Current Zoology 60 (3): 351–358, 2014

Evaluating preservation medium for the storage of DNA in African lion *Panthera leo* faecal samples

Talatu TENDE[1,2*], Bengt HANSSON[2], Ulf OTTOSSON[1], Staffan BENSCH[2]

[1] A. P. Leventis Ornithological Research Institute, P. O. Box 13404, Jos, Nigeria

[2] Department of Biology, Lund University, Ecology Building, SE-223 62 Lund, Sweden

Abstract Lion faecal samples, collected in the field between 1 hour to 1 week after defecation were preserved in three different media (ethanol, ASL buffer and Two-step storage). The aim was to determine which faecal DNA field preservation method best enhances PCR amplification success. Samples stored in ethanol showed a significantly higher amplification success of microsatellite loci than samples stored in the other two media. In contrast, amplification success of a mitochondrial locus was similar among the samples stored in the three types of media. We reviewed twelve previous studies that employed different media for the storage of faeces, although patterns of success were not fully consistent among different media, ethanol storage was scored highest in the majority of these tests [*Current Zoology* 60 (3): 351–358, 2014].

Keywords Amplification success, Faecal sample DNA, Lion *Panthera leo*, PCR, Preservation medium

For small and elusive wildlife populations, non-invasive DNA sampling from faecal samples is a feasible method for obtaining genetic data (Kohn and Wayne, 1997). Several studies have employed this method to estimate and monitor wildlife (Kohn et al., 1999; Ernest et al., 2000; Banks et al., 2002, Flagstad et al., 2004, Bensch et al., 2006; Perez et al., 2006; Zhang et al., 2009). For elusive carnivores such as lions *Panthera leo* non-invasive sampling is especially suitable because faecal samples are easily encountered and recovered along trails where they defecate to mark territory boundaries (Macdonald, 1980). However, studies that employ non-invasive sampling are confronted with pitfalls such as low quantity and quality DNA which can result in low genotyping success and frequent genotyping errors (Taberlet et al., 1997).

The amount and quality of DNA initially present in faeces samples at the time of collection may change during the period of storage and will determine the amount and quality of DNA that can be extracted and used in downstream analyses. It is therefore important to transfer samples into a storage condition that best preserves the DNA prior to the laboratory analysis. The choice of a good preservation medium should be of prime importance for every genetic study, especially when collected samples cannot be processed immediately at the collection site. The identification of effective preservation methods can enhance PCR amplification success rate, increase genotyping accuracy and increase the feasibility of using faecal DNA for genetic studies.

Faecal DNA has so far mainly been used in studies of mammals and less often for studies of birds (Idaghdour et al., 2003; Regnaut et al., 2006). In mammals, DNA has been recovered from faeces that were preserved dried (Höss et al., 1992; Kohn et al., 1995; Foran et al., 1997; Taberlet et al., 1997), in 70% ethanol (Höss, 1992; Kohn et al., 1995), in 99%–100% ethanol (Gerloff et al., 1995) and frozen at -20°C (Reed et al., 1997).

Several studies have evaluated the relative genotyping success from two or more preservation media (Wasser et al., 1997; Frantzen et al., 1998; Murphy et al., 2002; Panasci et al., 2011). These studies span an array of mammal species (Appendix 1) including several species of carnivores (e.g. Wasser et al., 1997; Murphy et al., 2002; Santini et al., 2007; Panasci et al., 2011; Reddy et al., 2012). A few studies comparing storage methods in tropical environments have been conducted mostly on primates (Gerloff et al., 1995; Frantzen et al., 1998; Bayes et al., 2000; Nsubuga et al., 2004; Vallet et al., 2007). Frantzen et al. (1998) attempted to evaluate the success of preserving baboon *Papio cynocephalus urainus* DNA in four different media and pointed out that the optimal methods are variable according to species and conditions at study sites.

Although some studies have evaluated the relative

Received May 27, 2013; accepted Oct. 8, 2013

* Corresponding author. E-mail: talatu.tende@biol.lu.se

success of the same preservation media (Table 1) they have reached different conclusions on their performance. This suggests that effectiveness varies among taxa, environmental conditions and possibly other variables. These conflicting findings call for more studies to be conducted in order to establish the medium that is best for the preservation of faecal samples. In particular, there are relatively few studies from dry tropical regions because most tests have been using fresh faeces (i.e. less than 12 hrs old), therefore we still know relatively little on the storage conditions that are best for the most commonly encountered faeces in the field, i.e. those defecated days to weeks before collection.

