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SHORT COMMUNICATION

# Standardised protocol for primate faecal analysis

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**Abstract** Macroscopic analysis of primate faeces as a way to study diet is well established, but lack of standardisation of methods may handicap comparative studies of the resulting data. Here we present a proven technique, including equipment and supplies, protocol and procedure, that yields quantitative data suitable for systematic investigation within and across primate taxa. As the problems of habituation become more obvious, the application of such indirect methods may increase in usefulness.

**Keywords** Diet · Faecal analysis · Field methods · Scat · Dung

## Introduction

Macroscopic analysis of primate faecal specimens in order to infer diet has been done for at least 40 years by field primatologists (e.g. Goodall 1968), yet no standard method for processing faecal samples has emerged (Moreno-Black 1978). (Here, faeces is used as a synonym for dung, scat, stool, or jobbie.) The closest to a standard account is Tutin and Fernandez's (1993) comparative study of chimpanzee and gorilla diet at Lope, Gabon. A recent, comprehensive edited volume of 343 pages (Setchell and Curtis 2003) devoted just five paragraphs to the subject and gave no specific details on how to collect or process faecal specimens (Dew 2003, pp. 177–178; Ozanne and Bell 2003, pp. 218–219). Much more space was given over to faecal analyses for endoparasites or genetic material. A search of PrimateLit (http://primatelit.library.wisc.edu/) revealed only 11 references to f(a)ecal analysis for diet, whereas the total number of references generated for diet was 1526.

Why does this matter? In seeking to answer this, we focus on the chimpanzee (*Pan troglodytes*), but the points made apply across the board to all primate species. Chimpanzees have been studied at more than 50 field sites, but only eight of these have fully habituated subjects that yield behavioural data on feeding. That means that, for the vast majority, diet must be inferred from indirect sources, primarily feeding remnants and faeces. As subjects of study become habituated to the extent that close-range, all-day "follows" are possible, then direct observational data tend to replace the less-satisfactory indirect measures. However, habituation may take years—if it succeeds at all—and faecal analysis may provide valuable preliminary data in the meantime. A primatological pioneer provides an example: early research at Gombe prominently featured faecal analysis (Goodall 1968), but later it dropped out (Goodall 1986). For every new ecological study that seeks to become ethological, there will be an early stage when faecal analysis is the primary source of data about diet.

More recently, several researchers studying chimpanzee populations in unprotected areas have explicitly declined to seek habituation on the grounds that it may put their subjects at risk (Morgan and Abwe 2006; Deblauwe and Janssens 2008). That is, apes lulled into tolerance of humans by scientists may be vulnerable to hunting or to disease transmission. If this resolution becomes the norm, then faecal analysis will retain its utility, perhaps for the duration of even long-term studies.

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In principle, precise, comparative study of data from faecal samples ought to be easy. The data source is concrete and straightforward, and the contents are readily accessible and potentially discernable, in contrast to microscopic or molecular studies of faeces. In practice, methods vary, so that cross-study quantitative comparisons are difficult, e.g. wet vs. dry or fresh vs. preserved samples; data recorded at nominal, ordinal, or interval levels. How is one to compare results from fresh, wet samples rated on a three-point scale with results from preserved, dry ones with percentages that have been assigned by percentage of total volume? Here we present some suggested procedures that are proven under field conditions, based on lessons learned over more than 30 years. We focus on the sluice-and-sieve technique.

This modest attempt at methodological standardisation for processing of primate faecal samples does not deal with either the preceding stage (that is, collecting faecal specimens) or the succeeding stage (that is, identifying and quantifying recovered items in the laboratory). These aspects will be dealt with in a later article.

## Equipment and supplies

The key item is a flat-bottomed sieve, with a square or circular frame, at least 20 cm in diameter and at least 8 cm deep; the standard mesh size is 1 mm. (Meshes of greater size may be useful for preliminary screening, but smaller sizes are of little use as they clog up, although they may catch tiny objects, e.g. *Ficus* seeds, chitin fragments, etc.) Sieves can be homemade from metal (not plastic) fly-screening and wooden frames, as an economy measure, but the best ones are the geological sieves with heavy gauge mesh and solid brass frames (e.g. Endecotts Ltd. laboratory test sieves).

The sluicer (see below) should wear disposable latex surgical gloves or reusable ordinary dish-washing gloves, the more tightly fitting the better for precision gripping. She should have at least two pairs of forceps, one needle-nosed and one flat-nosed, for picking up items left in the sieve. A magnifying glass or hand-lens is needed to scrutinise tiny items, e.g. insect parts. Sluicing is a sit-down job, so a folding tripod stool or a foam seating pad are useful, as are gumboots (as most sluicing is done by stream- or riverside) and a hat with a neck-flap or head-net (for defense against tsetse flies, bees, etc.).

The scribe (see below) needs a data-recording instrument, whether retractable pencils (e.g. Bic) and waterproof notebook (e.g. Rite-in-the-Rain) or a handheld computer. He should have to hand a fine-pointed permanent marker pen with black ink, screwtop glass vials (from 5 to 30 ml), clear plastic, ziplock, freezer-weight bags of varying sizes, and 100% ethanol. The scribe is best suited for taking photographs of sieved contents or items to be stored for reference. He also can serve as a replicating rater/scorer for intercoder reliability. Finally, a fan to wave away pesky insects from the sluicer is much appreciated.

