Species identification of archaeological dung remains: A critical review of potential methods

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Dung, macroscopically recognisable as such or not, can more commonly be found in archaeological contexts than is perhaps realised. Up to now, identification of dung to the species which produced it is usually either tenuous, or is not possible. However, species identification can be very informative and is necessary before any further studies can be conducted on the dung, for example on health and hygiene in the past and palaeoecology. This study presents a review of potential methods by which species identifications of archaeological dung can be undertaken. Criteria for identification can be divided into three broad categories: morphometric features of the dung; the content of dung and contextual evidence. Overall, the chances of a precise identification are high; however, a combination of different criteria and techniques will often be necessary to establish a secure identification. Moreover, preservation issues may exclude the application of some criteria while several criteria require more research and the expansion of reference collections of recent material. The overall aim is to move towards standardised methods for species identification of archaeological dung.

Keywords: Dung, Archaeobotany, Archaeozoology, Lipid biomarkers, Stable isotopes, Ancient DNA

Introduction

The possible origins of dung at archaeological sites are manifold. Dung can, of course, represent *in situ* production by human inhabitants themselves. In other cases, dung may have been used as manure, fuel or as a building material (Moreno-Garcia and Pimenta 2011), alternatively, it could have been left accidentally by domestic or wild animals, corralled at the site or freely roaming across it.

Various taphonomic processes make dung difficult to recognise during the excavation of archaeological contexts. However, several types of evidence have been used to identify dung deposits, for example, stable isotope analyses (e.g. Shahack-Gross *et al.* 2003, 2008, 2011), the presence of faecal biomarkers (Bull *et al.* 1999, 2001, 2005; Shillito *et al.* 2011; Simpson *et al.* 1999a, 1999b) and micromorphological studies, for example, through the presence of spherulites (Brochier *et al.* 1992; Brochier 1994). Under special circumstances, including permanently dry, waterlogged and frozen conditions, dung can be preserved more or less in its original state, thanks to the absence of bacterial and fungal activity. Moreover, at Neolithic sites in the Fayum oasis in Egypt (cal 5th millennium BC), the first author has found dung pellets that appeared to have preserved their shape, but that had become rock hard and non-dispersible in water (Fig. 1). These dung pellets were recovered from a hearth context and showed a greyish colour, and their state of preservation may, thus, largely be due to processes during heating. Owing to its high content of phosphates and nitrates, dung can also be preserved in a mineralised state, especially in sites with dry sediments exposed to alternating wet and

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Figure 1 Archaeological dung remains from hearth context at Kom K (ca. cal 4500 BC) in the Fayum oasis, Egypt. Dung identified from morphometric criteria (Linseele, unpublished data): probably domestic ovicaprines.

dry seasons. Such preservation is rather common in prehistoric sites under continental climatic conditions, and occurs, for example, frequently in Neolithic sites from Bulgaria (cal 6100–4900 BC), where dung is known to have built up layers with a thickness of over 10 cm (Marinova 2007). The likeliness of dung being preserved is also influenced by the diet of the animals that were responsible for it with, for example, obvious differences between herbivores and bone-eating carnivores (Retallack 1984; Bryant and Dean 2006). In whatever state preserved, once the presence of dung has been established, the second question that needs to be solved is which species produced it.

Dung has often been neglected as an archaeological find category. Certainly when archaeological sediment is not sieved, it may easily be confused with or obscured by lumps of earth, and therefore remain unnoticed. Sieving, and especially wet sieving, can of course cause damage to dung or even destroy it completely. Therefore, the most appropriate sampling strategy should be established on a case-by-case basis. However, it is our personal experience that dung starts to appear frequently, once one has developed an eye for it. Even when recognised, archaeological dung is rarely reported or identified. When identification is given, it is usually without a clear and well-investigated basis. On no more than the general appearance of a few recent samples, Rasmussen (1993) considered, for example, that sheep (*Ovis orientalis* f. *aries*) pellets are round and large, while those of goat (*Capra aegagrus* f. *hircus*) are smaller and pointed. However, as will be discussed below, the pellets of ovicaprines actually show a lot of variation and these criteria cannot be applied. In some cases, there is even no discussion on why archaeological dung was attributed to a certain species, for example, Darmon (1989), who described 'goat' dung from Neolithic caves in Israel (cal mid-7th to early 5th millennium BC).

A reliable identification of the species responsible for archaeological dung can be very significant. Dung may be the only evidence for the presence of species that are not represented among the bone remains of a certain site. This becomes especially important when the species in question is an early domesticate. Dung layers in rock shelters have, for example, been cited as proof for the presence of domestic ovicaprines in the Negev desert by cal 6000 BC, despite the absence of bone remains (Rosen *et al*. 2005). Dung, particularly in large concentrations, can also suggest that a certain animal must have been numerically more important at a site than indicated by the composition of its bone assemblage, as argued, for example, for ovicaprines in cal 6th millennium BC contexts at Sodmein and the Tree Shelter, in the Eastern Desert of Egypt (Linseele *et al*. 2010). The presence of dung in settlement contexts could be indicative of animal husbandry practices and the spatial organisation of these sites (Kühn and Hadorn 2004; Kühn *et al*. 2013). Species identification of dung is also preferably performed before any other studies, for example, macrobotanical or palynological, are conducted on it. On the other hand, some of these studies can also provide arguments for identification. The great potential of dung for palaeoecological interpretations is for example shown in the overview of Savinetsky *et al*. (2012).

What follows is a summary of different methods for species identification of excrements. We have tried to bring together information on all possible animal groups and animals, including humans. However, with the exception of lipid biomarkers and DNA studies, there is a clear emphasis in the literature on herbivore dung. We hope that this critical summary will ultimately lead to a generally accepted and common methodology for reliable identification of dung, which is a frequent, and potentially very informative, archaeological find category.

Almost a decade ago, a standard system for the description of archaeological animal dung was proposed (Jouy-Avantin *et al*. 2003). Although representing a very good guide, it never seems to have been actually applied. According to the designers of this system, the main identification criteria for dung are its external, macroscopic characteristics and its content. This corresponds well with our main group of identification criteria, as described below: macroscopic features and diverse contents of the dung, to which we have also added contextual, archaeological evidence.

Morphometric Features

An obvious starting point for archaeological dung identification are field or wildlife guides. Most of these concern the identification of fresh dung based on external criteria, like shape, size, colour and smell, but also the natural environment in which it was found (e.g. Walker 1996). A summary of such criteria, mainly morphometric, for dung of terrestrial mammals from North America, Eastern and Southern Africa, Europe and Brazil, can be found in Chame (2003). Apparently, the error margin of identification in the field of fresh dung can be relatively large, especially for dung of related species. Genetic studies have, for example, indicated that only 58–76% of antelope dung from a game area in Tanzania was correctly identified in this way (Bowkett *et al*. 2009). Using the field guides in archaeological contexts entails even more risk, because the environmental criteria cannot be used and also because the features of the dung itself can change over time, not only colour and smell, but also shape and size. Moreover, recent droppings are studied by pellet-group, which is a certain number of pellets collected in one place and usually produced by one defecating animal, while archaeological dung usually has to be studied by individual specimen, since deposits may be mixed. A last, but major problem is that the manuals do not discuss domestic species. Despite the various problems with field and wildlife guides, they may be very useful in the classification of archaeological dung into broad categories. In the case of Sodmein and the Tree Shelter, for example, they allowed the conclusion to be drawn that the dung found there was deposited by bovids and not by other herbivores living in the area, like hare (*Lepus capensis*) or dassie (*Procavia capensis*) (Linseele *et al*. 2010).

