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MICROSATELLITE LETTERS

Using next-generation sequencing methods to isolate and characterize 24 simple sequence repeat loci in mandrills (*Mandrillus sphinx*)

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Abstract Mandrill is a vulnerable Old World primate living in the rain-forests of central Africa. This species is currently facing two major human encroachments: habitat destruction and bush-meat trade. The total population size remains unknown in the wild, but it is suspected to have recently declined. We developed and characterized 24 new polymorphic microsatellite markers from the next-generation sequencing data using 66 individuals from a wild population. The number of alleles per locus ranged from 2 to 9 and the observed heterozygosity from 0.12 to 0.92. Conveniently, the developed markers did not amplify human DNA avoiding cross-species contamination. These microsatellites will be especially useful for studies based on sensible DNA, including population genetics analyses to studies in behavioral ecology.

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Mandrill (*Mandrillus sphinx*) is a forest-dwelling primate endemic to a restricted part of the Equatorial forests of central Africa, extending from Southern Cameroon through Equatorial Guinea and Gabon, to Southern Congo (Oates and Butynski 2008). Mandrills are classified as Vulnerable (status: A2cd) by the IUCN (Oates and Butynski 2008) and face two major threats: destruction and fragmentation of their natural habitat and bush-meat trade. Because studying mandrills in the wild is challenging (closed habitat, nomadic lifestyle), the total population size remains unknown, but it is suspected to have recently declined (Oates and Butynski 2008).

From 2002 to 2006, 65 captive-born mandrills from a medical research centre (CIRMF) were released into a private park in Southern Gabon (Lekedi Park, Bakoumba). These released individuals reproduced with wild mandrills as early as 1 year post-release (Peignot et al. 2008). In 2012, a long-term project was initiated to study this unique habituated population ('Mandrillus Project': http://www.cefe.cnrs.fr/mandrillus/presentation), and in 2014, the population numbered about 120 individuals, including less than 20 % of captive-born founders.

As in many other non-human primates, microsatellite amplifications of DNA from captive mandrills were initially performed with human primers (Charpentier et al. 2005). Despite their high availability, they generally amplify only weakly with mandrill DNA because of the genetic distance that separates this primate from humans. Consequently, the proportion of loci yielding replicable and reliable results was generally low.

In 2012 and 2013, 66 animals of the study population were anaesthetized by blowpipe intramuscular injection of

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Table 1 Genetic information of 24 new primers

Locus id	Multiplex id	Primer sequence (left)	Primer sequence (right)	Repeat motif	Allele size	N	Na	Ho/He	Genbank accession No
MaCh0007	1	TGGAATTTAGTCAGGGGTTCC	TGCCAGCTTCCATAATCACA	TCTA	184–208	66	6	0.82/0.79	KJ881176
MaCh0063	1	TAGGTGGGTGAATGAATGCG	AAACACTGCCTACATGACTCG	TGGA	89–109	66	6	0.76/0.80	KJ881177
MaCh0070	1	CTATCGTGGAACCTTGCGAT	CTATTTTTCACCCTGCCCAA	TATC	181–213	63	7	0.49/0.76*	KJ881178
MaCh0184	1	ATGGCAAGGATGTGACCTTT	AGGGTTACCCGTAGAACTGAG	AC	212–230	65	7	0.75/0.76	KJ881181
MaCh0409	1	AGCTCTTGCCCTCTCCTTTC	CAAGCTGGATGCTGTGAAGA	CTAT	176–200	66	6	0.70/0.71	KJ881186
MaCh0705	1	TCCAAAAGGAAATTAAGCTGG	GCTGAGGAATGTGCCTGATT	AAC	88–106	42	6	0.14/0.67*	KJ881192
MaCh0868	1	TCATCTGTCATTATCTGTCTGACTGT	GGCGGAATGAATAGATAGAGAC	TCTA	85–117	64	7	0.41/0.53*	KJ881174
MaCh0891	1	TGGATGATTGATGATGGATGA	TAGTGGTATGGGTGCAAAGC	ATGG	98–102	66	2	0.53/0.49	KJ881175
MaCh0303	2	CCCTGCATCTATCCGTCATT	TGTATCCCTGGAGTGCCTTT	TCCA	226–238	62	4	0.56/0.67	KJ881183
MaCh0312	2	GCATGCACCTCTGTCTCAAA	TGTGCATGTAAAGGTTAGTACATCA	AC	225–247	58	9	0.38/0.85*	KJ881184
MaCh0419	2	ATGAAGCTGCCATTTCAACC	CTATGTCCCATCCATCCACC	ATGG	127–147	65	5	0.60/0.55	KJ881187
MaCh0625	2	TTTGCTGTTTGAATCCTCCC	ACTACCCCACGGGTCTCTTT	TTG	111–132	59	3	0.37/0.40*	KJ881190
MaCh0661	2	GAGCCAATATCGTTGAGGCT	TCAAGATGAATGCTTCTTTGTAT	AC	149–161	34	6	0.12/0.70*	KJ881191
MaCh0726	2	TTCCATCTGTCCATCCTTTCTT	GATCCCAGTGACCTAGCCTG	TCCA	141–183	65	8	0.78/0.80	KJ881193
MaCh0799	2	CTTTGGGAGCCAGTTTTCAC	TGGAATTGAGATTTGTTTGTGAC	TATC	220–236	65	5	0.71/0.73	KJ881194
MaCh0834	2	TGTCTGCGACCCATGAGTAT	AGCCCAACTGAGACTGCCTA	GTT	232–247	65	5	0.42/0.71*	KJ881172
MaCh0129	3	AGTGCAATGTGGGTAGGCTC	CCAGGCGGTTTTGAGAATTA	CAT	162–171	62	4	0.44/0.66*	KJ881179
MaCh0141	3	CTGAGGGCCTAACAGGAACA	GCCTGGCCTACAAAGGTACA	CATC	228–260	64	8	0.81/0.86	KJ881180
MaCh0262	3	AGGACCCCTCTTGCAAGTTT	CCTGGCTAGCAGTCAGCTCT	TTG	234–252	65	7	0.92/0.81	KJ881182
MaCh0372	3	TCACAAAGGCACAAAGAACG	AAACTCTTTGCCAAGACCGA	CA	243–273	65	8	0.68/0.73	KJ881185
MaCh0581	3	CACTCACTTCCTTTTTCGTG	AGATCTAGTGTGGCAGAAAG	CCAT	153–185	62	7	0.56/0.77	KJ881188
MaCh0600	3	ATCCATTCCCCAGTTCTTCC	GCCTGGGCTAAAAGAAGTGA	TTG	249–261	52	3	0.37/0.63*	KJ881189
MaCh0824	3	GGGAGAGGTGGAAGTAGCTG	GGCTCCCTTAGAATTCTGCC	GATA	158–170	64	4	0.44/0.47	KJ881171
MaCh0866	3	GATGCTGAGTTTTCTGGAAGC	CAGTTGTCTTTGGATTGCCC	TAGA	147–171	65	7	0.88/0.73	KJ881173