All of this kit should be carried in a waterproof bag, which also contains a portable reference collection of preserved items recovered from previous samples (the obvious advantage of having the reference collection close at hand is convenience; the obvious drawback is its vulnerability to loss or damage). Both sluicer and scribe should be dressed in loose-fitting, long-sleeved shirts and trousers, or even waterproofs, to seek to minimise bites and distraction from insects.

## Protocol

Ideally, at least two persons take part; if others are available, they best serve as extra sluicers or as preparators. The sluicer processes the specimens by swirling water through the sieve, so that the matrix is washed away downstream in the current, leaving only the undigested proportion of the gut contents. These items range for plants from exudate to shells to skins, to whole or partial seeds, fruits, leaves, flowers, stems, bark, etc.; for animals from exoskeleton to wings, feathers, hair, bone, skin, whole organisms (e.g. ants), etc.; for nonorganic from clay to stones.

The sluicer handles the specimens and dictates findings to the scribe, while the preparator readies the bagged specimens and cleans the bags. Thus an assembly line is created (although regular changing of roles is advised). Sluicers should have manual dexterity and keen eyesight; scribes should have neat handwriting and sketching abilities. Sketch drawings as well as verbal descriptions, e.g. size, shape, colour, texture, etc., are needed to record data, especially on unidentified items. Use of common referents and descriptors is helpful, e.g. “resembles a pumpkin seed”, “has the consistency of toothpaste”, etc.

The ideal site for sluice-and-sieving is a flat streambed, e.g. a sandbar, with enough room to spread out gear, next to a clear, flowing (of medium velocity) stream with a sandy bottom. The best combination is shade to work in, with sunny spots in which to scrutinise items. Musical accompaniment (background music) is nice for longer sessions, but not so loud as to mask the approach of large animals, e.g. elephants. Needless to say, if the watercourse also serves other purposes, e.g. bathing, washing, laundry, etc., then the sluicing site should be downstream of these activities. If running water is absent, then nonflowing pools will do, with the obvious constraint of increasing contamination with repeated sluicing, and so there is the need to constantly wear rubber gloves. If surface water is lacking

altogether, then a plastic or metal tub can be used, with a preparator fetching and pouring water from a bucket through the sieve. This is tedious, tiring, and time-consuming, especially if water is in short supply.

## Procedure

Line up the faeces-containing specimen bags in numerical/chronological order on the substrate, and work systematically down the line. Weigh each bag to the nearest 10 g using a spring balance (e.g. Salter spring scale, 1500 × 25 g); a digital balance is more accurate but may not be logistically feasible in the field. (The Kenex KX digital scale is pocket-sized and precise, 400 × 0.1 g, but it requires a flat surface and consumes batteries.) Then, half-fill each bag with water in order to soak the faeces, squashing big pieces of bolus between thumb and forefinger while still in the bag. Ideally this reduces the faeces to a slurry. Decant the first bag into a sieve. (If the faecal sample is overly large, then it can be divided between two sieves. This also provides an opportunity for interobserver, split-half reliability testing.) Refill the bag with water and pour into the sieve; repeat until the bag's interior is clear of all solid matter, then set the bag aside. Sluice the contents of the sieve in the running water, kneading any remaining large lumps until they disappear; a soft-bristle artist's brush can speed up this process. Agitate the contents by swirling, bouncing, sliding, etc., until all the matrix is washed away, i.e. until the liquid draining through the sieve is clear. Now the contents are ready to scrutinse (Fig. 1).

Remove extraneous foreign matter, e.g. accidental dead leaves, twigs, etc., and dung beetles, and discard. Pick out and count the large seeds; if identified, dictate the number and species to the scribe; if not identified, use an agreed descriptor (e.g. "barrel-shaped, big and brown"). This removal of large seeds should clear space on the mesh surface, facilitating the viewing of the smaller items. Re-swirl frequently to spread the contents evenly over the mesh surface, and use flotation to capture chitin. Count or rate all seeds, fibre, petioles, leaf fragments, etc. of plants, heads, mandibles, legs, carapace fragments, etc. of invertebrates, and hair, feather, scales, skin, soft tissue, etc. of vertebrates. (Ratings vary: a four-point scale of abundant, common, few, rare, Tutin and Fernandez 1993; a three-point scale of abundant, common, rare, Yamagiwa et al. 1993.) When in doubt, remove an item and put it aside to return to later, for joint scrutiny. Discard known items (further freeing up space in the sieve), but retain unknown items in labelled vials or small plastic bags of ethanol. These are added to the reference collection in sufficient numbers to show variation and to allow subsets to be sent away for identification. Preserve all helminths in ethanol. If overwhelmed with insect parts, try to preserve intact specimens, or if in fragments, give preference to heads.

When all bags have been processed, empty and rinse the sieves, using the current to float away the debris. Refill each bag and check for leaks. If leaky, discard. If intact, rinse clean for reuse. The best way to clean a bag is to turn it inside-out and rub the inner surface of the bag underwater on a sandy bottom in the watercourse. Rinse all other equipment that has been in contact with the faeces, then lay it out to dry. Bags are best dried fully open, inside-out, ideally hanging on a drying line.

**Fig. 1** Pamela Baldwin doing a solo faecal analysis session at Assirik, Senegal. Photograph by W.C. McGrew

## Conclusion

Faecal analysis remains a useful and informative means of gaining data about diet and other aspects of the natural lives of primates. However, the potential for comparison across taxa and populations will be realised only when data collection is thorough and systematic. We commend these procedures to our colleagues.

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