

# **Microsatellite repeat motif and amplicon length affect amplification success of degraded faecal DNA**

Alida de Flamingh<sup>a</sup>, Catherine L. Sole<sup>b</sup>, Rudi J. van Aarde<sup>a</sup>

<sup>a</sup> Conservation Ecology Research Unit, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

<sup>b</sup> Scarab Research Group, Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

**INDEXING KEYWORDS:** repeat motif, amplicon length, degraded DNA, faecal DNA, African elephant, *Loxodonta africana*

**CORRESPONDING AUTHOR:** Alida de Flamingh

**POSTAL ADDRESS:** Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa

**FAX:** +27 12 420 4523

**TELEPHONE:** +27 12 420 4522

**EMAIL:** adeflamingh@gmail.com

**RUNNING TITLE:** Repeat motif and amplicon length affect degraded DNA amplification

**WORD COUNT:** 942 (including the abstract, body and acknowledgements and figure legends)

## **ABSTRACT**

Degradation reduces DNA quality and quantity in faecal samples and leads to low amplification success. We investigated the influence of repeat motif and amplicon length by comparing the amplification success of five dinucleotide, five tetranucleotide, and two compound microsatellite markers for African elephant (*Loxodonta africana*) faecal DNA samples. We found that both repeat motif and amplicon length influenced amplification success, and suggest the use of simple microsatellite markers containing alleles with small amplicon sizes to maximise amplification success of degraded DNA.

## **INTRODUCTION**

Faecal samples often contain low quality and quantity of DNA (Murphy et al. 2007) and therefore require the use of methods that may increase amplification success (e.g. Piggot et al. 2004; Lampa et al. 2008). Marker repeat motif and amplicon length have inconsistent effects on amplification success (Broquet et al. 2007; Ishida et al. 2012). It may be necessary to choose markers with the highest likelihood of amplification success. The influence of repeat motif and amplicon length on amplification success may dictate marker choice. Our case study on faecal samples from free-ranging African elephants (*Loxodonta africana*) (categorised as vulnerable according to the IUCN redlist) therefore investigates the influence of marker repeat motif and amplicon length on the amplification success of five dinucleotide, five tetranucleotide, and two compound microsatellite markers.

## METHODS

### *Laboratory Analysis*

One-hundred African elephant faecal samples, collected (following Vidya and Sukumar, 2005) in the Kavango-Zambezi region (southern Africa), were genotyped for 12 microsatellite markers (Table 1 Online Resources). Our DNA extraction protocol followed the Qiagen (Southern Cross Biotechnology, Cape Town, SA) DNA stool mini kit protocol for human stool, but included increased vortex, centrifuge and incubation times. We used the Qiagen Multiplex PCR kit and Applied Biosystems 3130 xl and 3500 xl automated sequencers (Life Technologies, Carlsbad, US) for fragment analysis, and GeneMapper ® Software version 3.7 (Applied Biosystems, Foster City, USA) to view and score data. The genotyping process (PCR and fragment analysis) was repeated up to four times, after which unclear genotypes were scored as missing data. We calculated amplification success as the number of individuals for which genotypes could not be determined divided by the total number of individuals that were genotyped per locus (n=100), where amplification success decreases as missing data increases.

### *Statistical Analysis*

We used linear regression analysis to assess amplification success as a function of amplicon length (in base pairs of the largest allele size). We used unpaired two-tailed t-tests to determine if dinucleotide and tetranucleotide amplicon length differed significantly, and to compare the amplification success of simple<sup>1</sup> (dinucleotide markers (n=5)) and complex markers (tetranucleotide (n=5) and compound markers (n=2)). Statistical analyses were done using GraphPad Prism version 3.00 (GraphPad Software, San Diego California, USA). We identified

