A practive faeces collection protocol for multidisciplinary research in wildlife science

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Faecal samples have become an important non-invasive source of information in wildlife biology and ecological research. Despite regular use of faeces, there is no universal protocol available for faeces collection and storage to answer various questions in wildlife biology. In this study we collected 1408 faeces from ten different species using a dry sampling approach, and achieved 77.49% and 75.25% success rate in mitochondrial and nuclear marker amplifications respectively. We suggest a universal framework to use the same samples to answer different questions. This protocol provides an easy, quick and cheap option to collect non-invasive samples from species living in different environmental conditions to answer multidisciplinary questions in wildlife biology.

Keywords: Non-invasive wildlife research, species biology, dry sampling, variable habitats, field logistics.

NON-INVASIVE samples, especially faeces, have become a regular choice in wildlife biology, population monitoring and ecological research globally. Advantages of faecal sample-based wildlife research include easy collection, access to large sample sizes and spatio-temporal coverage. Historically, large-scale use of faeces in wildlife biology started with dietary analysis of animals[1], but the introduction of advanced molecular tools added a new dimension to non-invasive research. These molecular tools have allowed biologists to examine questions regarding population genetics[2,3], species distribution[4], demography[5,6], evolutionary biology[7] and wildlife forensics[8]. In more recent times, faecal samples have been used to address various questions related to wildlife physiology, including endocrinology and reproductive capacity[9,10], along with parasitology[11,12], disease dynamics[13] and conservation genomics[14]. The sampling and storage demands of various questions in non-invasive wildlife research have led to a gradual development of faecal sampling and storage protocols. Several logistical factors including collector's safety, storage in the field, shipping samples from remote field areas in different environmental conditions, etc. have been considered with a gradual development of these protocols.

Over the years, a number of faeces collection and storage approaches have been used in wildlife research that are broadly classified into three categories: (i) dry sampling (e.g. simple drying[15], silica preservation[16]); (ii) wet sampling (ethanol collection[17], TNE and DMSO buffer[18], DETs solution[19], RNAlater[20]) and (iii) two-step approach[21,22] (Table 1). While all these approaches have been used in wildlife research, they have several logistical limitations making their implementation in the field challenging. For example, sampling with silica beads has advantages in post-collection sample transport and storage[23], but is not cost-effective as it requires large amounts of silica beads to keep the samples moisture-free in humid areas. Similarly, ethanol preservation, the most widely used wet sampling approach is also expensive, requires specific training to collect samples and is often problematic during the shipping of samples from remote areas[21]. Currently no universal sampling protocol is available and limited work has focused on testing faeces sampling and storage protocols to answer different questions in non-invasive wildlife research[17,23,24]. Most of such experiments were conducted on captive animals[15,23,25] or studies were performed under favourable environmental conditions for faecal sampling, where frozen samples were collected from the field[26,27].

Here we describe a simple and cost-effective dry sampling approach for faeces collection and storage that overcomes the above-mentioned limitations and helps in answering different questions in faecal sample-based wildlife research. We followed this sampling approach to collect faecal samples of several carnivore and herbivore species living in different environmental conditions. Following sampling, we conducted molecular species identification and microsatellite amplification to demonstrate the efficacy of this approach for genetic work. Finally we have proposed a universal framework to use the faecal samples for various research purposes. We believe that the simplicity of the approach, ease of sample collection in the field and downstream use of the samples to answer various ecological questions will make this protocol useful in studying cryptic, elusive wild species across different environmental conditions globally.

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Table 1. Details of different faecal sampling protocols and their downstream research use

