Quaternary Science Reviews 138 (2016) 1–5

Contents lists available at ScienceDirect

Quaternary Science Reviews

journal homepage: www.elsevier.com/locate/quascirev

A protocol for subsampling Late Quaternary coprolites for multi-proxy analysis

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ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form
15 February 2016
Accepted 16 February 2016
Available online xxx

Keywords:
Analyses
Coprolite
Protocols
Sampling

ABSTRACT

The study of Late Quaternary coprolites can provide unique insights into various aspects of the biology and ecology of extinct species and prehistoric humans. Coprolite studies are becoming increasingly multi-disciplinary, allowing a greater amount of information to be obtained from individual specimens. Subsampling is a critical part of multi-proxy coprolite analysis, yet no standardised subsampling protocols exist, and details of subsampling methods have rarely been reported in published studies. Here, we outline a procedure for the subsampling of coprolites for multi-proxy analysis. The method is designed to minimise the risk of sample contamination for sensitive analyses (e.g. ancient DNA, palynology), thereby maximising the robustness of interpretations made from the results. We also stress the need for voucher samples to be retained to ensure the repeatability of results and allow for further analytical methods to be applied to specimens in the future.

1. Introduction

Desiccated coprolites are frequently encountered whilst excavating Late Quaternary archaeological and palaeontological deposits, and offer the potential to gain unique insights into certain aspects of the biology, ecology and behaviour of prehistoric human and animal populations. Such insights, often unattainable through study of other types of remains, include details about diet, agricultural practices, seasonal migration, health and ecological interactions between species (e.g. Hofreiter et al., 2000; Gilbert et al., 2008; Riley, 2008; Wood et al., 2012, 2013). Although dry caves and rockshelters tend to provide the most favourable environments for preservation of Late Quaternary coprolites, specimens also occur in a range of other sites including sand dunes (Sutton, 1993; Horrocks et al., 2002), dry soils (Vinton et al., 2009), swamp margins (Irwin et al., 2004), latrine fills (Reinhard and Bryant, 1992; Horrocks and Best, 2004), small mammal middens (Betancourt et al., 1990; Pearson and Betancourt, 2002; Chase et al., 2012; Diaz et al., 2012), and *in situ* with mummified or skeletal remains (Reinhard et al., 1992; van Geel et al., 2008; Rasmussen et al., 2009).

As many of the analytical techniques used to study coprolites are destructive, it is important to maximise the amount of data that is obtained from each specimen. To this end, the study of Late Quaternary coprolites is becoming increasingly multi-disciplinary, with studies often combining expertise in several different fields of research (e.g. palynology and palaeobotany, palaeozoology, chemical analyses, molecular analyses). The use of multiple analytical techniques in the study of a coprolite presents the need for a rigorous subsampling protocol. Such a protocol is particularly important in view of some analytical methods, such as ancient DNA (aDNA) analysis or palynology, which are particularly susceptible to sample contamination. Contamination risk can originate from several sources, including the sediment in which the coprolite was buried, the environment in which it was excavated, the subsequent storage environment, handling by researchers, and the environment in which it was subsampled. Despite the potential issues of contamination, which can confound later interpretations of results, details of subsampling protocols used in coprolite studies are seldom reported. To help rectify this we outline here a protocol for subsampling coprolites for multi-proxy analysis (Fig. 1). The protocol aims to ensure that the maximum amount of metadata is recorded about the specimen prior to sampling, and that the sampling method minimises the risk of sample contamination for sensitive analyses and maximise the robustness of interpretations made from the results.

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http://dx.doi.org/10.1016/j.quascirev.2016.02.018
0277-3791/

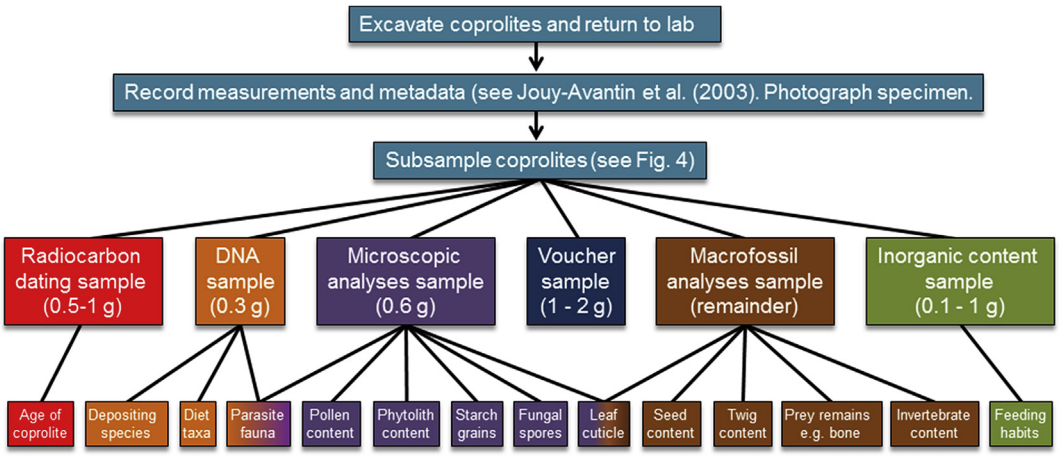

Fig. 1. Flow diagram for a multi-proxy coprolite analysis, showing approximate minimum subsample sizes required for different analytical techniques, and the types of information that can be gained from each.

