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Adapting Methodology Used on Captive Subjects for Estimating Gut Passage Time in Wild Monkeys

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Digestive markers · Frugivores · Mean retention time · Seed dispersal · Transit time · Wild guenon

Abstract

Gut passage time of food has consequences for primate digestive strategies, which subsequently affect seed dispersal. Seed dispersal models are critical in understanding plant population and community dynamics through estimation of seed dispersal distances, combining movement data with gut passage times. Thus, developing methods to collect in situ data on gut passage time are of great importance. Here we present a first attempt to develop an in situ study of gut passage time in an arboreal forest guenon, the samango monkey (*Cercopithecus albogularis schwarzi*) in the Soutpansberg Mountains, South Africa. *Cercopithecus* spp. consume large proportions of fruit and are important seed dispersers. However, previous studies on gut passage times have been conducted only on captive *Cercopithecus* spp. subjects, where movement is restricted, and diets are generally dissimilar to those observed in the wild. Using artificial digestive markers, we targeted provisioning of a male and a female samango monkey 4 times over 3 and 4 days, respectively. We followed the focal subjects from dawn until dusk following each feeding event, collecting faecal samples and recording the date and time of deposition and the number of markers found in each faecal sample. We recovered 6.61 ± 4 and 13 ± 9% of markers from the male and the female, respectively, and were able to estimate a gut passage window of 16.63–25.12 h from 3 of the 8 trials. We discuss methodological issues to help future researchers to develop in situ studies on gut passage times.

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Introduction

The length of time food remains in the gastrointestinal (GI) tract (gut passage time) of animals has consequences for digestive strategies and how animals access energy and nutrients from the food they consume [Blaxter et al., 1956; Lambert, 2002]. In frugivores, gut passage time influences seed dispersal distance [Link and Di Fiore, 2006], which has important ecological implications for the recruitment, range expansion, genetic structure and gene flow in plant populations [Traveset, 1998; Nathan and Muller-Landau, 2000], as well as affecting germination success [Robertson et al., 2006]. Among primates, frugivorous species play an important role in seed dispersal in many communities [Andresen et al., 2018; Razafindratsima et al., 2018]. In seed dispersal models, dispersal kernels combine movement data with gut passage time to infer the statistical distribution of postdispersal locations relative to the seeds' point of origin [Nathan et al., 2012]. These data allow for predictions of seed dispersal, whilst removing the effort required in measuring actual dispersal distances in the field [Bullock et al., 2006]. Therefore, reliable estimates of gut passage time from animals in situ are critical in estimating dispersal kernels [Chapman and Onderdonk, 1998; Lambert and Chapman, 2005]. Nevertheless, studies measuring gut passage time in wild animals are scarce.

Gut passage time is a measure of gut function and reflects the length of time food items are retained in the GI tract, subject to mechanical and chemical digestive processes such as the breakdown of food, microbial fermentation and absorption, before undigested matter is eliminated through faeces [Cabre-Vert and Feistner, 1995]. Several indices are used to calculate gut passage times including transit time (TT), defined as the time of the first appearance of the focal elements in faeces, time of last appearance (TLA), defined as the time of the last appearance of the focal elements in faeces, and mean retention time (MRT), defined as the mean gut passage time of the focal elements from ingestion to excretion [Blaxter et al., 1956; Warner, 1981].

Gut passage time has been widely studied in primates [Cabre-Vert and Feistner, 1995; Lambert, 1998; Norconk et al., 2002; Remis and Dierenfeld, 2004; Tsuji et al., 2015; Bai et al., 2019] and can vary considerably [Lambert, 1998]. It is thought that both body size and digestive strategy can explain the large variation observed in primates [Lambert, 1998; Clauss et al., 2008; Blaine and Lambert, 2012]. There is a general trend of increasing gut passage time observed between the smallest and the largest-sized primates [Lambert, 1998]. There is also a general pattern for frugivorous primates, whose diets contain greater quantities of simple carbohydrates such as glucose and fructose, to display reduced gut passage times compared with folivorous and exudativorous primates, whose diets consist of greater quantities of complex structural carbohydrates such as cellulose [Lambert, 2002; Clauss et al., 2008; Cabana et al., 2017]. Cellulose is a major constituent of plant cell walls, and many primates rely on non-fruit plant matter as a major source of energy. Unlike simple carbohydrates, digestion of cellulose depends on fermentation, which, like in other herbivorous vertebrates, occurs in the primate GI tract and can increase gut passage time [Chivers and Hladik, 1980].

Within primates, *Cercopithecus* spp. show increased gut passage time compared to similar-sized primate species [Lambert, 1998, 2002], with studies reporting gut passage times between 21.4 h in *C. mona mona* (crested Mona monkey) [Poulsen et al., 2001] and 40.6 h in *C. mitis* (blue monkey) [Clemens and Phillips, 1980] (Table 1).