Sampling approach	Sample collection protocol	Advantages	Disadvantages	Downstream use			
				DNA	Diet	Hormone	Parasite
Wet sampling	Ethanol[17,28,39]	Better amplification success, reduced collector health hazards, easy availability	Expensive, logistical issues during transportation	Yes	Yes	Yes	No
	Queen's College lysis buffer[23]	Cheap ingredients, easy preparation in the laboratory	Logistical issues during transportation	Yes	Yes	No	No
	20% DMSO in TNE buffer[23]	Cheap ingredients, easy preparation in the laboratory	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	DETs solution[19]	Cheap ingredients, easy preparation in the laboratory	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	RNAlater[20,40]	Easy availability	Expensive, health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	LST buffer[23]	Simple, low cost of preparation	Health hazards to collector, logistical issues during transportation	Yes	Yes	No	No
	Formalin[41]	Cheap and easily available	Health hazards to collector, logistical issues during transportation	No	No	No	Yes
Dry sampling	Drierite desiccant[23]	Easy to store, easy transportation	Expensive, required in large quantities	Yes	Yes	Yes	No
	Freeze dry[23]	Easy availability in the laboratory, better amplification success	Difficult to maintain equipment in remote field areas	Yes	Yes	Yes	No
	Oven dry[23]	Easy availability in the laboratory	Difficult to maintain equipment in remote field areas	Yes	Yes	Yes	No
	Silica desiccant[23]	Easy availability, no transport and storage issues	Expensive, required in large quantities	Yes	Yes	Yes	No
	Direct collection[23]	Very cheap, no transport issues, works well for temperate conditions	Not been extensively tested in tropical and subtropical conditions	Yes	Yes	Yes	Yes
Two-step sampling	Dry and wet: ethanol then silica[20,21]	Reduced collector health hazards, easy availability, no transport and storage issues	Expensive, requires a long time to process samples in the field, difficult to implement in remote field areas	Yes	Yes	No	No

Methods

Research permissions

All required permissions for our surveys and collection of biological samples were provided by the Forest Departments of Uttarakhand (Permit no.: 90/5-6 and 978/6-32/56), Uttar Pradesh (UP; Permit no.: 1127/23-2-12(G) and 2233/23-2-12 (G)) and Maharashtra (Permit no.: 09/2016).

Study habitats and species

In this study our focus was to develop a faecal sampling protocol that could be used to answer different ecological questions (DNA, diet, parasite, hormone, etc.) for terrestrial species. To test our protocol, we collected samples from both herbivores (elephants and other wild ungulates) as well as carnivores (both small and large) occupying various habitats ranging from sub-alpine forests of the Lesser Himalayas, dry alpine scrub forests of trans-Himalayas, moist-deciduous forests and swampy grassland of Terai-Arc landscape in northwestern India and dry-deciduous forests of the central Indian landscape. Sampling was conducted during different seasons across the states of Uttarakhand, Uttar Pradesh and Maharashtra, where environmental conditions (ambient temperature, precipitation, humidity, etc.) are varied.

Collection and storage of faecal samples

We adopted a simple, cheap but effective field sampling protocol that involves inexpensive and easily available material. Instead of standard use of absolute ethanol, silica gel, RNA later or other similar approaches, we collected faecal samples in butter paper (wax paper) and stored them individually in sterile zip-lock bags. The samples were stored inside dry, dark boxes in the field till they were transferred to the laboratory (within a maximum time of two months duration in this study). In the laboratory, the samples were stored in –20°C freezers till further processing. All samples were collected with the respective GPS locations and other associated field information. We collected a total of 1408 faecal samples of various carnivore and herbivore species across different habitats between December 2015 and May 2017. During collection, the samples were categorized into their respective groups (large carnivore, small carnivore and herbivore respectively) based on the morphological characteristics (physical characters and signs) in the field. Table 2 provides details of species-wise sample size.

Faecal DNA extraction

To check the DNA quality following this dry sampling approach, we tested two different DNA extraction protocols in the laboratory. Both methods were initially tested with few faecal samples collected from different habitat types before being employed in large-scale sample processing. Our first approach was a slightly modified version of faecal swabbing protocol described in Ball *et al.*[26]. This approach is advantageous over the others as it retains most of the host cells from the top layer and reduces the inhibitors present inside the faecal samples. Frozen faecal samples were thawed at room temperature and the upper layer was swabbed with phosphate buffer saline (PBS) (Sigma-Aldrich, USA) saturated gamma-sterilized cotton applicators (HiMedia, Catalogue no.: PW1136-1x500NO). Each sample was swabbed twice separately and immediately stored in separate 2 ml microcentrifuge tubes in –20°C freezers till further processing. During extraction, 30 µl of proteinase K (20 mg/ml) and 300 µl of ATL buffer (Qiagen Inc., Mississauga, Ontario) were added into each tube containing swab and incubated overnight at 56°C, followed by Qiagen DNAeasy tissue DNA kit extraction protocol. DNA was eluted twice in 100 µl preheated 1× TE buffer. For every set of 22 samples, two extraction negatives were taken to monitor any possible contaminations.