In this study we intend to compare the effectiveness of three preservation media, ethanol, ASL buffer and two-step storage (i.e. ethanol and silica gel) for the storage and preservation of lion faecal DNA obtained in Yankari Game Reserve, central North-East Nigeria. The aim is to determine the preservation method that best enhances PCR amplification success of both the mitochondrial and nuclear DNA. This study is part of an ongoing research project that uses DNA from lion faecal samples to monitor lion populations in protected areas in Nigeria (Tende et al., 2010). We hypothesise that the PCR amplification success will differ between the preservatives used. The outcome of our test was evaluated in relation to a literature compilation of other studies testing preservation media.

1 Material and Methods

1.1 Study site

The study was conducted in Yankari Game Reserve (the reserve), central North-East Nigeria (9°50′ N and 10°30′ E), with a landmass of 2,244 km^2. The reserve lies in the Sudan Savannah zone and the vegetation is composed mainly of dry savannah woodland with a narrow floodplain, bordered by patches of gallery and riparian forests (Geerling, 1973; Crick and Marshall, 1981; Green, 1989). Temperature ranges between 18–24°C during the wet / harmattan season and 30–45°C during the dry hot season.

1.2 Faecal sampling

We conducted daily faeces surveys between January and April 2011 within the reserve, by visiting tracks both in the mornings and evenings. We collected 23 faecal samples of similar age, determined to be between 1hr and 1 week old because we often travelled these tracks more than once per day and at least on a weekly basis. Efforts were made to collect samples that were as fresh as possible (c.f. Piggott, 2004; Murphy et al., 2002). The freshness of faeces was determined based on moisture content, appearance and strength of odour (Vynne et al., 2011). We collected our samples from the outer layer of each faeces, according to Frenando et al. (2003). Each of the twenty-three samples were preserved in the three different media (n=69); 95% ethanol, ASL buffer (Qiagen) and Two-step storage (ethanol and silica beads; e.g. Wasser et al., 1997; Reddy et al., 2012). Wherever a sample was encountered a small portion from the outer part of the faeces (c.f. Bidlack et al., 2007; Ferrando et al., 2008; Stenglein et al., 2010) was collected using dry sticks and immediately put into ethanol and ASL buffer. Each stick was discarded after each sample collection to avoid contamination. Samples

Table 1 Summary of overview

Authors (year)	Climate	Preservation methods								
		Ethanol	2-step	Buffers				Silica	Drying	Freezing
				ASL	DETs	RNA*later*	GUS			
This study	Tropical, dry	1	3	2	-	-	-	-	-	-
Reddy et al., 2012	Temperate, dry	2	1	-	-	-		3	-	-
Roeder et al., 2004	Temperate	1	1		-	-	-	2	-	-
Calderon et al., 2009	Tropical, humid	2	-	-	-	1	-	3	-	-
Santini et al., 2007	Temperate, dry	2	-	-	-	-	1	-	3	3
Frantz et al., 2003	Temperate	1	-	-	2	-	-	-	-	3
Piggot & Taylor, 2003	Temperate	1	-	-	2	-	-	-	1	1
Murphy et al., 2002	Temperate	1	-	-	-	-	-	2	3	-
Frantzen et al., 1998	Temperate – Sub-tropical	1	-	-	1	-	-	-	-	2
Wasser et al., 1997	Temperate	2	-	-	-	-	-	1	-	3
Nsubuga et al., 2004	Tropical,humid	-	1	-	2	2	-	2	-	-
Panasci et al., 2011	Temperate, dry	1	-	2		-	-	-	-	-

The figures 1–3 denotes the within study results for preservation, where 1=best, 2= second best, 3= third best

preserved with the Two-step storage method were first collected in 95% ethanol and kept for 24 hours at ambient temperature. Afterwards the ethanol was carefully poured off and the dry solid faecal sample was transferred into the tube containing silica beads (c.f. Roeder et al., 2004; Reddy et al., 2012). The top of the tube containing the dry silica gel and faecal sample was then carefully stuffed with cotton wool to make it air tight. All collected samples were stored at room temperature, thereafter transported to the laboratory and stored at -33°C prior to DNA extraction.

DNA extraction was carried out in a separate room exclusively dedicated to faeces and hair extractions. DNA from faeces was extracted using the stool DNA extraction protocols in QIAamp® (Qiagen) according to the manufacturer's instructions. All DNA extractions and PCRs contained a negative control (reagents only) to monitor for contamination at any step along the way.