Table 1. Mean marker transit time (TT), time of last appearance (TLA) and gut retention time (±SD) (MRT) of *Cercopithecus* spp. reported in ex situ studies

Sample			Measures of gut passage			Marker information			Study details	
trials per subject	*n*/sex	body weight, kg	TT	TLA	MRT	type	size, mm	recovery, %	faecal collection	diet/ location
Cercopithecus ascanius[a]										
4	1 M	5.7	19.9 (4.6)	35.6 (9.3)	24.9 (6.6)	Plastic beads	4×2×1	90	Day: continuous collection Night: estimated by appearance	CFP/zoo
4	1 F	4.7	19.4 (0.1)	42.1 (5.7)	29.4 (9.8)					
Cercopithecus ascanius[b]										
2	1 M	4.2	20.6 (0.5)	48.7 (6.7)	26.7 (3.7)	Plastic ribbon	5×0.09	NA	Continuous collection	CFCP/ research colony
Cercopithecus erythrotis[b]										
2	1 M	4.2	20.6 (0.5)	48.7 (6.7)	26.7 (3.7)	Plastic ribbon	5×0.09	NA	Continuous collection	CFCP/ research colony
Cercopithecus l'hoesti[c]										
3	1 M	3.6	23.25 (0.35)	41.05 (7)	26.6 (3.14)	Plastic beads	1×2×1	5–45	Day: continuous collection Night: estimated by appearance	CFP/zoo
3	1 F		23.95 (1.48)	33 (9.19)	25.43 (0.81)			5–20		
Cercopithecus mitis[d]										
1	3 NA	6.2	NA	NA	40.6	Plastic tube	2×2	NA	every 12 h	C/not reported
Cercopithecus mitis stuhlmanni[a]										
4	1 M	9.8	17.2 (2.9)	54.8 (12.7	29.7 (14.6)	Plastic beads	4×2×1	80	Day: continuous collection Night: estimated by appearance	CFP/zoo
4	1 F	7.4	16.5 (3.4)	42.8 (19.8)	20.6 (12.8)					
Cercopithecus mona mona[e]										
1	1 M	5.1	NA	NA	21.4 (6.9)	Seeds/ food	NA	NA	Continuous collection	NFP/zoo
1	1 F	NA			21.7 (7.4)					
Cercopithecus mona pogonias[b]										
2	2 M	4.5	16.6 (2.6)	43.7 (6)	26.6 (6.7)	Plastic ribbon	5×0.09	NA	Continuous collection	CFCP/ research colony
Cercopithecus neglectus[a]										
4	1 M	6.9	21.7 (2.5)	56 (12.7)	33.9 (10.8)	Plastic beads	4×2×1	78	Day: continuous collection Night: estimated by appearance	CFP/zoo
4	1 F	6.1	19.1 (3.4)	63.1 (10)	34.4 (16.6)					
Cercopithecus nictitans nictitans[e]										
1	1 M	6.7	NA	NA	23.8 (4.8)	Seeds/ food	NA	NA	Continuous collection	NFP/zoo
1	1 F				22.8 (2.4)					

NA, not reported. Times are presented with study sample size/sex and body weight, marker type, size (mm) and recovery (%), faecal collection method (continuous collection – samples collected at time of deposition; estimated by appearance – samples collected in the morning and then time of deposition estimated by degree of desiccation), subjects' diet (CFP, commercial and fresh produce; CFCP, commercial and fresh and cooked produce; C, commercial only; NFP, natural fruits and fresh produce) and location. [a] Lambert [2002]. [b] Maisels [1994]. [c] Blaine and Lambert [2012]. [d] Clemens and Phillips [1980]. [e] Poulsen et al. [2001].

Cercopithecines exhibit considerable feeding flexibility with consistently large proportions of both fruit and non-fruit plant parts in their diets [Blaine and Lambert, 2012]. It is suggested that *Cercopithecus* spp. digestive strategies include extended retention time of food for fermentation and extraction of nutrients from a diet high in fibrous material [Lambert, 1998, 2002].

Prior research on *Cercopithecus* spp. gut passage times have all been conducted in captivity, predominantly in zoos, where subjects' diets consist of commercial food pellets supplemented with domestic fruits and vegetables, and where movement is limited (Table 1). However, wild animals are generally more active than captive animals, and energy expenditure can also influence gut passage times [Blaine and Lambert, 2012]. Captive diets are also not necessarily representative of the diets of wild counterparts. For example, in slow loris (*Nycticebus* spp.), subjects fed a natural wild diet had significantly longer gut passage rates than those fed a captive diet [Cabana et al., 2017]. Furthermore, the "captivity effect" [Martin et al., 1985], whereby the GI tract can become reduced in captivity, can reduce gut passage rates [Milton, 1984; Martin et al., 1985; Blaine and Lambert, 2012]. As such, captive studies may paint a misleading picture of gut passage time in primates adapted to high-fibrous and flexible diets.

