MacPhee RDE, *et al.* 2011. Paper II – dirt, dates and DNA: OSL and radiocarbon chronologies of perennially frozen sediments in Siberia, and their implications for sedimentary ancient DNA studies. *Boreas* **40**: 417–445.

Austin JJ, Ross AJ, Smith AB, *et al.* 1997. Problems of reproducibility – does geologically ancient DNA survive in amber-preserved insects? *Proceedings of the Royal Society of London Series B* **264**: 467–474.

Banks NG, Hoblitt RP. 1981. Summary of temperature studies of 1980 deposits. In *The 1980 Eruptions of Mount St. Helens, Washington*, Lipma PW, Mullineaux DR (eds). *Geological Survey, Professional Paper* **1250**: 295–313.

Bennett KD, Parducci L. 2006. DNA from pollen: principles and potential. *The Holocene* **16**: 1031–1034.

Birks HH, Birks HJB. 2006. Multi-proxy studies in palaeolimnology. *Vegetation History and Archaeobotany* **15**: 235–251.

Birks HJB, Heiri O, Seppä H, *et al.* 2010. Strengths and weaknesses of quantitative climate reconstructions based on late-Quaternary biological proxies. *Open Ecology Journal* **3**: 68–110.

Bissett A, Gibson JAE, Jarman SN, *et al.* 2005. Isolation, amplification, and identification of ancient copepod DNA from lake sediments. Limnology and oceanography. *Methods* **3**: 533–542.

Boessenkool S, Epp LS, Haile J, *et al.* 2012. Blocking human contaminant DNA during PCR allows amplification of rare mammal species from sedimentary ancient DNA. *Molecular Ecology* **8**: 1806–1815.

Boessenkool S, McGlynn G, Epp LS, *et al.* 2014. Use of ancient sedimentary DNA as a novel conservation tool for high-altitude tropical biodiversity. *Conservation Biology* **28**: 446–455.

Bohmann K, Evans A, Gilbert MTP, *et al.* 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution* **29**: 358–367.

Brotherton P, Endicott P, Sanchez JJ, *et al.* 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Research* **35**: 5717–5728.

Bunce M, Worthy TH, Phillips MJ, *et al.* 2009. The evolutionary history of the extinct ratite moa and New Zealand Neogene paleogeography. *Proceedings of the National Academy of Science of the United States of America* **106**: 20646–20651.

Calvignac-Spencer S, Merkel K, Kutzner N, *et al.* 2013. Carrion fly derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. *Molecular Ecology* **22**: 915–924.

Campos PF, Willerslev E, Mead JI, *et al*. 2010. Molecular identification of the extinct mountain goat, *Oreamnos harringtoni* (Bovidae). *Boreas* **39**: 18–23.

Cano RJ, Poinar H, Poinar G. 1992. Isolation and partial characterisation of DNA from the bee *Proplebeia dominicana* (Apidae: Hymenoptera) in 25–40 million year old amber. *Medical Science Research* **20**: 249–251.

Chan YL, Lacey EA, Pearson OP, *et al*. 2005. Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent. *Biology Letters* **1**: 423–426.

Churchman GJ, Lowe DJ. 2012. Alteration, formation, and occurrence of minerals in soils. In *Handbook of Soil Sciences*. 2nd edn. Vol. 1, Huang PM, Li Y, Sumner ME (eds). CRC Press: Boca Raton; 20.1–20.72.

Clack AA, MacPhee RDE, Poinar HN. 2012a. *Mylodon darwinii* DNA sequences from ancient fecal hair shafts. *Annals of Anatomy* **194**: 26–30.

Clack AA, MacPhee RDE, Poinar HN. 2012b. Case study: ancient sloth DNA recovered from hairs preserved in paleofeces. In *Ancient DNA. Methods in Molecular Biology 840*, Shapiro B, Hofreiter M (eds). Springer: New York; 51–56.

Clarkson BR, Patel RN, Clarkson BD. 1988. Composition and structure of forest overwhelmed at Pureora, central North Island, New Zealand, during the Taupo eruption (c. A.D. 130). *Journal of the Royal Society of New Zealand* **18**: 417–436.

Coffey KT, Schmitt AK, Ford A, *et al*. 2014. Volcanic ash provenance from zircon dust with an application to Maya pottery. *Geology* **42**: 595–598.

Coolen MJL, Gibson JAE. 2009. Ancient DNA in lake sediment records. *PAGES News* **17**: 104–106.

Cooper A, Poinar HN. 2000. Ancient DNA: do it right or not at all. *Science* **289**: 1139.

Cooper A, Mourer-Chauviré C, Chambers GK, *et al*. 1992. Independent origins of New Zealand moas and kiwis. *Proceedings of the National Academy of Science of the United States of America* **89**: 8741–8744.

Corinaldesi C, Beolchini F, Dell'anno A. 2008. Damage and degradation rates of extracellular DNA in marine sediments: implications for the preservation of gene sequences. *Molecular Ecology* **17**: 3939–3951.

Danišík M, Shane P, Schmitt AK, *et al*. 2012. Re-anchoring the Late Pleistocene tephrochronology of New Zealand based on concordant radiocarbon ages and combined $^{238}U/^{230}Th$ disequilibrium and (U–Th)/He zircon ages. *Earth and Planetary Science Letters* **349–350**: 240–250.

D'Anjou RM, Bradley RS, Balascio NL, *et al*. 2012. Climate impacts on human settlement and agricultural activities in northern Norway revealed through sediment biogeochemistry. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 20332–20337.

D'Costa VM, King CE, Kalan L, *et al*. 2011. Antibiotic resistance is ancient. *Nature* **477**: 457–461.

de Bruyn M, Hoelzel AR, Carvalho GR, *et al*. 2011. Faunal histories from Holocene ancient DNA. *Trends in Ecology and Evolution* **26**: 405–413.

de la Fuente C, Flores S, Moraga M. 2013. DNA from human ancient bacteria: a novel source of genetic evidence from archaeological dental calculus. *Archaeometry* **55**: 766–778.

Demuro M, Arnold LJ, Froese DG, *et al*. 2013. OSL dating of loess deposits bracketing Sheep Creek tephra beds, northwest Canada: dim and problematic single-grain OSL characteristics and their effect on multi-grain age estimates. *Quaternary Geochronology* **15**: 67–87.

Drummond AJ, Rambaut A, Shapiro B, *et al*. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution* **22**: 1185–1192.

Epp LS, Boessenkool S, Bellemain EP, *et al*. 2012. New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. *Molecular Ecology* **21**: 1821–1833.

Fridriksson S. 1987. Plant colonization of a volcanic island, Surtsey, Iceland. *Arctic and Alpine Research* **19**: 425–431.

Giguet-Covex C, Pansu J, Arnaud F, *et al*. 2014. Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nature Communications* **5**: 3211.

Gilbert MTP, Bandelt HJ, Hofreiter M, *et al*. 2005. Assessing ancient DNA studies. *Trends in Ecology and Evolution* **20**: 541–5544.

Gilbert MTP, Tomsho LP, Rendulic S, *et al*. 2007. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* **317**: 1927–1930.

Gilbert MTP, Jenkins DL, Götherstrom A, *et al*. 2008. DNA from pre-Clovis human coprolites in Oregon, North America. *Science* **320**: 786–789.

Gilbert MTP, Jenkins DL, Higham TFG, *et al*. 2009. Response to comment by Poinar *et al*. on 'DNA from pre-Clovis human coprolites in Oregon, North America'. *Science* **325**: 148.

Goldberg P, Berna F, Macphail RI. 2009. Comment on 'DNA from pre-Clovis human coprolites in Oregon, North America'. *Science* **325**: 148.