In the second approach, we scraped the top layer of faecal samples with a sterile blade and stored it in 2 ml microcentrifuge tubes for further processing. DNA was extracted using slightly modified QIAamp DNA stool mini kit (Qiagen Inc.) protocol described in Mondol *et al.*[28]. In brief, the scraped faecal layers were lysed overnight at 56°C with a mix of 300 µl of ASL buffer (Qiagen Inc.) and 30 µl of proteinase K (20 mg/ml). Following lysis, the standard stool DNA extraction protocol mentioned in the kit was followed. Final elution was carried out twice with 100 µl preheated 1× TE buffer. All DNA extractions were conducted in an exclusive faecal DNA extraction room.

Molecular data generation

Field-collected non-invasive samples often generate DNA of poor quantity and quality for downstream molecular work[29]. Here we tested the efficacy of sampling and DNA extraction protocols through molecular assignment of species (using mitochondrial DNA markers) and amplification of nuclear DNA (microsatellites) from faecal DNA samples collected in the field during the study.

Species identification (using mitochondrial DNA): We have adopted a number of approaches currently available for assignment of faecal samples to species. These involve both species-specific PCRs as well as sequencing-based methods. Table 2 provides the details of species-specific approaches used for the identification of species. We did not perform molecular species identification for elephants as they were easily identified in the field from their size.

Table 2. Details of faecal sample collection and species-wise success rates in molecular species identification

Targeted order/species	Area/landscape	No. of samples collected	DNA extraction protocol	Species confirmation method	Confirmed species	Species identified samples	Success rate (%)
Large carnivore	Terai-Arc landscape, India	1260	Swab	Species-specific PCR-electrophoresis[42]	Tiger (*Panthera tigris tigris*)	567	75.95
	Terai-Arc landscape, India,		Swab	Species-specific PCR-electrophoresis[42–44]	Leopard (*Panthera pardus fusca*)	259	
	Central Indian landscape		Swab and scrape	Species-specific PCR-electrophoresis[45]	Dhole (*Cuon alpinus*)	126	
	Trans Himalayas		Swab	Carnivore-specific PCR-sequencing[46]	Red fox (*Vulpes vulpes*)	5	
Small carnivore	Lesser Himalayas	21	Swab and scrape	Carnivore-specific PCR-sequencing[46]	Jungle cat (*Felis chaus*)	15	100
			Swab and scrape	Carnivore-specific PCR-sequencing[46]	Leopard cat (*Prionailurus bengalensis*)	6	
Herbivore	Terai-Arc landscape, India	127	Swab	Visual observation	Elephant (*Elephas maximus*)	11	88.98
	Terai-Arc landscape, India		Swab	Ungulate-specific PCR-sequencing[47]	Swamp deer (*Rucervus duvaucelii*)	71	
	Terai-Arc landscape, India		Swab	Ungulate-specific PCR-sequencing[47]	Chital (*Axis axis*)	22	
	Middle Himalayas		Swab	Ungulate-specific PCR-sequencing[47]	Himalayan tahr (*Hemitragus jemlahicus*)	9	
Total		1408				1091	77.49

Table 3. Details of microsatellite marker amplification success rates on species-identified samples

Species confirmed	Species-identified samples	Samples with microsatellite loci data	Microsatellite loci used	Average success rate (%)
Tiger (*P. tigris tigris*)	567	408	13 (refs 43 and 48)	66.23
Leopard (*P. pardus fusca*)	259	159	12 (refs 43 and 48)	62.5
Dhole (*C. alpinus*)	126	126	4 (refs 49 and 50)	62.5
Red fox (*V. vulpes*)	5	5	4 (refs 49 and 50)	69
Jungle cat (*F. chaus*)	15	4	4 (refs 43and 48)	100
Leopard cat (*P. bengalensis*)	6	4	4 (refs 43and 48)	100
Elephant (*E. maximus*)	11	11	3 (ref. 51)	100
Swamp deer (*R. duvaucelii*)	71	71	3 (ref. 52)	100
Chital (*A. axis*)	22	11	3 (ref. 52)	100
Himalayan tahr (*H. jemlahicus*)	9	9	3 (ref. 52)	100
Total	1091	821		75.25

