

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,

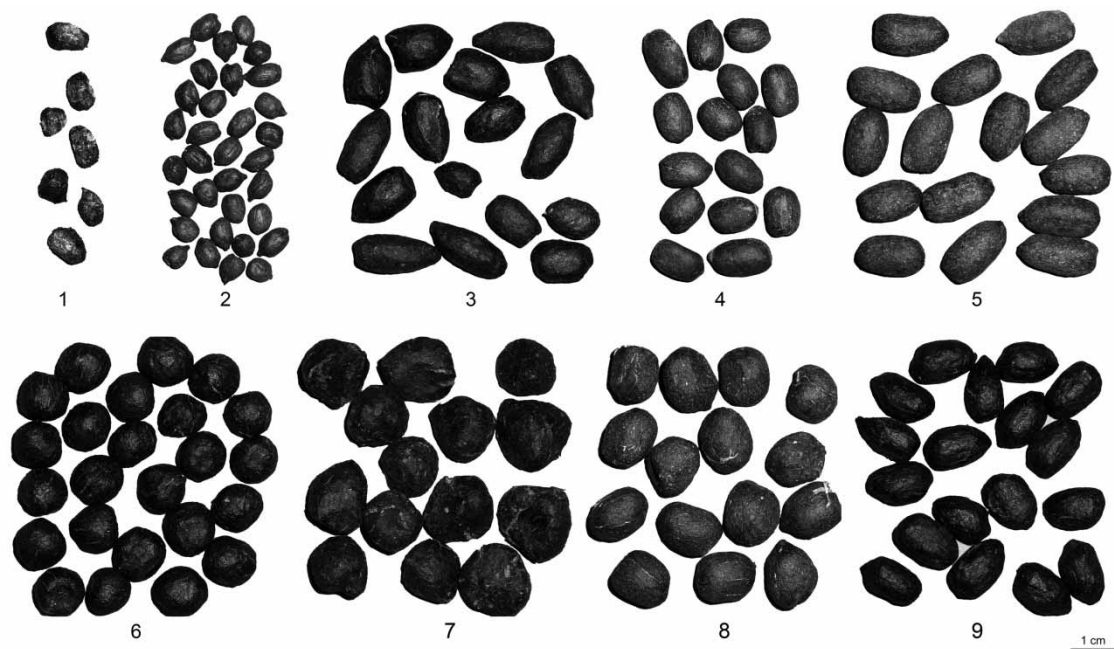


Figure 2 Pellet samples of species relevant for the Egyptian deserts (University of Cologne reference collection): 1, Dassie (*Procavia capensis*); 2, Dorcas gazelle (*Gazella dorcas*); 3, Addax (*Addax nasomaculatus*); 4, Nubian ibex (*Capra ibex nubiana*); 5, Barbary sheep (*Ammotragus lervia*); 6, 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

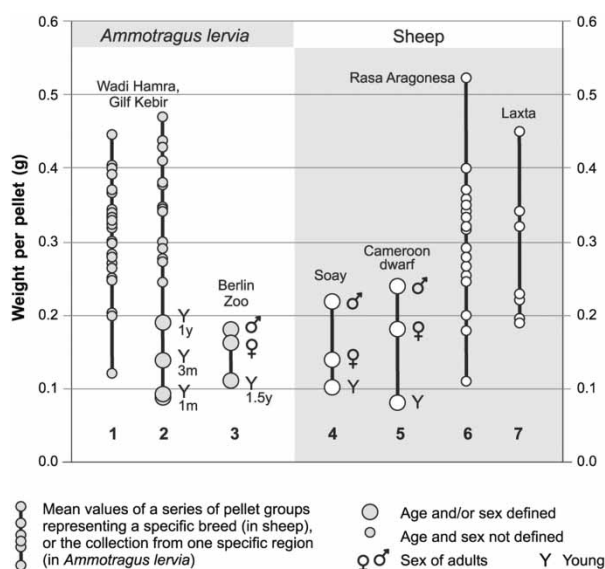


Figure 3 Correlations between pellet weight and body weight according to variation in breed, age and sex, based on data from the reference collection of recent dung at the University of Cologne (see also Riemer 2011). Short-statured sheep (*Ovis orientalis f. aries*) breeds (Soay and Nigerian/Cameroon dwarf) produce significantly lighter pellets than 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 (Gifl 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 5 β -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.