Gould BA, León B, Buffen AM, *et al*. 2010. Evidence of a high-Andean, mid-Holocene plant community: an ancient DNA analysis of glacially preserved remains. *American Journal of Botany* **97**: 1579–1584.

Green RE, Krause J, Ptak SE, *et al*. 2006. Analysis of one million base pairs of Neanderthal DNA. *Nature* **444**: 330–336.

Green RE, Briggs AW, Krause J, *et al*. 2009. The Neandertal genome and ancient DNA authenticity. *EMBO Journal* **28**: 2494–2502.

Green RE, Krause J, Briggs AW, *et al*. 2010. A draft sequence of the Neandertal genome. *Science* **328**: 710–722.

Gugerli F, Parducci L, Petit RJ. 2005. Ancient plant DNA: review and prospects. *New Phytologist* **166**: 409–418.

Gugerli F, Alvarez N, Tinner W. 2013. A deep dig – hindsight on Holocene vegetation composition from ancient environmental DNA. *Molecular Ecology* **22**: 3433–3436.

Haile J. 2012. Ancient DNA extraction from soils and sediment. In *Ancient DNA. Methods in Molecular Biology 840*, Shapiro B, Hofreiter M (eds). Springer: New York; 57–63.

Haile J, Holdaway R, Oliver K, *et al*. 2007. Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology and Evolution* **24**: 982–989.

Haile J, Froese DG, MacPhee RDE, *et al*. 2009. Ancient DNA reveals late survival of mammoth and horse in interior Alaska. *Proceedings of the National Academy of Science of the United States of America* **106**: 22352–22357.

Haouchar D, Haile J, McDowell MC, *et al*. 2014. Thorough assessment of DNA preservation from fossil bone and sediments excavated from a late Pleistocene–Holocene cave deposit on Kangaroo Island, South Australia. *Quaternary Science Reviews* **84**: 56–64.

Hansen AJ, Mitchell DL, Wiuf C, *et al*. 2006. Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments. *Genetics* **173**: 1175–1179.

Hebsgaard MB, Phillips MJ, Willerslev E. 2005. Geologically ancient DNA: fact or artefact? *Trends in Microbiology* **13**: 212–220.

Hebsgaard MB, Gilbert MTP, Arneborg J, *et al*. 2009. 'The farm beneath the sand' – an archaeological case study on ancient 'dirt' DNA. *Antiquity* **83**: 430–444.

Herrera A, Cockell CS. 2007. Exploring microbial diversity in volcanic environments: a review of methods in DNA extraction. *Journal of Microbiological Methods* **70**: 1–12.

Hewitt AE. 2010. *New Zealand Soil Classification*, 3rd edn. Landcare Research Science Series **1**: 1–136.

Higham TFG, Anderson AJ, Jacomb C. 1999. Dating the first New Zealanders: the chronology of Wairau Bar. *Antiquity* **73**: 420–427.

Higuchi RG, Bowman B, Freiberger M, *et al*. 1984. DNA sequences from the quagga, an extinct member of the horse family. *Nature* **312**: 282–284.

Hofreiter M, Poinar HN, Spaulding WG, *et al*. 2000. A molecular analysis of ground sloth diet through the last glaciation. *Molecular Ecology* **9**: 1975–1984.

Hofreiter M, Betancourt JL, Sbriller AP, *et al*. 2003a. Phylogeny, diet, and habitat of an extinct ground sloth from Cuchillo Cura, Neuquén Province, southwest Argentina. *Quaternary Research* **59**: 364–378.

Hofreiter M, Mead JI, Martin P, *et al*. 2003b. Molecular caving. *Current Biology* **13**: R693–R695.

Hogg AG, Higham TFG, Lowe DJ, *et al*. RM 2003. A wiggle-match date for Polynesian settlement of New Zealand. *Antiquity* **77**: 116–125.

Hogg AG, Lowe DJ, Palmer JG, *et al*. 2012. Revised calendar date for the Taupo eruption derived by ^{14}C wiggle-matching using a New Zealand kauri ^{14}C calibration data set. *The Holocene* **22**: 439–449.

Holmsgaard PN, Norman A, Hede SC, *et al*. 2011. Bias in bacterial diversity as a result of Nycodenz extraction from bulk soil. *Soil Biology and Biochemistry* **43**: 2152–2159.

Huang Y-T, Churchman GJ, Lowe DJ, *et al*. 2012. Evaluating the character and preservation of DNA within allophane clusters in buried soils on Holocene tephras, northern New Zealand. In *Proceedings, Combined Australian Regolith Geoscientists Association and Australian Clay Minerals Society Conference: Mildura (Victoria, Australia), 7–9 February 2012*, Churchman GJ, Cresswell R, Singh B (eds); 121–124.

Huang Y-T, Lowe DJ, Churchman GJ, *et al*. 2014. Carbon storage and DNA adsorption in allophanic soils and paleosols. In *Soil Carbon. Progress in Soil Science Series*, Hartemink AE, McSweeney K (eds). Springer: New York; 163–172.

Hudspith VA, Scott AC, Wilson CJN, *et al*. 2010. Charring of woods by volcanic processes: an example from the Taupo ignimbrite, New Zealand. *Palaeogeography, Palaeoclimatology, Palaeoecology* **291**: 40–51.

Iñiguez AM, Araújo A, Ferreira LF, *et al*. 2003a. Analysis of ancient DNA from coprolites: a perspective with random amplified polymorphic DNA-polymerase chain reaction approach. *Memorias do Instituto Oswaldo Cruz* **98** (Suppl I): 63–65.

Iñiguez A, Reinhard KJ, Araujo A, *et al*. 2003b. *Enteriobius vermicularis*: ancient DNA from North and South American human coprolites. *Memorias do Instituto Oswaldo Cruz* **98** (Suppl I): 67–69.

Iñiguez AM, Reinhard K, Carvalho Gonçalves MLC, *et al*. 2006. SL1 RNA gene recovery from *Enterobius vermicularis* ancient DNA in pre-Columbian human coprolites. *International Journal for Parasitology* **36**: 1419–1425.

James HF, Burney DA. 1997. The diet and ecology of Hawaii's extinct flightless waterfowl: evidence from coprolites. *Biological Journal of the Linnean Society* **62**: 279–297.

Jenkins DL, Davis LG, Stafford TW, *et al*. 2012. Clovis age Western Stemmed projectile points and human coprolites at the Paisley Caves. *Science* **337**: 223–228.

Johnson SS, Hebsgaard MB, Christensen TR, *et al*. 2007. Ancient bacteria show evidence of DNA repair. *Proceedings of the National Academy of Science of the United States of America* **104**: 14401–14405.

Jørgensen T, Haile J, Möller P, *et al*. 2012a. A comparative study of ancient sedimentary DNA, pollen and macrofossils from permafrost sediments of northern Siberia reveals long-term vegetational stability. *Molecular Ecology* **21**: 1989–2003.

Jørgensen T, Kjaer KH, Haile J, *et al*. 2012b. Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA meta-barcoding. *Molecular Ecology* **21**: 1980–1988.

Kemp BM, Monroe C, Smith DG. 2006. Repeat silica extraction: a simple technique for the removal of PCR inhibitors from DNA extracts. *Journal of Archaeological Science* **33**: 1680–1689.

Knapp M, Hofreiter M. 2010. Next generation sequencing of ancient DNA: requirements, strategies and perspectives. *Genes* **1**: 227–243.

Knauth S, Schmidt H, Tippkötter R. 2013. Comparison of commercial kits for the extraction of DNA from paddy soils. *Letters in Applied Microbiology* **56**: 222–228.

Krüttli A, Bouwman A, Akgül G, *et al*. 2014. Ancient DNA analysis reveals high frequency of European lactase persistence allele (T-13910) in medieval central Europe. *PLOS ONE* **9**: e86251.

