Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects

NICOLAS J. RAWLENCE, DAVID J. LOWE, JAMIE R. WOOD, JENNIFER M. YOUNG, G. JOCK CHURCHMAN, YU-TUAN HUANG and ALAN COOPER

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

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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, tephas, 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

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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., 2013), 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 origins 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 (14C) 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
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 Gurkerli 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 (cpDNA) 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 cpDNA 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 invertebrates (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 (Giguert-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 14C 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) |
|  | Bacteria, fungi, bryophytes, plants, invertebrates (insects), vertebrates (mammals, birds) | 2 – < 600 ka | Shi et al. (1997), Willerslev et al. (2001, 2004), Lydolph et al. (2005), Vishniveltsevskaya et al. (2006), Johnson et al. (2007), Somstebel 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) |
|  | Fungi, protists, plants, invertebrates (insects) | 0.3 – < 800 ka | Willerslev et al. (1999, 2007), Ma et al. (2000) |
| **Palaeoenvironmental or material** | **Target taxa** | **Age range** | **Examples (references)** |
| Ice | Plants, invertebrates (parasites), vertebrates (mammals, birds) | 10.8–0.6 14C ka | Hofreiter et al. (2003b), Willerslev et al. (2003), Thomsen et al. (2009), Haouchar et al. (2014) |
| Lacustrine | Diatoms, plants, invertebrates (crustaceans, copepods) | 13 cal ka–modern | Limburg and Weider (2002), Bissett et al. (2005), Parducci et al. (2005, 2013), Marković 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) |
| **Palaeoenvironmental or material** | **Target taxa** | **Age range** | **Examples (references)** |
| Cave deposit | Plants, invertebrates (insects), vertebrates (mammals, birds) | 10.1 14C ka | Kuch et al. (2002), Jørgensen et al. (2012a) |
|  | Bacteria Mesolithic, Neolithic, Bronze Age, Medieval | | Haile et al. (2009) |
|  | Vertebrates (mammals, birds) | 7.6–11.76 14C ka | Poinar et al. (1998, 2001), Hofreiter et al. (2000, 2003a), Reinhard et al. (2008), Wood et al. (2008, 2012a,b,c), 2013a,b |
| **PALAEOECOLOGY: MEGAFANAAL EXTINCTION** | | | |
| Permafrost | Vertebrates (mammals, birds) | ≤45 ka | Paffetti et al. (2007), Lejzerowicz et al. (2013) |
| **PALAEOECOLOGY: PALAEO DIETARY RECONSTRUCTION** | | | |
| Coprolite | Plant, invertebrates (parasites), vertebrates (mammals, birds) | 32–0.6 14C ka | Rollo et al. (2002), van Geel (2008, 2009), Leles et al. (2010), Giguet-Covex et al. (2009), Jenkins et al. (2009), Poinar et al. (2008), Madeja et al. (2008), Rasmussen et al. (2009), Jenkins et al. (2012) |
| **Intestinal contents** | Plants, vertebrates (mammals, birds) | 41.9–5.1 14C ka | Adler et al. (2013), De la Fuente et al. (2013), Metcalf et al. (2014), Warinner et al. (2014) |
| **Dental calculus (calculated plaque)** | Bacteria | Mesolithic, Neolithic, Bronze Age, Medieval | | |
| **PALAEOECOLOGY: TAXONOMY** | | | |
| **Coprolite** | Vertebrates (mammals) | Late Pleistocene to <3 14C ka | Poinar et al. (2003), Bunce et al. (2009), Clack et al. (2012a,b) |
| **Hairs in coprolite** | Vertebrates (mammals) | 13 ka | Campos et al. (2010) |
| **ARCHAEOLOGICAL HISTORY** | | | |
| **Cave deposit** | Plants, vertebrates (mammals, birds) | 12.3 14C 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 | Matisco-Smith et al. (2008), Madeja et al. (2010), Giguët-Covex et al. (2014) |
| **Soil** | Vertebrates (mammals) | 0.9–0.39 14C ka | Hebsgaard et al. (2009) |
| **Gravel, sand, gyttja, peat** | Bacteria | 10–2.9 14C ka | Madeja et al. (2009) |
| **Coprolites** | Vertebrates, invertebrates (parasites), vertebrates (mammals, birds) | 12.3 14C ka – 0.6 cal ka | Loreille et al. (2001), Iniguez 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** | 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. (Alley et al., 2010) extracted PalEnDNA from frozen plant material uncovered from 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 Creek 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 palaeoecology 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 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 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; Lezkerowicz 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 palaeoenvironmental 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 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 BP, long after mammoth became extinct in the region. Dating of permafrost sediments containing mammoth aDNA using both 14C for organic fractions and optically stimulated luminescence (OSL) for inorganic fractions showed significant mismatches between the ages obtained (14C: ca. 19 000–5000 cal BP; OSL: mean age 5700 a BP). These results suggest that extensive reworking can occur even in some permafrost settings, 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, Helsgaard 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 (Williams 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 Cameliadæ coprolite from Paisley Cave, Oregon, with a macrofossil age of 12 125 ± 30 14C a BP, which was found to have a water-soluble fraction age of 11 315 ± 25 14C a BP, a difference of around 800 14C years. Three human coprolites were excavated in close proximity to the Cameliadæ coprolite but these had concordant macrofossil and water-soluble fraction 14C ages. Jenkins et al. (2012) suggested that DNA leaching had occurred in the Cameliadæ 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 homogenous 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.

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 14C dating (if in range), or other methods where appropriate, such as (U–Th)/He or U–Pb dating (e.g. Danišk 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 hencebound, DNA fraction. In contrast, 14C dating can potentially date the organic and unbound DNA fraction. The same sort of split applies to macrofossil versus water-soluble-fraction 14C 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 10Be/36Cl 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 palaeodiary 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 extraction 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.

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 minimize 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’ metabar-code genetic markers. These metabar-codes are highly variable, allowing taxonomic discrimination, but they are flanked by regions conserved across multiple taxa. Commonly used metabar-codes in PalEnDNA research are listed in Table S3. However, many metabar-codes have resolution problems. The short plant rbcL (p6-loop) and trnL (p6-loop) metabar-codes 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 metabar-codes 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 metabar-codes 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; Calvig nic-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 metabar-code 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 subfossil 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 Gugeri 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; Jorgensen 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 examination 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).

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. Jorgensen 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

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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 palaeodietsary information (Jorgensen et al., 2012a; Wood et al., 2012a). Figure from Wood et al. (2012a, p. 10).
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.

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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.

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