Table 4. Cost comparison between various sampling protocols

Consumables required	Faecal sampling approach and associated cost (INR) per sample			
	Dry sampling (INR) (direct collection)	Dry sampling (INR) (silica method)	Dry sampling (INR) (Drierite desiccant)	Wet sampling (INR) (ethanol preservation)
Zip-lock bag	5	5	5	Not required
Plastic container	Not required	Not required	Not required	50 (Tarsons Products Private Ltd, Kolkata)
Butter paper	5	Not required	Not required	Not required
Silica beads	Not required	325 (Sigma-Aldrich, USA)	50 (W.A. Hammond Drierite Company, Ltd, USA)	Not required
Ethanol	Not required	Not required	Not required	250 (MilliporeSigma, USA)
Cardboard box	25	25	25	Not required
Approximate cost	35	355	80	300

Nuclear DNA (microsatellite) amplification: Nuclear DNA amplification from non-invasive samples is challenging due to poor quantity and quality of DNA[29]. In this study we have also amplified nuclear microsatellite markers from our field-collected and species-identified faecal samples. We used a number of microsatellite markers to test the quality of DNA from field-collected samples from different species (Table 3). Species-wise cumulative amplification success rates for all tested loci were calculated.

Results

We considered species identification and nuclear microsatellite amplification success rates from both swabbing and scraping protocols as efficacy of our faecal sampling approach for non-invasive wildlife genetic research. Initially we tested both approaches with 100 field-collected carnivore faecal samples (50 were swabbed and 50 were scraped) and achieved 100% success rates in species identification. As both approaches resulted in high success rate from field-collected faeces we compared other factors such as cost of consumables, ease of extraction protocol, time required, etc. for both methods and finally adopted the swabbing approach for larger sample size. Subsequently, we swabbed the remaining 1308 faecal samples of different carnivore and herbivore species (Table 2) collected from different habitats across India. We ascertained 10 different species from these field-collected faeces, including four large carnivore species ($n = 957$ samples), two small carnivore species ($n = 21$ samples) and four herbivore species ($n = 113$ samples). Overall, success rate was 75.95%, 100% and 88.98% for large carnivores, small carnivores and herbivores respectively (Table 2). The species identified were tiger (*Panthera tigris tigris*), leopard (*Panthera pardus fusca*), dhole (*Cuon alpinus*), red fox (*Vulpes vulpes*), jungle cat (*Felis chaus*), leopard cat (*Prionailurus bengalensis*), elephant (*Elephas maximus*), swamp deer (*Rucervus duvaucelii*), chital (*Axis axis*) and Himalayan tahr (*Hemitragus jemlahicus*) (Table 2).

Following species identification we amplified multiple nuclear microsatellites for all ten species (Table 3). We successfully amplified 821 of the total 1091 samples of different species, with an average success rate of 75.25% (see Table 3 for species wise details).

Discussion

Here, we describe a simple, quick and cost-effective faecal sampling approach for non-invasive wildlife research. We have tested this method on ten different species that are found in a variety of different habitats. Development of field-suitable sampling and storage protocol is a progressive approach in non-invasive wildlife research as faeces degradation in the wild is accelerated by exposure to various environmental conditions including sunlight (UV), humidity, temperature and rainfall, thus posing a challenge to generate useful information for any target species. In comparison to other studies involving field-sampling and storage protocol standardization[17,22–26], we collected a large number of samples ($n = 1408$) from multiple species covering a wide variety of habitats to test this protocol. Depending on the regions, the samples were stored in field conditions for up to two months before processing in the laboratory. High amplification success in species identification and nuclear marker amplification from field-collected samples indicate the efficacy of the approach for DNA-based research. To the best of our knowledge, no previous studies have used such a large sample size from varied species to test faecal sampling and storage protocol. Given our sampling from a wide variety of habitats and range of species with different ecologies, we believe that this protocol would work well in other species living in different habitats across the globe. This approach is much cheaper than other commonly used sampling protocols (e.g. silica gel, drierite, ethanol, etc.) (Table 4 provides a cost comparison of the widely used protocols), takes less time in the field and does not require specific training of field staff for implementation. However, we strongly suggest appropriate