Several different insoluble particulate markers have been used for gut retention studies. The majority of cercopithecine studies have used artificial markers such as 2- to 3-mm plastic beads and plastic ribbon (Table 1) [e.g. Maisels, 1994; Lambert, 2002]. Of the 10 studies conducted on cercopithecines so far, 6 did not report the percentage of markers recovered (Table 1). However, reported recovery of these markers from faeces following ingestion is highly variable. For example, Blaine and Lambert [2012] reported marker recovery between 5–20% and 5–45% in *C. l'hoesti* (L'Hoest's monkey), and Lambert [2002] reported 80% marker recovery in *C. mitis* (blue monkey), 78% in *C. neglectus* (De Brazza's monkey) and 90% in *C. ascanius* (red-tailed monkey) [Lambert, 2002]. Low marker recovery in *Cercopithecus* spp. has been attributed to oral processing in which markers may have been crushed by high-crowned molars [Lambert, 2001]. In other animals, particulate markers associated with food particles, such as chromium oxide [e.g. Cabre-Vert and Feistner, 1995] have been used. Other particulate markers have included glitter [Cabana et al., 2017] and polystyrene and cellulose acetate beads [Power and Oftedal, 1996]. Such markers are not biodegradable, and their use in situ presents environmental concerns such as plastic pollution from uncollected markers. Enrichment of seed coats during the developmental phase with stable isotope ^{15}N-urea has been used to identify parent plants of dispersed seeds [Carlo et al., 2009], which could be used to estimate gut retention time of seeds in situ, although this would require several months of preparation between application and ingestion. Several in situ studies in other primates, for example, bonobo (*Pan paniscus*) [Beaune et al., 2013b], spider monkeys (*Ateles belzebuth*) [Link and Di Fiore, 2006], woolly monkeys (*Lagothrix lagothricha*) [Stevenson, 2000] and white-faced capuchins (*Cebus capucinus*) [Valenta and Fedigan, 2010], have used seeds from fruit species that were not further consumed between the time of the first feeding event on that species and the first defaecation event of seeds from that species to estimate gut retention times. For species for which gut retention time is relatively short, such as capuchins [Valenta and Fedigan, 2010] and tamarins (*Saginus* spp.) [Oliveira and Ferrari, 2000], DNA fingerprinting of dispersed seeds has shown this method to be highly reliable [Hey-

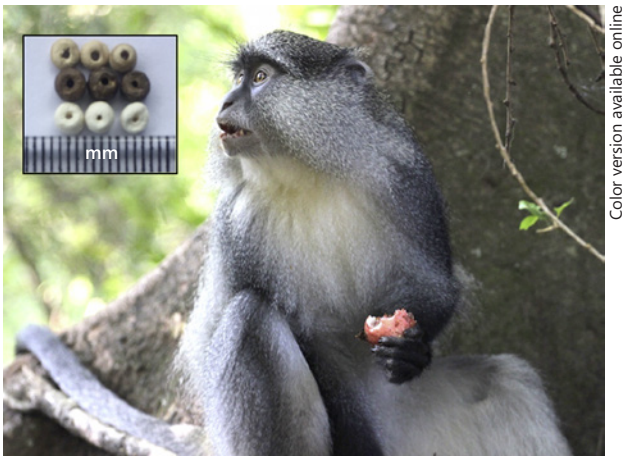
mann et al., 2012]. However, for species for which gut retention time is longer, such as guenons, this observation-based method can be extremely difficult and much less reliable [Heymann et al., 2012]. Relying on infrequently ingested fruit means ensuring continuous observation of the focal animal for the entire sampling period to avoid replicated feeding on focal tree species [Stevenson, 2000]. Depending on the number of fruits consumed, as well as the number of seeds actually swallowed, this method also has a high risk of missing the collection of faeces containing the focal seeds.

Our aim was to test, for the first time, gut retention experimental methods developed in captivity, in the field. We measured the gut passage rate of samango monkeys (*Cercopithecus albogularis schwarzi*) in situ following published methodology used ex situ [Lambert, 2002], but using a novel marker made from natural material and with lower environmental impact than previous plastic markers. Specifically, our study aimed to determine a gut passage time window that could be used to estimate TT, TLA and MRT in a wild primate. Samango monkeys are South Africa's only true forest primate and are restricted to pockets of indigenous forest [Linden et al., 2016; Nowak et al., 2017]. The subspecies at Lajuma, *C. a. schwarzi* [Dalton et al., 2015], is classified as endangered due to indigenous forests being converted for agriculture and other human activities [Linden et al., 2016]. Investigations into the relationship between samango monkeys and indigenous forests may be vital in decisions regarding forest protection and therefore samango monkey survival.

Methods

We conducted trials at Lajuma (29°26' E, 23°01' S) in the western Soutpansberg Mountains, Limpopo Province, South Africa. The study site is a mountainous environment with an altitudinal range of between 1,100 and 1,747 m above sea level. Local vegetation is characterised by a complex mosaic of vegetative and structural elements of forest, thicket, grassland and savannah biomes [Maltitz et al., 2003; Mucina and Rutherford, 2006]. We conducted gut retention trials on two well-habituated, easily recognisable wild samango monkeys (Fig. 1) from the Barn Group [Coleman and Hill, 2014a; Nowak et al., 2014], one male and one female, during February and May 2018, respectively. We used 2 × 3 mm beads made from natural materials (Fig. 1) as artificial digestive markers for these experiments. Similar-sized plastic markers have been used in ex situ studies on *Cercopithecus* spp. (Table 1) and previous research reported that samango monkeys swallowed seeds between <1 × <1 mm and 6 × 10 mm [Linden et al., 2015]. We used distinct marker types differing in material (coconut shell or wood), colour (white, natural or dark) and shape (flat or round edged) for each trial. The average (mean ± SD) weight of the coconut shell markers was 0.019 ± 0.002 g, and wooden markers weighed 0.014 ± 0.0003 g on average. We tested the resilience of the markers to chewing and gut passage in a preliminary study in September 2017, and successfully collected and identified different coloured intact markers from faecal samples. We did not find any partial segments of damaged beads in faecal samples. We conducted 4 trials on each subject using 50 markers per trial, totalling 200 markers for each subject.