1.3 Amplification of mitochondrial DNA

In order to evaluate the amplification success of the mitochondrial DNA, all extracted samples were PCR amplified using primers LIHYF (5′-ATGACCAACAT-TCGAAAATCWC-3′) and LIHYR (5′-ATGTGGGTS-ACTGATGAG-3′). These primers are designed to amplify a short portion of the mitochondrial *cytochrome b* gene (206 bp) and enhance the detection of the target species, while avoiding the amplification of human and ungulate DNA (Tende et al., 2010). All amplifications were carried out using a 2X Qiagen multiplex PCR kit in 10 µl reaction volumes; containing 5 µl Qiagen multiplex PCR buffer mix, 0.2 µM forward primers (Applied Biosystems), 0.2 µM reverse primer, 2.6 µl of water and 2 µl of DNA extract. The PCR profile consisted of the following program: a hot start at 95°C for 15 min; 35 cycles at 90°C for 30 s, annealing of 52°C for 30 s, and an elongation of 72°C for 30 s. A blank control (reagents only) from the extracted DNA process was included in all PCRs to monitor for contamination. The results of the PCR were evaluated by electrophoresis using 2% agarose gels and GelRed™ (Biotium) staining.

All samples that were successfully amplified were sequenced using the forward primer (BigDye sequencing kit; Applied Biosystems, Foster City, CA, USA) in an ABI Prism® 3100 capillary sequencer (Applied Biosystems) for species identification. This is necessary to ensure that the faeces used in the study are from the lion and not from other carnivores such as the spotted hyena *Crocuta crocuta* and striped hyena *Hyena hyena* present in the reserve. Sequences were aligned against reference sequences of lion, spotted hyena and striped hyena obtained from GenBank. All samples assessed to belong to the lion were selected for further analysis.

1.4 Amplification of nuclear DNA

To evaluate amplification success and genotyping accuracy of the nuclear DNA, all positive samples found to originate from lion were PCR amplified using six polymorphic microsatellite primers (FCA001, FCA026, FCA031, FCA077, FCA506 and FCA567; Menotti-Raymond et al., 1999). PCR amplifications were performed in 6 µl multiplex reactions containing 0.12 µl (concentration: 10 µM) dye-labelled (6-Fam or Hex) F-primer, 0.12 µl unlabelled R-primer (concentration: 10 µM), 3 µl of 2X Qiagen Master mix, 0.76 µl double distilled water and 2 µl DNA extract. PCRs were done in a GeneAmp 9700 thermocycler (Applied Biosystems) with the following program: 95°C for 15 min; 40 cycles at 94°C for 30 s, 52°C for 90 s and 72°C for 90 s; followed by an elongation period at 72°C for 10 min. Primers were multiplexed together in two batches based on differences in fragment length and dye. The primer combinations were as follows: FCA001-FCA026, FCA031-FCA567, FCA077-FCA506. Each sample and locus was PCR amplified three times in order to ascertain the results. Alleles of the PCR products were separated using capillary electrophoresis in an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Alleles were sized relative to GS500 ROX size standard and proof read and scored in Gene Mapper 4.0 (Applied Biosystems). No allele was accepted unless it was detected at least twice in the three independent PCRs.

1.5 Data analysis

Direct and parallel comparison of the amplification success of the samples obtained from the three different media was made from the genotype result obtained from the six microsatellites, by direct count of the number of successful amplifications. Success rate was then estimated as the number of samples that amplified at a locus from each medium. We tested if the probability of success or failure (1, 0) at the six different loci is dependent on preservation method by building a generalized linear mixed model (GLMM) with a binomial error structure with the probability of success as the response variable, preservation method (Ethanol, ASL buffer or Two-step storage) and loci (1–6) as explanatory variables and sample replicates as a random factor, thus:

Success (1/ 0) = preservation method (Ethanol, ASL buffer, Two-step storage) + loci (1-6) + replicates (random factor)

The model also included two-way interactions of the

explanatory variables and using a stepwise backward elimination process, the final model which best explains the variability in the data was selected based on the value of the Akaike Information Criterion (AIC). The best-fit model was the one with the lowest AIC value.

2 Results

Of the 23 samples collected, the *cytochrome b* gene was successfully amplified in all three preservation methods, except for one sample that was only amplified from the two-way storage medium. There was no apparent difference in the strengths of the bands amplified from the samples in the three different methods of preservation. The sequencing confirmed that in 20 of the samples the amplified *cytochrome b* gene matched to lion (after aligning our sequences to reference sequences of the lion, spotted and striped hyenas obtained from the GenBank) whereas three samples matched to spotted hyena.