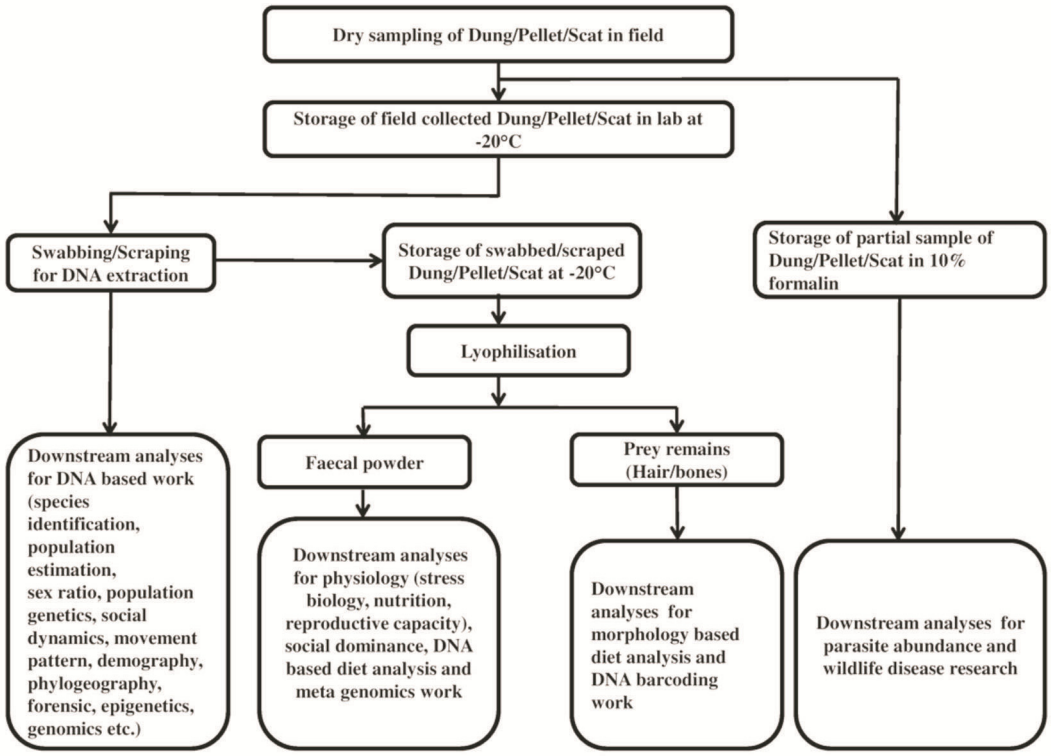

Figure 1. Flowchart showing a framework of various uses of faecal samples collected through the dry sampling approach described in this study.

safety protocols (mask, gloves, protective gears, etc.) during sample collection and processing for dry sampling approaches as exposure to potential pathogens is possible from dry faeces.

Testing two different DNA extraction protocols during this study provided important insights on generating good-quality DNA data from samples of differing quality. We performed swabbing and scraping DNA extraction approaches on a set of 100 carnivore samples. Carnivore scat samples were specifically chosen for standardization as they often yield poor results due to the presence of prey DNA[30]. Given similar success rates achieved from both approaches and considering low cost of consumables, extraction time and ease of the protocol, swabbing was used for the remaining samples. While earlier studies have shown great efficiency of this approach[25–27,31,32], swabbing was mostly conducted with fresh (≤24 h)[19,23], captive[15,25] and frozen (≤0°C)[26,27] faecal samples. Due to higher success rates with a large number of faeces from multiple species in this study, we recommend the use of swabbing approach in future non-invasive genetic research. However, it is also important to point out that the scraping approach would be useful for comparatively older (≥ 2 weeks) faeces where the outer layer is disturbed and for samples collected from dry/dusty regions where swabbing the top layer is not feasible. Though we have tested both approaches with a reasonably large number of carnivore faecal samples, any new study should test both approaches either with a few field-collected samples of the target species, or decide on a specific approach based on the sample conditions and physical characteristics (strata, dryness, availability of faeces top layer, etc.) of the study area.