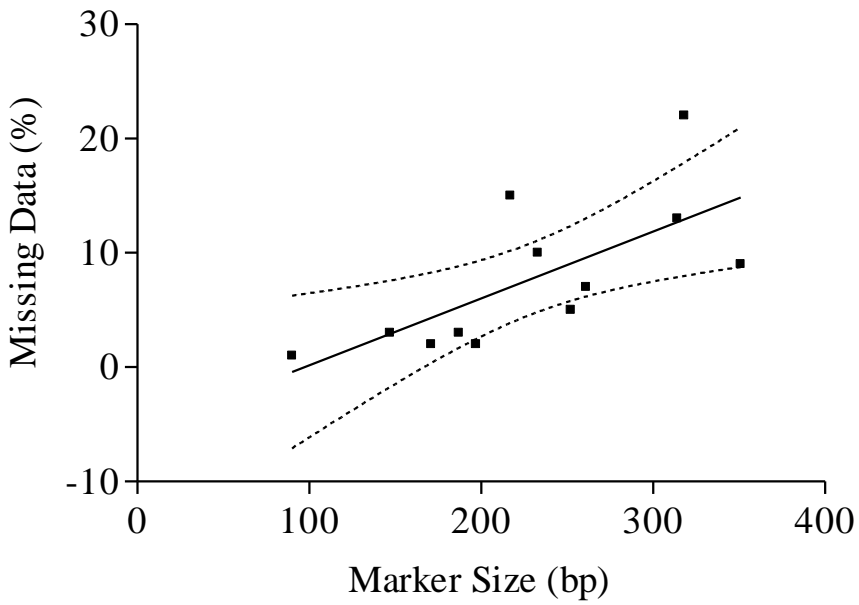
---

<sup>1</sup>In the context of this paper, we use the word “simple” to refer to the less complicated repeat motif of dinucleotide markers as compared to more complex repeat motifs of tetranucleotide or compound microsatellite markers.

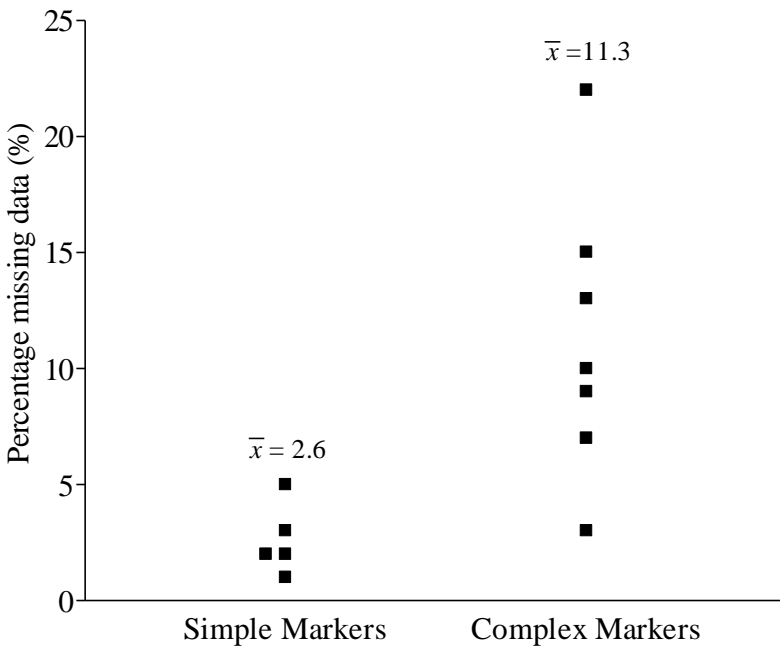
genotype errors by comparing observed and expected allele frequencies and calculated significance values using Fisher's probability test in the program MICROCHECKER<sup>®</sup> version 2.2.3 (Van Oosterhout et al., 2004). We excluded these genotype errors in the amplification success calculations.

## **RESULTS**

Missing data increased with amplicon length (Slope=0.059 ± 0.019;  $F_{10}=9.33$ ;  $p<0.05$ ) and nearly half ( $r^2=0.48$ ) of the variability in missing data could be explained by differences in the marker amplicon length (Fig. 1). There was no significant difference ( $t=1.56$ ;  $df=8$ ;  $p>0.5$ ) between the length of dinucleotide (mean=171.4;  $SD=59.94$ ) and tetranucleotide (mean=232.6;  $SD=64.47$ ) amplicons (Fig. 1 Online Resources). Simple microsatellite markers (mean=2.6,  $SD=1.52$ ) had significantly fewer missing data ( $t=3.06$ ;  $df=10$ ;  $p<0.05$ ) than complex markers (mean=11.3;  $SD=6.13$ ) (Fig. 2). Five markers showed excessive homozygotes (Table 2 Online Resources), but no locus showed evidence of scoring error due to stuttering or large allele dropout. Two loci (Lat18 and FH19 – Table 2 Online Resources) had excessive homozygotes at more than one size class.