On the next level, the same or similar criteria as in the field manuals may be applied in a more rigorous, statistical manner. Such applications focus on the identification of particular species, for example recent dung of Reeves muntjac (*Muntiacus reevesi*) in England (Chapman 2004), or red (*Cervus elaphus*) and fallow deer (*Dama dama*) in Spain (Alvarez 1994). Another example of such a study is the one designed by Landsberg *et al*. (1994) to distinguish between recent feral sheep and goat dung pellet groups in an Australian rangeland context, based on the shape, size (greatest length and greatest breadth) and weight of individual pellets. In areas where no wild bovids in the same size category occur, this method can be sufficient to identify sheep or goat dung, but usually, at least, a few wild species have to be considered as well. Such is the case in Egypt, where gazelle, mostly dorcas gazelle (*Gazella dorcas*), Barbary sheep (*Ammotragus lervia*), ibex (*Capra ibex*), addax (*Addax nasomaculatus*) and oryx (*Oryx dammah*) can also be expected. Riemer (2011) expanded the method of Landsberg *et al*. (1994) to include wild small bovid species of northeastern Africa, based on the study of a reference collection of recent pellets, obtained from animals living in the wild as well as from zoo animals (Fig. 2). Later on,

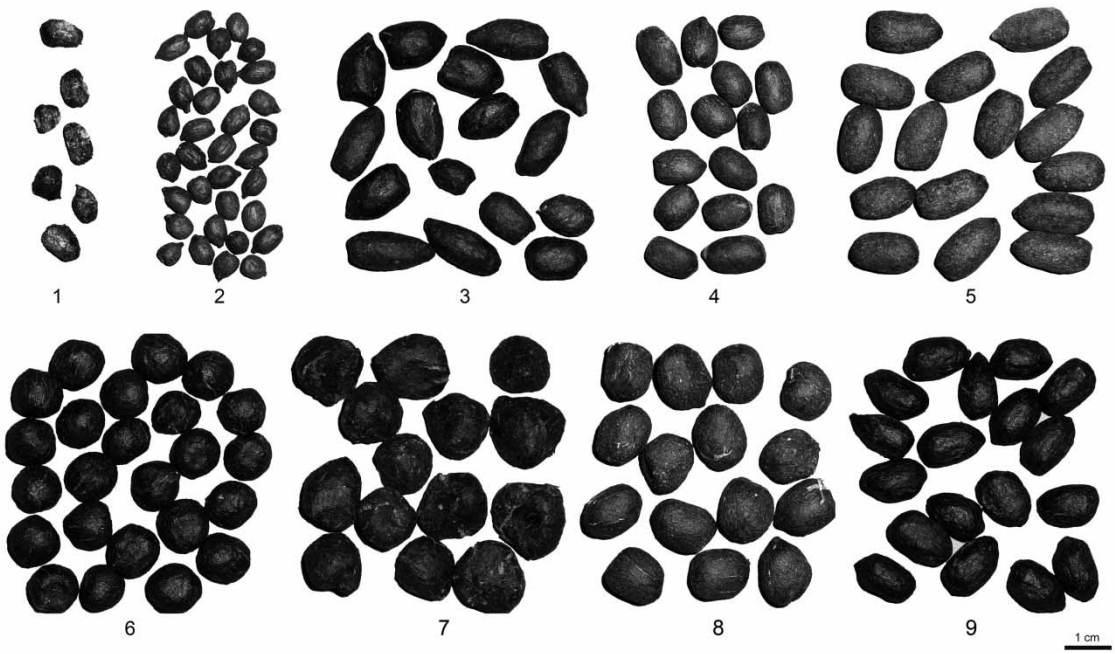

Figure 2 Pellet samples of species relevant for the Egyptian deserts (University of Cologne reference collection): 1, Dassie (*Procavia capensis*); 2, Dorcas gazelle (*Gazella dorcas*); 3, Addax (*Addax nasomaculatus*); 4, Nubian ibex (*Capra ibex nubiana*); 5, Barbary sheep (*Ammotragus lervia*); 6, Sheep (*Ovis orientalis* f. ammon), Nigerian dwarf breed; 7, Sheep, Rasa Aragonesa breed; 8, Goat (*Capra aegagrus* f. hircus), Nigerian dwarf breed; 9, Goat, Corsican breed.

reference material available at the Royal Belgian Institute of Natural Sciences was added to the dataset (Linseele *et al*. 2010). An overall conclusion of these analyses was that shape is a difficult criterion to distinguish between species. More importantly they showed that the greatest length and breadth of the recent pellets, as well as the weights, cluster by species. This is mainly true for the wild bovids. Domestic sheep and goat show a large variation and there is much overlap between the two and with the wild small bovids. Actually, overlap between sheep and goat pellets had already been shown by Landsberg *et al*. (1994), although the new studies have shown that the overlap is more extensive than previously thought. The large variation in the dung pellets of domestics is probably related to the large variation in breeds and animal sizes, as well as in their diet and the environments they occur in. The second author was able to demonstrate a correlation between pellet weight and body weight, varying with animal age, sex and breed, for domestic sheep and goat (Fig. 3). A similar correlation had already been found between the body weight of 11 wild African bovid species and pellet dry weight (Coe and Carr 1983). Factors like environment, diet, breed and body size are usually too difficult to estimate for archaeological animal populations to allow for comparisons with dung of recent animals that display similar features. In any case it is clear that this would require very large reference collections. The main value of macroscopic studies probably lies, therefore, in identifying or excluding certain wild bovid species as dung producers. The dung from Sodmein and the Tree Shelter was, for example, considered too large and too heavy for gazelle, while on the other hand most dung from the cal 3rd millennium BC rock shelter El Kharafish in the Western desert of Egypt (Riemer 2011) was attributed to gazelle based on its size and weight.

Owing to water loss, archaeological specimens may have shrunk and become lighter compared with fresh specimens (Liebenberg 1990, 14–15). However, our Egyptian case studies have shown that, at least there, the changes of archaeological pellets compared with recent ones are minor. Taking into account the necessary caution, morphometric criteria, designed on the basis of recent dung pellets, are therefore applicable to the archaeological specimens (see also Fig. 4). Moreover, experiments have shown that under dry and sunny conditions soft goat pellets lose 99% of water content within less than 1 week after defecation (Riemer 2011). Concerning the effects of fire on dung pellets, the analysis of complete specimens from the archaeological deposit at El Kharafish suggests that

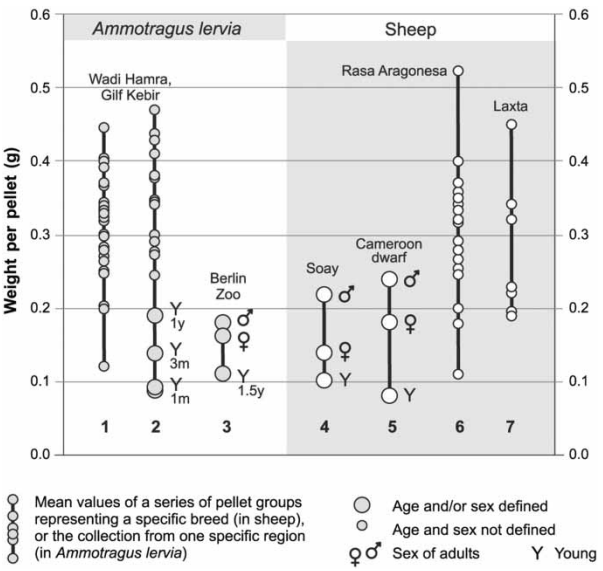

Figure 3 Correlations between pellet weight and body weight according to variation in breed, age and sex, based on data from the reference collection of recent dung at the University of Cologne (see also Riemer 2011). Short-statured sheep (*Ovis orientalis* f. aries) breeds (Soay and Nigerian/Cameroon dwarf) produce significantly lighter pellets then the middle to large-sized sheep breeds from the Spanish Pyrenees (Rasa Aragonesa and Laxta). Dung pellets of young (around 0.5 years old) sheep are lighter than those of adults (sex dimorphism is indicated in Nigerian dwarf, and pronounced in Soay). Sex dimorphism is indicated in Soay, and pronounced in Nigerian dwarf. The systematic collection of 25 pellet groups of Barbary sheep (*Ammotragus lervia*) in Wadi Hamra (Gilf Kebir massif, Western Desert, Egypt) shows the full weight range of droppings, making a good comparison with the middle to large-sized sheep.

Figure 4 Dung pellets from the shelter excavation Chufu 01/8 in the Egyptian Western Desert. Dung identified from morphometric criteria (Riemer, unpublished data): 1, middle-sized sheep (*Ovis orientalis* f. aries) or goat (*Capra aegagrus* f. hircus), or Barbary sheep (*Ammotragus lervia*); 2, supposed young or small goat; (3) small gazelle (*Gazella* sp.). Chufu 01/8 showed a clear stratigraphy of two well-separated layers: Gazelle droppings, which were predominantly found in the lower layer composed of compacted sand and rock gravel, radiocarbon dated to an age older than cal 4500 BC, and the small goat/sheep and larger caprinae droppings, dominating the upper layer, which consisted of an ashy and significantly finer material, directly dated to around cal 1250 BC (Riemer, unpublished data).

pellets affected by fire exhibit a reduced weight, while there is no significant effect on size (Riemer 2011). Firing experiments on sheep droppings carried out therefore have shown that the pellets quickly catch fire and, once they get glowing, completely disintegrate to ashes. During this process the pellets lose up to 50% of their weight before they start to disintegrate. Therefore, 'burned' pellets found in deposits can be regarded as dung that was only shortly exposed to fire and these pellets should be excluded from weight measurements to identify animal species.