The overall microsatellite amplification success was 50% for samples stored in ethanol, 30% for ASL buffer and 20% for Two-step (n=20 individuals, 6 loci). Ethanol storage showed the highest amplification success at all six individual microsatellite loci and this effect was significant when compared to Two-step storage for five of the six loci (Fig. 1). A logistic regression (Table 2) confirmed the main effect of storage method ($P < 0.001$) and also showed a significant difference between the loci ($P < 0.001$). On average, the highest amplification success was obtained at locus FCA077. The non-significant interaction between preservative method and loci (Table 2) indicates that the probability of obtaining a positive outcome at any locus was not influenced by the preservation method used.

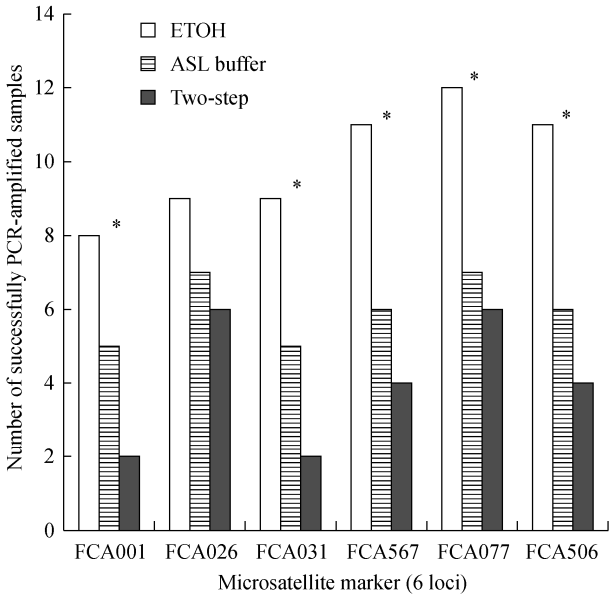

Fig. 1 Number of successfully genotyped samples after three repeated independent PCRs from the different medium: ethanol (ETOH), ASL buffer (ASL) and Two-step storage, respectively

Stars indicate pair-wise significant ($P < 0.05$) differences (Chi-square).

Table 2 Logistic regression showing probability of amplification success of lion samples (n = 20) preserved in three different media

	Wald chi-squared	*df*	*P*
Intercept	80.462	1	<0.001
Preservation method	41.663	2	<0.001
Locus	27.878	5	<0.001
Preservation method*locus	11.385	10	0.328
Replicates	1.087	6	0.982

3 Discussions

Several studies have evaluated methods for preserving DNA in faecal samples from mammals (Table 1). Although most studies compared some of the different media commonly used, storage in ethanol scored high in the majority of studies where it was tested. This pattern agrees with our study of lions.

We found that mtDNA was amplified with a high success (97%) irrespective of the media. However for nuclear markers (the microsatellites) that are the most valuable markers for studies of individual identification and population comparisons we found the highest success for samples preserved in ethanol. This is suggesting that ethanol (>90%) is an ideal medium for preserving DNA in faecal samples from lions in studies aiming for individual identification, population monitoring and population size estimates. Our findings are thus consistent with Murphy et al. (2002) who found that silica gel beads produced poorer results compared to ethanol, and therefore recommended the use of absolute ethanol for the preservation of faecal sample DNA. They also found that brown bear *Ursus arctos* samples preserved in DETs buffer performed equally well as samples preserved in high percentage ethanol. Moreover, this study also reported that oven-dried samples produced better results than samples stored in silica beads or preserved by microwave drying. In the present study of lions in Nigeria, the climate is often very hot (up to +45°C) and dry during times when faeces are found. The faeces therefore tend to dry quickly (<24 hrs) thus making it

unnecessary to dry the samples before storage.

Panasci et al. (2011) showed that coyote faeces preserved in 95% ethanol and DETs buffer performed equally well and both performed better than samples stored in lysis buffer. Wasser et al. (1997) and Panasci et al. (2011) advised against the use of lysis buffer for the preservation of faecal DNA because it could result in the digestion of ingested hair from consumed prey that thereby could act as a potential source of DNA contamination. Also, it is possible that faeces collected in ASL buffer get lysed if stored at room temperature prior to deposition in the freezer. This might have affected DNA quality in our case, since the collected faeces were not deposited immediately in the freezer. Other preservation methods might be useful for faecal DNA storage depending on storage conditions. For example, Wasser et al. (1997) found silica gel to perform well especially when stored at room temperature.

We reviewed 12 different studies that had evaluated the effects of storage medium for DNA in faecal samples based on amplification success both on mtDNA and nDNA (Appendix 1). Although these studies have not evaluated the same methods a striking pattern is that storage in ethanol (70%–95%) proved to be the best medium in seven out of the twelve studies reviewed (Table 1). The two-step storage was found to be the best in three out of four studies (Table 1). Two buffers (GUS lysis buffer and RNA*later)* worked well in the few studies that tested them however; this needs to be investigated further.