N: number of individuals that successfully amplified. Note that two primers (MaCh0705 and MaCh0661) showed a lower rate of amplification success than the 22 others. Na: number of alleles. Ho/He: observed and expected heterozygosities (*: significantly deviating from Hardy–Weinberg equilibrium)

Ketamine (Imalgène 1000) and Xylazine (Rompun) and subsequently anti-sedated to facilitate awakening. Various physiological data, including blood samples, were collected. Blood samples were centrifuged in situ and DNA extractions were performed from buffy coats using QIAamp DNA Blood Mini Kits (Hilden, Germany).

An enriched DNA library was obtained by Genoscreen (Lille, France), by coupling multiplex microsatellite enrichment and sequencing on 454 GS-FLX Titanium platforms according to the method described in (Malausa et al. 2011). Enrichment of total DNA and the pipeline implemented in the program QDD (Meglecz et al. 2010) yielded primer sequences for 1,031 microsatellite loci. We chose 64 primer pairs based on results from this implementation, a large expected numbers of microsatellite repeats and an expected size of the fragments between 80 and 320 bp. Initial amplifications by PCR were performed in a final volume of 10 µl containing 5 µl of Multiplex Master Mix (Qiagen, Hilden, Germany), 0.2 µmol of each primer, 4 µl of pure water, and 1 µl of DNA extract. PCR conditions were as follows: 5 min at 95 °C, 30 cycles including 30 s at 94 °C, 1 min at 72 °C, and 1 min at 72 °C, and then 7 min at 72 °C. Amplification products were checked by electrophoresis in a 1 % agarose gel. At this stage, we selected 24 markers that amplified successfully (Table 1; we excluded one primer that also amplified human DNA to avoid cross-contamination). PCR products were sized using an ABI PRISM 3500XL sequencer (Applied Biosystems, Foster City, USA) with fluorescent dye-labeled primers and the 500 LIZ GenScan size standard. Alleles were scored with the software GENEMAPPER v. 5.0 (Applied Biosystems, Foster City, USA) and double-checked manually.

We performed basic population genetic analyses using GenAlex (Peakall & Smouse 2012). The number of alleles ranged from 2 to 9 (mean across loci: 5.8). Nine loci significantly deviated from Hardy–Weinberg equilibrium with a deficit in heterozygotes (Table 1), as expected in species where reproduction is not random. None of the loci exhibited any linkage disequilibrium. This is the first time that microsatellite loci were developed for this understudied and endangered primate species. These markers are especially useful for researchers working with sensible DNA, highly contaminated by human DNA. Future applications of these microsatellites are therefore numerous, ranging from population genetic studies with applications in conservation, to pedigree-based analyses of questions in social behavior and phylogeography.

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