Potentially, morphometric criteria are useful not only to identify small herbivore dung, but also the excrements of other animal groups, provided that they preserve their shape in archaeological contexts. We would expect that morphometrics are applicable to carnivore dung. Although it was not systematically verified on modern material, Horwitz and Goldberg (1989) suggested, for example, that archaeological excrements of striped (*Hyaena hyaena*) and spotted hyaena (*Crocuta crocuta*) can be distinguished based on their shape and size. On the other hand, cattle (*Bos primigenius* f. taurus) dung would be more difficult or impossible to recognise from morphometric features, as it has no particular, recognisable shape.

Content

Plant Remains

Archaeobotanical studies can contribute to the recognition and identification of dung, but much less to the specific attribution of dung to a particular animal group. Herbivore faeces are most likely to be the focus of botanical research, especially if they contain diverse, well-preserved palaeobotanical remains. The botanical information and potential for dung identification depend to a great extent on the preservation conditions. Dry conditions, like in deserts, rock shelters or inside building structures of wattle and daub or mud brick, offer the best conditions for preservation. In such cases, the intact plant content of the dung could provide information on the diet of the animal that produced the dung (Ghosh *et al*. 2008; Linseele *et al*. 2010). From the diet, one may indirectly deduce if humans intentionally fed the animal or not. Thus, one can differentiate between dung of wild animals, and of domestic animals or animals that were held in captivity (Marinova *et al*. 2013). One of the most prominent examples of plants used for fodder is the chaff of cultivated cereals. Such remains, like spikelet forks of Einkorn and Emmer, are well recognisable even in dung preserved in charred and mineralised state. However, the content of dung from domestic animals may also be almost completely dominated by wild growing vegetation, such as in the case of sheep/goat dung pellets found in mud bricks from Coptic monasteries (4th to 8th century AD) in Middle Egypt (Marinova *et al*. 2011).

It has long been recognised that animal dung, especially from livestock, is a major source of charred plant remains on sites in arid regions of the Eastern Mediterranean. In these sites, with dry preservation conditions and in arid areas where dung fuel was used, the archaeobotanical samples typically reveal small seeds passing through digestion, chaff remains and other plant remains typical for dung (see Miller 1984; Moens and Wetterstrom 1988; Charles 1998; Anderson and Ertug-Yaras 1998; Valamoti and Charles 2005). The digestion of different herbivores leads to a different representation of ingested seeds/fruits and plant matter (for discussion, see Wallace and Charles, 2013). Therefore, this knowledge could be used while evaluating the general composition of archaeobotanical assemblages derived from dung fuel. In sites with waterlogged plant remains, when the dung is not intact, it is very difficult to distinguish between dung and organic deposits in the cultural layers. Nevertheless, Kühn and Hadorn (2004) show the potential of careful observations and meticulous sampling during excavations to recognise the presence of cow dung.

In historical European contexts, macroscopic plant contents may also be useful to distinguish between human faeces and other waste in cesspits. Inside faeces, various uncharred plant remains can be expected, such as seed coat and pericarp fragments of cereals, linseeds, poppy seeds, fragments of field weeds, small seeds/fruits, fragments of fruit epidermis, pericarps of apples and the epiderms of vegetables. On the other hand, carbonised remains of plants, larger quantities of chaff and pods, nut shells, bigger fruitstones and charcoal are typically absent (Charles *et al*. 2009).

Plant microfossils can also be used for identification of dung and its producer. Pollen analyses can help to identify dung in organic rich deposits, or at least to determine the presence of dung, through the overrepresentation of entomophilous plant taxa, and the presence of indicators for dung like fungal spores (van Geel 2001). Analyses of recent dung samples by Powers *et al*. (1989) have shown that phytolith contents, as microscopic remnants of plants that were consumed, may be useful to distinguish between cattle and sheep dung, and this has extensively been applied to Early Iron Age (ca. 200–1000 AD) contexts in South Africa (see summary in Badenhorst 2009). However, the usefulness of phytoliths seems to be context dependent as other attempts to distinguish between cattle and sheep or goat based on phytolith morphology were unsuccessful (Shahack-Gross *et al*. 2003). Pollen is usually also related to dietary intake and thus to the species plants consumed by dung

producers/animals. Analysis of pollen preserved in dung pellets found near the Neolithic iceman, Ötzi, dated between cal 5400 and 2000 BC, indicated that they were more likely produced by game, for example ibex (*Capra ibex*), rather than by domestic sheep or goat (Oeggl *et al*. 2009). Palynological studies of dung from Barbary sheep (*Ammotragus lervia*), helped to identify attempts to manage herds of these animals (Di Lernia 2001; Mercuri 2008) in the Libyan Sahara during the Early Holocene.

Generally, the botanical approaches for the study of animal dung can be helpful in the recognition of dung remains and, in special cases, such as those illustrated above, in the identification of the animals producing dung. Thereby, the usually fragmentary character of the botanical evidence should be taken into account: seeds/fruits pass selectively through digestion, phytoliths are produced only by certain plant taxa, pollen needs special anoxic or very arid conditions for preservation etc. However, in order to obtain reliable results the best approach is to apply these studies in combination with further analyses, which also depend on the state of preservation and origin of the analysed deposits (Lancelotti and Madella 2012; Portillo *et al*. 2012).

Animal Remains

Dung may also contain a variety of remains from animals that were consumed, such as bones, hair and pieces of hooves, in which case the animal that produced the excrements was probably an omnivore or carnivore (Walker 1996). Other animal remains may have been unintentionally ingested. Panagiotakopulu (1999) found puparial fragments of Diptera in dung samples from presumed animal pens at the 18th Dynasty Egyptian site of Amarna (mid-14th century BC). The fragments were too small for secure identification but they were tentatively attributed to Calliphoridae, maggots that feed on carrion. The dung producers that occupied the animal pens therefore seem to have eaten meat, and were probably domestic pigs (*Sus scrofa* f. domestica). Other cases are known in which lice specific for a certain host, and presumably swallowed accidentally, were used to identify dung. Through the presence of remains of *Damalinia ovis*, the sheep louse, Schelvis and Koot (1995) were able to attribute dung deposits from Middendelfland, an Iron Age site in the Netherlands, to sheep, while other deposits contained remains of cattle louse (*Damalinia bovis*) and were attributed to cattle. In Sodmein, attempts were made to retrieve parasitic mites in order to identify the dung-producing species, but none of the recovered arthropod remains belonged to mites (Schelvis 1999). Predatory mites may also invade faeces at any time after it was deposited (Baker 2009). Apparently, certain predatory mites are specific to the excrements of certain animals, such as cattle, sheep, horse, pig and poultry (Schelvis 1992). Based on this type of evidence, Schelvis (1998) was, for example, able to prove the use of chicken excrement in wool processing at medieval Ypres (Belgium).

Endoparasites

Animal dung may contain different types of endoparasites, helminth and intestinal protozoa, as well as their cysts and eggs (ova). The latter two, in particular, have good preservation chances in archaeological contexts. Apparently parasite eggs are very durable, because they have a comparable resistance to decomposition as pollen grains (Reinhard 1992). The assemblage of ova may be indicative of a certain host species (Jones 1982), but contamination with recent parasite ova needs to be excluded first. According to Schelvis (1992) the use of endoparasites is mainly useful in identification of human dung, as there are many problems involved in specific identification of the ova for domestic animals. However, there seem to be several cases in which domestic animals were successfully identified through their endoparasites (Reinhard 1992). Naturally, only the dung of infected individuals can be recognised.

DNA Analyses

Ancient DNA in dung

In recent years, genetic analysis of ancient dung has proven to be a valuable tool to investigate various aspects of the ecology of animal species. Retrieving ancient DNA (aDNA) sequences from dung enables identification of the defecator's species, and potentially provides relevant insights into its diet, particularly in the case of plants that cannot be morphologically identified after mastication and passage through the gastrointestinal tract (Poinar *et al*. 1998; Hofreiter *et al*. 2000).

Degradation of DNA molecules and contamination represent serious challenges in the genetic analysis of ancient biological samples. From the first phase of decomposition, a major degrading action is microbial attack by external micro-organisms and commensal bacteria (e.g. gut flora). In addition, a number of physico-chemical parameters have been proven to affect DNA preservation, leading to fragmentation and modifications of the nucleotide chain which may affect genetic analysis (Hoss *et al*. 1996; Hofreiter *et al*. 2001; Briggs *et al*. 2007; Brotherton *et al*. 2007; Gilbert *et al*. 2007). Temperature plays a pivotal role, as it is exponentially linked to the rate of degradation (Smith *et al*. 2001, 2003). Nevertheless, other factors like proximity of free water, environmental salt content or exposure to radiation may affect the rate of DNA decay (Lindahl 1993), and this means that modelling such a process is extremely arduous.

Furthermore, in materials that are rich in organics and particularly in dung, molecular links between polynucleotides and peptides are commonly formed and these will hinder the amplification of aDNA through polymerase chain reaction. As a general rule, low temperatures and dry environments turn out to be more suitable for DNA preservation in archaeological samples.

