Conservation Genetics **1:** 173–176, 2000.
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Testing the reliability of microsatellite typing from faecal DNA in the savannah baboon

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Received 3 February 2000; accepted 11 April 2000

Key words: baboons, faeces, microsatellites, non-invasive sampling, primates

Considerable debate has surrounded the potential of faecal material for non-invasive genetic studies of wild animal populations (e.g. Taberlet et al. 1996; Taberlet and Waits 1998). Whilst faecal material is becoming a more widely used and accepted source of DNA for mitochondrial DNA amplification, concern has been expressed about its suitability for the amplification of single copy nuclear DNA markers such as microsatellite loci. While several studies have used high-quality DNA sources such as blood as a species control (Kohn et al. 1995; Gerloff et al. 1995; Van der Kuyl et al. 1996; Paxinos et al. 1997), we present one of the few tests using matched comparisons between faeces and high-quality DNA sources of known individuals (Reed et al. 1997; Wasser et al. 1997; Launhardt et al. 1998). Further, only two other studies have performed Mendelian checks on known mother-offspring pairs (Launhardt et al. 1998; Smith et al. 2000). Here we report the results of genotyping 12 individuals at 8 loci, measuring amplification success rate and genotype fidelity (especially 'allelic dropout'; Gagneux et al. 1997) in faecal DNA, by comparing DNA extracted from faeces and blood in savannah baboons from Amboseli, Kenya.

Blood samples were collected and stored as described in Altmann et al. (1996). Faecal samples were collected within minutes of defecation, stored in 95% ethanol at room temperature in the field, and then stored at −70 °C. DNA was extracted from blood using standard methods (Bruford et al. 1998). DNA was extracted from faeces using the QIAamp® DNA Stool Mini Kit (Qiagen® GMBH, Hilden, Germany), on spin columns following the manufacturer's protocol. DNA was eluted in 100 μl of TE buffer. From this 2 μl were used for each PCR reaction. When PCR reactions failed to produce a product, template DNA solutions were vacuum dried and resuspended in 50 μl of TE buffer. Extracted DNA was stored at −20 °C for the long term or at 4 °C for immediate use.

Twenty-one human microsatellite primer sets (D1S207, D1S533, D1S548, D1S550, D2S141, D3S1768, D4S243, D4S431, D5S1457, D6S271, D6S311, D7S503, D7S817, D10S611, D11S925, D13S159, D14S306, D16S402, D16S420, D17S791, and D21S441) which were known to amplify DNA derived from baboon blood were tested on faecally-derived DNA. Eight of these: D4S243, D5S1457, D6S271, D7S503, D10S611, D13S159, D14S306, and D16S402 (see Altmann et al. (1996) for PCR protocols) amplified polymorphic DNA derived from faeces. Primers were end-labeled with fluorescent dyes for visualisation on an ABI 377 genetic analyser (for further details see Ciofi et al. 1998).

All blood extractions originated from a single blood sample per individual and each individual was typed 1–6 times per locus (x = 2, s.d. ± 1 times per locus). Faecal extractions originated from 1–5 distinct faecal samples per individual.

To compare resulting genotypes from different source materials a total of 96 unique (individual by locus) genotypes were obtained using both blood and

Table 1. Total number of successful amplifications for this study (n = 407). Where there is in inconsistency in scoring the cell is highlighted. Adjacent to each genotype is the number of times the true genotype was achieved. Cells denoted '–' indicate where no successful amplifications were produced. Cells in bold italics indicate where spurious bands were produced