Reddy et al. (2012) found that the amount of amplifiable DNA in faeces declined with time. This is probably because environmental conditions (e.g. sun exposure, temperature, humidity) facilitate the degradation of DNA in faeces after deposition (Morin and Woodruff, 1996; Farrell et al., 2000; Goossens et al., 2000; Lucchini et al., 2002; Friedberg, 2003; Nsubuga et al., 2004). The degradation of DNA in faeces appears to be faster during warm compared to cool seasons (Lucchini et al., 2002; Vynne et al., 2011). Wasser et al. (1997) and Vynne et al. (2011) found that this rate of DNA degradation is dependent on both the ambient temperature and humidity. It is therefore vital to recover faeces as quickly as possible (Piggott, 2004; Murphy et al., 2007). Faeces freshness can be determined based on moisture content, appearance and strength of odour (Rutledge et al., 2008; Vynne et al., 2011). Vynne et al. (2011) carried out a study in the Brazilian Cerrado, a seasonally dry tropical environment, to determine factors that cause DNA degradation in faeces of maned wolf *Chrysocyon brachyurus*. They found environmental condition as one of the predictors of the amplification success of mtDNA. Thus, the time interval between scat deposition and collection, as well as sample storage duration can influence the amplification success apart from preservation method (Wasser et al.,1997; Frantzen et al.,1998; Goossens et al., 2000). Although efforts were made during our survey period to recover faeces as soon as possible to minimize exposure to environmental degradation, the samples were not analyzed immediately. Thus it is possible that the duration of sample storage in the freezer before analysis (>1 month) could impact our results. This needs to be investigated further in the same species by analyzing samples of different storage periods.

Previous studies have demonstrated that the specific part of the faeces collected may affect the DNA quality and the amplification success (Rutledge et al., 2008; Gobush et al., 2009; Stenglein et al., 2010; Vynne et al., 2011; Wasser et al., 2011). This is because DNA from faeces is obtained from sloughed epithelial cells of the intestinal lining of the focal species (Albaugh et al., 1992). These cells are often not homogenously distributed in the faeces (Wasser et al., 1997). The different techniques used to address the problem of non-uniform distribution of DNA are: 1) swabbing the surface of the faeces (Lampa et al., 2008; Rutledge et al., 2008; Vynne et al., 2011), 2) scraping the surface (Kohn et al., 1999; Fernando et al., 2000; Livia et al., 2007), 3) washing the surface (Banks et al., 2002; Maudet et al., 2004; Piggott, 2004; Bhagavatula and Singh, 2006; Perez et al., 2006), 4) sampling from the outside (Ferrando et al., 2008; Gobush et al., 2009) or 5) homogenizing the faeces before taking a sample (Wasser et al., 1997; Frantzen et al., 1998; Puechmaille et al., 2007). Samples from the surface of the faeces will thus be more likely to contain the sloughed intestinal cells and yield good DNA quality (Reed et al., 2004; Prugh et al., 2005).

In conclusion, our results are in agreement with most studies (e.g. Santini et al., 2007; Calderon et al., 2009; Reddy et al., 2012) that ethanol is the best method for preservation of DNA. However, it is necessary to point out certain pitfalls associated with the use of ethanol. For example, tubes may leak during transportation when the caps are not properly tightened which may erode/ destroy labelling. Also, there may be transportation restrictions by air because ethanol is highly flammable. As mentioned previously, the amplification success in relation to preservation method might vary depending on study species and condition at study sites. We there-

fore advise that multiple storage media are used until a pilot study has helped in deciding the most promising method.

Acknowledgments We would like to thank the Leventis foundation for providing funds for the field work. We will also like to thank the director and staff of A.P. Leventis Ornithological Research Institute (APLORI) Jos for logistic supports. A big thank you to all the staff of APLORI research centre Yankari Game Reserve; Mr Taiwo Crossby Omotoriogun who was our faithful field assistant at some points during this survey, Haladu Idi and Mr Onoja Joseph for assistance in the field. We also thank the management of Yankari Game Reserve for allowing us to carry out this survey, Dr Yahkat Barshep for statistical advice, and Dr Richard Bergl and Shomboro Karau Dauda who kindly provided the silica gel beads. Finally, we thank Cassandra Silverio for proofreading and editing the final version of this paper. This is contribution no. 69 from A.P.Leventis Ornithological Research Institute.