For each trial, we split a firm, peeled, yellow banana into 5 pieces, inserted 10 markers into each piece and positioned the pieces in the path of the approaching target subject (feeding event). We either left pieces on the floor or dropped them from an upside-down container upon the approach of the target individual by means of a pulley. We timed each feeding event to occur when the target subject was not in close proximity to other individuals in the group, nor human observers, to facilitate targeted provisioning and minimise the association between hu-



Color version available online

Fig. 1. *Cercopithecus albogularis schwarzi* at Lajuma in the Soutpansberg Mountains, Limpopo Province, South Africa, feeding on broom cluster fig (*Ficus sur*) fruit. Inset: examples of the beads used as artificial digestive markers for the experiments.

mans and food. During the experiment with the male, feeding events occurred once on day 1 at 16:30, twice on day 2 at 06:20 and 13:00 and once on day 3 at 07:00. During the experiment with the female, feeding events occurred once per day between 12:00 and 15:00 over 4 days. The timing of feeding events was coordinated to control for the natural daily variation in food items, which might affect gut passage time. We also wanted to ensure that following each feeding event, we had sufficient time (from 16 h after ingestion) to collect markers from faecal samples within the passage times reported in ex situ studies (Table 1). We recorded the time of ingestion as the time when the monkey placed the final banana piece into the mouth. We followed focal subjects from dawn until dusk following each feeding event, with the male subject being followed for 4 successive days and the female subject for 5 successive days. If the target individual was briefly lost during a follow day, we continued to search until it had been relocated.

During daily follows we collected faecal samples from observed defaecation events and recorded the date and time of deposition. We could not collect samples at night. We washed samples using a sieve with a 0.5-mm diameter mesh at the end of each follow day and recorded the number and type of each marker that we removed from the remaining undigested matter. We recorded the times at which we located the troop each morning and left them each evening, as well as the times and duration of periods during which the focal subject was out of view. We used these data to identify estimates of TT and TLA if we could be confident that we collected all possible faecal samples, because we had successfully followed the subject continuously at least within the potential TT window of 16–24 h reported in ex situ studies (Table 1), and if we retrieved clear faecal samples (containing no markers) immediately before (TT) or after (TLA) samples with markers. We set out to measure MRT [Blaxter et al., 1956; Warner, 1981], which is the standard measure of gut passage times, but low marker return meant we did not have sufficient data to calculate this index.

Results

Marker Ingestion and Focal Animal Observation

In the first trial with each subject, a non-target individual consumed 1 of the 5 pieces of banana, reducing the number of available markers to 40 for that trial. Both the male and female subjects placed the remaining banana into their mouths within 30 s. During 5 out of 8 trials, we observed the focal animals spitting markers directly

Table 2. Reliable (*) estimates of transit time (TT) and time to last appearance (TLA) of markers (h), of the male and female *Cercopithecus albogularis schwarzi* subjects at Lajuma, Soutpansberg Mountains, South Africa

Subject/trial	Marker recovery			Measures of gut passage, h	
	collected spat	recovered from faeces	unaccounted	TT	TLA
Male					
1	4	0	36	–	–
2	6	2	42	24.88*	25.48
3	32	2	16	16.73*	23.18
4	26	1	23	–	27.92
Female					
1	0	6[a]	34	21.37	26.73
2	0	4	46	18.73	25.12*
3	6	2[b]	42	–	27.27
4	0	12	38	16.63*	23.43
Species	74	29[a]	277	16.63	27.92

Presented with the number of markers spat out, recovered from faeces and unaccounted for. Remaining values are not definitive first and last appearance but presented for information as potential TT and TLA. [a] Excluding an anomaly retrieved at 4.25 h after ingestion. [b] Both markers retrieved from one faecal sample.

in the location of the feeding event and attempted to collect all of these markers (Table 2).

Due to difficulties in following arboreal animals in undulating, natural environments, especially high canopy forests and semi-deciduous woodlands with thick understoreys, the time that the focal animal was followed after each feeding event differed between subjects. On average (mean ± SD), the male was lost 2.5 ± 1.7 times per day and the female 2.2 ± 1.9 times per day. The average (mean ± SD) time to relocation was 55 ± 49 min for the male subject and 49 ± 48 min for the female subject, with a maximum time to relocation for each sex of 3 h (only recorded once for each sex). Total focal observation time was 30.25 h for the male (3 full days) and 37.92 h for the female (4 full days), and mean (±SD) daily observation time was 9.95 h (±1.56) and 9.48 h (±1.58), respectively. On the fifth day of the experiment with the female, the group slept on land that we were not permitted to enter and so data collection ended. The group could not be found on the fifth day during the experiment with the male; hence, we terminated the experiment at the end of the fourth day. Although finishing earlier than planned, the staggered nature of the trials meant that we followed both individuals for at least 3 days from the initial feeding trial.

Marker Recovery and Gut Passage Time

On average (mean ± SD), we observed the male defaecating 6 ± 1 times per day, with an average defaecation interval of 1 h 45 min during daily follow time. We ob-

served the female defaecating 10 ± 3.46 times per day, with an average defaecation rate of 46 min during daily follow time. Overall, we collected 18 faecal samples from the male and 45 faecal samples from the female. Of these, 5 (27.8%) male faecal samples and 15 (33.33%) female faecal samples contained markers.