**Fig. 1** Missing data per locus is significantly positively related (Slope= $0.59 \pm 0.019$ ;  $F_{10}=9.33$ ;  $P<0.05$ ;  $r^2 = 0.48$ ) to amplicon length. The regression line (least squares line) is indicated by the solid black line with the 95% CI indicated by the dotted lines.



**Fig. 2** Simple microsatellite markers (n=5; mean =2.60, SD=1.52) had significantly less missing data ( $t=3.06$ ;  $df=10$ ;  $p<0.05$ ) than complex markers (n=7; mean=11.33; SD=6.13).

## **DISCUSSION**

Amplification success decreased with increasing amplicon length, thus supporting earlier findings of Ishida et al. (2012) who redesigned microsatellite primers to produce shorter amplicons that were more effective in amplifying faecal DNA than the original primers were. In our study repeat motif also influenced amplification success and simple markers had significantly fewer missing data than complex markers. Complex markers may have more difficulty amplifying short fragmented strands of degraded DNA. The similarity in amplicon lengths of dinucleotide and tetranucleotide markers suggests that complexity, rather than length alone, influences amplification success.

We do not know if the excessive homozygotes present in five of our markers occurred naturally in the population or whether they were due to genotype errors. The latter may require the incorporation of allele adjustments provided in MICROCHECKER<sup>®</sup> and/or the elimination of markers Lat18 and FH19 for future research.

We recognise that repeat motif and amplicon length are only two of a multitude of factors that influence amplification success. For example, field conditions (Murphy et al. 2007), preservation methods (Frantzen et al. 1998), and animal diet (e.g. PCR inhibitors in plants (Huber et al. 2002)) may also influence amplification success.

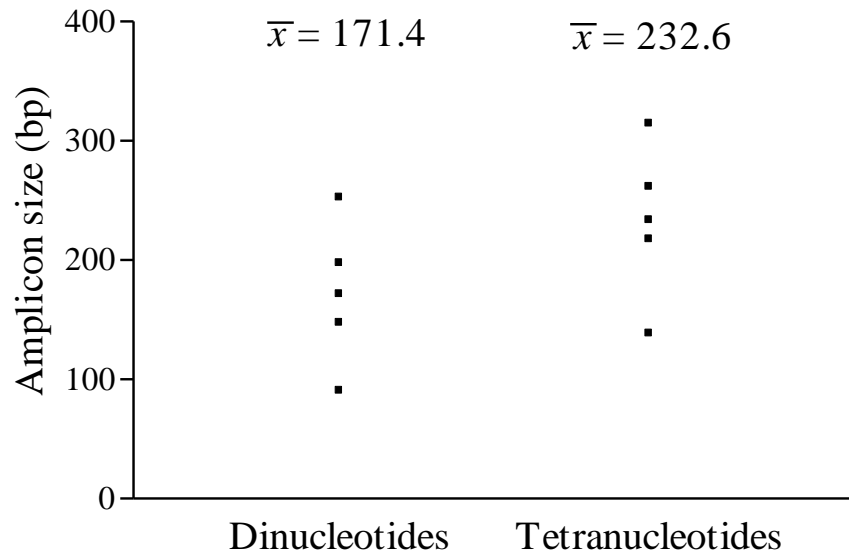
## **ACKNOWLEDGEMENTS**

Thanks to the Conservation Ecology Research Unit team, the International Fund for Animal Welfare, the Zambian Wildlife Authority, the Department of Wildlife and National Parks (Botswana), Elephants Without Borders, and the Conservation Foundation (Zambia) for facilitating sample collection and funding this project.