References

Albaugh GP, Iyengar V, Lohani A Malayeri M, Bala S et al., 1992. Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. Intl. J. Cancer 52: 347–350.

Banks SC, Piggott, MP, Hansen, BD, Robinson NA, Taylor AC, 2002. Wombat coprogenetics: Enumerating a common wombat population by microsatellite analysis of faecal DNA. Aust. J. Zool. 50: 103–204.

Bayes MK, Smith KL, Alberts SC, Altmann J, Bruford MW, 2000. Testing the reliability of microsatellite typing from faecal DNA in the savannah baboon. Con. Gen. 1: 173–176

Bensch S, Andren H, Hansson B, Pedersen H-C, Sand H et al., 2006. Selection for heterozygosity gives hope to a wild population of inbred wolves. PLoS ONE 1(1): e72.doi:10.1371/journal.pone.0000072.

Bhagavatula J, Singh L, 2006. Genotyping faecal samples of Bengal tiger *Panthera tigris tigris* for population estimation: A pilot study. BMC Genetics. 7: 48.

Bidlack AL, Reed SE, Palsbøll PJ, Getz WM, 2007. Characterization of a western North American carnivore community using PCR-RFLP of cytochrome *b* obtained from faecal samples. Cons. Gen 8: 1511–1513.

Crick HQP, Marshall PJ, 1981. The birds of Yankari Game Reserve, Nigeria: Their abundance and seasonal occurrence. Malimbus 3: 103–114.

Ernest HB, Penedo MCT, May BP, Syvaren M, Boyce WM, 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: Genetic analysis using microsatellites and faecal DNA. Mol. Ecol. 9: 433–441.

Ferrando A, Lecis R, Domingo-Roura X, Ponsa M, 2008. Genetic diversity and individual identification of reintroduced otters *Lutra lutra* in north-eastern Spain by DNA genotyping of spraints. Con. Gen. 9: 129–139.

Fernando P, Pfrender ME, Encalada SE, Lande R, 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. Heredity 84: 362–372.

Foran DR, Crooks, KR, Minta SL, 1997. Species identification from scat: An unambiguous genetic method. Wildl. Soc. Bull. 25: 835–839.

Frantz AC, Pope LC, Carpenter PJ Roper TJ, Wilson GJ et al., 2003. Reliable microsatellite genotyping of the Eurasian badger *Meles meles* using faecal DNA. Mol. Ecol. 12: 1649–1661.

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH, 1998. Empirical evaluation of preservation methods for faecal DNA. Mol. Ecol. 7: 1423 –1428.

Friedberg EC, 2000. DNA damage and repair. Nature 421: 436–440.

Geerling C, 1973. The Vegetation of Yankari Game Reserve, Its Utilization and Condition. Ibadan, Nigeria: Department of Forestry, University of Ibadan.

Gerloff U, Schlötterer C, Rassmann K, RAMBOLD I, HOHMANN G al., 1995. Amplification of hypervariable simple sequence repeats (microsatellits) from excremental DNA of wild living bonobos *Pan paniscus*. Mol. Ecol. 4: 515–518.

Gobush K, Kerr B, Wasser S, 2009. Genetic relatedness and the disrupted social structure in a poached population of African elephants. Mol. Ecol. 18:722–734.

Green AA, 1988. Avifauna of Yankari Game Reserve, Nigeria: New records and observations. Malimbus 11: 61–72.

Höss M, Kohn M, Knauer F, Schröder W, Pääbo S, 1992. Excrement analysis by PCR. Nature 359: 199.

Idaghdour Y, Broderick D, Korrida A, 2003. Faeces as a source of DNA for molecular studies in a threatened population of great bustards. Con. Gen. 4: 789–792

Kohn M, Wayne RK, 1997. Facts from faeces revisited. TREE 12: 223–227.

Kohn M, Knauer HF, Stoffella A, Schröder W, Pääbo S, 1995. Conservation genetics of the European brown bear: A study using excremental PCR of nuclear and mitochondrial sequences. Mol. Ecol. 4: 95–103.

Kohn MH, York EC, Kamradt DA, Haught G, Sauvajot RM et al., 1999. Estimating population size by genotyping faeces. Proc. R. Soc. Lond. Bull. 266: 657–663.

Lampa S, Gruber B, Henle K, Hoehn M, 2008. An optimization approach to increase DNA amplification success of otter faeces. Con. Gen. 9: 201–210.