We recovered no markers from the first trial with the male and on average (mean ± SD), we recovered 6.61 ± 4% of markers from 3 trials with the male and 13 ± 9% of markers from 4 trials with the female (Table 2). We recovered 1 marker after 4.25 h from the female subject during an aggressive between-troop encounter. As we encountered this TT on just 1 occasion, we regarded it as an anomaly. Two trials out of 4 for each subject provided reliable estimates of either TT or TLA (Table 2), and we were, therefore, able to estimate a preliminary gut passage time window of between 16.63 and 25.12 h. We were unable to reliably estimate TT and TLA from a single trial, nor were we able to reliably estimate an overall MRT. This was due to potentially missing faecal samples when each subject was out of sight and the low number of returned markers was insufficient to calculate average passage times; however, we are confident in our estimation of a gut passage time window for the following reasons.

From the male subject, we were able to confidently estimate a TT of between 16.73 and 24.88 h from the third and second trials, respectively (Table 2; Fig. 2). In the second trial, we successfully followed the male from 3.17 to 12.65 h following ingestion of the markers, except for a 40-min period between 10.34 and 11 h after ingestion, collecting 5 clear faecal samples. We left him at the groups' sleep site overnight, relocating him 23 h after marker ingestion and collecting a clear sample at 05:58, 23.63 h after ingestion. The peak time of defaecation in other *Cercopithecus* spp. has been documented as 06:00–09:00 [Lambert, 2002; Blaine and Lambert, 2012], and we are confident that this was the first defaecation after waking. We successfully followed him and collected a further 3 faecal samples without losing him from sight. The first of these contained markers from the second trial at 24.88 h, which we can be confident was the TT of these markers. As we lost him for 1.42 h after collecting the third of these samples, we could not be confident that the second marker retrieved at 25.48 h after ingestion was the TLA of markers from this trial. In the third trial with the male subject, we collected 2 clear faecal samples at 14.88 and 16.13 h after marker ingestion and retrieved the first marker from this trial at 16.73 h after ingestion. In the fourth trial, we were unable to locate the male between 22.53 and 23.37 h following ingestion the morning after marker delivery and could not be certain that we collected the first faecal sample defaecated by the male that day. As we also lost him between 24.95–26.12 h and 32.7–33.2 h after marker ingestion, we have not included the marker we recovered at 27.92 h after ingestion as a reliable TT, including it instead as a potential TLA because it indicates markers were retained in the gut for at least this period of time.

From the female subject, we were able to confidently estimate a TT of 16.63 h and TLA of 25.12 h from the fourth and second trials, respectively (Table 2; Fig. 3). Following ingestion of the markers in the fourth trial, we relocated the group at the sleep site 14.92 h after marker ingestion and collected a clear sample at 16.08 h and a sample containing 3 markers 16.63 h after ingestion. Both of these faecal samples were collected before 09:00, and we are confident they were the first samples defecated by the female that day. We are confident in our estimate of the 25.12-h TLA of markers from the second trial with the female, as we retrieved a further 7 clear faecal samples between 25.5 and 30.23 h after marker ingestion. We are confident that these