Kuch M, Rohland N, Betancourt JL, *et al*. 2002. Molecular analysis of an 11700-year-old rodent midden from the Atacama Desert, Chile. *Molecular Ecology* **11**: 913–924.

Kuch M, Poinar HN. 2012. Extraction of DNA from paleofeces. In *Ancient DNA. Methods in Molecular Biology 840*, Shapiro B, Hofreiter M (eds). Springer: New York; 37–42.

Kuzmina SA, Sher AV, Edwards ME, *et al*. 2011. The Late Pleistocene environment of the eastern west Beringia based on the principal section at the Main River, Chukotka. *Quaternary Science Reviews* **30**: 2091–2106.

Larson G, Cucchi T, Fujita M, *et al*. 2007. Phylogeny and ancient DNA of *Sus* provides insights into Neolithic expansion in island Southeast Asia and Oceania. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 4834–4839.

Lejzerowicz F, Esling P, Majewski W, *et al*. 2013. Ancient DNA complements microfossil record in deep-sea subsurface sediments. *Biology Letters* **9**: 20130283.

Leles D, Araújo A, Ferreira LF, *et al*. 2008. Molecular paleoparasitological diagnosis of *Ascaris* sp. from coprolites: new scenery of ascariasis in pre-Colombian South America times. *Memorias do Instituto Oswaldo Cruz* **103**: 106–108.

Letts B, Fulton TL, Stiller M, *et al*. 2012. Ancient DNA reveals genetic continuity in mountain woodland caribou of the Mackenzie and Selwyn Mountains, Northwest Territories, Canada. *Arctic* **65** (Suppl 1): 80–94.

Limburg PA, Weider LJ. 2002. 'Ancient' DNA in the resting egg bank of a microcrustacean can serve as a palaeolimnological database. *Proceedings of the Royal Society of London Series B* **269**: 281–287.

Lindahl T. 1993a. Instability and decay of the primary structure of DNA. *Nature* **362**: 709–715.

Lindahl T. 1993b. Recovery of antediluvian DNA. *Nature* **365**: 700.

Lloyd-Jones G, Hunter DWF. 2001. Comparison of rapid DNA extraction methods applied to contrasting New Zealand soils. *Soil Biology and Biochemistry* **33**: 2053–2059.

Loreille O, Roumat E, Verneau O, *et al*. 2001. Ancient DNA from *Ascaris*: extraction amplification and sequences from eggs collected in coprolites. *International Journal for Parasitology* **31**: 1101–1106.

Lowe DJ. 1988. Stratigraphy, age, composition, and correlation of Late Quaternary tephras interbedded with organic sediments in Waikato lakes, North Island, New Zealand. *New Zealand Journal of Geology and Geophysics* **31**: 125–165.

Lowe DJ. 2011. Tephrochronology and its application: a review. *Quaternary Geochronology* **6**: 107–153.

Lowe DJ, Palmer DJ. 2005. Andisols of New Zealand and Australia. *Journal of Integrated Field Science* **2**: 39–65.

Lowe DJ, Wilson CJN, Newnham RM, *et al*. 2010. Dating the Kawakawa/Oruanui eruption: comment on "Optical luminescence dating of a loess section containing a critical tephra marker horizon, SW North Island of New Zealand" by R. Grapes et al. *Quaternary Geochronology* **5**: 493–496.

Lowe DJ, Blaauw M, Hogg AG, *et al*. 2013. Ages of 24 widespread tephras erupted since 30 000 years ago in New Zealand, with re-evaluation of the timing and palaeoclimatic implications of the late-glacial cool episode recorded at Kaipo bog. *Quaternary Science Reviews* **74**: 170–194.

Luciani S, Fornaciari G, Rickards O, *et al*. 2006. Molecular characterization of a pre-Columbian mummy and in situ coprolite. *American Journal of Physical Anthropology* **129**: 620–629.

Lydolph MC, Jacobsen J, Arctander P, *et al*. 2005. Beringian paleoecology inferred from permafrost-preserved fungal DNA. *Applied and Environmental Microbiology* **71**: 1012–1017.

Ma LT, Rogers SO, Catranis CM, *et al*. 2000. Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* **92**: 286–295.

Madeja J, Wacnik A, Zyga A, *et al*. 2009. Bacterial ancient DNA as an indicator of human presence in the past: its correlation with palynological and archaeological data. *Journal of Quaternary Science* **24**: 317–321.

Madeja J, Wacnik A, Wypasek E, *et al*. 2010. Integrated palynological and molecular analyses of Late Holocene deposits from lake Milkowskie (NE Poland): verification of local human impact on environment. *Quaternary International* **220**: 147–152.

Magyari EK, Major A, Bálint M, *et al*. 2011. Population dynamics and genetic changes of *Picea abies* in the south Carpathians revealed

by pollen and ancient DNA analyses. *BMC Evolutionary Biology* **11**: 66.

Marková S, Černý M, Rees DJ, *et al*. 2006. Are they still viable? Physical conditions and abundance of *Daphnia pulicaria* resting eggs in sediment cores from lakes in the Tatra mountains. *Biologia* **61** (Suppl): S135–S146.

Matisoo-Smith E, Roberts K, Welikala N, *et al*. 2008. Recovery of DNA and pollen from New Zealand lake sediments. *Quaternary International* **184**: 139–149.

McClelland E, Wilson CJN, Bardot L. 2004. Paleotemperature determinations for the 1.8-ka Taupo ignimbrite, New Zealand, and implications for the emplacement history of a high-velocity pyroclastic flow. *Bulletin of Volcanology* **66**: 492–513.

McDaniel PA, Lowe DJ, Arnalds O, *et al*. 2012. Andisols. In *Handbook of Soil Sciences*. 2nd edn, Vol. 1, Huang PM, Li Y, Sumner ME (eds). CRC Press: Boca Raton; 33.29–33.48.

Metcalf JL, Ursell LK, Knight R. 2014. Ancient human oral plaque preserves a wealth of biological data. *Nature Genetics* **46**: 321–323.

Meyer M, Kircher M, Gansauge MT, *et al*. 2012. A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**: 222–226.

Mitchell D, Willerslev E, Hansen AJ. 2005. Damage and repair of ancient DNA. *Mutation Research* **571**: 265–276.

Mitchell KJ, Llamas B, Soubrier J, *et al*. 2014. Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution. *Science* **344**: 898–900.

Munch K, Boomsma W, Huelsenbeck JP, *et al*. 2008a. Statistical assignment of DNA sequences using Bayesian phylogenetics. *Systematic Biology* **57**: 750–757.

Munch K, Boomsma W, Willerslev E, *et al*. 2008b. Fast phylogenetic DNA barcoding. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **363**: 3997–4002.

Newnham RM, de Lange PJ, Lowe DJ. 1995. Holocene vegetation, climate, and history of a raised bog complex, northern New Zealand, based on palynology, plant macrofossils and tephrochronology. *Holocene* **5**: 267–282.

Orlando L, Ginolhac A, Zhang G, *et al*. 2013. Recalibrating *Equus* evolution using the genome sequence of an early middle Pleistocene horse. *Nature* **499**: 74–78.

Oskam CL, Haile J, McLay E, *et al*. 2010. Fossil avian eggshell preserves ancient DNA. *Proceedings of the Royal Society of London Series B* **277**: 1991–2000.

Pääbo S. 1985. Molecular cloning of ancient Egyptian mummy DNA. *Nature* **314**: 644–645.

Pääbo S, Poinar H, Serre D, *et al*. 2004. Genetic analyses from ancient DNA. *Annual Review of Genetics* **38**: 645–679.