Lindahl T, 1993. Instability and decay of the primary structure of DNA. Nature 362: 709–715

Livia L, Francesca V, Antonella P, Fausto P, Bernardino R, 2007. APCR-RFLP method on faecal samples to distinguish *Martes martes*, *Martes foina*, *Mustela putorius* and *Vulpes vulpes*. Con. Gen. 8: 757–759.

Lucchini V, Fabbri E, Marucco F, Ricci S, Boitani L et al., 2002. Noninvasive molecular tracking of colonizing wolf *Canis lupus* packs in western Italian Alps. Mol. Ecol. 11: 857–868.

Macdonald DW, 1980. Patterns of scent marking with unique urine and faeces amongst carnivore communities. Symp. Zool. Soc. Lond. 45: 107–139.

Maudet C, Luikart G, Dubray D, von Hardenberg A, Taberlet P, 2004. Low genotyping error rates in wild ungulate faeces sampled in winter. Mol. Ecol. Notes. 4: 772–775.

Menotti-Raymond M, David VA, Lyons LA, Schaffer AA, Tomlin JF et al., 1999. A genetic linkage map of microsatellites in the domestic cat *Felis catus*. Genomics 57: 9–23.

Morin PA, Woodruff DS, 1996. Noninvasive genotyping for ver-

tebrate conservation. In: Smith TB, Wayne RK ed. Molecular Genetic Approaches in Conservation. Oxford: Oxford University Press, 298–313.

Murphy MA, Waits LP, Kendall KC, 2000. Quantitative evaluation of faecal drying methods for brown bear DNA analysis. Wildl. Soc. Bull. 28: 951–957.

Murphy MA, Waits LP, Kendall KC, Wasser SK, Higbee JA et al., 2002. An evaluation of long-term preservation methods for brown bear *Ursus arctos* faecal DNA samples. Conserv. Gen. 3: 435–440.

Nsubuga AM, Robbins MM, Roeder AD, Morin A, Boesch C et al., 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Mol. Ecol. 13: 2089–2094.

Panasci M, Ballard WB, Breck S, Rodriguez D, Densmore III LD et al., 2011. Evaluation of faecal DNA preservation techniques and effects of sample age and diet on genotyping success. J. Wildl. Mgt. 75(7): 1616–1624

Perez I, Geffen E, Mokady O, 2006. Critically endangered Arabian leopards *Panthera pardus nimr* in Israel: Estimating population parameters using molecular scatology. Oryx 40: 295–301.

Piggott MP, 2004. Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildl. Res. 31: 485–493.

Piggott MP, Taylor AC, 2003. Extensive evaluation of faecal preservation and DNA extraction methods in Australian native and introduced species. Austr. J. Zool. 51: 341–355.

Prugh LR, Ritland CE, 2005. Molecular testing of observer identification of carnivore faeces in the field. Wildl. Soc. Bul. 33: 189–194.

Puechmaille SJ, Mathy G, Petit EJ, 2007. Good DNA from bat droppings. Acta Chiropterologica 9: 269–276.

Reed JZ, Tollit DJ, Thompson PM, Amos W, 1997. Molecular scatology: The use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Mol. Ecol. 6: 225–234.

Reddy PA, Bhavanishankar M, Bhagavatula J, Harika K, Ranjeet SM et al., 2012. Improved methods of carnivore faecal sample preservation, DNA extraction and quantification for accurate genotyping of wild tigers. PLoS ONE DOI:10.1371/journal.pone.0046732.g003.

Regnaut S, Francoise SL, Fumagalli L, 2006. DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. Con. Gen. 7: 449–453.

Rutledge LY, Holloway JJ, Patterson BR, White BN, 2008. An improved field method to obtain DNA for the identification from wolf scat. J. Wildl. Mgt. 73(8) 1430–1435.

Santini A, Lucchini V, Fabbri E, Randi E, 2007. Ageing and environmental factors affect PCR success in wolf *Canis lupus* excremental DNA samples. Mol. Ecol. Notes. 7(6): 955–961.

Stenglein JL, deBarba M, Ausband DE, Waits LP, 2010.Impacts of sampling location within a faeces on DNA quality in two carnivore species. Mol. Ecol. Res.10: 109–114.

Taberlet P, Camarra JJ, Griffin S Uhrès E, Hanotte O et al., 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear. Mol. Ecol. 9: 869–876.

Taberlet P, Waits LP, Luikart G, 1999. Non-invasive genetic sampling: Look before you leap. TREE. 14: 323–327.

Tende T, Ottosson U, Hansson B, Åkesson M, Bensch S, 2010. Population size of lions in Yankari Game Reserve as revealed by faecal DNA sampling. Afr. J. Ecol. 59: 7–12.