## REFERENCES

- Broquet T, Ménard N, Petit E (2007) Noninvasive population genetics: a review of sample source, diet. Fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conserv Genet* 8:249-260
- Frantzen MAJ, Silk JB, Ferguson JWH et al (1998) Empirical evaluation of preservation methods for faecal DNA. *Mol Ecol* 7:1423-1428
- Huber S, Burns U, Arnold W (2002) Sex determination of red deer using polymerase chain reaction of DNA from feces. *Wildl Soc Bull* 30:208-212
- Ishida Y, Demeke Y, van Coeverden de Groot PJ et al (2012) Short amplicon microsatellite markers for low quality elephant DNA. *Conserv Genet Resour* 4:491-494
- Lampa S, Gruber B, Henle K, Hoehm M (2008) An optimisation approach to increase DNA amplification success of otter faeces. *Conserv Genet* 9:201-210
- Murphy MA, Kendall KC, Robinson A, Waits LP (2007) The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. *Conserv Genet* 8:1219-1224
- Piggot MP, Bellemain E, Taberlet P, Taylor AC (2004) A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conserv Genet* 5:417-420
- Vidya TNC, Sukumar R (2005) Amplification success and feasibility of using microsatellite loci amplified from dung to population genetic studies of the Asian elephant (*Elephas maximus*). *Current Science*, 88:489-492

## Supplementary Material



**Fig. 1** The length (bp) of the dinucleotide marker amplicons (n=5; mean=171.4; SD=59.94) did not differ significantly ( $t=1.56$ ;  $df=8$ ;  $p>0.1$ ) from the length of tetranucleotide amplicons (n=5; mean=232.6; SD=64.47).

**Table 1** Microsatellite marker names and individual marker characteristics

Marker Name	Repeat Motif	Length (bp)	Missing Data (%)	Marker Source
LaT08	(TAGA)16	181-217	15%	Archie et al., 2003
Lat13	(CATC)21	237-261	7%	Archie et al., 2003
Lat17	(GGAT)15...	323-351	9%	Archie et al., 2003
Lat18	(GGAT)10 (CCAT)22	286-314	13%	Archie et al., 2003
Lat24	(GGAT)22	201-233	10%	Archie et al., 2003
FH1	(CA)12	76-90	1%	Comstock et al., 2000
LA3	(CA)10	163-171	2%	Eggert et al., 2000
FH39	(CA)18	232-252	5%	Comstock et al., 2000
FH102	(CT)11(CA)14	169-187	3%	Comstock et al., 2000
FH19	(CA)15	173-197	2%	Comstock et al., 2000
LA5	(CA)13	131-147	3%	Eggert et al., 2000
Lat25	(CCAT)15	298-138	22%	Archie et al., 2003

**Table 2** The total observed and expected homozygotes (as a percentage of the alleles) calculated using a cumulative binomial distribution (Weir 1996) (observed) and randomised genotypes (expected) for five microsatellite loci that



showed possible null allele amplification through a significant excess of homozygotes (Fisher's combined probability < 0.05). Marker names in **bold** are those loci that showed an excess of homozygotes at more than one size-class (allele).

Marker Name	Expected homozygotes (% of alleles)	Observed homozygotes (% of alleles)
Lat13	28.32	41
<b>Lat18</b>	15.41	30
FH39	20.80	34
<b>FH19</b>	30.40	45
Lat25	17.62	29

### Supplementary Material References

Archie EA, Moss CJ, Alberts SC (2003) Characterization of tetranucleotide microsatellite loci in the African Savannah Elephant (*Loxodonta africana africana*). Mol Ecol 3:244-246

Comstock KE, Wasser SK, Ostrander EA (2000) Polymorphic microsatellite DNA loci identified in the African elephant (*Loxodonta africana*). Mol Ecol 9:993-1011

Eggert LS, Ramakrishnan U, Mundy NI, Woodruff DS (2000) Polymorphic microsatellite DNA markers in the African elephant (*Loxodonta africana*) and their use in the Asian elephant (*Elephas maximus*). Mol Ecol 9:2155-2234

Weir BS (1996) Genetic data analysis II: methods for discrete population genetic analysis, Sinauer Associates, Sunderland, Massachusetts