Another major advantage of this dry sampling approach is the ability to use the same samples to generate additional information apart from DNA data at species/individual levels. We propose a useful framework to showcase different uses of the same samples in addressing various important biological questions in wildlife biology (Figure 1). For example, following swabbing/scraping for DNA, the frozen sample can be lyophilized to separate faecal powder and the remaining prey hairs/plant products[16]. Morphological analyses of hair/plant material can provide information on diet[33,34]. Similarly, the faecal powder could be subsequently used in understanding physiological parameters (stress[35,36], reproductive fitness[9,10,37], social dominance[38] and diet[16]). During field sampling, a part of the faeces can be collected in formalin to study parasite abundance[11]. In conclusion, our dry faecal sampling method provides an easy and cheap option to collect non-invasive samples from terrestrial wild animals. This universal protocol can be used to collect samples from species living in different environmental conditions and answer various questions related to genetics, genomics, physiology, diet, health, etc. Along with other ecological information, these

parameters would help develop informed conservation plans for any target species.

1. Storr, G., Microscopic analysis of faeces, a technique for ascertaining the diet of herbivorous mammals. *Aust. J. Biol. Sci.*, 1961, **14**, 157–164.
2. O'Brien, S. J. and Johnson, W. E., Big cat genomics. *Annu. Rev. Genomics Hum. Genet.*, 2005, **6**, 407–429.
3. Broquet, T., Ray, N., Petit, E. and Fryxell, J. M., Genetic isolation by distance and landscape connectivity in the American marten (*Martes americana*). *Landsc. Ecol.*, 2006, **21**, 877–889.
4. Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W., Ten species in one : DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 14812–14817.
5. Flagstad, O. *et al.*, Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. *Mol. Ecol.*, 2003, **12**, 869–880.
6. Miller, C. R. and Waits, L. P., The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implfications for conservation. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 4334–4339.
7. Price, T. D. *et al.*, Niche filling slows the diversification of Himalayan songbirds. *Nature*, 2014, **509**, 222–225.
8. Wasser, S. K. *et al.*, Genetic assignment of large seizures of elephant ivory reveals Africa's major poaching hotspots. *Science*, 2015, **349**, 84–87.
9. Moreira, N. *et al.*, Reproductive steroid hormones and ovarian activity in felids of the Leopardus genus. *Zoo Biol.*, 2001, **20**, 103–116.
10. Rolland, R. M., Hunt, K. E., Kraus, S. D. and Wasser, S. K., Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. *Gen. Comp. Endocrinol.*, 2005, **142**, 308–317.
11. Marathe, R. R., Goel, S. S., Ranade, S. P., Jog, M. M. and Watve, M. G., Patterns in abundance and diversity of faecally dispersed parasites of tiger in Tadoba National Park, central India. *BMC Ecol.*, 2002, **2**, 6.
12. Barutzki, D. and Schaper, R., Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. *Parasitol. Res.*, 2011, **109**, 45–60.
13. Batchelor, D. J., Tzannes, S., Graham, P. A., Wastling, J. M., Pinchbeck, G. L. and German, A. J., Detection of endoparasites with zoonotic potential in dogs with gastrointestinal disease in the UK. *Transbound. Emerg. Dis.*, 2008, **55**, 99–104.
14. Shehzad, W. *et al.*, Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Mol. Ecol.*, 2012, **21**, 1951–1965.
15. Murphy, M. A., Waits, L. P. and Kendall, K. C., Quantitative evaluation of fecal drying methods for brown bear DNA analysis. *Wildl. Soc. Bull.*, 2000, **28**, 951–957.
16. Shores, C., Mondol, S. and Wasser, S. K., Comparison of DNA and hair-based approaches to dietary analysis of free-ranging wolves (*Canis lupus*). *Conserv. Genet. Resour.*, 2015, **7**, 871–878.
17. Murphy, M. A., Waits, L. P., Kendall, K. C., Wasser, S. K., Hibgee, J. A. and Bogden, R., An evaluation of long-term preservation methods for brown bear (*Ursus arctos*) faecal DNA samples. *Wildl. Soc. Bull.*, 2002, **3**, 435–440.