Parducci L, Suyama Y, Lascoux M, *et al*. 2005. Ancient DNA from pollen: a genetic record of population history in Scots pine. *Molecular Ecology* **14**: 2873–2882.

Parducci L, Matetovici I, Fontana SL, *et al*. 2013. Molecular- and pollen-based vegetation analysis in lake sediments from central Scandinavia. *Molecular Ecology* **22**: 3511–3524.

Paffetti D, Vettori C, Caramelli D, *et al*. 2007. Unexpected presence of *Fagus orientalis* complex in Italy as inferred from 45,000-year-old DNA pollen samples from Venice lagoon. *BMC Evolutionary Biology* **7** (Suppl): S6.

Park JS, Vreeland RH, Cho BC, *et al*. 2009. Haloarchaeal diversity in 23, 121 and 419 MYA salts. *Geobiology* **7**: 515–523.

Pedersen MW, Ginolhac A, Orlando L, *et al*. 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews* **75**: 161–168.

Poinar HN, Hofreiter M, Spaulding WG, *et al*. 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**: 402–406.

Poinar HN, Kuch M, Sobolik KD, *et al*. 2001. A molecular analysis of dietary diversity for three archaic Native Americans. *Proceedings of the National Academy of Science of the United States of America* **98**: 4317–4322.

Poinar H, Kuch M, McDonald G, *et al*. 2003. Nuclear gene sequences from a Late Pleistocene sloth coprolite. *Current Biology* **13**: 1150–1152.

Poinar H, Fiedel S, King CE, *et al*. 2009. Comment on 'DNA from pre-Clovis human coprolites in Oregon, North America'. *Science* **325**: 148.

Ponnamperuma C, Young RS, Caren LD. 1967. Some chemical and microbiological studies of Surtsey. *Surtsey Research Progress Report (Surtsey Research Society, Iceland)* **3**: 70–780.

Prüfer K, Racimo F, Patterson N, *et al*. 2014. The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**: 43–49.

Rai H, Yokoyama S, Hashimoto S, *et al*. 2010. A restriction factor of direct DNA extraction from volcanic ash soils. In *Proceedings 19th World Congress of Soil Science*, Symposium 2.5.1 Extracellular proteins and nucleic acids in soil, Gilkes RJ, Prakongkep N (eds); 1–4. Website http://www.iuss.org/.

Rasmussen M, Cummings LS, Gilbert MTP, *et al*. 2009. Response to Comment by Goldberg et al. on "DNA from Pre-Clovis Human Coprolites in Oregon, North America". *Science* **325**: 148.

Rawlence NJ, Cooper A. 2013. Youngest reported radiocarbon age of a moa (Aves: Dinornithiformes) dated from a natural site in New Zealand. *Journal of the Royal Society of New Zealand* **43**: 100–107.

Rawlence NJ, Wood JR, Armstrong KN, *et al*. 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa. *Proceedings of the Royal Society of London Series B* **276**: 3395–3402.

Reinhard KJ, Chaves SM, Jones JG, *et al*. 2008. Evaluating chloroplast DNA in prehistoric Texas coprolites: medicinal, dietary, or ambient ancient DNA? *Journal of Archaeological Science* **35**: 1748–1755.

Reyes AV, Zazula GD, Kuzmina S, *et al*. 2011. Identification of last interglacial deposits in eastern Beringia: a cautionary note from the Palisades, interior Alaska. *Journal of Quaternary Science* **26**: 345–352.

Rizzi E, Lari M, Gigli E, *et al*. 2012. Ancient DNA studies: new perspectives on old samples. *Genetics, Selection, Evolution: GSE* **44**: 1–19.

Rollo F, Ubaldi M, Ermini L, *et al*. 2002. Otzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. *Proceedings of the National Academy of Science of the United States of America* **99**: 12594–12599.

Saeki K, Sakai M, Wada S. 2010. DNA adsorption on synthetic and natural allophanes. *Applied Clay Science* **50**: 493–497.

Sawyer S, Krause J, Guschanski K, *et al*. 2012. Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLOS ONE* **7**: e34131.

Shapiro B, Drummond AJ, Rambaut A, *et al*. 2004. Rise and fall of the Beringian Steppe Bison. *Science* **306**: 1561–1565.

Shi T, Reeves RH, Gilichinsky DA, *et al*. 1997. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. *Microbial Ecology* **33**: 169–179.

Sirocko F, Dietrich S, Veres D, *et al*. 2013. Multi-proxy dating of Holocene maar lakes and Pleistocene dry maar sediments in the Eifel, Germany. *Quaternary Science Reviews* **62**: 56–76.

Smith HG. 1985. The colonization of volcanic tephra on Deception Island by protozoa: long-term trends. *British Antarctic Surveys Bulletin* **66**: 19–33.

Smith CI, Chamberlain AT, Riley MS, *et al*. 2001. Not just old, but old and cold. *Nature* **410**: 771–772.

Smith CI, Chamberlain AT, Riley MS, *et al*. 2003. The thermal history of human fossils and the likelihood of successful DNA amplification. *Journal of Human Evolution* **45**: 203–217.

Sønstebø JH, Gielly L, Brysting AK, *et al*. 2010. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular Ecology Resources* **10**: 1009–1018.

Speller CF, Kemp BM, Wyatt SD, *et al*. 2010. Ancient mitochondrial DNA analysis reveals complexity of indigenous North American turkey domestication. *Proceedings of the National Academy of Science of the United States of America* **107**: 2807–2812.

Stoof-Leichsenring KR, Epp LS, Trauth MH, *et al*. 2012. Hidden diversity in diatoms of Kenyan Lake Naivasha: a genetic approach detects temporal variation. *Molecular Ecology* **21**: 1918–1930.

Suyama Y, Kawamuro K, Kinoshita I, *et al*. 1996. DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. *Genes and Genetic Systems* **71**: 145–149.

Suyama Y, Gunnarsson U, Parducci L. 2008. Analysis of short DNA fragments from Holocene peatmoss samples. *The Holocene* **18**: 1003–1006.

Taberlet P, Coissac E, Pompanon F, *et al*. 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research* **35**: e14.

Taberlet P, Coissac E, Hajibabaei M, *et al*. 2012a. Environmental DNA. *Molecular Ecology* **21**: 1789–1793.

Taberlet P, Prud'Homme SM, Campione E, *et al*. 2012b. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology* **21**: 1816–1820.

Thomsen PF, Elias S, Gilbert MTP, *et al*. 2009. Non-destructive sampling of ancient insect DNA. *PLOS ONE* **4**: e5048.

Thomson VA, Lebrasseur O, Austin JJ, *et al*. 2014a. Using ancient DNA to study the origins and dispersal of ancestral Polynesian chickens across the Pacific. *Proceedings of the National Academy of Sciences* **111**: 4826–4831.

Thomson VA, Lebrasseur O, Austin JJ, *et al*. 2014b. Reply to Beavan, Bryant, and Storey and Matisoo-Smith: Ancestral Polynesian 'D' haplotypes reflect authentic Pacific chicken lineages. *Proceedings of the National Academy of Sciences* **111**: E3585–E3586.

Tito RY, Knights D, Metcalf J, *et al*. 2012. Insights from characterizing extinct human gut microbiomes. *PLOS ONE* **7**: e51146.

Tringe SG, von Mering C, Kobayashi A, *et al*. 2005. Comparative metagenomics of microbial communities. *Science* **308**: 554–557.