2. Before subsampling

2.1. Coprolite storage

The risk of sample contamination begins with collection of coprolites in the field. Coprolites are best stored individually in bags or boxes to prevent cross-contamination between samples, and all relevant details (e.g. location, stratigraphic position, etc.) should be kept with each specimen. Upon return to the laboratory, the samples can be transferred to suitable packaging for long-term storage in a cool room or refrigerator at 4 °C. Storage at cool stable temperatures will help slow degradation of DNA in the specimens. If the coprolites are relatively hard, and were excavated from a very dry site, then plastic zip lock bags should be sufficient for storage. However, if there is any moisture in the specimens, fungal growth can be a problem if stored in plastic. In such instances the specimens are better being gently air dried before storage in breathable paper bags.

2.2. Coprolite description

As coprolite subsampling is a destructive process, it is important to record as much metadata about each coprolite before beginning. A standard method for the description of coprolites, and details of the attributes that can be recorded, is provided by Jouy-Avantin et al. (2003). In addition to a written description and measurements, 3-dimensional images of coprolites can be recorded. There are a variety of methods for doing this, including high-resolution approaches such as micro-CT scanning or laser tomography. However, basic stereophotography using a tripod-mounted camera (with macro lens for small coprolites) may be suitable for most studies. The coprolite can be placed on a sheet of paper with a scale, which is photographed twice, with the paper being slid eye-distance apart between photographs (Fig. 2). The two photographs can be converted to anaglyphs using freely available software such as StereoPhotomaker (Ver. 5.10, http://stereo.jpn.org/eng/stphmkr/, March 2015).

For large collections of coprolites it can be useful to set up a customised database to record and manage the metadata and photographs associated with each specimen. Through amassing records for a large number of samples whose identities have been verified by aDNA analysis, it may be possible to determine whether any morphological characters can be used in future to identify the depositing species (e.g. Chame, 2003; Wood and Wilmshurst, 2014).

3. Subsampling protocol

3.1. Subsampling environment

It is best practice to subsample coprolites in a clean, sterile environment. This is particularly critical if aDNA analyses are to be performed. DNA can be retrieved from coprolites that are thousands of years old (e.g. Hofreiter et al., 2000; Wood et al., 2012), however DNA from such specimens is likely to be degraded and in very small quantities, and therefore at high risk of contamination by modern DNA in the subsampling environment. Particular care is required if working on human coprolites, as there are significant contamination risks from anyone directly handling such specimens. However, contamination risks are not strictly confined to aDNA, and most microfossil techniques (e.g. pollen analysis), also require relatively clean subsampling environments.

Purpose-built aDNA laboratories offer the cleanest environments for subsampling work, due to their design features for maintaining low-levels of background contamination. However, it may not always be possible to use an aDNA facility, and in such circumstances protocols for aDNA-standard cleanliness (Cooper and Poinar, 2000; Fulton, 2012; Knapp et al., 2012) should be followed as closely as possible. A design for a simple Perspex box that can provide a clean, still-air environment for subsampling is shown in Fig. 3.

The interior and exterior of the box, and surrounding benchtops, can be cleaned thoroughly with detergent and bleach prior to each use, and between different coprolites. After each cleaning, a UVC tube mounted inside the box can be used as a final sterilization step (Cone and Fairfax, 1993). This is only necessary when aDNA analyses will be performed, but is good practice in all cases (particularly as voucher samples may be used for aDNA analyses in the future). The irradiation time required to effectively sterilize the coprolite surface will be unique for each box, and should be determined using an experimental approach (e.g. Cone and Fairfax, 1993). The box should be placed in a clean laboratory space that has not previously been used for DNA amplification or post-amplification work (due to the extreme contamination risk that these pose). To help reduce air movement, all windows should be closed during sampling, unfiltered air conditioning turned off, and a sign placed on the door to advise other people not to enter while subsampling is taking place. Researchers or technicians performing the subsampling may also reduce the risk of sample contamination by wearing cleansuits (e.g. Tyvek), facemasks and, most importantly, latex gloves (which should be changed regularly).