18. Wasser, S. K., Shedlock, A. M., Comstock, K., Ostrander, E. A, Mutayoba, B. and Stephens, M., Assigning African elephant DNA to geographic region of origin: applications to the ivory trade. *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 14847–14852.
19. Frantzen, M. A. J., Silk, J. B., Ferguson, J. W. H., Wayne, R. K., and Kohn, and M. H., Empirical evaluation of preservation methods for faecal DNA. *Mol. Ecol.*, 1998, **7**, 1423–1428.
20. Nsubuga, A. M., Robbins, M. M., Roeder, A. D., Morin, P. A., Boesch, C. and Linda, V., Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Mol. Ecol.*, 2004, **13**, 2089–2094.
21. Roeder, A. D., Archer, F. I., Poinar, H. N. and Morin, P. A., A novel method for collection and preservation of faeces for genetic studies. *Mol. Ecol. Notes*, 2004, **4**, 761–764.
22. Reddy, P. A., Bhavanishankar, M., Bhagavatula, J., Harika, K., Mahla, R. S. and Shivaji, S., Improved methods of carnivore faecal sample preservation, DNA extraction and quantification for accurate genotyping of wild tigers. *PLoS ONE*, 2012, **7**, 1–7.
23. Wasser, S. K., Houston, C. S., Koehler, G. M., Cadd, G. G. and Fain, S. R., Techniques for application of faecal DNA methods to field studies of Ursids. *Mol. Ecol.*, 1997, **6**, 1091–1097.
24. Hunt, K. E. and Wasser, S. K., Effect of long-term preservation methods on faecal glucocorticoid concentrations of grizzly bear and African elephant. *Physiol. Biochem. Zool.*, 2003, **76**, 918–928.
25. Ramón-Laca, A., Soriano, L., Gleeson, D. and Godoy, J. A., A simple and effective method for obtaining mammal DNA from faeces. *Wildl. Biol.*, 2015, **21**, 195–203.
26. Ball, M. C. *et al.*, Characterization of target nuclear DNA from faeces reduces technical issues associated with the assumptions of low-quality and quantity template. *Conserv. Genet.*, 2007, **8**, 577–586.
27. Wasser, S. K., Keim, J. L., Taper, M. L. and Lele, S. R., The influences of wolf predation, habitat loss, and human activity on caribou and moose in the Alberta oil sands. *Front. Ecol. Environ.*, 2011, **9**, 546–551.
28. Mondol, S., Ullas Karanth, K., Samba Kumar, N., Gopalaswamy, A. M., Andheria, A. and Ramakrishnan, U., Evaluation of non-invasive genetic sampling methods for estimating tiger population size. *Biol. Conserv.*, 2009, **142**, 2350–2360.
29. Broquet, T., Ménard, N. and Petit, E., Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conserv. Genet.*, 2007, **8**, 249–260.
30. Taberlet, P. and Luikart, G., Non-invasive genetic sampling and individual identification. *Biol. J. Linn. Soc.*, 1999, **68**, 41–55.
31. Rutledge, L. Y., Holloway, J. J., Patterson, B. R. and White, B. N., An improved field method to obtain DNA for individual identification from wolf scat. *J. Wildl. Manage.*, 2009, **73**, 1430–1435.
32. Cullingham, C. I., Curteanu, M., Ball, M. C. and Manseau, M., Feasibility and recommendations for swift fox faecal DNA profiling. *J. Wildl. Manage.*, 2010, **74**, 849–859.
33. Mukherjee, S., Goyal, S. P. and Chellam, R., Refined techniques for the analysis of Asiatic lion *Panthera leo persica* scats. *Acta Theriol.*, 1994, **39**, 425–430.
34. De, R., Joshi, B. D., Shukla, M., Pandey, P., Singh, R. and Goyal, S. P., Understanding predation behaviour of the tiger (*Panthera tigris tigris*) in Ranthambore tiger Reserve, Rajasthan, India: use of low-cost gel based molecular sexing of prey hairs from scats. *Conserv. Genet. Resour.*, 2018, 97–104.
35. Wasser, S. K., Bevis, K., Gina, K. and Hanson, E., Noninvasive physiological measures of disturbance in the northern spotted owl. *Conserv. Biol.*, 1997, **11**, 1019–1022.
36. Palme, R., Rettenbacher, S., Touma, C., El-bahr, S. M. and Mostl, E., Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in faecal samples. *Ann. NY Acad. Sci.*, 2005, **1040**, 162–171.
37. Schwarzenberger, F., Möstl, E., Palme, R. and Bamberg, E., Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Anim. Reprod. Sci.*, 1996, **42**, 515–526.
38. Creel, S., Social dominance and stress hormones. *Trends Ecol. Evol.*, 2001, **16**, 491–497.