The other major challenge to face in aDNA analyses is contamination from exogenous modern samples, which leads to a strict series of precautions to be taken in order to reduce its impact during sampling and laboratory procedures (Cooper and Poinar 2000; Pääbo *et al*. 2004; Gilbert *et al*. 2005). When dealing with dung samples, particularly in relatively wet temperate settings, a further potential source of exogenous contamination is represented by leaching of DNA, mainly through the vertical migration of DNA from younger to older stratigraphic layers (Haile *et al*. 2007). Parallel genetic tests conducted on sediment samples together with the analysis of faunal and floral composition in the depositional context of dung may be useful to rule out potential contamination due to DNA leaching in ancient faeces samples (Gilbert *et al*. 2008).

Molecular markers for defecating animal species

In terms of number of species, the amount of endogenous genetic information in a dung sample is high, namely DNA from the defecating animal, commensal microbes of the intestinal tract and plant and animal species consumed. In conditions where DNA extracted from biological samples contain an endogenous mixture of many species, the possibility to detect and discriminate between them is crucial. Such an approach is strictly linked to the concept of DNA barcoding, defined as accurate and automatable species identification by using a standardised DNA region as a tag (Hebert and Gregory 2005). Taberlet *et al*. (2007) have summarised the criteria a DNA barcoding system should ideally meet. It is worth noting that particularly in archaeological samples a large fraction of the nucleic acid content is represented by the exogenous component stemming from the depositional context (e.g. microbes and fungi of the soil), which constitutes 'environmental contamination'.

Mitochondrial gene cytochrome oxidase subunit I (*COI* or *COXI*) as well as 12S and 16S rRNA encoding DNA, turned out to be useful barcoding markers for animal species identification in dung (Poinar *et al*. 1998, 2001; Hofreiter *et al*. 2000) and sediment samples (Willerslev *et al*. 2003, 2007; Haile *et al*. 2007). Detecting animal DNA in ancient faeces may reveal the species of the defecator, as well as give indications about animal species consumed (Poinar *et al*. 2001; Gilbert *et al*. 2008). Nevertheless, it is worth mentioning that relying exclusively on genetic assays of dung does not offer the possibility to discriminate the DNA molecules that stem from the defecator from those of the consumed animal species. When possible, the identification of the animal that produced the dung may be ascertained by retrieving homologous DNA sequences from skeletal remains found in the same context as the dung (Hofreiter *et al*. 2000). Furthermore, a multidisciplinary approach appears to always be crucial for species identification. Information about potential defecators gathered from preliminary analyses (e.g. ecological, morphological, biochemical) makes possible to narrow the genetic screening to lower taxonomic groups (e.g. genus or even species) and to adopt genetic assays targeting specific markers of the putative defecator.

Recently, an attempt to retrieve DNA from dung deposits at Sodmein Cave, in the Egyptian Eastern Desert, has been carried out at the Center for Archaeological Sciences (KU Leuven). Based on morphometric criteria and contextual evidence, the dung samples were putatively assigned to domestic ovicaprine, and a genetic assay was meant to confirm the identification and possibly to specify the defecator species as sheep or goat. With this regard, a short fragment in the mtDNA cytochrome b gene was targeted (Loreille *et al*. 1997), but no successful amplification of DNA by means of polymerase chain reaction was obtained after multiple attempts. The age of these samples (cal 6th millennium BC and later) together with high mean annual temperature, 28°C, in the area (Griffiths and Soliman 1972) may be responsible for the poor preservation of DNA in the dung samples from Sodmein.

Lipid Biomarkers

The development and identification of archaeological biomarkers have received widespread attention in the field of organic residue analysis, as they provide diagnostic criteria to pinpoint the nature and origin of amorphous organic residues. Archaeological biomarkers can be defined, from an organic geochemical point of view, as organic molecules that are resistant to diagenetic processes and indicative of their original biogenic source (Bull *et al*. 2002). The discovery of 5β-stanols and bile acids in ancient faecal remains has enabled their use as faecal biomarkers in archaeological studies (Lin *et al*. 1978; Knights *et al*. 1983). They have been isolated from contexts such as coprolites, middens, ditches, latrines and cesspits (Lin *et al*. 1978; Knights *et al*. 1983; Pepe *et al*. 1989; Pepe and Dizabo 1990; Bethell *et al*. 1994; Bull *et al*. 2003, 2005; Shillito *et al*. 2011; Baeten *et al*. 2012). In addition, faecal biomarkers are highly valuable in cases where dung remains are expected but macroscopic evidence is lacking, like in manured soils and

putative animal pens (Bull *et al*. 1999, 2001, 2005; Simpson *et al*. 1999a, 1999b; Birk *et al*. 2011; Shillito *et al*. 2011). Important in the light of this study is the fact that faecal biomarkers are furthermore diagnostic for certain animal species due to differences in diet, digestion and metabolism.

5β-stanols

The first group of faecal biomarkers are 5β-stanols such as coprostanol and 5β-stigmastanol. These compounds are products of microbial sterol reduction in the intestines of animals (Macdonald *et al*. 1983). This is illustrated for some common sterols in Fig. 5. As 5β-stanols might also be present in small concentrations in sediments, Grimalt *et al*. (1990) proposed that the (5β: (5β + 5alpha)) stanol ratio can be used to assess the faecal origin of 5β-stanols, with ratios above 0.7 indicating faecal pollution (see Bull *et al*. 2002; Birk *et al*. 2011; Baeten *et al*. 2012 for further discussion).

A thorough understanding of faecal stanol distributions is a prerequisite to assess the source specificity of faecal stanols. Leeming *et al*. (1996) found that the distribution of faecal stanols is governed by three factors. Firstly, faecal stanol composition highly depends on the intestinal microbial population (Macdonald *et al*. 1983). For example, excreta of dogs and birds contain only trace amounts of 5β-stanols which is probably due to a lack of sterol-reducing bacteria (Leeming *et al*. 1996). Secondly, animals having a low dietary intake of cholesterol, for instance herbivores and vegetarians, synthesise cholesterol *de novo* and secrete this sterol into their intestines, thus leading to substantial amounts of coprostanol in their faeces (Leeming *et al*. 1996; Reddy *et al*. 1998). Thirdly, the sterol composition of an animal's diet, and the amount of plant material in particular, have a clear impact on stanol profile. As animal and plant sterols are chemically distinct from each other and are both subject to intestinal reduction, significant differences can be observed in the faecal stanol profile of animals. For instance, faeces of carnivores are highly enriched in animal-derived stanols such as coprostanol, while herbivore dung contains significant quantities of plant-derived stanols such as 5β-campestanol and 5β-stigmastanol (Leeming *et al*. 1996). In this regard, the ratio of coprostanol to 5β-stigmastanol can be used to distinguish between omnivore and herbivore excrements (Bull *et al*. 2001; Shillito *et al*. 2011; Baeten *et al*. 2012).

Bile acids

The second group of faecal biomarkers are bile acids. In vertebrates, primary bile acids are synthesised from cholesterol in the liver. Upon passage through the intestines, these primary bile acids undergo a set of microbial transformation reactions (MacDonald *et al*. 1983). The most important reaction is dehydroxylation at carbon atom 7, which yields secondary bile acids (see Fig. 6). A portion of these secondary bile acids is excreted via the faeces. Certain animal taxa, such as fish, amphibians and a few early evolving bird and mammal species, produce bile alcohols rather than bile acids (Hofmann *et al*. 2010). Unlike 5β-stanols, the composition of the faecal bile acid pool is not influenced significantly by diet, but instead is determined by the biochemical pathways in bile acid synthesis of the host organism (Hagey *et al*. 2010a, 2010b). Bile acids and alcohols show a remarkably high structural diversity across vertebrate species (Hofmann *et al*. 2010). The major primary bile acids of placental mammals are cholic acid and