VanderHoek R, Nelson RC. 2007. Ecological road blocks on a constrained landscape: the cultural effects of catastrophic Holocene volcanism on the Alaska Peninsula, southwest Alaska. In *Living Under the Shadow: Cultural Impacts of Volcanic Eruptions*, Grattan J, Torrence R (eds). Left Coast Press: Walnut Creek; 133–152.

van Geel B, Aptroot A, Baittinger C, *et al*. 2008. The ecological implications of a Yakutian mammoth's last meal. *Quaternary Research* **69**: 361–376.

van Geel B, Guthrie RD, Altmann JG, *et al*. 2011. Mycological evidence of coprophagy from the feces of an Alaskan late glacial mammoth. *Quaternary Science Reviews* **30**: 2289–2303.

van Geel B, Fisher DC, Rountrey AN, *et al*. 2012. Palaeo-environmental and dietary analysis of intestinal contents of a mammoth calf (Yamal Peninsula, northwest Siberia). *Quaternary Science Reviews* **30**: 3935–3946.

van Geel B, Protopopov A, Bull I, *et al*. 2014. Multiproxy diet analysis of the last meal of an Early Holocene Yakutian bison. *Journal of Quaternary Science* **29**: 261–268.

Vestheim H, Jarman SN. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples: a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology* **5**: 12.

Vishnivetskaya TA, Petrova MA, Urbance J, *et al*. 2006. Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods. *Astrobiology* **6**: 400–414.

Vreeland RH, Rosenzweig WD, Powers DW. 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature* **407**: 897–900.

Wales N, Andersen K, Cappellini E, *et al*. 2014. Optimization of DNA recovery and amplification from non-carbonized archaeobotanical remains. *PLOS ONE* **9**: e86827.

Wall JD, Kim SK. 2007. Inconsistencies in Neanderthal genomic DNA sequences. *PLOS Genetics* **3**: 1862–1866.

Warinner C, Rodrigues JFM, Vyas R, *et al*. 2014. Pathogens and host immunity in the ancient human oral cavity. *Nature Genetics* **46**: 336–344.

Willerslev E, Cooper A. 2005. Ancient DNA. *Proceedings of the Royal Society of London Biological Series* **272**: 3–16.

Willerslev E, Hansen AJ, Christensen B, *et al*. 1999. Diversity of Holocene life forms in fossil glacier ice. *Proceedings of the National Academy of Science of the United States of America* **96**: 8017–8021.

Willerslev E, Hansen AJ, Binladen J, *et al*. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**: 791–795.

Willerslev E, Hansen AJ, Poinar HN. 2004. Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends in Ecology and Evolution* **19**: 141–147.

Willerslev E, Cappellini E, Boomsma W, *et al*. 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* **317**: 111–114.

Willerslev E, Davison J, Moora M, *et al*. 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* **506**: 47–51.

Wilmshurst JM, Anderson AJ, Higham TFG, *et al*. 2008. Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 7676–7680.

Wilmshurst JM, Moar NT, Wood JR, *et al*. 2014. Use of pollen and ancient DNA as conservation baselines for offshore islands in New Zealand. *Conservation Biology* **28**: 202–212.

Wood JR. 2007. Moa gizzard content analyses: further information on the diets of *Dinornis robustus* and *Emeus crassus*, and the first evidence for the diet of *Pachyornis elephantopus* (Aves: Dinornithiformes). *Records of the Canterbury Museum* **21**: 27–39.

Wood JR, Rawlence NJ, Rogers GM, *et al*. 2008. Coprolite deposits reveal the diet and ecology of the extinct New Zealand mega-herbivore moa (Aves, Dinornithiformes). *Quaternary Science Reviews* **27**: 2593–2602.

Wood JR, Wilmshurst JM, Wagstaff SJ, *et al*. 2012a. High-resolution coproecology: using coprolites to reconstruct the habits and habitats of New Zealand's extinct upland moa (*Megalapteryx didinus*). *PLOS ONE* **7**: e40025.

Wood JR, Wilmshurst JM, Worthy TH, *et al*. 2012b. First coprolites evidence for the diet of *Anomalopteryx didiformis*, an extinct forest ratite from New Zealand. *New Zealand Journal of Ecology* **36**: 164–170.

Wood JR, Wilmshurst JM, Worthy TH, *et al*. 2012c. A lost link between a flightless parrot and a parasitic plant and the potential role of coprolites in conservation paleobiology. *Conservation Biology* **26**: 1091–1099.

Wood JR, Wilmshurst JM, Rawlence NJ, *et al*. 2013a. A megafauna's microfauna: gastrointestional parasites of New Zealand's extinct moa (Aves: Dinornithiformes). *PLOS ONE* **8**: e57315.

Wood JR, Wilmshurst JM, Richardson SJ, *et al*. 2013b. Resolving lost herbivore community structure using coprolites of four sympatric moa species (Aves: Dinornithiformes). *Proceedings of the National Academy of Sciences of the United States of America* **110**: 16910–16915.

Woodward SR, Weyand NJ, Bunnell M. 1994. DNA sequence from Cretaceous period bone fragments. *Science* **266**: 1229–1232.

Xu Z-, Jiang XD, Wang GZ, *et al*. 2011. DNA extraction, amplification and analysis of the 28S rRNA portion in sediment-buried copepod DNA in the Great Wall Bay and Xihu Lake, Antarctica. *Journal of Plankton Research* **33**: 917–925.

Yoccoz NG, Bråthen KA, Gielly L, *et al*. 2012. DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular Ecology* **21**: 3647–3655.

Young JM, Rawlence NJ, Weyrich LS, *et al*. 2014. Limitations and recommendations for successful DNA extraction from forensic soil samples: a review. *Science and Justice: Journal of the Forensic Science Society* **54**: 238–244.

Zischler H, Hoss M, Handt O, *et al*. 1995. Detecting dinosaur DNA. *Science* **268**: 1192–1193.

JQS 2740. Supporting information, Tables S1 to S3

Table S1. A more comprehensive list of PalEnDNA publications to supplement Table 2 in the main article.

Table S2. A selection of ancient DNA laboratories that have published PalEnDNA research within the last 5 years.

Table S3. Universal metabarcoding genetic markers and PCR primers used in PalEnDNA-based research.

References for Tables S1-S3 are listed in the main article in *Journal of Quaternary Science* (2014), vol. 29 (7) pp. 610-626.

Table S1. A more comprehensive list of PalEnDNA publications to supplement Table 2 in the main article. Studies have been organised chronologically within general Quaternary research areas. Taxa in **bold type** were identified morphologically.