Individual	Sample type	Sample number	D4S243		D5S1457		D6S271		D7S503		D10S611		D13S159		D14S306		D16S402	
A	Blood		164/172	1/1	130/138	1/1	166/180	1/1	154/162	1/1	152/184	1/1	168/178	1/1	176/180	1/1	146/154	2/2
A	Faeces	1	164/172	1/1	130/138	1/1	166/180	3/3	154/162	4/4	152/184	1/1	168/178	2/2	–	–	146/154	1/1
A	Faeces	2	164/172	1/1	–	–	166/180	1/1	154/162	4/4	–	–	168/178	3/4	176/180	1/1	146/154	2/2
A	Faeces	3	164/172	1/1	130/138	1/1	–	–	154/162	2/2	–	–	168/178	1/1	176/180	1/1	–	–
A	Faeces	4	164/172	1/1	–	–	–	–	154/162	2/2	–	–	–	–	–	–	–	–
A	Faeces	5	164/172	1/1	–	–	–	–	154/162	1/1	–	–	–	–	–	–	–	–
B	Blood		164/172	3/3	112/126	2/2	188/196	2/2	154/162	3/3	184/188	1/1	168/178	6/6	172/176	1/1	146/146	2/2
B	Faeces	1	164/172	2/2	–	–	–	–	154/162	3/3	184/188	2/2	168/178	1/1	172/176	2/2	***146/192***	0/1
B	Faeces	2	164/172	1/1	112/126	2/2	196/196	0/1	154/162	2/3	–	–	168/178	2/2	–	–	146/146	3/3
B	Faeces	3	–	–	–	–	188/196	1/1	154/162	1/1	–	–	168/178	1/1	–	–	146/146	1/1
C	Blood		168/172	2/2	126/130	1/1	170/198	1/1	164/168	3/3	184/184	1/1	166/172	2/2	176/176	2/2	146/160	1/1
C	Faeces	1	168/172	1/1	–	–	–	–	164/164	0/1	184/184	2/2	166/172	2/2	176/176	3/3	–	–
C	Faeces	2	168/172	1/1	126/130	1/1	170/198	2/3	164/168	1/1	–	–	–	–	–	–	146/160	2/2
D	Blood		166/170	1/1	118/122	2/2	180/180	2/2	154/154	3/3	152/188	2/2	166/172	3/3	176/184	1/1	146/154	1/1
D	Faeces	1	166/170	2/2	118/122	1/1	180/180	1/1	–	–	152/188	1/1	166/172	3/3	–	–	146/146	0/1
D	Faeces	2	166/170	2/2	118/122	1/1	180/180	1/1	***154/154***	2/3	152/152	2/2	–	–	–	–	146/154	2/2
D	Faeces	3	–	–	118/122	2/2	–	–	–	–	–	–	166/172	1/1	176/184	1/2	–	–
E	Blood		146/164	2/3	122/130	1/1	166/188	1/1	158/164	2/2	184/184	1/1	168/178	3/3	172/176	1/1	146/146	1/1
E	Faeces	1	146/164	1/1	–	–	166/188	1/1	158/164	1/1	184/184	3/3	168/178	1/1	176/176	0/1	146/146	2/2
E	Faeces	2	146/164	1/1	122/130	2/2	166/188	1/1	158/164	1/1	–	–	168/178	1/1	172/176	3/3	–	–
F	Blood		164/164	1/1	122/130	1/1	170/188	2/2	160/164	3/3	184/192	1/1	168/178	2/2	176/180	1/1	146/146	A
F	Faeces	1	164/164	1/1	–	–	–	–	160/164	1/1	–	–	168/178	2/2	–	–	–	–
F	Faeces	2	164/164	1/1	122/130	1/1	170/188	2/3	–	–	184/192	2/2	168/178	1/1	176/180	2/2	146/146	2/2
G	Blood		168/172	3/3	130/134	2/2	166/172	1/1	154/154	1/1	184/192	1/1	164/170	1/2	180/180	2/2	146/146	1/1
G	Faeces	1	172/172	0/1	–	–	–	–	154/154	2/2	–	–	164/170	1/1	180/180	1/1	***146/146***	1/2
G	Faeces	2	–	–	130/134	2/2	166/172	2/2	–	–	184/192	2/2	–	–	–	–	146/146	2/2
H	Blood		168/172	1/1	122/126	1/1	164/164	1/1	164/168	3/3	152/188	2/2	166/168	1/1	176/180	1/1	146/146	1/1
H	Faeces	1	168/172	1/1	122/126	1/1	–	–	–	–	152/188	1/1	166/168	1/1	176/180	1/1	146/146	2/2
H	Faeces	2	–	–	122/126	1/1	164/164	2/2	164/168	2/2	–	–	166/168	1/1	–	–	–	–
I	Blood		176/180	3/3	134/138	1/1	182/182	1/1	154/156	1/1	180/196	1/1	170/170	4/4	150/180	1/1	146/146	2/2
I	Faeces	1	176/180	3/3	134/138	2/3	–	–	154/156	2/3	–	–	170/170	2/2	150/180	1/2	146/146	2/2
I	Faeces	2	–	–	–	–	182/182	2/2	–	–	180/196	1/1	–	–	–	–	–	–
J	Blood		164/180	1/1	130/138	1/1	166/170	1/1	168/168	1/1	152/192	2/2	162/170	2/2	172/180	1/1	154/174	3/3
J	Faeces	1	–	–	–	–	–	–	168/168	3/3	–	–	170/170	0/1	172/180	2/2	154/174	2/3
J	Faeces	2	164/180	1/1	130/138	3/3	166/170	2/2	–	–	152/192	2/2	–	–	–	–	–	–
K	Blood		164/164	2/2	134/138	6/6	166/166	2/2	154/168	3/3	184/188	1/1	166/178	6/6	172/180	1/1	146/154	2/2
K	Faeces	1	–	–	134/138	1/1	–	–	154/168	1/1	184/188	1/1	166/178	1/1	–	–	146/154	2/3
K	Faeces	2	164/164	3/3	134/138	2/2	166/166	3/3	–	–	–	–	166/178	1/1	172/180	1/1	–	–
L	Blood		172/176	1/1	126/130	1/1	166/170	2/2	158/168	1/1	188/188	1/1	164/178	6/6	172/180	1/1	146/146	1/1
L	Faeces	1	–	–	126/130	1/1	166/170	2/4	158/168	1/1	188/188	2/2	164/178	3/3	172/180	1/1	146/146	2/2
L	Faeces	2	172/176	1/1	–	–	–	–	–	–	–	–	164/178	2/2	–	–	–	–