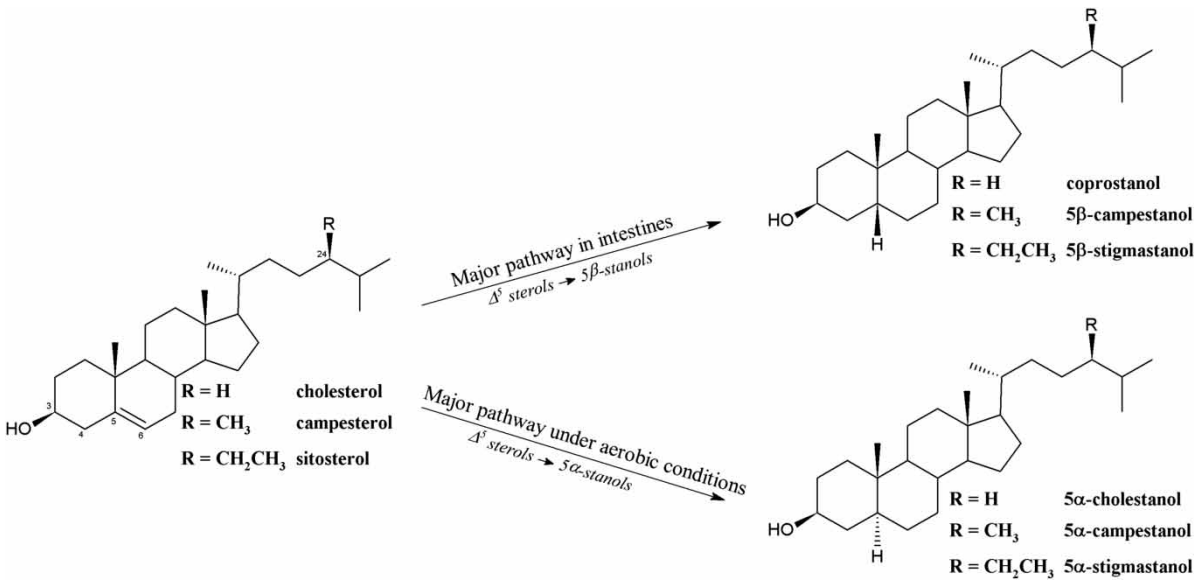

Figure 5 Formation of common 5β- and 5alpha-stanols, from their sterols precursors, in the mammalian gut and in the natural environment.

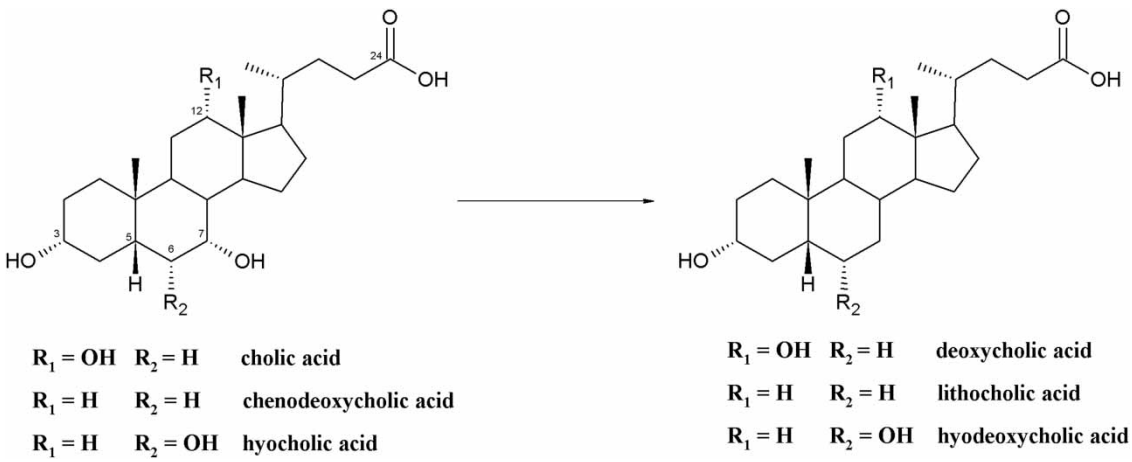

Figure 6 Formation of secondary bile acids, from their primary bile acid precursors, in the mammalian gut.

chenodeoxycholic acid (Fig. 5). Apart from these widely dispersed bile acids, certain animals produce very specific bile acids. For instance, pigs have a unique 6α-hydroxylation pathway, while mice and rat of the Muridae family produce characteristic 6β-hydroxylated bile acids (Haslewood 1967). Furthermore, certain cervids and equids produce C_{27} bile alcohols and C_{27} bile acids, respectively along with common C_{24} bile acids (Hagey *et al*. 2010a, 2010b). Rhinoceroses (Rhinocerotidae) and paenungulates, including elephants, manatees and hyraxes, are unique in that they exclusively produce C_{27} bile alcohols (Hagey *et al*. 2010a, 2010b). This has been corroborated by the observation that bile acids were completely absent in mammoth coprolites (van Geel *et al*. 2008, 2011).

Preservation issues

It has been shown that 5β-stanols and bile acids are very persistent in the archaeological record, even if they are scattered such as in anthropogenic soils. Still, the efficiency of recovery from archaeological contexts can be fairly variable. The parameters that affect the preservation of these compounds, however, are poorly understood. In general, losses of lipids primarily depend on microbial activity and on environmental parameters that affect these microbiota (Bethell *et al*. 1994; Bull *et al*. 2000). Some authors have claimed that bile acids are more recalcitrant than 5β-stanols (Elhmmali *et al*. 1997; 2000; Bull *et al*. 2003). However, bile acids might not be detected in well-percolated sediments due to their higher water solubility (Baeten *et al*. 2012). Moreover, since bile acids are excreted in lower amounts in faeces than 5β-stanols, failure to detect them may be due to detection close to the instrumental limit of detection (Elhmmali *et al*. 2000; Lin and Conner 2001). Other factors that affect the preservation of lipids are clay content, temperature and particle size distribution (Bethell *et al*. 1994). However, the impact of these parameters on the preservation of 5β-stanols and bile acids in soils still requires rigorous examination.

Recent developments

While new bile alcohols and bile acids are still being discovered (Hofmann *et al*. 2010), identification of dung remains can be achieved based on the aforementioned faecal biomarkers. However, differentiation between certain classes of animals remains difficult. For example, ruminant species such as cattle and ovicaprines remain indistinguishable. However, attempts have been made to increase the diagnostic capacity of biomarker analyses. For instance, the lipid composition of woolly mammoth (*Mammuthus primigenius*) coprolites was consistent with a herbivorous diet consisting of grasses and sedges (van Geel 2008, 2011). The coprolites of another extinct animal, the ground sloth (*Nothrotheriops shastensis*), also contained molecular evidence relating to dietary preferences (Gill *et al*. 2009). Furthermore, Gill *et al*. (2010) proposed archaeol, a membrane lipid of methanogenic archaea, as a new biomarker for foregut-fermenting herbivores, such as cattle, sheep, deer (*Cervus elaphus*), camel (*Camelus bactrianus*), alpaca (*Llama peruana*) and giraffe (*Giraffa camelopardalis*).

Stable Isotopes

The assessment of dietary preferences by means of stable isotope analysis can further support species identification of dung remains. Stable nitrogen isotope ratios can be used as trophic level indicator (Minagawa and Wada 1984), while stable carbon isotopes can differentiate between different photosynthetic pathways (DeNiro and Epstein 1978). For example, livestock enclosures and manured grasslands can be identified based on the fact that dung is enriched in ^{15}N relative to fodder (Simpson *et al*. 1999a; 1999b; Shahack-Gross *et al*. 2008). In addition, Shahack-Gross *et al*. (2008) were able to differentiate between grazers feeding on C4 grasses (e.g. cattle),

and browsers feeding also on C3 bushes or tree leaves (e.g. goat). It should be noted, however, that such inferences can be made only taking into account the ancient botanical environment. For instance, Carr *et al*. (2010) identified higher plant wax alkanes in ancient hyrax (*Procavia capensis*) middens from southern Africa. These alkanes were enriched in ^{13}C indicating a C4 plant input. However, since C4 plants were virtually absent in the studied region, it was argued that the enrichment was due to an input of crassulacean acid metabolism plants (e.g. succulents).

Spherulites

The presence of spherulites in soil deposits is one of the methods to identify dung layers (Brochier *et al*. 1992). Apparently soil pH is one of the main factors determining their preservation, with values below 7 being especially detrimental, while sometimes dissolution is also observed when values are as high as pH 8 (Canti 1999). Differences in shape between the spherulites of animal groups have not yet been described, but the quantities in which spherulites are produced are known to be very variable. They are influenced by feeding and digestive strategies, as well as the pH of the soils which the animals are living on (Canti 1999). Ruminants, including all bovids, produce the largest numbers of spherulites, but spherulites are more numerous in sheep than in goat dung (Brochier 1996). Low numbers are produced by omnivorous and carnivorous species and spherulites are absent from caecal digesters, including hare. Other than excluding certain species, spherulites seem as yet not very helpful in species identification of dung.