DEPOSIT OR MATERIAL	TAXA	AGE	LOCATION	REFERENCE
PALAEOECOLOGY: PALAEOENVIRONMENTAL RECONSTRUCTION				
Peat deposit	Plant (*Pincea abies*)	155 ka	Japan	Suyama *et al.* (1996)
Permafrost (fluvial)	Viable bacteria	2 ^{14}C ka–3 Ma	Kolyma-Indigirka lowland, Siberia, Russia	Shi *et al*. (1997)
Ice	Fungi, protists, plants	2–4 ^{14}C ka	Greenland	Willerslev *et al*. (1999)
Ice	Fungi	0.3–140 ka	Greenland	Ma *et al.* (2000)
Coprolite/rodent midden	Rodent (*Phyllotis limatus*), mammal, birds, plants	10.1 ^{14}C ka	Atacama Desert, Chile	Kuch *et al*. (2002)
Lacustrine	Microcrustacean (*Daphnia* sp.)	Modern–0.2 ^{14}C ka	Belauer See, Germany	Limburg and Weider (2002)
Cave deposit	Sloth, condor, ringtail cat, rodent, human	10.8 ^{14}C ka	Arizona, USA	Hofreiter *et al*. (2003b)
Cave deposit, sand	Birds, plants	0.6–3 ^{14}C ka	New Zealand	Willerslev *et al*. (2003)
Permafrost (tundra soil, fluvial/lacustrine, unknown)	Vertebrates, plants	10.4 ^{14}C ka–400 ka	Lena-Kolyma River region, Sibera, Russia	Willerslev *et al*. (2003)
Permafrost (unknown)	Bacteria	< 400–600 ka	Northeast Siberia, Russia	Willerslev *et al*. (2004)
Lacustrine	Copepod	0–9.9 cal ka	Antarctica	Bissett *et al*. (2005)
Permafrost (tundra soil, fluvial/lacustrine, unknown)	Fungi	<400 ka	Lena-Kolyma River region, Sibera, Russia	Lydolph *et al*. (2005)
Lacustrine	Microcrustacean (*Daphnia* sp.)	Unknown	High Tatra Mountains, Western Carpathians, Europe	Markova *et al*. (2006)
Lacustrine	Scots pine	0.1–10 ka	Holtjarnen, Sweden	Parducci *et al*. (2005); Bennett and Parducci (2006)
Cave deposit (soil, tephra)	Moa, sheep, duck, insect, plants	< 3.2 ^{14}C ka	New Zealand	Haile *et al.* (2007); Thomsen *et al*. (2009)
Permafrost (marine, lacustrine, alluvium, palaeosol)	Bacteria	5 ka–3 Ma*	Kolyma lowland, Siberia, Russia	Vishnivetskay *et al*. (2006)
Marine	Plants (*Fagus* sp.)	ca. 45 ka	Venice	Paffetti *et al*. (2007)
Permafrost (tundra soil, fluvial/lacustrine, ice rich/silty sediment, unknown)	Bacteria, plants	< 400 ka	Lena-Kolyma River region, Mayn River, Southern Chukotka, Khomus-Yuryakh River, Sibera, Russia	Johnson *et al*. (2007)
Ice (silt-rich)	Insects, plants	< 800 ka	Greenland	Willerslev *et al.* (2007)
Peat deposit	Peat moss (*Sphagnum fascum*)	Modern–0.45 ka	Norway	Suyama *et al*. (2008)
Glacial (fluviogravel and moraine)	Plants	4.5–5.2 cal ka	Peru	Gould *et al.* (2010)
Permafrost (ice rich, silty sediment)	Plants	15.8–22.9 ^{14}C ka	Mayn River, Southern Chukotka, Siberia, Russia	Sonstebo *et al*. (2010)
Lacustrine	Plants	< 4.6 ^{14}C ka	Great Lakes, North America	Anderson Carpenter *et al*. (2011)
Permafrost (peat, clay, silt, sand)	Mammals	4.47–50.6 ^{14}C ka	Taimyr Peninsula and north-central Siberia, Russia	Arnold *et al*. (2011)

Lacustrine, marine	Copepods	Unknown	Antarctica	Xu *et al.* (2011)
Permafrost	Bacteria	30 cal ka	Alaska	D'Costa *et al*. (2011)
Soil	Mammals, birds	Modern	Norway	Anderson *et al*. (2012)
Permafrost (silt, fine sand)	Mammals (incl. woolly rhino)	15.8–>50 ^{14}C ka	Main River, Southern Chukotka, Duvanny Yar, Kolyma River, Siberia, Russia	Kuzmina *et al*. (2011); Boessenkool *et al*. (2012)
Lacustrine	Norway spruce	10.7–13 cal ka	Retezat Mountains, South Carpathians, Europe	Magyari *et al*. (2011)
Silt, sand, organic deposit (silt-soaked)	Plants	12–45 ^{14}C ka	Taymir Peninsula, Siberia, Russia	Jorgensen *et al*. (2012a)
Soil, sediment	Plants	Modern–5.5 cal ka	J.A.D. Jemsen's Nunataks, Greenland	Jorgensen *et al*. (2012b)
Permafrost (silt, fine sand)	Plants, bryophytes, fungi, beetles, birds	15.8–> 50 ^{14}C ka	Main River, Southern Chukotka, Duvanny Yar, Kolyma River, Siberia, Russia	Epp *et al*. (2012)
Lacustrine	Diatoms	0–0.2 ka	Lake Naivasha, Kenya	Stoof-Leichsenring *et al.* (2012)
Lacustrine	Plants	0.342–9.52 cal ka	Lake Comarum, Greenland	Pendersen *et al*. (2013)
Cave deposit	Mammal, marsupial, reptile, plant	6.8–>20 ka	Kelly Hill Caves, Kangaroo Island, South Australia	Haouchar *et al*. (2014)
Lacustrine, mire	Plants	1980 AD–2790 BC	Mt. Gahinga, Mt. Muhavura, Albertine Rift, East Africa	Boessenkool *et al*. (2014)
Lacustrine	Mammals (*Bos, Ovis, Equus*), plants (*Pinus, Alnus*)	Present – ca. 4000 BC	Lake Anterne, France	Giguet-Covex *et al*. (2014)
Permafrost (various), coprolites, gut contents	Plants	Present – 50 ka	Arctic region	Willerslev *et al*. (2014)
Soil	Plants	Present–ca. 236 AD	Tawhiti Rahi, Poor Knights Islands, New Zealand	Wilmshurst *et al*. (2014)
PALAEOECOLOGY: MEGAFAUNAL EXTINCTION				
Permafrost (loess, palaeosol)	Mammals (especially mammoth, horse)	7.6–11.76 ^{14}C ka	Steven's Village, Yukon Flats, Alaska	Haile *et al*. (2009)
PALAEOECOLOGY: PALAEODIETARY RECONSTRUCTION				
Coprolite	Shasta ground sloth, plants	19.8 ^{14}C ka	Nevada, USA	Poinar *et al*. (1998)
Coprolite	Various extinct species	10–32 ^{14}C ka	SW USA, Chile	Poinar *et al*. (1998)
Coprolite	Shasta ground sloth, plants	11–28.5 ^{14}C ka	Nevada, USA	Hofreiter *et al*. (2000)
Coprolite	Human, mammal, plants	2.1–2.2 ^{14}C ka	Texas, USA	Poinar *et al*. (2001); Reinhard *et al*. (2008)
Intestinal contents	Mammal, plants	5.1–5.2 cal ka	Italy	Rollo *et al*. (2002)
Coprolite	Cuchillo Cura ground sloth, plants	14.6 ^{14}C ka	Argentina	Hofreiter *et al*. (2003a)
Coprolite	**Mammoth**, plants	18.5 ^{14}C ka	Yakutia, Siberia, Russia	van Geel *et al*. (2008)
Coprolite	Moa, plants, parasites	0.6-1 ^{14}C ka	New Zealand	Wood *et al*. (2008, 2012a, 2012b, 2013a, 2013b)
Coprolite	**Mammoth**, plants, fungi	12.3 ^{14}C ka	Cape Blossom, Alaska, USA	van Geel *et al*. (2011)
Intestinal contents	**Mammoth**, plants, **fungi**	41.9 ^{14}C ka	Yamal Peninsula, northwest Siberia, Russia	van Geel *et al*. (2012)