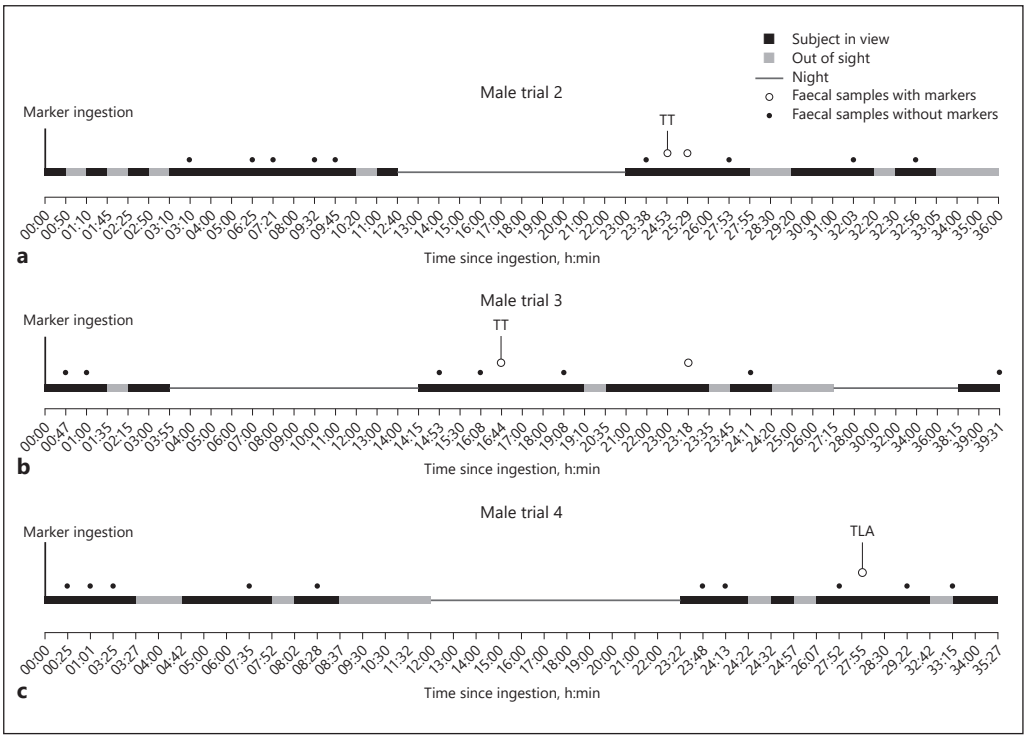


Fig. 2. Timelines of three gut passage time trials (**a–c**) conducted on a male *Cercopithecus albogularis schwarzi* subject at Lajuma, Soutpansberg Mountains, South Africa, presenting times since ingestion of times the subject was in view, out of view and night hours, faecal samples collected with and without markers, and indication of reliable estimates of either transit time (TT) or time of last marker appearance (TLA). Note the *x* axis time since ingestion is not to scale.

7 faecal samples represented all of the samples deposited by the female during this time as we followed her continuously during the collection period, with only two 15-min periods in which we could identify her as being in a small group of travelling monkeys, but could not identify her individually. We were away from the female between 5.5 and 20.83 h after ingestion of the markers in the first trial, which included the peak defaecation period between 06:00 and 09:00, and lost her between 28.25 and 29.33 h after marker ingestion. As such, although we collected 6 faecal samples collectively containing 7 markers between 20.83 and 28.25 h after ingestion, we were unable to use these data to reliably estimate TT or TLA. Likewise, we lost the female several times between 17.58 and 24.67 h after ingestion of the markers in the third trial and, even though we collected 5 clear samples between 21.98 and 26.58 h after ingestion, we have included the 27.27 gut passage time as a potential, but not reliable, estimate of TLA (Table 2).

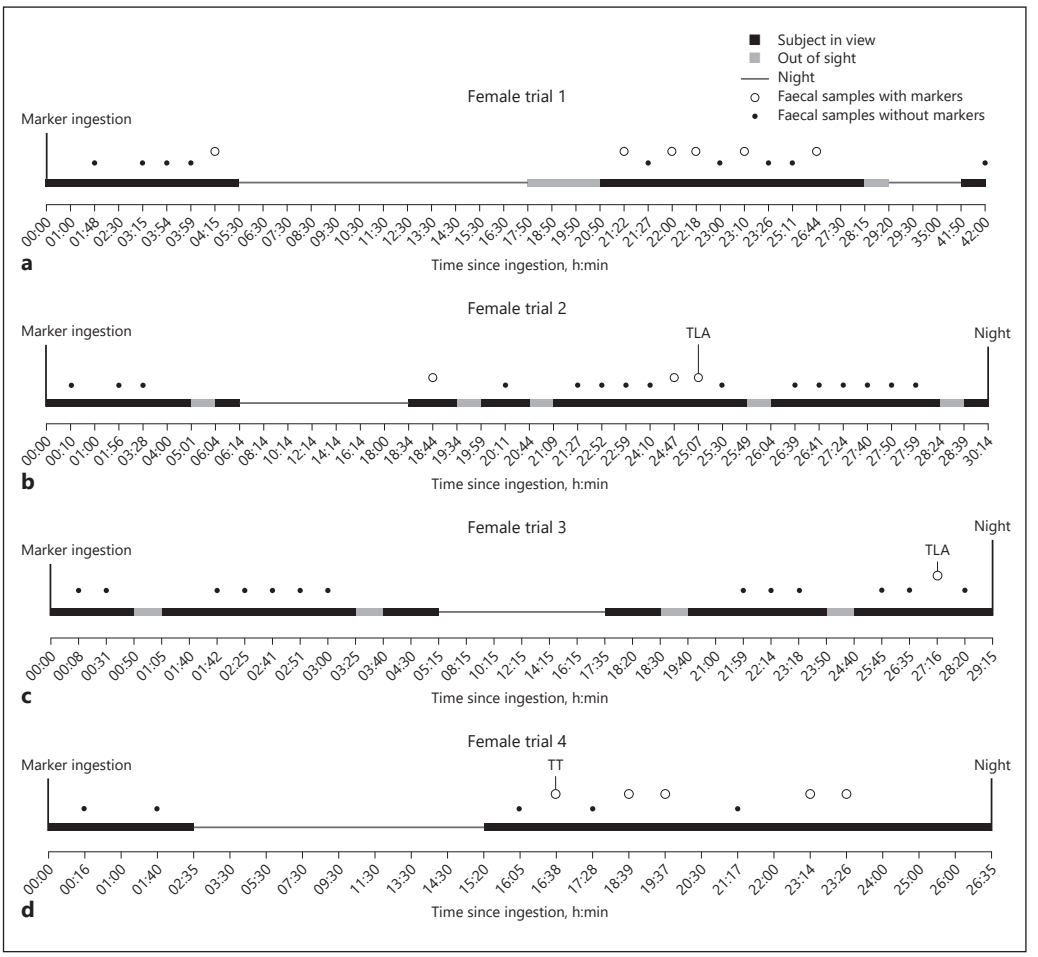


Fig. 3. Timelines of four gut passage time trials (**a–d**) conducted on a female *Cercopithecus albogularis schwarzi* subject at Lajuma, Soutpansberg Mountains, South Africa, presenting times since ingestion of times the subject was in view, out of view and night hours, faecal samples collected with and without markers, and indication of reliable estimates of either transit time (TT) or time of last marker appearance (TLA). Note the *x* axis time since ingestion is not to scale.