Archaeological Context

In addition to the criteria described above the archaeological context in which dung was found can also be used to argue for certain species. In the case of dung from cesspits for example, the most likely producers are the human inhabitants themselves or their livestock. The species composition of animal bone remains from a certain site, can also help to narrow down the possible animals that produced dung found at the same location. Very large deposits of animal dung, larger than is known to be produced in natural circumstances, point to species under human control, especially when hearths and artefacts are also present in the deposits (e.g. Rosen *et al*. 2005). In such cases we will usually, but not necessarily, be dealing with domesticated animals. It seems, for example, that in the Uan Afuda Cave in the Libyan Acacus large dung deposits were formed through penning of wild Barbary sheep (*Ammotragus lervia*), about 8000 years ago (Di Lernia 2001). Also, the location of dung deposits can be informative. Gazelles are, for example, not known to defecate underneath rock shelters, although human actions, like the collection of dung pellets as fuel, can cause accumulations to form in such places (Riemer 2011). It is known from ethnographic contexts that artificial stone enclosures, for example under rock overhangs, are used to keep goats and sheep, in particular the young (c.f. Brochier *et al*. 1992), and dung deposits in places of this nature can thus probably be attributed to domesticates (e.g. Riemer 2011). For the dung layers at the Sodmein Cave, apart from the size and weight of the excrements, which were not diagnostic, the dimensions of the layers and the presence of hearths and artefacts inside them, as well as the species represented in the bone assemblage from the site, brought us to the conclusion that the dung was deposited by domestic ovicaprines (Linseele *et al*. 2010). In any case, it is clear that the circumstantial evidence that can be used for identification has to be judged on a site by site basis and can be quite variable.

Conclusions

Dung is a more common find category on archaeological sites than often realised, and its study can moreover be very informative from several palaeoecological and palaeoeconomic perspectives. Its presence is not always obvious and careful sampling and specific analytical techniques may be necessary to trace it. Well-argued identification of the animal that produced the discovered excrements, adds much to the informative value of archaeological dung. There are several criteria that potentially allow the identification of archaeological animal dung. Ideally, identification should rely on more than one method, as most methods in themselves do not allow a very high precision nor are sufficient to be decisive. All methods also have their own requirements and constraints and, moreover, preservation issues often do not allow their application. Although direct evidence is to be preferred, circumstantial evidence may at the moment often still be necessary for species identification of archaeological dung.

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References

Alvarez, G. 1994. Morphological variability and identification of deer pellets in central Spain. *Folia Zoologica* **43**, 25–37.

Anderson, S. and Ertug-Yaras, F. 1998. Fuel fodder and faeces: an ethnographic and botanical study of dung fuel use in Central Anatolia. *Environmental Archaeology* **1**(1), 99–109.

Badenhorst, S. 2009. Phytoliths and livestock dung at early iron age sites in Southern Africa. *South African Archaeological Bulletin* **64**, 45–50.

Baeten, J., Marinova, E., De Laet, V., Degryse, P., De Vos, D. and Waelkens, M. 2012. Faecal biomarker and archaeobotanical analyses of sediments from a public latrine shed new light on ruralisation in Sagalassos, Turkey. *Journal of Archaeological Science* **39**(4), 1143–59.

Baker, A. 2009. Acari in archaeology. *Experimental and Applied Acarology* **49**, 147–60.

Bethell, P. H., Goad, L. J. and Evershed, R. P. 1994. The study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material. *Journal of Archaeological Science* **21**, 619–32.

Birk, J. J., Teixeira, W. G., Neves, E. G. and Glaser, B. 2011. Faeces deposition on Amazonian anthrosols as assessed from 5β-stanols. *Journal of Archaeological Science* **38**(6), 1209–20.

Bowkett, A., Plowman, A., Stevens, J., Davenport, T. and Van Vuuren, B. 2009. Genetic testing of dung identification for antelope surveys in the Udzungwa Mountains, Tanzania. *Conservation Genetics* **10**, 251–5.

Briggs, A. W., Stenzel, U., Johnson, P. L., Green, R. E., Kelso, J., Prufer, K., *et al.* 2007. Patterns of damage in genomic DNA sequences from a Neandertal. *Proceedings of the National Academy of Sciences* **104**, 14616–21.

Brochier, J. L. 1994. Etude de la sedimentation anthropique. La strategie des ethnofacies sedimentaires en milieu de constructions en terre. *Bulletin de Correspondance Hellenique* **118**, 619–45.

Brochier, J. L. 1996. Feuilles ou fumiers? Observations sur le rôle des poussières sphérolithiques dans l'interprétation des dépôts archéologiques holocènes. *Anthropozoologica* **24**, 19–30.

Brochier, J. E., Villa, P. and Giacomarra, M. 1992. Shepherds and sediments: geo-ethnoarchaeology of pastoral sites. *Journal of Anthropological Archaeology* **11**, 47–102.

Brotherton, P., Endicott, P., Sanchez, J. J., Beaumont, M., Barnett, R., Austin, J., *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**, 5717–28.

Bryant, V. M. and Dean, G. W. 2006. Archaeological coprolite science: the legacy of Eric O. Callen (1912–1970). *Palaeogeography Palaeoclimatology Palaeoecology* **237**(1), 51–66.

Bull, I. D., Betancourt, P. P. and Evershed, R. P. 2001. An organic geochemical investigation of the practice of manuring at a Minoan site on Pseira island, Crete. *Geoarchaeology* **16**, 223–42.

Bull, I. D., Elhmmali, M. M., Perret, V., Matthews, W., Roberts, D. J. and Evershed, R. P. 2005. Biomarker evidence of faecal deposition in archaeological sediments at Çatalhöyük, pp. 415–20 in Hodder, I. (ed.), *Inhabiting Çatalhöyük: Reports from 1995–1999 Seasons* (McDonald Institute Monographs). Cambridge: McDonald Institute for Archaeological Research, University of Cambridge.

Bull, I. D., Elhmmali, M. M., Roberts, D. J. and Evershed, R. P. 2003. The application of steroidal biomarkers to track the abandonment of a roman wastewater course at the agora (Athens, Greece). *Archaeometry* **45**, 149–61.

Bull, I. D., Lockheart, M. J., Elhmmali, M. M., Roberts, D. J. and Evershed, R. P. 2002. The origin of feces by means of biomarker detection. *Environment International* **27**, 647–54.

Bull, I. D., Simpson, I. A., Dockrill, J. and Evershed, R. P. 1999. Organic geochemical evidence for the origin of ancient anthropogenic soil deposits at Tofts Ness, Sanday, Orkney. *Organic Geochemistry* **30**, 535–56.

Bull, I. D., van Bergen, P. F., Nott, C. J., Poulton, P. R. and Evershed, R. P. 2000. Organic geochemical studies of soils from the Rothamsted classical experiments – V. The fate of lipids in different long-term experiments. *Organic Geochemistry* **31**, 389–408.

Canti, M. G. 1999. The production and preservation of faecal spherulites: animals, environment and taphonomy. *Journal of Archaeological Science* **26**, 251–8.

Carr, A. S., Boom, A. and Chase, B. M. 2010. The potential of plant biomarker evidence derived from rock hyrax middens as an indicator of palaeoenvironmental change. *Palaeogeography, Palaeoclimatology, Palaeoecology* **285**, 321–30.

Chame, M. 2003. Terrestrial mammal feces: a morphometric summary and description. *Memorias do Instituto Oswaldo Cruz* **98**(Suppl. 1), 71–94.

Chapman, N. G. 2004. Faecal pellets of Reeves' muntjac, *Muntiacus reevesi*: defecation rate, decomposition period, size and weight. *European Journal of Wildlife Research* **50**, 141–5.

Charles, M. 1998. Fodder from dung: the recognition and interpretation of dung-derived plant material from archaeological sites. *Environmental Archaeology* **1**(1), 111–22.

Charles, M., Crowther, A., Ertug, F., Herbig, C., Jones, G., Kutterer, J., *et al.* 2009. Archaeobotanical Online Tutorial http://archaeobotany.dept.shef.ac.uk/wiki/index.php/

Coe, M. and Carr, R. D. 1983. The relationship between large ungulate body weight and faecal pellet weight. *African Journal of Ecology* **21**, 165–74.

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

Darmon, F. 1989. Etude de l'environnement de la Grotte de Nahal Hemar dans le désert de Judée au Néolithique ancien d'après l'analyse des croprolithes de chèvres. *Comptes Rendus de l'Academie des Sciences* **308**, 1759–64.

DeNiro, M. J. and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

Di Lernia, S. 2001. Dismantling dung: delayed use of food resources among early Holocene foragers of the Libyan Sahara. *Journal of Anthropological Archaeology* **20**, 408–41.

Elhmmali, M. M., Roberts, D. J. and Evershed, R. P. 1997. Bile acids as a new class of sewage pollution indicator. *Environmental Science and Technology* **31**, 3663–8.

Elhmmali, M. M., Roberts, D. J. and Evershed, R. P. 2000. Combined analysis of bile acids and sterols/stanols from riverine particulates to assess sewage discharges and other fecal sources. *Environmental Science and Technology* **34**, 39–46.