Coprolite	Kakapo, **plants**	< 3 ^{14}C ka	New Zealand	Wood *et al*. (2012c)
Intestinal contents	Bison	10.5 cal ka BP	Yakutia, Siberia, Russia	Van Geel *et al*. (2014)
ARCHAEOLOGICAL HISTORY				
Cave deposit	Human, mammal, birds, plants	12.3 ^{14}C ka	Oregon, USA	Gilbert *et al*. (2008, 2009); Goldberg *et al*. (2009); Poinar *et al*. (2009); Rasmussen *et al*. (2009); Jenkins *et al*. (2012)
Lacustrine	'Human', rat, fish, bacteria**	ca. 1700 cal a BP	New Zealand	Matisoo-Smith *et al*. (2008)
Palaeosol (anthropogenic)	Human, mouse, reindeer, sheep, goat, cattle	0.39–0.9 ^{14}C ka	Greenland	Hebsgaard *et al*. (2009)
Gyttja, gravel, sand, detritus, moss peat, herbaceous peat	Bacteria	2.8–10 ka	Poland	Madeja *et al.* (2009)
Lacustrine	Bacteria	2.9–0.6 ka	Poland	Madeja *et al*. (2010)
Coprolite	Parasite (*Ascaris* sp.)	0.6 cal ka	Belgium	Loreille *et al*. (2001)
Coprolite	Human intestinal parasites	< 7 ka	Brazil, Chile	Iniquez *et al*. (2003a)
Coprolite	Human, Pinworm	4110 BC–900 AD	Chile, USA	Iniquez *et al*. (2003b)
Coprolite	Pinworm	4110 BC–900 AD	Chile, USA	Iniquez *et al.* (2006)
Coprolite	Human	0.7–2 ka	California, USA	Kemp *et al.* (2006)
Coprolite	Intestinal bacteria (incl. *Haemophylus parainfluenzae*)	980–1170 AD	Peru	Luciani *et al*. (2006)
Coprolite	Human, mammal, birds, plants	12.3 ^{14}C ka	Oregon, USA	Gilbert *et al*. (2008, 2009); Goldberg *et al*. (2009); Poinar *et al*. (2009); Rasmussen *et al*. (2009); Jenkins *et al*. (2012)
Coprolite	Human, parasite (*Ascaris* sp.)	0.95–8.8 ^{14}C ka	Brazil, Chile, Peru	Leles *et al*. (2008)
Coprolite	Turkey	200 BC–1800 AD	USA	Speller *et al*. (2010)
Coprolite	Human	1.4–ca. 8 ka	USA, Mexico, Chile	Tito *et al*. (2012)
Dental calculus	Human, bacteria	Mesolithic, Neolithic, Bronze Age, Medieval	Poland, Germany, England	Adler *et al*. (2013)
Dental calculus	Human, bacteria, virus, mammals, plants	Medieval	Dalheim, Germany	Metcalf *et al*. (2014); Warriner *et al*. (2014);
DNA DAMAGE				
Permafrost (unknown)	All taxa, bacteria	10–600 cal ka	Region between Lena and Kolyma River, Siberia, Russia	Mitchell *et al*. (2005); Hansen *et al*. (2006)
TAXONOMY				
Coprolite	Shasta ground sloth	> 10 ^{14}C ka	Nevada, USA	Poinar *et al*. (2003)

Coprolite	Moa	< 3 ^{14}C ka	New Zealand	Bunce *et al*. (2009)
Coprolite	Harrington's mountain goat	11.2–14.4 ^{14}C ka, Late Pleistocene	Grand Canyon, USA	Campos *et al*. (2010)
Hairs in coprolite	Darwin's ground sloth (*Mylodon darwinii*)	13 ka	Milodon Cave, Chile	Clack *et al*. (2012a, 2012b)

*We question the veracity of this older age limit

**Cloned bacterial DNA sequences from sediments aged ca. 1700 cal a BP produced a 98% similarity match with human gut bacterial genus *Prevotella*. This genus, however, is found in a range of animal (including insect) guts and so postulated evidence for possible human presence is equivocal (see text of main article).

Table S2. A selection of ancient DNA laboratories that have published PalEnDNA research within the last 5 years.

North America	
Paleo-DNA Laboratory	Lakehead University, Thunder Bay, Ontario, Canada
McMaster Ancient DNA Centre	Department of Anthropology and Biology, McMaster University, Hamilton, Ontario, Canada
Department of Archaeology	Simon Fraser University, Burnaby, Canada
Department of Anthropology	University of Oklahoma, Norman, USA
New Zealand	
Landcare Research Long-Term Ecology Lab	Landcare Research, Lincoln (Canterbury)
Otago Zoology Palaeogenetics Lab	Department of Zoology, University of Otago, Dunedin
Australia	
Australian Centre for Ancient DNA	School of Earth and Environmental Science, University of Adelaide, SA
Trace and Environmental DNA Laboratory	Curtin University, Perth, WA
Europe	
School of Biological Sciences	Royal Hollaway University of London, Egham, UK
Molecular Taxonomy Laboratory	Natural History Museum of Budapest, Hungary
Evolutionary Biology Centre	Department of Ecology and Genetics, Uppsala University, Sweden
Institute of Botany	Department of Palaeobotany, Jagiellonian University, Krakow, Poland
Institute of Palaeobiology	Polish Academy of Sciences, Warsaw, Poland
National Centre for Biosystematics	Natural History Museum, University of Oslo, Norway
Centre for Geogenetics	Natural History Museum of Denmark, University of Copenhagen, Denmark

Table S3. Universal metabarcoding genetic markers and PCR primers used in PalEnDNA-based research.

Taxon	Forward primer	Sequence (5`-3`)	Reverse primer	Sequence (5`-3`)	Marker	Size (bp)	Reference
Universal primers (eukaryotes)							
Eukaryotes	BMBC-R	GTACACACCGCCCGTCG	NS8	TCCGCAGGTTCACCTACGGA	18S	125	Lydolph *et al*. (2005)
Eukaryotes	SR7R	AGTTAAAAAGCTCGTAGTTG	SR5	GTGCCCTTCCGTCAATT	18S	510	Lydolph *et al*. (2005)
Eukaryotes	Uni1	TCCCCAACAAACTAGGAGG	Uni2	ACTGGTTGTCTCCAATTCA	cyt b	178	Matisoo-Smith *et al*. (2008)
Protists, fungi and higher plants							
Protists, fungi and higher plants	NS8	TCCGCAGGTTCACCTACGGA	nu-SSU-1627-31-r	GRACACACCGCCCGT	18S	160	Willerslev *et al*. (2007)
Diatoms	Diat rbcL 705F	AACAGGTGAAGTTAAAGGTTCATAYTT	Diat rbcL 808R	TGTAACCCATAACTAAATCGATCAT	rbcL	103	Stoof-Leichsenring *et al.* (2012)
Diatoms	Diat rbcL 708F	AGGTGAAGTTAAAGGTTCATACTTDAA	Diat rbcL 802R	CCCATAACTAAATCGATCATAAYRAT	rbcL	94	Stoof-Leichsenring *et al*. (2012)
Fungi	KW3	TCCAGCTCCAATAGCGTATA	SL43	GAACCACACGTCCTATTC	18S	210	Lydolph *et al*. (2005)
Fungi	ITS5	GGAAGTAAAAGTCGTAACAAGG	5.8S fungi	CAAGAGATCCGTTGTTGAAAGTT	ITS1	50-100	Epp *et al.* (2012)
Bryophytes	Bryo P6F	GATTCAGGGAAACTTAGGTTG	Bryo P6R	CCATTGAGTCTCTGCACC	trnL	50-100	Epp *et al.* (2012)
Plants							
Plants	rbcL h1aF	GGCAGCATTCCGAGTAACTCCTC	Hp2R	CGTCCTTTGTAACGATCAAG	rbcL	120-130	Willerslev *et al*. (2003)
Plants	rbcLZ1	ATGTCACCACAAACAGAGACTAAAGCAAGT	rbcL h1aR	GAGGAGTTACTCGGAATGCTGCC	rbcL	132	Poinar *et al.* (2001)
Plants	rbcL h1aF	GGCAGCATTCCGAGTAACTCCTC	Hp2R	CGTCCTTTGTAACGATCAAG	rbcL	137	Hofreiter *et al.* (2000)
Plants	Z1aF	ATGTCACCACCAACAGAGACTAAAGC	19bR	CTTCTTCAGGTGGAACTCCAG	rbcL	157	Hofreiter *et al.* (2000)
Plants	rbcLZ1	ATGTCACCACAAACAGAGACTAAAGCAAGT	19bR	CTTCTTCAGGTGGAACTCCAG	rbcL	157	Poinar *et al.* (2001)
Plants	rbcLZ1	ATGTCACCACAAACAGAGACTAAAGCAAGT	rbcL19	AGATTCCGCAGCCACTGCAGCCCCTGCTTC	rbcL	183	Poinar *et al.* (2001)
Plants	rbcL h1aF	GGCAGCATTCCGAGTAACTCCTC	rbcL h1aR	GAGGAGTTACTCGGAATGCTGCC	rbcL	?	Poinar *et al.* (1998)
Plants	Z1aF	ATGTCACCACCAACAGAGACTAAAGC	Hp2R	CGTCCTTTGTAACGATCAAG	rbcL	?	Hofreiter *et al.* (2000)
Plants	trnLg	GGGCAATCCTGAGCCAA	trnLh	TTGAGTCTCTGCACCTATC	trnL	59-100	Taberlet *et al*. (2007)
Plants	trnLc	CGAAATCGGTAGACGCTACG	trnLd	GGGGATAGAGGGACTTGAAC	trnL	ca. 450	Taberlet *et al*. (2007)
Plants	trnLg	GGGCAATCCTGAGCCAA	*TrnL*gh	CCATTGAGTCTCTGCACCTATC	trnL	83-103	Haile *et al*. (2007)
Plants	trnLg	GGGCAATCCTGAGCCAA	Plant_trnL_49863R	GGGGATAGAGGGACTTGAAC	trnL	438	Willerslev *et al*. (2007)
Insects/Invertebrates							
Insects	InsCOIf1	TTATGCTATATTANCTATTGG	InsCOIr1	GTAAAGTAAGCTCGTGTATC	COI	97	Willerslev *et al*. (2007)
Insects	InsCOIR	GTAAAGTAAGCTCGTGTATC	Ins3L	AAAGAAACATTTGGAGCTTTAGGA	COI	125	Thomsen *et al.* (2009)
Insects	Ins3R	TCCTGTTGGAACAGCAATAAT	Ins3L	AAAGAAACATTTGGAGCTTTAGGA	COI	159	Thomsen *et al.* (2009)
Coleoptera	Coleop 16Sc	TGCAAAGGTAGCATAATMATTAG	Coleop 16Sd	TCCATAGGGTCTTCTCGTC	16S	50-100	Epp *et al*. (2012)
Enchytraeidae	Ench 12Sa	GCTGCACTTTGACTTGAC	Ench 12Sc	AGCCTGTGTACTGCTGTC	12S	50-100	Epp *et al*. (2012)
Intestinal parasites							
Ascaridoidea	Asc8	ATACATGCACCAAAGCTCCG	Asc9	GCTATAGTTATTCAGAGTCACC	18S	99	Lorielle *et al*. (2001)