Discussion

To our knowledge, our study is the first to present data on the gut passage time of *Cercopithecus* spp. in situ using artificial digestive markers. We were able to confidently estimate a gut passage time window of between 16.63 and 25.12 h, which provides valuable data for future studies to compare in situ estimates of *Cercopithecus* spp. gut passage times. Our results concur with other published studies that indicate an extended gut passage rate in *Cercopithecus* spp. [Maisels, 1994; Lambert, 1998], which is indicative of digestive adaptation for fermentation of plant parts [Lambert,

2002]. It has previously been reported that forest cercopithecines exhibit considerable feeding flexibility [Blaine and Lambert, 2012], and previous studies using time budget analysis have reported samango monkeys to spend 51.7–72% of feeding time consuming fruit, 17–52% of feeding time consuming leaves and 4.4–11% of feeding time consuming other items [Coleman and Hill, 2014a, b; Linden et al., 2015].

In captive studies, TT for *Cercopithecus* spp. is estimated to be between 16.05 and 23.95 h after marker ingestion (Table 1). Our finding of 16.63–25.12 h TT for samango monkeys is remarkably consistent with results from captivity and, as such, provides an important ecological validation. Whilst captivity can reduce gut passage rates [Milton, 1984; Martin et al., 1985; Blaine and Lambert, 2012] through the "captivity effect" [Martin et al., 1985] and invariable diets [Cabana et al., 2017], our results suggest that other factors, such as site-specific behaviours, may influence gut passage time in wild subjects. For example, aggressive between-troop encounters may influence gut passage time, as observed from the retrieval of one of our markers 4.25 h after ingestion. Additionally, daily and seasonal changes in diet can also influence gut passage times [Lambert, 1998; Tsuji et al., 2015]. These behavioural traits and changes in diet can only be observed in situ, are likely to be site specific and may differ from behaviours displayed in captive subjects. Hence, whilst our results obtained in situ are consistent with those obtained in captivity, our results demonstrate the importance of in situ measures of gut passage time where behaviours linked to the social and physical environment may influence gut passage times. In species where gut retention time is relatively short, simpler observation-based methods exist that are efficient and reliable for estimating gut passage times [Heymann et al., 2012]. One such method relies on a single feeding event on a particular fruit species followed by no further feeding on that species before the first defaecation of seeds from that species [Heymann et al., 2012]. For species of which gut retention time is longer, observation-based methods can be much less reliable [Heymann et al., 2012], and our study provides valuable data on which to build more efficient techniques in situ.

In this regard, we highlight some caveats to our study. First, as is typical of gut retention studies, we had a low sample size (one animal of each sex), although this is in line with other published studies (Table 1) [Maisels, 1994; Poulsen et al., 2001; Lambert, 2002; Blaine and Lambert, 2012]. Secondly, we had low faecal marker recovery. Low faecal marker recovery has been previously documented in *Cercopithecus* spp. studies and has been attributed to cercopithecine use of cheek pouches and oral processing, which includes seed predation through crushing by high-crowned molars [Lambert, 2001]. For example, Blaine and Lambert [2012] reported 5–45% marker recovery from a male *C. l'hoesti* subject and 5–20% marker recovery from a female subject. With our markers being made of natural materials, it may be that some markers were crushed prior to swallowing, although we did not find fragments of markers in any of the faecal samples we collected. Although we watched each subject closely following each feeding event, it is possible that more markers were spat out following feeding events than we observed. Alternatively, we could have waited for the ingestion of infrequently consumed food items, a practice adopted in a few seed dispersal studies [e.g. Stevenson, 2000; Link and Di Fiore, 2006; Beaune et al., 2013a]. However, this may happen rarely across species and is not predictable. Such practice relies on either locally scarce or clumped food resources that minimise replicated feeding on focal tree species [Stevenson, 2000]. Depending on the number of fruits consumed, as well as the number of seeds actually swallowed, this method also has a high risk of missing

the collection of faeces containing the focal seeds. As such, the use of artificial markers may reduce the error and loss of data potentially associated with this method. Finally, we were only able to conduct one set of trials in our study, and so our results only reflect the various food items that were present during that season. As the degree of frugivory may be both seasonal and individual, future studies should aim to capture data that span the spectrum of variation in frugivory and therefore the range of gut passage times within their study systems.

Adapting a methodology such as we have, from a captive to an in situ setting, was partially successful in estimating a gut passage time window, although we were not able to calculate MRT. There is currently no minimum for the number of retrieved markers required to estimate MRT; however, such calculations require reliable and consistent collection of faeces clear of markers before and after the first and last marker, respectively. Given the challenges in the study of wild animals, especially those associated with arboreal primates and the level of habituation required for such intense focal observations [e.g. Souza-Alves and Ferrari, 2010], it would be nearly impossible to expect to collect all of the markers spat out or defaecated following ingestion, as well as being entirely sure that all faecal samples during the night were collected. However, as we have shown, as long as the first and last appearance of markers in faeces can be captured from across trials, researchers should be encouraged to develop and refine methods which prevent markers being spat out and that will allow for the identification of faecal samples deposited at night from focal individuals.