Ghosh, R., Gupta, S., Bera, S., Jiang, H. E., Li, X. and Li, C. S. 2008. Ovi-caprid dung as an indicator of paleovegetation and paleoclimate in northwestern China. *Quaternary Research* **70**, 149–57.

Gilbert, M. T. P., Bandelt, H. J., Hofreiter, M. and Barnes, I. 2005. Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20**, 541–4.

Gilbert, M. T., Binladen, J., Miller, W., Wiuf, C., Willerslev, E., Poinar, H., *et al.* 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Research* **35**, 1–10.

Gilbert, M. T., Jenkins, D. L., Gotherstrom, A., Naveran, N., Sanchez, J. J., Hofreiter, M., *et al.* 2008. DNA from pre-Clovis human coprolites in Oregon, North America. *Science* **320**, 786–9.

Gill, F. L., Crump, M. P., Schouten, R. and Bull, I. D. 2009. Lipid analysis of a ground sloth coprolite. *Quaternary Research* **72**, 284–8.

Gill, F. L., Dewhurst, R. J., Dungait, J. A., Evershed, R. P., Ives, L., Li, C. S., *et al.* 2010. Archaeol – a biomarker for foregut fermentation in modern and ancient herbivorous mammals? *Organic Geochemistry* **41**, 467–72.

Griffiths, J. F. and Soliman, K. H. 1972. The northern desert Sahara, pp. 75–132 in Griffiths, J. F. (ed.), *World Survey of Climatology 10: Climates of Africa*. Amsterdam: Elsevier.

Grimalt, J. O., Fernández, P., Bayona, J. M. and Albaigés, J. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. *Environmental Science and Technology* **24**, 357–63.

Hagey, L. R., Vidal, N., Hofmann, A. F. and Krasowski, M. D. 2010a. Complex evolution of bile salts in birds. *Auk* **127**, 820–31.

Hagey, L. R., Vidal, N., Hofmann, A. F. and Krasowski, M. D. 2010b. Evolutionary diversity of bile salts in reptiles and mammals, including analysis of ancient human and extinct giant ground sloth coprolites. *BMC Evolutionary Biology* **10**, 133.

Haile, J., Holdaway, R., Oliver, K., Bunce, M., Gilbert, M. T., Nielsen, R., *et al.* 2007. Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology and Evolution* **24**, 982–9.

Haslewood, G. A. D. 1967. Bile salt evolution. *Journal of Lipid Research* **8**, 535–50.

Hebert, P. D. and Gregory, T. R. 2005. The promise of DNA barcoding for taxonomy. *Systematic Biology* **54**, 852–9.

Hofmann, A. F., Hagey, L. R. and Krasowski, M. D. 2010. Bile salts of vertebrates: structural variation and possible evolutionary significance. *Journal of Lipid Research* **51**, 226–46.

Hofreiter, M., Jaenicke, V., Serre, D., Haeseler Av, A. and Pääbo, S. 2001. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Research* **29**, 4793–9.

Hofreiter, M., Poinar, H. N., Spaulding, W. G., Bauer, K., Martin, P. S., Possnert, G., *et al.* 2000. A molecular analysis of ground sloth diet through the last glaciation. *Molecular Ecology* **9**, 1975–84.

Horwitz, L. K. and Goldberg, P. 1989. A study of Pleistocene and Holocene hyaena coprolites. *Journal of Archaeological Science* **16**, 71–94.

Hoss, M., Jaruga, P., Zastawny, T. H., Dizdaroglu, M. and Paabo, S. 1996. DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Research* **24**, 1304–7.

Jones, A. K. G. 1982. Human parasite remains: prospects for a quantitative approach, pp. 66–70 in Hall, A. R. and Kenward, H. K. (eds.), *Environmental Archaeology in the Urban Context*. York: Council for British Archaeology.

Jouy-Avantin, F., Debenath, A., Moigne, A. M. and Moné, H. 2003. A standardized method for the description and the study of coprolites. *Journal of Archaeological Science* **30**, 367–72.

Knights, B. A., Dickson, C. A., Dickson, J. H. and Breeze, D. J. 1983. Evidence concerning the Roman military diet at Beardsen, Scotland, in the 2nd century AD. *Journal of Archaeological Science* **10**, 139–52.

Kühn, M. and Hadorn, P. 2004. Pflanzliche Makro- und Mikroreste aus Dung von Wiederkäuern, pp. 227–350 in Jacomet, S., Leuzinger, U. and Schibler, J. (eds.), *Die Jungsteinzeitliche Seeufersiedlung Arbon – Bleiche 3* (Umwelt und Wirtschaft. Archälogie im Thurgau 12). Thurgau: Departement für Erziehung und Kultur des Kantons.

Kühn, M., Maier, U., Herbig, C., Ismail-Meyer, K., Le Bailly, M. and Wick, L. 2013. Methods for the examination of cattle, sheep and goat dung in prehistoric wetland settlements with the examples of the sites Alleshausen-Täschenwiesen and Alleshausen-Grundwiesen (around 2900 cal. BC) at Lake Federsee, South West Germany. *Environmental Archaeology* **18**(1), 43–57.

Lancelotti, C. and Madella, M. 2012. The 'invisible' product: developing markers for identifying dung in archaeological contexts. *Journal of Archaeological Science* **39**, 953–63.

Landsberg, J., Stol, J. and Müller, W. 1994. Telling the sheep (dung) from the goats'. *Rangeland Journal* **16**, 122–34.

Leeming, R., Ball, A., Ashbolt, N. and Nichols, P. 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. *Water Research* **30**, 2893–900.

Liebenberg, L. 1990. *A Field Guide to the Animal Tracks of Southern Africa*. Cape Town and Johannesburg: David Philip Publishers.

Lin, D. S. and Conner, W. E. 2001. Fecal steroids of the coprolite of a Greenland Eskimo mummy, AD 1475: a clue to dietary sterol intake. *American Journal of Clinical Nutrition* **74**, 44–9.

Lin, D. S., Conner, W. E., Napton, L. K. and Heizer, R. F. 1978. The steroids of 2000-year-old human coprolites. *Journal of Lipid Research* **19**, 215–21.

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. *Nature* **362**, 709–15.

Linseele, V., Marinova, E., Van Neer, W. and Vermeersch, P. M. 2010. Sites with Holocene dung deposits in the Eastern Desert of Egypt: visited by herders? *Journal of Arid Environments* **74**, 818–28.

Loreille, O., Vigne, J. D., Hardy, C., Callou, C., Treinen-Claustre, F., Dennebouy, N., *et al.* 1997. First distinction of sheep and goat archaeological bones by the means of their fossil mtDNA. *Journal of Archaeological Science* **24**, 33–7.

Macdonald, I. A., Bokkenheuser, V. D., Winter, J., McLernon, A. M. and Mosbach, E. H. 1983. Degradation of steroids in the human gut. *Journal of Lipid Research* **24**, 675–700.

Marinova, E. 2007. Archaeobotanical data from the early Neolithic of Bulgaria, pp. 93–109 in Connolly, J. and Colledge, S. (eds.), *Early Neolithic in Southwest Asia and Europe: Archaeobotanical Perspectives in Neolithic Plant Economies*. London: UCL-Press.

Marinova, E., De Meyer, M., van Loon, G. and Willems, H. 2011. Plant economy and land use in Middle Egypt during the late Antique/early Islamic period – archaeobotanical analysis of mud bricks and mud plasters from the area of Dayr al Barsha, pp. 120–36 in Fahmy, A. G., Kahlheber, S. and D'Andrea, A. C. (eds.), *Windows on the African Past: Current Approaches to African Archaeobotany* (Reports in African Archaeology 3). Frankfurt-am-Main: Africa Magna Verlag.

Marinova, E., Ryan, P., Van Neer, W. and Friedman, R. 2013. Animal dung from arid environments and archaeobotanical methodologies for its analysis: an example from animal burials of the Predynastic elite cemetery HK6 at Hierakonpolis, Egypt. *Environmental Archaeology* **18**(1), 58–71.

Mercuri, A. M. 2008. Plant exploitation and ethnopalynological evidence from the Wadi Teshuinat area (Tadrart Acacus, Libyan Sahara). *Journal of Archaeological Science* **35**(6), 1619–42.

Miller, N. F. 1984. The use of dung as fuel: an ethnographic example and an archaeological application. *Paléorient* **10**(2), 71–9.

Minagawa, M. and Wada, E. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between δ15N and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–40.

Moens, M.-F. and Wetterstrom, W. 1988. The agricultural economy of an Old Kingdom town in Egypt's West Delta: insights from the plant remains. *Journal of Near Eastern Studies* **3**, 159–173.