faeces (12 individuals by 8 loci). To investigate the reproducibility between faecal samples we collected faeces from a number of individuals on different days (2–5 samples per individual), and were able to compare consistency between samples for 33 genotypes. For within-blood comparisons of reproducibility, 42 genotypes were typed 2–6 times (Table 1).

To establish the true genotype, all loci were typed repeatedly for each individual using DNA from both source materials. A unique genotype was resolved by accepting the score of the majority of repeats. In accordance with Taberlet et al. (1996), if the only source of discrepancy between the two tissue sources was the absence of one allele, we considered the resolved genotype to comprise both alleles. For each faecal sample, the majority genotype for that sample was used for the comparison so as not to include multiple, potentially non-independent values

per sample. The consistency across samples is indicated in Table 1 where the number of replicates per sample follows the resolved genotype, as described in the table footnote.

A total of 515 amplifications were performed (407 successful) to produce the 96 genotypes for which both DNA sources were used and for which heterozygotes were amplified at least twice and homozygotes were amplified at least three times. As expected, the proportion of amplifications from which genotypes could be scored was significantly lower for faecally-derived DNA (70%, 238/340) than for blood (97%, 169/175) when all amplifications were considered (binomial test, nominal $p < 10^{-3}$). When considering the incidence of allelic dropout in independent samples there was a significant difference (binomial test, $p < 10^{-2}$) between the two sample types; 8% (18/238) of faecal amplifications and 1% (2/169) of blood amplifications were subject to allelic dropout (Table 1, individual E at D4S243 for example). In addition to allelic dropout, we also found three cases where spurious bands were amplified from faecally-derived DNA (Table 1, see individuals B and G at D16S402 and individual D at D7S503). Genotype inconsistencies between the two DNA sources were due to allelic dropout and occurred when only one amplification was carried out with each source (Table 1, G at D4S243 and J at D13S159).

We were able to perform Mendelian checks on 48 of the 96 genotypes analysed. All Mendelian checks in this study passed; for example individuals E (daughter) and F (mother) share an allele at all 8 loci. Of the further 40 genotypes, all offspring shared at least one allele with their mothers. These Mendelian checks further support the 96 tissue-matched genotypes resolved in this study.