39. Frantz, A. C. *et al.*, Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. *Mol. Ecol.*, 2003, **12**, 1649–1661.
40. Vlčková, K., Mrázek, J., Kopečný, J. and Petrželková, K. J., Evaluation of different storage methods to characterize the fecal bacterial communities of captive western lowland gorillas (*Gorilla gorilla gorilla*). *J. Microbiol. Meth.*, 2012, **91**, 45–51.
41. Crawley, J. A. H., Chapman, S. N., Lummaa, V. and Lynsdale, C. L., Testing storage methods of faecal samples for subsequent measurement of helminth egg numbers in the domestic horse. *Vet. Parasitol.*, 2016, **221**, 130–133.
42. Mukherjee, N., Mondol, S., Andheria, A. and Ramakrishnan, U., Rapid multiplex PCR based species identification of wild tigers using non-invasive samples. *Conserv. Genet.*, 2007, **8**, 1465–1470.
43. Mondol, S., Kumar, N. S., Gopalaswamy, A., Sunagar, K., Karanth, K. U. and Ramakrishnan, U., Identifying species, sex and individual tigers and leopards in the Malenad–Mysore Tiger Landscape, Western Ghats, India. *Conserv. Genet. Resour.*, 2015, **7**, 353–361.
44. Maroju, P. A., Yadav, S., Kolipakam, V., Singh, S., Qureshi, Q. and Jhala, Y., Schrodinger's scat: a critical review of the currently available tiger (*Panthera tigris*) and leopard (*Panthera pardus*) specific primers in India, and a novel leopard specific primer. *BMC Genet.*, 2016, **17**, 37.
45. Modi, S., Mondol, S., Ghaskadbi, P., Hussain, Z., Nigam, P. and Habib, B., Non-invasive DNA-based species and sex identification of Asiatic wild dog (*Cuon alpinus*). *J. Genet.*, 2018, **97**, 1457–1461.
46. Farrell, L. E., Roman, J. and Sunquist, M. E., Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Mol. Ecol.*, 2000, **9**, 1583–1590.
47. Gupta, S. K., Kumar, A. and Hussain, S. A., Novel primers for sequencing of the complete mitochondrial cytochrome b gene of ungulates using non-invasive and degraded biological samples. *Conserv. Genet. Resour.*, 2014, **6**, 499–501.
48. Mondol, S., Thatte, P., Yadav, P. and Ramakrishnan, U., A set of miniSTRs for population genetic analyses of tigers (*Panthera tigris*) with cross-species amplification for seven other Felidae. *Conserv. Genet. Resour.*, 2012, **4**, 63–66.
49. Ostrander, E. A., Sprague, G. F. and Rine, J., Identification and characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. *Genomics*, 1993, **16**, 207–213.
50. Ostrander, E. A, Mapa, F. A, Yee, M. and Rine, J., One hundred and one new simple sequence repeat-based markers for the canine genome. *Mammal Genome*, 1995, **6**, 192–195.
51. Kongrit, C., Siripunkaw, C., Brockelman, W. Y., Akkarapatumwong, V., Wright, T. F. and Eggert, L. S., Isolation and characterization of dinucleotide microsatellite loci in the Asian elephant (*Elephas maximus*). *Mol. Ecol. Resour.*, 2008, **8**, 175–177.
52. Queirós, J., Godinho, R., Lopes, S., Gortazar, C., de la Fuente, J. and Alves, P. C., Effect of microsatellite selection on individual and population genetic inferences: an empirical study using cross-specific and species-specific amplifications. *Mol. Ecol. Resour.*, 2015, **15**, 747–760.

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