Moreno-Garcia, M. and Pimenta, C. M. 2011. Animal dung: rich ethnographic records, poor archaeozoological evidence, pp. 20–8 in Albarella, U. and Trentacoste, A. (eds.), *Ethnozooarchaeology. The Present and Past of Human-animal Relationships*. Oxford: Oxbow Books.

Oeggl, K., Schmidl, A. and Kofler, W. 2009. Origin and seasonality of subfossil caprine dung from the discovery site of the Iceman (Eastern Alps). *Vegetation History and Archaeobotany* **18**, 37–46.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Despres, V., Hebler, J., Rohland, N., *et al.* 2004. Genetic analyses from ancient DNA. *Annual Review of Genetics* **38**, 645–79.

Panagiotakopulu, E. 1999. An examination of biological materials from coprolites from XVIII Dynasty Amarna, Egypt. *Journal of Archaeological Science* **26**, 547–51.

Pepe, C. and Dizabo, P. 1990. Étude d'une fosse du 13ème siècle par les marqueurs biogéochimiques: chantier archéologique du Louvre (Paris). *Revue d'Archéométrie* **14**, 23–8.

Pepe, C., Dizabo, P., Scribe, P., Dagaut, J., Fillaux, J. and Saliot, A. 1989. Les marqueurs biogéochimiques: application à l'archéologie. *Revue d' Archéométrie* **13**, 1–12.

Poinar, H. N., Hofreiter, M., Spaulding, W. G., Martin, P. S., Stankiewicz, B. A., Bland, H., *et al.* 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**, 402–6.

Poinar, H. N., Kuch, M., Sobolik, K. D., Barnes, I., Stankiewicz, A. B., Kuder, T., *et al.* 2001. A molecular analysis of dietary diversity for three archaic Native Americans. *Proceedings of the National Acadademy of Sciences USA* **98**, 4317–22.

Portillo, M., Valenzuela, S. and Albert, R. M. 2012. Domestic patterns in the Numidian site of Althiburos (northern Tunisia): The results from a combined study of animal bones, dung and plant remains. *Quaternary International* **275**, 84–96.

Powers, A. H., Padmore, J. and Gilbertson, D. D. 1989. Studies of late prehistoric and modern opal phytoliths from coastal sand dunes and machair in northwest Britain. *Journal of Archaeological Science* **16**, 27–45.

Rasmussen, P. 1993. Analysis of sheep/goat faeces from Egolzwil 3, Switzerland: evidence for branch and twig foddering of livestock in the Neolithic. *Journal of Archaeological Science* **20**, 479–502.

Reddy, S., Sanders, T. A. B., Owen, R. W. and Thompson, M. H. 1998. Faecal pH, bile acid and sterol concentrations in premenopausal Indian and white vegetarians compared with white omnivores. *British Journal of Nutrition* **79**, 495–500.

Reinhard, K. J. 1992. Parasitology as an interpretative tool in archaeology. *American Antiquity* **57**, 231–45.

Retallack, G. J. 1984. Completeness of the rock and fossil record: some estimates using fossil soils. *Palaeobiology* **10**, 59–78.

Riemer, H. 2011. Animal dung, pp. 146–61 in Riemer, H. (ed.), *El Kharafish. The Archaeology of Sheikh Muftah Pastoral Nomads in the Desert around Dakhla Oasis (Egypt)* (Africa Praehistorica 25). Cologne: Heinrich Barth Institute.

Rosen, S. A., Savinetsky, A. B., Plakht, Y., Kisseleva, N. K., Khassanov, B. F., Pereladov, A. M., *et al.* 2005. Dung in the desert: preliminary results of the Negev Holocene ecology project. *Current Anthropology* **46**, 317–27.

Savinetsky, A. B., Babenko, A. N., Debella, H. J., Khassanov, B. F., Kiseleva, N. K., Krylovich, O. A., *et al.* 2012. Zoogenic deposits in rock shelters from the Old World (experience for palaeoecological purposes), pp. 25–31 in Lefèvre, K. (ed.), *Proceedings of the General Session of the 11th International*

Council for Archaeozoology Conference (Paris, 23–28 August 2010) (BAR International Series 2354). Oxford: Archaeopress.
Schelvis, J. 1992. The identification of archaeological dung deposits on the basis of remains of predatory mites (Acari; Gamasida). *Journal of Archaeological Science* **19**, 677–82.
Schelvis, J. 1998. Remains of sheep ectoparasites as indicators of wool processing in the past, pp. 89–110 in Dewilde, M., Ervynck, A. and Wielemans, A. (eds.), *Ypres and the Medieval Cloth Industry in Flanders.* Asse/Zellik: Instituut voor het Archeologisch Patrimonium.
Schelvis, J. 1999. Verslag van het waarderingsonderzoek aan resten van ongewervelden afkomstig uit veertien bodemstalen verzameld te Sodmein-Cave, Egypt. Scarab-report 61. Unpublished report.
Schelvis, J. and Koot, C. 1995. Sheep or goat? Damalinia deals with the dilemma. *Proceedings of the section Experimental and Applied Entomology of the Netherlands Entomological Society* **6**, 161–2.
Shahack-Gross, R. 2011. Herbivorous livestock dung: formation, taphonomy, methods for identification, and archaeological significance. *Journal of Archaeological Science* **38**(2), 97–107.
Shahack-Gross, R., Marshall, F. and Weiner, S. 2003. Geo-ethnoarchaeology of pastoral sites: the identification of livestock enclosures in abandoned Maasai settlements. *Journal of Archaeological Science* **30**, 439–59.
Shahack-Gross, R., Simons, A. and Ambrose, S. H. 2008. Identification of pastoral sites using stable nitrogen and carbon isotopes from bulk sediment samples: a case study in modern and archaeological pastoral settlements in Kenya. *Journal of Archaeological Science* **35**, 983–90.
Shillito, L. M., Bull, I. D., Matthews, W., Almond, M. J., Williams, J. M. and Evershed, R. P. 2011. Biomolecular and micromorphological analysis of suspected faecal deposits at Neolithic Çatalhöyük, Turkey. *Journal of Archaeological Science* **38**(8), 1868–77.
Simpson, I. A., Bol, R., Bull, I. D., Evershed, R. P., Petzke, K. J. and Dockrill, S. J. 1999a. Interpreting early land management through compound specific stable isotope analyses of archaeological soils. *Rapid Communications in Mass Spectrometry* **13**, 1315–9.
Simpson, I. A., van Bergen, P. F., Perret, V., Elhmmali, M. M., Roberts, D. J. and Evershed, R. P. 1999b. Lipid biomarkers of manuring practice in relict anthropogenic soils. *Holocene* **9**, 223–9.
Smith, C. I., Chamberlain, A. T., Riley, M. S., Cooper, A., Stringer, C. B. and Collins, M. J. 2001. Neanderthal DNA. Not just old but old and cold? *Nature* **410**, 771–2.
Smith, C. I., Chamberlain, A. T., Riley, M. S., Stringer, C. and Collins, M. J. 2003. The thermal history of human fossils and the likelihood of successful DNA amplification. *Journal of Human Evolution* **45**, 203–17.
Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., *et al.* 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Research* **35**, e14.
Valamoti, S. and Charles, M. 2005. Distinguishing food from fodder through the study of charred plant remains: an experimental approach to dung-derived chaff. *Vegetation History and Archaeobotany* **14**(4), 528–33.
van Geel, B. 2001. Non-pollen palynomorphs, pp. 99–119 in Smol, J., Birks, J. and Last, W. (eds.), *Traking Environmental Changes using Lake Sediments. Vol.3: Terrestrial, Algal and Siliceous Indicators.* Dordrecht: Kluwler Academic Press.
van Geel, B., Aptroot, A., Baittinger, C., Birks, H. H., Bull, I. D., Cross, H. B., *et al.* 2008. The ecological implications of a Yakutian mammoth's last meal. *Quaternary Research* **69**, 361–76.
van Geel, B., Guthrie, R. D., Altmann, J. G., Broekens, P., Bull, I. D., Gill, F. L., *et al.* 2011. Mycological evidence of coprophagy from the feces of an Alaskan Late Glacial mammoth. *Quaternary Science Reviews* **30**, 2289–303.
Walker, C. 1996. *Signs of the Wild.* Cape Town: Struik Publisher.
Wallace, M. and Charles, M. 2013. What goes in doesn't always come out: the impact of the ruminant digestive system of sheep on plant material, and its importance for the interpretation of dung-derived archaeobotanical assemblages. *Environmental Archaeology* **18**(1), 18–30.
Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, M.T., Shapiro, B., *et al.* 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**(5620), 791–5.
Willerslev, E., Cappellini, E., Boomsma, W., Nielsen, R., Hebsgaard, M.B., Brand, T.B., *et al.* 2007. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* **317**(5834), 111–4.