We show a difference between blood and faecal DNA as efficient sources for microsatellite amplification, in both initial amplification success rates and instances of allelic drop out. However, despite this difference, our amplification success rate for faecally-derived material is high enough to justify the use of faeces in this and other studies of wild populations. Further, since the level of agreement among genotypes generated in the same individual using blood and faeces was 98% (94/96 genotypes), and because faecal genotype fidelity was 91% (217/238 genotypes), faeces proved to be a viable source of DNA. Given the higher levels of allelic dropout observed here and in other studies when using faeces, the need for repeated amplification as a general validation tool is recommended, but the extent to which this needs to be carried out will vary on a case-by-case basis and is likely to be locus-specific. In this case, to reliably genotype each individual, we type each heterozygote twice and each homozygote at least three times for all loci.

It is important to highlight the fact that here samples were routinely collected within minutes of defecation, and all individuals were identifiable. Therefore, repeat samples could be ascribed to the same source, and the material was generally in good condition. Other populations and species, where it is less easy to identify individuals or where faeces are more difficult to find, may prove more difficult to type non-invasively.

Acknowledgements

For permissions and support during fieldwork, we are grateful to the Office of the President, Republic of Kenya; Kenya Wildlife Services, its Amboseli staff and Wardens; the Institute of Primate Research, R. Leakey, D. Western, J. Else, C.S. Bambra, M. Isahakia, and to C. Mlay. Colleagues who collected additional samples were R. Altmann, G. Marinka, S. Mwtengere, R. Mututua, S. Sayialel, D. Shimizu, K. Warutere, and E. Whittaker. Carole Ober provided valuable insights. Other financial support was provided primarily by the Zoological Society of London and the Chicago Zoological Society, NSF IBN-9223335, NSF IBN-9422013, and NSF IBN-9729586.

References

Altmann J, Alberts S, Coote T et al. (1996) Behavior predicts genetic structure in a wild primate group. *Proc. Nat. Acad. Sci. USA*, **93**, 5797–5801.

Bruford MW, Hanotte O, Brookfield JFY, Burke T (1998) Multi- and single locus DNA fingerprinting. In: Molecular *Analysis of Populations: A Practical Approach*, 2nd edn (ed. Hoelzel AR), pp. 287–336. IRL Press, Oxford.

Ciofi C, Funk SM, Coote T et al. (1998) Genotyping with microsatellite markers. In: *Molecular Tools for Screening Biodiversity: Plants and Animals* (eds. Karp A, Isaac PG, Ingram DS), pp. 195–201. Chapman and Hall, London.

Gagneux P, Boesch C, Woodruff DS (1997) Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Mol. Ecol*., **6**, 861–868.

Gerloff U, Schlotterer C, Rassmann E et al. (1995) Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Mol. Ecol*., **4**, 515–518.

Kohn M, Knauer F, Stoffella A et al. (1995) Conservation genetics of the European brown bear – a study using excremental PCR of nuclear and mitochondrial sequences. *Mol. Ecol.*, **4**, 95–103.

Van der Kuyl AC, Dekker JT, Goudsmit J (1996) St. Kitts green monkeys originate from West Africa: genetic evidence from feces. *Am. J. Primatol.*, **40**, 361–364.

Launhardt K, Epplen C, Epplen JT, Winkler P (1998) Amplification of microsatellites adapted from human systems in faecal DNA of wild Hanuman langurs, *Presbytis entellus*. *Electrophoresis*, **19**, 1356–1361.

Paxinos E, McIntosh C, Ralls K, Fleischer R (1997) A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. *Mol. Ecol.*, **6**, 483–486.

Reed JZ, Tollit DJ, Thompson PM et al. (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Mol. Ecol.*, **6**, 225–234.

Smith KL, Alberts SC, Bayes MK et al. (2000). Cross-species amplification, non-invasive genotyping, and non-Mendelian inheritance of human STRPs in savannah baboons. *Am. J. Primatol.*, **51**, 219–227.

Taberlet P, Griffin S, Goossens B et al. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res.*, **24**, 3189–3194.

Taberlet P, Camarra JJ, Griffin S et al. (1997) Non-invasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.*, **6**, 869–876.

Wasser SK, Houston CS, Koehler GM, Cadd GG, Fain SR (1997) Techniques for application of faecal DNA methods to field studies of Ursids. *Mol. Ecol.*, **6**, 1091–1097.