We can make several recommendations for future in situ gut retention studies. Firstly, as a novel and potentially high-value food item, the banana we used for the delivery of the markers may have elicited retrieve-and-retreat behaviour in response to feeding competition [Smith et al., 2008], especially in the female who may have stored the banana in her cheek pouches temporarily. In cercopithecines, adult males dominate other group members, whereas subordinates use cheek pouches to store high-value contestable food items [Smith et al., 2008]. Ideally, replacing the banana with a native locally available fruit would be preferable to such a high-value food item, although we note that high-value items may increase the probability of marker delivery compared with low-value readily available items that are commonly available in the surrounding environment. In addition, local fruits must be soft enough to allow removal of the seed and/or large enough to accommodate markers, neither of which were available at our study site.

Secondly, following arboreal animals in natural environments is intrinsically difficult, especially in high canopy forests and semi-deciduous woodlands with thick understoreys, and we lost the male and female subjects occasionally, which could account for the low marker recovery. Although we collected an average of 10 faecal samples per day from the female subject, faecal sample collection was considerably lower for the male subject, from whom we collected 6 per day on average. There is a possibility that the male subject especially defaecated out of sight of the observer. Future studies should be optimised to maximise the likelihood of continuous observation through careful planning of the timing and location of their experiments. This should include additional observers strategically placed to monitor established travel paths during experiments.

Thirdly, we could not collect faecal samples at night and markers excreted at night have not been included in our analyses. However, a first step should be the iden-

tification of the gut passage time window by establishing reliable estimates of TT and TLA, followed by repeated trials designed to maximise faecal sample collection within this target window. Future studies should aim to stagger the delivery of different marker types, as we did, throughout the day to increase the likelihood of marker retrieval within a day's follow. Traps underneath sleeping sites, such as fine mesh cloth, may aid in the collection of faecal samples in locations during the night to capture samples with markers. Time of deposition can then be read from a camera trap or estimated by appearance [Lambert, 2002]. Notwithstanding, the staggered nature of our trials enabled us to follow both individuals for at least 4 days from the initial feeding trial. We collected clear faecal samples and successfully followed each subject sufficiently to obtain estimates of TT and TLA for samango monkeys in our study system.

Our results indicate a wide range in gut passage times for samango monkeys, which may also have consequences for models of primate seed dispersal distances. Dispersing seeds away from parent plants can reduce the negative effects of conspecific density-dependent competition and natural enemies [Janzen, 1970; Connell, 1971; Comita et al., 2014] and can influence range expansion [Howe and Smallwood, 1982]. However, this would be dependent on mean annual home range, daily path length, direction and speed of travel, and tortuosity. For example, longer retention times of seeds in the digestive tract may mean that although seeds are transported over the daily path length, they may be deposited close to the original source, depending on home range size and tree revisitation rates. For samango monkeys at our study site, the mean (±SE) annual home range has been estimated to be between 0.56 km^2 (±0.07 km^2) and 0.60 km^2 (±0.13 km^2) from 2012 to 2016 [Parker, 2018], and between 0.67 and 0.97 km^2 in 2018 (Stringer, unpubl. data). Whilst samango monkeys may, therefore, be able to cover a wide area of their home range per day, they often utilise different locations for sleeping sites which may increase variability in dispersal distance. Dispersal kernels can be used to infer the statistical distribution of seed dispersal distances by combining gut passage time with movement data [Nathan et al., 2012; Fuzessy et al., 2017]. Inaccurate estimation of gut passage time could thus produce dispersal kernel models that over- or underestimate dispersal distances [Côrtes and Uriarte, 2013], and studies such as ours, that aim to ascertain retention times in situ, provide valuable data from which to estimate dispersal kernels, especially where the use of captive animals for estimating gut passage time is restricted.

In conclusion, *C. a. schwarzi* follow the general trend in cercopithecines of a relatively long gut retention time. This can be attributed to the inclusion of large proportions of non-fruit plant parts in their diet and the need for longer fermentation of these food items. Our study is the first to report a gut passage time window of a *Cercopithecus* species in situ. Whilst our study goes some way to validate similar results reported from ex situ studies, we encountered methodological issues in retrieving all of the markers following ingestion by the monkeys and were unable to estimate MRT. However, in situ studies are critical in providing ecologically valid estimates of gut passage times requisite for models of seed dispersal distances and which may be necessary when the use of captive animals is restricted. Therefore, moving forward, we highlight the need for discussion in implementing a standardised protocol for future studies investigating gut retention time in situ and hope that our study encourages similar attempts to study gut passage rates on naturally foraging primates.

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Statement of Ethics

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. We adhered to the Association for the Study of Animal Behaviour guidelines for the ethical treatment of animals throughout the study. We carried out all procedures with ethical approval from the Animal Welfare and Ethical Review Board at Liverpool John Moores University (application No.: NK_SS/2016-1) and the Animal Welfare Ethical Review Board at Durham University and with permission from the Limpopo Department of Economic Development, Environment and Tourism, South Africa.

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The authors have no conflict of interest to declare.

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Author Contributions

S.S., R.H. and N.K. conceived and designed the experiments. S.S. performed the experiments. S.S. analysed the data and wrote the manuscript; R.H., L.S. and N.K. revised manuscript drafts.

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