5 β -stanols

The first group of faecal biomarkers are 5 β -stanols such as coprostanol and 5 β -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 5 β -stanols might also be present in small concentrations in sediments, Grimalt *et al.* (1990) proposed that the (5 β : (5 β + 5 α)) stanol ratio can be used to assess the faecal origin of 5 β -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 5 β -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 5 β -campestanol and 5 β -stigmastanol (Leeming *et al.* 1996). In this regard, the ratio of coprostanol to 5 β -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 5 β -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

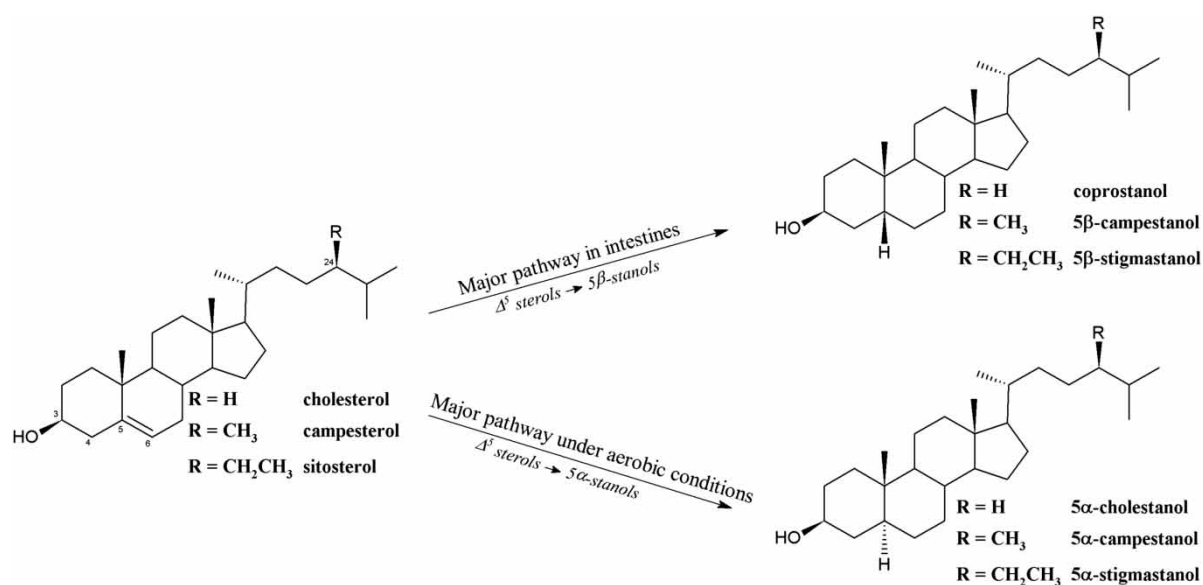


Figure 5 Formation of common 5 β - and 5 α -stanols, from their sterols precursors, in the mammalian gut and in the natural environment.

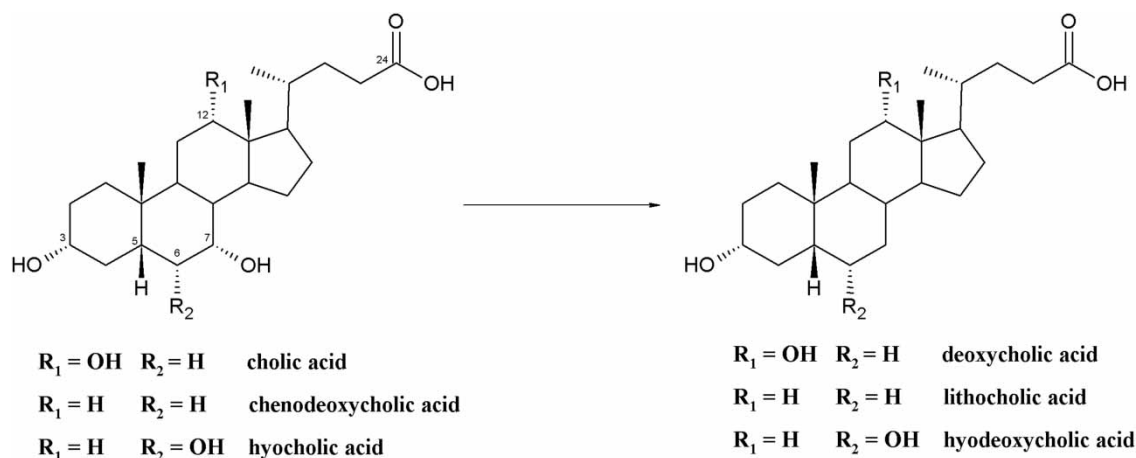


Figure 6 Formation of secondary bile acids, from their primary bile acid precursors, in the mammalian gut.

chenodeoxycholic acid (Fig. 5). Apart from these widely dispersed bile acids, certain animals produce very specific bile acids. For instance, pigs have a unique 6 α -hydroxylation pathway, while mice and rat of the Muridae family produce characteristic 6 β -hydroxylated bile acids (Haslewood 1967). Furthermore, certain cervids and equids produce C₂₇ bile alcohols and C₂₇ bile acids, respectively along with common C₂₄ bile acids (Hagey *et al.* 2010a, 2010b). Rhinoceroses (Rhinocerotidae) and paenungulates, including elephants, manatees and hyraxes, are unique in that they exclusively produce C₂₇ 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 5 β -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 5 β -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 5 β -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 5 β -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 ovicaprine 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 ¹⁵N 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 ovicaprids (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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