Ascaridoidea	Asc6	CGAACGGCTCATTACAACAG	Asc7	TCTAATAGATGCGCTCGTC	18S	123	Lorielle *et al*. (2001)
Ascaridoidea	Asc10	CCATGCATGTCTAAGTTCAA	Asc11	CARAAAWTCGGAGCTTTGGT	18S	147	Lorielle *et al*. (2001)
Nematodes	Nem18SF	ATTCCGATAACGARCGAGAC	Nem18SR	CCGCTKRTCCCTCTAAGAAGT	18S	40-120	Wood *et al*. (2013a)
Nematodes	Nem18SlongF	CAGGGCAAGTCTGGTGCCAGCAGC	Nem18SlongR	GACTTTCGTTCTTGATTAATGAA	18S	350-400	Wood *et al*. (2013a)
Ascaris	Asc1	GTTAGGTTACCGTCTAGTAAGG	Asc2	CACTCAAAAAGGCCAAAGCACC	cyt b	142	Lorielle *et al*. (2001)
Vertebrates							
Vertebrates	12ss	AATTTCGTGCCAGCCACCGCGGTCA	12st	AAGCTGTTGCTAGTAGTACTCTGGC	12S	?153-273	Poinar *et al*. (1998)
Vertebrates	12sd	TAAAGGACTTGGCGGTGCTTCAC	12sn	CCATTTCATAGGCTACACCTTGACC	12S	?153-273	Poinar *et al*. (1998)
Vertebrates	12shp	GCACAATTATTACTCATAAGC	12sb	TGACTGCAGAGGGTGACGGGCGGTGTGT	12S	?153-273	Poinar *et al*. (1998)
Vertebrates	12s ll	GCATAACTATTACCCATAAGTA	12sb	TGACTGCAGAGGGTGACGGGCGGTGTG T	12S	?153-273	Hofreiter *et al*. (2003a)
Vertebrates	12sa	CTGGGATTAGATACCCCACTAT	12Sm	GAGGATGGCGGTATATAGGCTG	12S	205	Kuch *et al*. (2002)
Vertebrates	16SNS1	CCTCCGAACGACTATGCGCCCA	16S7	TTGCGCTGTTATCCCTAGGGTAACT	16S	?35	Hofreiter *et al*. (2000)
Vertebrates	16S6	TTTCGGTTGGGGCGACCTCGGAG	16S7	TTGCGCTGTTATCCCTAGGGTAACT	16S	141	Poinar *et al*. (2001)
Mammals							
Mammals	16SA&M Fv2	TCACTATTTTGCNACATAGA	16SA&M Rv2	CCCCGAAACCAGACGAGCTA	16S	70	Rassmussen *et al*. (2009)
Mammals	16Smam3	TGGGGTGACCTCGGAGAAY	16Smam4	TCAACGGAMCAAGTTACCCTA	16S	78	Haile *et al*. (2009)
Mammals	16Smam1	CGGTTGGGGTGACCTCGGA	16Smam2	GCTGTTATCCCTAGGGTAACT	16S	120	Willerslev *et al*. (2003)
Human blocking probe	Human block1	GAGCTACCTAAGAACAGCTA	Human block2	TTTGCTACATAGACGGGTGT	16S	n/a	Rasmussen *et al*. (2009)
Human blocking probe		TTCTCGTCTTGCTGTGTCATGCC			16S	n/a	Epp *et al*. (2012)
Mammals	12Sa	CTGGGATTAGATACCCCACTAT	12So	GTCGATTATAGGACAGGTTCCTCTA	12S	151	Poinar *et al*. (2001)
Human	H9bpA	ATGCTAAGTTAGCTTTACAG	H9bpB	ACAGTTTCATGCCCATCGTC	tRNA-Lys	121	Matisso-Smith *et al*. (2008)
Mammals	L2638	CCTCAGGATAGCTGGCGCTCT	2639H	TCTAATCATTCGCTTTACCGGAT	28S	74	Poinar *et al*. (2002)
Birds							
Bird	12SHf	CCTTGACCTGTCTTGTTAGC	12SKr	CCTACATACCGCCGTCGCCAG	12S	85	Willerslev *et al*. (2007)
Bird	12SF5	CTAACAAGACAGGTCAAGGTAT	12SR4	CCTATTTTACTGCTAAATCCG	12S	125	Oskam *et al*. (2010)
Bird	12SE	CCCACCTAGAGGAGCCTGTTC	12SH	CCTTGACCTGTCTTGTTAGC	12S	153	Haile *et al*. (2007)
Bird	12Sa	CTGGGATTAGATACCCCACTA T	12Sh	CCTTGACCTGTCTTGTTAGC	12S	250	Oskam *et al*. (2010)
Bird	Aves 12Sa	GATTAGATACCCCACTATGC	Aves 12Sc	GTTTTAAGCGTTTGTGCTCG	12S	50-100	Epp *et al*. (2012)