Vallet D, Petit E, Gatti S, Levréro F, Ménard N, 2007. A new 2CTAB/PCI method improves DNA amplification success from faeces of Mediterranean (Barbary macaques) and tropical (lowland gorillas) Primates. Con. Gen. 9: 677–680

Vynne C, Baker MR, Breuer ZK, Wasser SK, 2011. Factors influencing degradation of DNA and hormones in maned wolf scat. Anil. Con. 15: 184–194

Wasser SK, Houston CS, Koehiler GM, Cadd GG, Fain SR, 1997. Techniques for applications of faecal DNA methods to field studies of Ursids. Mol. Ecol. 6: 1091–1097.

Wasser SK, Keim JL, Taper ML, Lele SR, 2011. The influences of wolf predation, habitat loss, and human activity on the caribou and moose in the alberta oil sands. Front Ecol. Env. 9 (10): 546–551.

Zhang HM, Guo Y, Li DS, Wang PY, Fang SG, 2009. Sixteen novel microsatellite loci developed for the giant panda *Ailuropoda melanoleuca*. Conser. Gen. 10: 589–592.

Appendix 1 Overview of studies comparing different preservation media for faecal sample DNA

Authors (Year)	Study species /Climate at study sites	Preservation methods	Relative success / (% success)
The present study	Lion *Panthera leo* Tropical, dry	i) 95% ethanol ii) Two-step storage* iii) Buffer ASL	Highest (50%) Intermediate (20%) Lowest (30%)
Reddy et al.(2012)	Tiger *Panthera pardus* Temperate, dry	i) Two-step storage* ii) Ethanol iii) Silica beads only	Highest Intermediate Lowest
Calderon et al.(2009)	Forest ungulates (*Cephalophus* spp.) Tropical, humid	i) RNA*later* ii) 95% Ethanol iii) Silica beads only	Highest Intermediate Lowest
Santini et al. (2007)	Wolf *Canis lupus* Temperate, dry	i) i) 95% Ethanol at freezer -20° ii) GUS lysis buffer** iii) Only freezer -20° iv) 95% Ethanol at room temperature	Highest (98%) Second best (92%) Intermediate (71%) Lowest (55%)
Nsubuga et al.(2004)	Mountain gorilla *Gorilla beringei beringei* Chimpanzee *Pan troglodytes versus* Tropical, humid	i) Two-step storage* ii) Silica beads only iii) RNA*later* solution	Highest Intermediate Intermediate
Roeder et al. (2004)	Gorilla *Gorilla gorilla* Temperate, dry	i) 90% Ethanol ii) Two-step storage* iii) Silica beads only	Highest Second best Lowest
Frantz et al. (2003)	Eurasian Badger *Meles meles* Temperate	i) 70% Ethanol ii) DETs *** iii) Freezing at -20°C	Highest (89%) Intermediate Lowest
Piggot & Taylor (2003)	Tasmanian pademelon *Thylogale billardierii* Temperate	i) Freezing at -20°C ii) 70% Ethanol iii) Dried at room temperature iv) DETs buffer***	High (55%) High (55%) High (55%) Lowest (40%)
Murphy et al. (2002)	Brown bear *Ursus arctos* Temperate	i) 90% Ethanol ii) Silica beads only iii) Oven-dried (stored at room temperature) iv) Oven-dried (stored at -20°C)	Highest (86%) Intermediate Lowest Lowest
Murphy et al. (2000)	Brown bear *Ursus arctos* Temperate	i) Freeze-drying ii) Oven drying iii) Silica beads only iv) Microwave drying	Highest (98%) Second best (95%) Lowest Lowest
Frantzen et al. (1998)	Baboons *Papio cynocephalus ursinus* Temperate - Subtropical	i) DETs buffer*** ii) Air-dried at room temperature iii) Frozen at -20°C iv) 70% ethanol	Highest (70%) Second best (67%) Intermediate (60%) Intermediate (61%)
Wasser et al. (1997)	American black bear *Ursus americanus* Sun bear *Helarctos malayanus* Temperate, dry	i) Silica beads only ii) Ethanol iii) Frozen at -20°C	Highest Intermediate Lowest
Panasci et al. (2011)	Coyote *Canis latrans*	i) 95% ethaol ii) DETs buffer*** iii) Lysis buffer	High High Lowest

*Ethanol and silica gel beads

** 3M guanidine thiocyanate, 50 mM Tris-HCl pH 7.0, 25 mM EDTA pH 8.0, 25% Triton X-100

***20% DMSO, 0.25 M EDTA, 100 mM Tris, pH 7.5 and NaCl to saturation